Scientific Opinion on the assessment of the control measures of the category A diseases of Animal Health Law: African Horse Sickness

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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 African Horse Sickness (AHS). 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 duration of measures in these zones. The general methodology used for this series of opinions has been published elsewhere; nonetheless, specific details of the transmission kernels used for the assessment of the minimum radius of the protection and surveillance zones are shown. Several scenarios for which these control measures were assessed were designed and agreed prior to the start of the assessment. In summary, sampling procedures described in the diagnostic manual for AHS were considered efficient for all Equidae considering the high case fatality rate expected. The monitoring period (14 days) was assessed as effective in every scenario, except for those relating to the epidemiological enquiry where the risk manager should consider increasing the monitoring period, based on the awareness of keepers, environmental conditions and the vector abundance in the region. The current protection zone (100 km) comprises more than 95% of the infections from an affected establishment. Both the radius and duration of the zones could be reduced, based on local environmental conditions and the time of year of the first index case. Recommendations provided for each of the scenarios assessed aim to support the European Commission in the drafting of further pieces of legislation relating to AHS.

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Keywords: Disease control measures, African Horse Sickness, Equidae, vector borne disease, sampling procedures, monitoring period, protection and surveillance zones

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Summary

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

- the clinical and laboratory examination in their capacity to detect disease (or estimate the disease prevalence within an establishment), either in suspect or confirmed animals in a single establishment, or in establishments within 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, was agreed on, and published in a separate technical report (EFSA, 2020).

A qualitative assessment was carried out, based on available literature, of the effectiveness of clinical examination for infection with African horse sickness (AHS) virus in Equidae. Given the expected high case fatality rate and non-pathognomonic signs, it was determined that clinical examination alone cannot be used to confirm disease. Laboratory testing should be targeted at fallen stock (dead animals) in the first instance and further testing of other Equidae present could be used for the epidemiological enquiry, alongside vector surveillance. As data are poor for the average size of establishments with Equidae, and given the effectiveness of targeting fallen stock, it is recommended that within the protection zone (PZ) and surveillance zone (SZ), fallen stock should be targeted for laboratory testing rather than a random serological survey (in the absence of vaccination). Derogations from killing susceptible animals can be applied where vector surveillance and vector control measures are effective to prevent the onward transmission via infected midge species.

To answer ToR 2, and to assess the minimum length of time measures should be implemented in the protection and surveillance zones (ToR 3.2), an extensive literature search (ELS) was carried out. This ELS aimed to assess the average, shortest and longest period between the earliest point of infection of an equid with AHS virus, and the time of reporting of a suspicion by the competent authority. The average time to the reporting of a suspicion report was used then to assess the effectiveness of the length monitoring period. For scenarios related to carrying out the epidemiological enquiry, the existing length of the monitoring period for AHS (14 days) was considered sufficient but would not be effective for every scenario, and therefore, this could be increased to 21 days, based on a risk assessment of the vector abundance, environmental conditions and awareness of keepers. The lack of data for the incubation period in Asinine species or wild Equidae in Europe is highlighted as an uncertainty but given the likely low numbers in comparison to kept equines it is not expected to substantially change the opinion.

To assess the effectiveness of the minimum length of time the measures should be applied in the protection and surveillance zones, the average and the longest time assessed via the ELS were used, respectively. In this regard, the minimum length of time the protection zone (12 months) must be in place for, when based on existing legislation, was considered effective however this length of time could be reduced based on the outcome of a risk assessment considering local vector activity, environmental conditions and the time of year of the first index case.

To assess the effectiveness of the minimum radius to be implemented in the protection and surveillance zones (ToR 3.1), transmission kernels were used. These kernels represent the relative risk of transmission to each individual establishment from the affected establishment. As African horse sickness is a vector borne disease, the transmission kernel was based on vector dispersal models. However, due to the paucity of information for European outbreaks of AHS, data for bluetongue virus transmission by Culicoides midges in Europe was used instead. The minimum radius to be implemented in the protection zone (100 km) and the surveillance zone (150 km) were considered effective. The probability of transmission beyond the 100 km protection zone was less than 0.03; therefore, the radius for both the PZ and SZ could be reduced. As the size of zones is so large, a high number of establishments will fall under restriction but provided surveillance focuses on laboratory testing of fallen stock (dead animals), not all premises will need to be surveyed. The conditions under which the radius could be reduced are considered in detail in the opinion. It is important to note that...
the transmission kernels presented cover only some of the risk pathways associated with spread of the index case and they do not take account of movements of live animals and products off the establishment prior to confirmation.

A number of uncertainties around the population size of establishments with horses and other Equidae, the susceptibility of Equidae in Europe and whether clinical signs would be observed in all species, were considered.

In summary, the case fatality rate is considered to be so high, that laboratory testing of fallen stock would be effective in detecting disease, rather than any surveillance based on clinical signs alone. The transmission of AHS is dependent on vector abundance and activity and would therefore be highly seasonal in different parts of the European Union; therefore, a ‘one size fits all’ approach cannot be used.
Table of contents

Abstract................................................................................................................................................... 1
Summary.................................................................................................................................................. 3
1. Introduction.......................................................................................................................................... 7
   1.1. Background and Terms of Reference as provided by the requestor.................................................. 7
      1.1.1. ToR 1: sampling of animals and establishments for the detection of category A diseases in terrestrial animals........................................................................................................ 7
      1.1.2. ToR 2: monitoring period............................................................................................................. 8
      1.1.3. ToR 3: minimum radius of restricted zones and duration of the disease control measures in restricted zones........................................................................................................... 8
      1.1.4. ToR 4: prohibitions in restricted zones and risk-mitigating treatments for products of animal origin and other materials ............................................................................................ 8
   1.2. Interpretation of the Terms of Reference....................................................................................... 9
2. Epidemiology, and geographical distribution of AHS ....................................................................... 9
   2.1. Epidemiology................................................................................................................................... 9
   2.2. Geographical distribution of the disease........................................................................................ 10
3. Data and methodologies.................................................................................................................... 13
3.1. Methodologies............................................................................................................................... 13
3.1.2. Methodology used in ToR 2.......................................................................................................... 13
3.1.3. Methodology used in ToR 3.......................................................................................................... 13
3.1.4. Uncertainty .................................................................................................................................. 13
3.2. Additional information.................................................................................................................... 13
4. Assessment......................................................................................................................................... 14
4.1. Assessment of sampling procedures in the event of suspicion or confirmation of African Horse Sickness (AHS).............................................................................................................. 14
   4.1.1. In the event of a suspicion of AHS in an establishment where animals of the listed species are kept ........................................................................................................................................... 14
   4.1.2. For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU) 2016/429 in an AHS affected and officially confirmed establishment ........................................................................ 16
   4.1.3. For granting a specific derogation from killing animals of the categories of article 13.2 of the Delegated Regulation in an AHS affected establishment ........................................................................... 17
   4.1.4. For the animals of non-listed species kept in an AHS affected establishment.............................. 18
   4.1.5. For wild animals of the listed species within the AHS affected establishment and its surroundings ................................................................................................................................. 19
   4.1.6. For animals of listed species in the non-affected establishments located in a protection zone, and for non-affected establishments located in a protection zone with a radius larger than 3 km ........................................................................ 20
   4.1.7. For non-affected establishments located in a surveillance zone.................................................. 21
   4.1.8. Assessment of sampling procedures to grant derogations for animal movements ................... 22
   4.1.8.1. 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 ........................................ 22
   4.1.8.2. 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 .................................................................................... 23
   4.1.8.3. 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..................................................................................................................... 24
   4.1.8.4. From an establishment in a surveillance zone to pastures situated within the surveillance zone ..... 25
   4.1.8.5. From an establishment in a surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone ................................................................................. 26
   4.1.8.6. 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 .................................................................................................................. 27
   4.1.9. Assessment of sampling procedures for repopulation purposes .................................................. 28
   4.1.9.1. For the animals that are kept for the repopulation prior to their introduction ................................ 28
   4.1.9.2. In the event of unusual mortalities or clinical signs being notified during the repopulation .... 29
   4.1.9.3. For animals that have been repopulated ..................................................................................... 30
   4.2. Assessment of the length of the monitoring period ...................................................................... 30
   4.2.1. Results ....................................................................................................................................... 31
   4.2.2. Assessment................................................................................................................................. 33
   4.3. Assessment of the minimum radius and time periods of the protection and surveillance zones set in place subsequent to a disease outbreak ........................................................................ 37
   4.3.1. Assessment of the minimum radius .............................................................................................. 37
4.3.2. Assessment of the minimum period ........................................................................................................ 40
4.3.3. Uncertainty analysis.................................................................................................................................. 40
5. Conclusions and Recommendations ............................................................................................................ 41
References ..................................................................................................................................................... 46
Abbreviations .................................................................................................................................................. 47
Annex A – Definitions in EU legislation ......................................................................................................... 49
Annex B – Scenarios of ToR 1 ......................................................................................................................... 51
Annex C – Sampling procedures for AHS ........................................................................................................ 58
Annex D – Scenarios of ToR 2 ......................................................................................................................... 65
Annex E – Minimum radius and minimum period of duration of protection and surveillance zones .......... 68
Annex F – Uncertainty ..................................................................................................................................... 69
1. **Introduction**

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

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

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

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

ToR1.1 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.

ToR1.2 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.

ToR1.3 Establishments 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.
ToR1.4 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.

ToR1.5 Animals for laboratory examinations to ensure the detection of the relevant category A disease for the repopulation of affected establishments 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,
b) if relevant, suggest new treatments or procedures that can be effective to mitigate or to eliminate such risk

1.2. Interpretation of the Terms of Reference

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

a) The publication of 14 individual opinions, one per each of the diseases included in the list of category A diseases for terrestrial animals, with each of these opinions providing the answer to ToRs 1, 2 and 3. The current document is one of the 14 opinions covering ToRs 1, 2 and 3 for African Horse Sickness (AHS).

b) The publication of a unique opinion covering ToR 4 for all diseases listed (i.e. ToR 4 is not covered in this opinion).

c) To address ToR 1 (effectiveness of sampling procedures), EFSA agreed with the European Commission on 21 scenarios (based on different articles of the Delegated Act) for which the effectiveness of the sampling procedures will be assessed (Annex B). Although these scenarios will be assessed independently, some of them may be merged if the assessment 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 performed by assessing its ability to ensure that specific actions can be carried out without posing a risk of disease spread, if the monitoring period is calculated 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 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.

f) Scenarios 10, 11, 16 and 17 in ToR 1 (Annex B) were not relevant for AHS (as they refer to poultry), and therefore were not included in the assessment.

g) The duration of the monitoring period for AHS as described in Annex II of the Delegated Regulation is 14 days.

h) The minimum length of the radius of the protection zone (PZ) and surveillance zone (SZ) for AHS as described in Annex V of the Delegated regulation are 100 and 150 km, respectively.

i) The minimum duration of the measures in the PZ and SZ for AHS as described in Annex X and XI of the Delegated Regulation is 12 months for both zones.

2. Epidemiology and geographical distribution of AHS

2.1. Epidemiology

African horse sickness (AHS) is an arthropod-borne viral disease caused by one or more of the nine different serotypes of AHS virus (AHSV) belonging to the Reoviridae family, genus Orbivirus (Zientara et al., 2015), infecting Equidae, which are defined as wild or domesticated animals of the equine (including zebras) or asinine species or the offspring of crossings of those species.

AHS is a notifiable viral disease because of its severity and the potential risk it poses for global spread (Mellor and Hamblin, 2004; European Commission, online). It affects horses and other equids such as donkeys and zebras (Zientara et al., 2015). The disease has also been reported in dogs, which are considered as dead-end hosts (Spickler, 2015; Robin et al., 2016). Although some recent evidence suggests that they could be infected with AHSV, whether they play a role in the spread of the disease is unclear (Zientara et al., 2015; Oura, 2018). AHS is transmitted by infected midges (Diptera; Culicoides genus; in particular Culicoides imicola (present in the EU) is
considered the major field vector, while there are evidences that other species such as *C. obsoletus*, *C. pulicaris* (both present in the EU) and *Culicoides bolitinos* (not present in the EU) can act also as vectors (Mellor et al., 1990; Venter et al., 2000; Spickler, 2015). *Culicoides imicola* is distributed across the world\(^1\) from South Africa to southern Europe and from southern USA to southern China (Leta et al., 2019). AHS outbreaks occur mostly from spring to autumn in temperate regions, and after the rainy season in subtropical regions, especially in humid environments such as riverbanks and swampy areas (Zientara et al., 2003). However, AHS outbreaks also occur in previously AHS-free countries, probably due to a combination of climate change affecting the vector’s habitat and increasing international movement of animals, including illegal or not well-controlled equid movement (Robin et al., 2016; Leta et al., 2019). There is evidence that some outbreaks have been caused by virulent revertants of AHS live-attenuated vaccines (Weyer et al., 2013).

There are three principal forms of AHS as well as a mixed form. The most severe is the pulmonary form (peracute) with sudden and high fever (41–42°C), sweating, coughing, anorexia, respiratory distress and possible frothy nasal discharge leading to death in 24–48 h after the onset of dyspnoea; the case fatality rate for the pulmonary form is up to 95%. In the cardiac form (subacute), fever onset is more progressive (39–40°C in 10–12 days) and is followed by oedema provoking swelling of the head, neck and supraorbital fossae and sometimes, by petechial haemorrhages in the eyes. The case fatality rate in this form is about 50%, with death occurring 3–10 days after the apparition of oedema. The mildest form is a subclinical form (AHS Fever), with low-grade fever, anorexia, depression and congestion of the mucous membranes. It is generally non-fatal and sick animals recover within 10–15 days. The most common form is the mixed form, a combination of the lung and heart forms with death that can occur within 3–6 days after fever onset and a case fatality rate of about 70% (Zientara et al., 2003, 2015; Dennis et al., 2019). The incubation period for AHS in equids ranges from 3 days to 2 weeks (usually < 9 days), the cardiac form typically developing later than the pulmonary form (Spickler, 2015). AHS is typically fatal in horses, with the severity of the disease being influenced by strain virulence and immune status (vaccination), while the case fatality rate is lower in mules (around 50%) and African donkeys and zebra (around 5–10%) (van Rijn, 2019). In European and Asian countries, however, donkeys are moderately susceptible with a 10% mortality (OIE, 2019).

The only way to confirm AHS definitively is through laboratory testing based on the identification of AHSV, virus nucleic acid, viral antigens or specific antibodies. AHSV can be isolated from blood collected during the early febrile stage, taking into account that the duration of viraemia is shorter in horses (4–8 days) than in donkeys and mules (10–27 days) (Zientara et al., 2015). For virus isolation, the other samples of choice for diagnosis are spleen, lung and lymph nodes, collected at necropsy. Currently, real-time reverse transcriptase polymerase chain reaction (RT–PCR) assays for AHSV are widely used as first-line diagnostic tool. Confirmation of the presence of specific genome sections could also be performed with Seg-2-based PCR assays in order to identify the virus serotype. Virus isolation including serotyping with virus neutralisation tests can be performed, although, isolation is not routinely used for diagnostic purposes because this method is laborious, time-consuming, less sensitive and may pose a potential biosecurity risk. Antibodies against AHSV are detected by ELISA specific for VP7 antibodies which confirms AHSV infection, but the use of this diagnostic method is limited in acute forms, since infected horses may die prior to seroconversion. High throughput ELISAs are routinely used and are highly sensitive and specific, detecting all nine AHSV serotypes (OIE, 2019; van Rijn, 2019). Antibody detection by ELISA is mainly useful for active surveillance purposes in AHS-free zones since current commercial tests cannot differentiate infected animals from horses vaccinated with a live vaccine (Zientara et al., 2015).

### 2.2. Geographical distribution of the disease

AHS is endemic in eastern and central Africa, and northern South Africa, and spreads regularly to southern Africa. The nine above-mentioned serotypes (1–9) occur in central, southern and eastern Africa. In endemic areas, different serotypes of AHS may be active simultaneously. The disease is also detected sporadically in North Africa, from where it has occasionally extended into the Middle East and the Iberian Peninsula. AHS has not been recorded in Madagascar or Mauritius. AHS was recorded in Egypt in 1928, 1943, 1953, 1958 and 1971; in Yemen in 1930; and in Palestine, Jordan, Lebanon and Syria in 1944. In 1959, AHS serotype 9 occurred in the south-eastern regions of Iran. Outbreaks

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\(^1\) EFSA Story maps: African horse sickness [https://efsa.maps.arcgis.com/apps/MapJournal/index.html?appid=df1fa0739f144112957d2557f9e60a58](https://efsa.maps.arcgis.com/apps/MapJournal/index.html?appid=df1fa0739f144112957d2557f9e60a58)
followed during 1960 in Cyprus, Iraq, Jordan, Lebanon and Syria, as well as in Afghanistan, India, Pakistan and Turkey and between 1959 and 1961, more than 300,000 equids died. In 1965, AHS occurred in Algeria, Libya, Tunisia and Morocco and subsequently spread to Spain in 1966. Between 1987 and 1990, AHS serotype 4 occurred in Spain, with the source of infection being zebras (Equus burchelli) imported from Namibia. AHS was also confirmed in southern Portugal in 1989 and Morocco between 1989 and 1991, as a result of spread from Spain. In 1989, an outbreak of AHS serotype 9 occurred in Saudi Arabia and Yemen. Most recently, in 2020, AHS was reported for the first time in Thailand when serotype 1 was detected in horses (King et al., 2020), and a single infected establishment in Malaysia of unknown serotype (possibly as the result of movement of infected midge vectors or horses from Thailand). The most likely route for the introduction into Thailand was the movement of zebras from South Africa. To date, Thailand has reported 15 affected premises over eight regions and has vaccinated several thousand horses in response. The distribution of recent outbreaks, OIE official status and endemic areas are shown in Figures 1, 2 and 3.

**Figure 1:** Map of countries with endemic African horse sickness virus (Source: Zientara et al. (2015) Zientara et al. (2015); © OIE)
Figure 2: Map of countries with the OIE official free status for African horse sickness, 2020 (Source: OIE; © OIE)

Figure 3: Map of countries where outbreaks of African horse sickness were reported between 2015 and 2020 (Data sources: ADNS and OIE)
3. **Data and methodologies**

3.1. **Methodologies**

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

3.1.1. **Methodology used in ToR 1**

The average, median and maximum horse establishment size was estimated using ADNS outbreak data of Equine Infectious Anaemia and West Nile virus infection recorded since 2006, by dividing the number of susceptible exposed equines by the number of outbreaks (corresponding to the number of establishments). There is no data for establishments with other Equidae.

Quantitative estimation of sample size was not needed; instead a qualitative assessment of the clinical and laboratory procedures was performed.

3.1.2. **Methodology used in ToR 2**

To answer ToR 2, an extensive literature search (ELS) was outsourced by EFSA via an existing Framework Contract (OC/EFSA/ALPHA/2020/02 – LOT 2). The aim of this ELS was to answer the epidemiological question: ‘what is the average, shortest and longest period of time (measured as the number of days from the earliest point of infection with AHSV, to the time of declaration of a suspicion by the competent authority after the clinical investigation by an official veterinarian) for an outbreak of AHS to be reported?’.

To answer this question, an ELS on case reports, papers describing outbreaks or epidemics of AHS, and any other relevant grey literature or data, was carried out. For the inclusion criteria in the ELS, the earliest point of infection had to have been estimated by carrying out an epidemiological investigation. Papers and other sources of data, where the earliest point of infection was determined purely by subtracting a known incubation period from the date of the suspicion of the outbreak, were excluded. The ELS was restricted to studies conducted in Europe or describing results obtained in Europe. If none or very few articles were retrieved (less or equal to 5) in the first search, the search was extended to the rest of the world. The general protocol used for the ELS is shown in Annex 5 of the Methodology report (EFSA, 2020).

3.1.3. **Methodology used in ToR 3**

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

The functional form, parameter estimates and the 95% confidence or credible intervals for the parameters (where provided) of the best-fitting kernel were extracted from each study.

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

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

To estimate the duration of measures in the protection and surveillance zones, the outputs obtained from the ELS described in Section 3.1.2 were used. Further details can be found in the Methodology report (EFSA, 2020).

3.1.4. **Uncertainty**

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

3.2. **Additional information**

Data on Animal Population
Horse and donkey population and movement data are not systematically collected by Member States (MSs). Many horse and donkey establishments are not required to be registered by the competent authorities in the way livestock establishments are, unless the animals are being raised for slaughter. Many animals may be kept in livery (i.e. where owners pay to keep their horses on a weekly or monthly basis). Movements, not only for competition and leisure but also for exercising the animals for welfare, are common. The exception to these generalisations is for horses kept as livestock for meat production and horses which move internationally for competition or for breeding.

The number of outbreaks of Equine Infectious Anaemia and West Nile virus recorded since 2006 is 4,713, and the average establishment size is six animals (min-max, SD). Uncertainties related to these figures are discussed in Annex F.

With the implementation of the Animal Health Law, the registration of where horses are habitually kept will be mandatory. This will not impact on the evaluation of surveillance methodology although it will reduce the uncertainty pertaining to the population of Equidae in the European Union.

Environmental and climate data

AHSV is transmitted by Culicoides biting midges, which life cycle and activity are driven by temperature, humidity and wind speed (Purse et al., 2015; Miranda, 2017). These factors influence the life span, the flying and feeding activities, the extrinsic incubation period and the biting rate of the vector. Data from experimental and modelling infection rates for Orbiviruses in Culicoides, including AHSV, show that the threshold for virus replication (EIP – extrinsic incubation period) in the insect is 12°C, meaning that the virus transmission is halted at this temperature (Carpenter et al., 2011).

4. Assessment

4.1. Assessment of sampling procedures

4.1.1. Assessment of sampling procedures in the event of suspicion or confirmation of African Horse Sickness (AHS)

4.1.1.1. In the event of a suspicion of AHS 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 AHS in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2)).

Summary of sampling procedures

Council Directive 92/35/EEC requires the suspect establishment to be placed under restriction to prevent any movement of Equidae off the establishment, and that an official census is carried out for the Equidae population and for vector breeding sites. All Equidae must be examined, and a detailed clinical examination or an autopsy on the suspect or dead animals must be carried out, with samples taken when necessary for laboratory examinations. There are no details on the type or number of samples to be taken or the type of tests to be used. However, Council Directive 92/35/EEC allows...
clinical or epidemiological results to be the basis of confirming disease in the event of an epidemic (i.e. once infection is confirmed in a territory). All animals on the establishment, which test positive or with clinical signs, must be culled immediately.

Assessment

The European Reference Laboratory (EURL) for AHS determines the methods and reagents used in the national reference laboratories.

Given the almost 100% case-fatality rate (pulmonary form) expected with infection with AHS in a naïve non-endemic Equidae population, all fallen stock of susceptible species showing pathognomonic clinical signs should be tested for the presence of the virus by PCR and/or virus isolation. The EURL recommends laboratory diagnosis of AHS as the clinical signs and lesions may be confused with other infections (Anthrax, Equine Infectious Anaemia, etc.), or other pathologies, to the untrained eye. Clinical samples (EDTA blood and tissues) should be stored appropriately and handled in a level 3 containment facility.

It is important for the competent authority to consider the local population of Culicoides and their host preference (if any), the potential species involved in the transmission of AHV (ideally knowing their vector competence for the circulating serotype), their seasonal dynamics, mechanisms of overwintering and the existence of any plausible seasonally vector-free period. Vector presence and dynamics should be measured using standardised methods (i.e. UV suction light traps) (Miranda, 2017). An absolute vector-free period may not exist in some areas in Europe and indeed the definition of a seasonal vector-free period (Regulation (EC) 1266/2007) is for complete absence of Culicoides imicola, and less than five parous females captured in light traps of other Culicoides species. Temperature conditions also affect vector activity, virus replication (EIP – Extrinsic Incubation Period) and feeding behaviour; at relevant temperature thresholds should be taken into account.

According to the work on bluetongue (EFSA AHAW Panel, 2017), where the same species of vectors are involved, there is no evidence that the use of insecticides and repellents reduces the transmission of bluetongue virus (BTV) in the field, although this may reduce host/vector contact (by a reduction in the adult females feeding activity). Subsequent to the publication of the above-mentioned opinion two other articles were published (Benelli et al., 2017; Meloni et al., 2018) where a decrease in the vector population was reported by applying adulticides and larvicides in farm premises; nonetheless, no decrease in transmission was observed. By using pour-on insecticides (e.g. Deltamethrin, Permethrin), the protection of animals (including horses) obtained is lower than that provided by vector-proof establishments. The need for frequent reapplication, low diffusion on the different body parts (i.e. legs, belly) and the cost, means this is likely to be unfeasible for any but high value animals. According to scientific literature, a high level of efficacy (up to 86%) of pour-on insecticides is difficult to achieve under field conditions and there are few data on the reduction of engorged females. In addition, application of insecticides in the environment to kill adult or larval Culicoides is unlikely to be effective in Europe for either controlling vector population or decreasing AHV transmission. Environmental control of Culicoides is very limited since there are no effective officially approved larvicides to be applied in breeding sites (Miranda, 2017). Physical removal or control of breeding sites is also of limited efficacy due to the widespread of potential breeding sites in both the establishment, and the surroundings. Adulticides applied to the environment would also be of little efficacy, and they may lead to a potential environmental health issue.

Among other control methods for reducing host/vector contact, it was concluded that stabling in a vector protected establishment is effective where a high level of containment can be attained, and that insecticide treated meshes applied over windows substantially reduce vector populations inside stables (mean reduction of 99.7% of Culicoides population compared to outdoor population).

Development of new procedures

Clinical examination alone is not sufficient to diagnose AHS in a susceptible population. Laboratory examination should always be used. For the purposes of confirming disease, EDTA (not clotted) whole blood from all live animals in the suspect establishment and tissue samples from the spleen, lung and lymph nodes at necropsy should be taken for real-time RT–PCR tests. Clotted blood can be used for serology and could be used where the chronic or per-acute infection is suspected, or in populations of Equidae which are less susceptible to acute infection. Two PCR tests for different genetic targets should be used, or a PCR test and virus isolation or in combination with positive serology for unvaccinated horses.

2 The criteria for the Vector Protected Establishment are laid down in Annex II of the bluetongue Regulation as amended by Commission Regulation (EC) No 456/2012 and are based on those in the OIE Terrestrial Animal Health Code (2011).
In an epidemic event, confirmation of suspicion on the basis of clinical diagnosis alone or on the basis of an epidemiological link (given this is a vector-borne disease) may occur, but this is an inexact procedure and laboratory confirmation would still be recommended, but should not delay culling if clinical suspicion is strong. Confirmation of the presence, the species composition and seasonality of Culicoides spp. around the establishment is also needed for assessing vector control methods and to define period of low or nil virus transmission.

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

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

Summary of sampling procedures

There are no rules or guidance for the sampling procedures and there is no EU diagnostic manual. The EURL has recommended tests for virus isolation and viral RNA detection.

Assessment

The only way to definitively confirm AHS is through laboratory testing based on the identification of AHSV, virus nucleic acid, viral antigens or specific antibodies. To identify the origin of infection, not only should all epidemiological links be identified, but sequence analysis of the virus strain may also contribute to identify a source or likely geographic origin.

Data on outbreaks in South Africa suggest in a herd of unvaccinated horses, between 10% and 20% become infected, based on annual sentinel surveillance (Grewar et al., 2020). In Ethiopia, about 34% of equids and up to 50% of donkeys have been exposed to AHSV (Spickler, 2015).

Development of new procedures

In order to inform the epidemiological enquiry, each animal (dead or killed) present should be tested for virus or viral RNA detection and antibody detection, where there are fewer than 60 animals...
present; this is the sample needed in order to detect a 5% prevalence with 95% confidence. Where there are more than 60 animals of listed species present, a statistical sample (to detect 5% prevalence with 95% confidence) could be taken to inform the epidemiological enquiry, focussing on those with the highest risk contact through potential vector transmission (shared stabling, shared pasture).

Guidance on clinical samples should include taking whole blood (not clotted) for viral RNA detection by real-time RT–PCR. Tissue samples of carcases from the spleen may also be used. In the event of listed species such as zebra, African donkeys or mules being present, as the case fatality rate is expected to be lower (10% for donkeys and 50% for mules), serology should also be undertaken using clotted blood samples to verify the extent of infection (as some animals might not show clinical signs).

In terms of preventive culling of Equidae, this is not considered effective in preventing spread when a vector borne disease is present and therefore should be considered based on a risk assessment. If preventive culling is carried out, confirmation should be done by virological testing of all culled animals (if more than 60 animals in the premise, a statistical sample to detect 5% prevalence with 95% confidence must be taken).

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 AHS 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.

**3rd Scenario of sampling procedures**

- ToR 1.1 and ToR 1.2 in accordance with Mandate
- Article 13(3)c of the Delegated Regulation

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

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

**Summary of sampling procedures**

Previous Council Directive 92/35/EEC requires all Equidae, which are infected with or present clinical signs for AHS to be culled immediately.

**Assessment**

Not all animals will become infected on an affected farm as it will depend on vector abundance, vector competence and capacity, activity and whether other animals can be protected from vector blood feeding. Data from South Africa outbreaks suggest between 10% and 20% (Spickler, 2015) of unvaccinated horses in an establishment become infected. Provided all infected animals are culled as quickly as reasonably achievable, so they are no longer a source of virus for the local vector
population, and provided effective vector control is implemented, it is possible that not all animals will become infected and therefore these animals can be kept alive.

In order to derogate from culling, all animals should be protected from vector attack as per recommendations in Section 4.1.1.1, and undergo regular laboratory testing as indicated below; only those with negative results and where vector transmission can be ruled out (by expert entomological advice and based on lack of suitable vectors, absence of vector activity, effectiveness of vector proofing etc) should be considered for a derogation on the basis of a risk assessment by the competent authority.

In the event that a derogation is considered for a PCR positive animal(s), these animals should be protected from vector attack as per recommendations in Section 4.1.1.1, and undergo regular laboratory testing as indicated below; a clinical examination should also be carried out each day as severe clinical cases may need to be euthanised. After 40 days minimum and once the animals receive negative PCR results and positive serology results and where vector transmission can be ruled out (by expert entomological advice and based on lack of suitable vectors, absence of vector activity, effectiveness of vector proofing etc.) the animals may be released from vector protected accommodation.

**Development of new procedures**

The incubation period for AHS in equids is 3 days to 2 weeks (usually < 9 days) (Spickler, 2015); therefore, testing should be carried out every 3–7 days as the minimum time required to capture the minimum incubation period after which an infected animal may test positive.

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

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

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

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

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

**Summary of sampling procedures**

There are no specific guidelines on sampling procedures for the examination of non-listed species.

**Assessment**

African horse sickness is species specific and there is no evidence that non-listed species are involved in transmission. The virus is not contagious therefore there is no scientific reason to test other animals present at the affected establishment.

Dogs can be infected by eating meat from infected horse carcases or through the bites of infected midges, but it is unclear whether they play a role in the epidemiology of disease (Spickler, 2015; Robin et al., 2016; Oura, 2018; OIE, 2019). The case fatality rate is very high in such dogs and any dead
dogs should be tested but any further actions should focus on tracing the infected/contaminated carcases to dispose of the products correctly by rendering. Therefore, no other testing is recommended in other species.

Development of new procedures

Development of new procedures is not needed as no animal species (other than the listed species) are considered epidemiologically relevant for the transmission of the virus (based on available scientific literature).

4.1.1.5. For wild animals of the listed species within the AHS 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.

Summary of sampling procedures

There are no specific guidelines on sampling procedures for the examination of wild animals of the listed species.

Assessment

Wild Equidae in the restricted zones must be considered.

There are no data on the susceptibility of rare native breeds of equids, such as Przewalski’s horse (Equus ferus przewalskii) or ponies, such as the Pottoka, the Exmoor pony, the Konik Polski, the Yakut pony and the Hucul (all breeds of Equus caballus) all of which are discrete wild populations interspersed throughout Europe. There are also several populations of ‘feral’ or ‘rewilded’ horses and ponies, such as the Camargue horse, the Karakachan horse, the Welsh and Dartmoor ponies.

Development of new procedures

For the wild or feral Equidae, as these various breeds are expected to, but may not show severe clinical signs and high case fatality rates, testing of dead animals of wild populations should be undertaken, and in the event of suspicion of infection in these populations, samples taken for laboratory tests. Suspicion of infection may be based on proximity to the index case, vector activity and the source of infection into the index case. Wildlife experts would be able to provide advice in these exceptional circumstances.
4.1.1.6. For animals of listed species in the non-affected establishments located in a protection zone, and for non-affected establishments located in a protection zone with a radius larger than 3 km

Here, Scenario 6 and 7 have been assessed together, providing an assessment of 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, and for the number of establishments to be sampled in the protection zone where the radius of the protection zone for AHS is larger than 3 km.

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

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

1) It concerns the examination and sampling of animals within the protection zone
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) On these establishments, 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 a category A disease

**7th Scenario of sampling procedures**
- ToR 1.3 in accordance with Mandate
- Article 26(5) of the Delegated Regulation

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

1) It concerns the sampling of establishments within a protection zone with radius larger than 3 km
2) Sampling of the non-affected establishments of kept animals of listed species in the protection zone
3) In a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km
4) In case of a radius larger than 3 km, official veterinarians may not visit all establishments, only a sample of those.
5) 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
6) The purpose of sampling procedure is to ensure the detection of the disease if the disease is present in any of these establishments

**Summary of sampling procedures**

Council Regulation 92/35/EEC establishes the length of the radius of the protection zone for AHS of at least 100 km. Under this regulation, all holdings containing Equidae within 100 km must be identified and the official veterinarian must conduct periodic visits to these holdings; a clinical examination of the Equidae including, if necessary, the collection of samples for laboratory examination must be also carried out. A record of visits and findings must be kept.

In this regulation, there are no specific guidelines on the number of animals to be sampled within these establishments.

**Assessment**

The most likely transmission pathway for AHS is through infected vectors (short as well as long-distance movement), provided live animal movements, movements of semen or use of contaminated biological products that could have occurred prior to the notification have been ruled out. In Section 4.3.1, an assessment of the effectiveness of the radius of the protection and surveillance
zones was carried out; the relative probability of transmission of AHS (if transmission occurs) beyond a radius of 75 km was found to be 5% or lower (depending on the specific kernel selected). Further, in this section it is shown that the probability of transmission within a radius of 50 km is 90%, compared to 10% beyond this radius. Based on this, it can be concluded that sampling all establishments (and all animals in these establishments) within a 75 km radius would allow the detection of AHS virus (if the virus is present) with a 95% level of confidence (assuming 100% sensitivity of the diagnostic method for virus detection), and with a 90% confidence if establishments within a 50 km radius were to be sampled.

Development of new procedures

When the disease is first introduced in a country, and given the importance of early detection, listed species present should receive a visit from veterinary services to conduct clinical examinations and take samples for laboratory detection of viral RNA. Given the incubation period is a minimum of 3 days, between 3–7 days should be used as the interval for sampling and testing in the absence of vaccination. As there may be few Equidae on each establishment, all animals of listed species should be tested, particularly given the likelihood of clustered cases as a result of vector distribution. Where the establishments have fewer than 60 animals, all animals should be sampled. Where there are 60 animals or more, in a single epidemiological group, a statistical sample to give 95% confidence there is less than 5% prevalence should be taken. This should take place in establishments within the 75 km from the affected establishment if a 95% confidence level is to be achieved (if 90% confidence is aimed, then all establishments within a 50 km radius should be sampled).

It might not be feasible to clinically examine all horses within a 100 km-PZ given the time taken to identify and visit premises, and the resources available to carry out an examination.

The sampling of establishments should be carried out prioritising establishments with plausible epidemiological links and those within a closer distance from the affected establishment, expanding the radius of the sampling from the index case. All keepers should be advised to report any unusual mortalities or clinical signs associated with AHS infection, this will also allow the veterinary services to target those establishments for laboratory tests of all listed species present.

Vector surveillance must be carried out to detect breeding sites, parous or blood-fed Culicoides females in and around establishments with listed species present.

All listed species should be housed and protected from vectors, particularly during vector active periods.

In the event that several outbreaks are detected and not all establishments within the 75 km zone can be sampled, periodic visits to all establishments within the 75 km should be carried out as described above. Laboratory sampling should focus on establishments closer to the index case with an outward increasing series of zones progressively tested; vector surveillance, i.e. abundance, nulliparous/parous rate, will determine the periods of risk of transmission of the virus, and also the radius over which sampling should be prioritised. Establishments with unusual mortalities and/or clinical signs associated with AHS should also be prioritised. If there are any establishments present that have listed species such as zebra, donkeys, asses or ponies which may be less likely to show clinical signs, these animals should be targeted for active surveillance with laboratory testing. Enhancing passive surveillance by increasing awareness among keepers, owners, the horse industry and the general public, would be of high importance. Vector surveillance is required to find out the periods and the likely species involved in transmission, which will also be relevant for the housing of animals.

In the event that vaccination is used as an emergency measure, other important facts should be considered, such as the fact that serology cannot be used unless there is a DIVA vaccine. PCR may also be affected by vaccination; therefore, the diagnostic protocol must be set up according to the vaccine used. As this opinion is presuming vaccination will not be used, this will not be considered further.

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

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

Council Regulation 92/35/EEC establishes the length of the radius of the surveillance zone for AHS on at least 150 km (extending 50 km from the protection zone). Under this regulation, all holdings containing Equidae within 150 km must be identified and the official veterinarian must conduct periodic visits to these holdings; a clinical examination of the Equidae including, if necessary, the collection of samples for laboratory examination must be also carried out. A record of visits and findings must be kept. In this regulation vaccination is prohibited in the surveillance zone.

Assessment

Based on the results presented in Table 4, Section 4.3.1 where an assessment of the effectiveness of the radius of the protection and surveillance zones is carried out, the probability of transmission (given that transmission occurs) beyond 100 and 150 km is very similar (between 0.1 and 3% beyond 100 km, and between 0.1 and 2% beyond 150 km). The kernels provided in this section, also show that although spread beyond the 150 km may happen, there is a very small relative probability for that to occur.

Development of new procedures

As described for the protection zone, periodic visits to all establishments within the 150 km should be carried out as described above. Laboratory sampling in the surveillance zone should focus on establishments with unusual mortalities and/or clinical signs associated with AHS. If there are any establishments present that have listed species such as zebra, donkeys, asses or ponies which may be less likely to show clinical signs, these animals should be targeted for active surveillance with laboratory testing. Enhancing passive surveillance by increasing awareness among keepers, owners, the horse industry and the general public would be of high importance. Vector surveillance is required to find out the periods and the likely species involved in transmission, which will also be relevant for the housing of animals.

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).
Summary of sampling procedures

Council Regulation 92/35/EEC allowed the movement of Equidae from an establishment directly for emergency slaughter to a slaughterhouse in the PZ or the SZ if none were available in the PZ, provided the slaughterhouse is designated by the competent authority and the transport is under official supervision. No testing requirements are explicitly stated, but all establishments in the PZ should be periodically visited and clinical examinations carried out with lab samples if necessary.

Assessment

As already stated, clinical examination of listed species is not sensitive enough to confirm disease. Official designation of slaughterhouses makes certain that records are kept, biosecurity is maintained, animals inspected ante- and post-mortem and meat would be stamped. As this is a vector borne disease, vector treatment of the transport and disposal/disinfection of the bedding, feed and water in the vehicle should be carried out.

Insecticide treatment of the animals themselves is not recommended due to the withdrawal period for human consumptions. Horse meat for pet food must be treated to prevent infection of dogs.

Development of new procedures

Testing to provide confidence of 95% of less than 5% prevalence in the establishment means taking laboratory samples from 60 animals for virus identification tests. Real-time RT–PCR on EDTA blood should be taken and give negative results for the animals before they move, and they should be housed in vector proof accommodation for at least 9 days (as the average incubation period).

Where there are fewer than 60 animals present in the single epidemiological unit, all animals should be tested.

The midge vectors are most active during dawn and dusk therefore transport to the abattoir and slaughterhouse should only happen with vector proofed transport vehicles, taking account of animal welfare transport regulations.

4.1.2.2. 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).
Council Regulation 92/35/EEC allowed the movement of Equidae from an establishment directly for emergency slaughter to a slaughterhouse in the PZ or the SZ if none were available in the PZ, provided the slaughterhouse is designated by the competent authority and the transport is under official supervision. No testing requirements are expressly stated, but all establishments in the PZ should be periodically visited and clinical examinations carried out with lab samples if necessary.

**Assessment**

As above, provided all necessary precautions to prevent vector feeding activity during transport and slaughter, and laboratory testing is carried out, the derogation can be applied.

**Development of new procedures**

As above testing to give 95% confidence of less than 5% prevalence, should be carried out prior to the move of the animals.

The midge vectors are most active during dawn and dusk; therefore, transport should only happen during the day. The slaughterhouse must have a vector-proof establishment.

4.1.2.3. 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.

**Summary of sampling procedures**

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

1) It concerns 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

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**12th Scenario of sampling procedures**

- ToR 1.4 in accordance with Mandate
- Article 28(5) and article 37 of the Delegated Regulation

**13th Scenario of sampling procedures**

- ToR 1.4 in accordance with Mandate
- Article 43(5) and article 44 of the Delegated Regulation

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

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

Council Regulation 92/35/EEC allowed the movement of Equidae from an establishment directly to a slaughterhouse in the SZ or the PZ if none were available in the SZ, provided the slaughterhouse is designated by the competent authority and the transport is under official supervision. No testing requirements are expressly stated, but all establishments in the SZ should be periodically visited and clinical examinations carried out with lab samples if necessary.

Assessment

As above, provided all necessary precautions to prevent vector feeding activity during transport and slaughter, and laboratory testing is carried out, the derogation can be applied. For movement of listed species from the free zone to a slaughterhouse in the Restriction Zones provided animals are killed immediately and a clinical examination is carried out (and no animals have clinical signs) there is no requirement for testing.

Development of new procedures

As above for testing to give 95% confidence of less than 5% prevalence. The midge vectors are most active during dawn and dusk; therefore, transport should only happen during the day.

4.1.2.4. From an establishment in a surveillance zone to pastures situated within 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 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.

- **14th Scenario of sampling procedures**
  - ToR 1.4 in accordance with Mandate
  - Article 43(5) and article 45(1) of the Delegated Regulation

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

1. It concerns kept animals of listed species from establishments located in the surveillance zone
2. To grant derogation for movement from the surveillance zone
3. 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
4. Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures

Council Regulation 92/35/EEC did not provide management measures for such a movement.

Assessment

As this is a vector borne disease, during the vector season, moving animals long distances to new pastures could potentially introduce infection to a new area. However, all the establishments within a specified zone are considered to be at the same risk of exposure to the pathogen. Therefore, this movement would not necessarily increase the risk for the whole SZ.

If the Equidae are in vector proofed accommodation prior to the move and have tested negative after the monitoring period, they could be moved in transport under official supervision with little risk of introducing disease.

In case the movement happened while the animal was incubating disease, on arrival it would usually be sensible to place the animal in vector proofed accommodation and retest. This is not possible if the animal is moving to pasture; therefore, the pre-movement accommodation, vector proofed transport and testing procedure must be applied, as described in Section 4.1.1.1.
Development of new procedures

Clinical examination alone is not effective to detect disease. Any animals to be moved permanently and not just for exercise should receive negative test results for the presence of the virus prior to movement. As above for testing to give 95% confidence of less than 5% prevalence.

The midge vectors are most active during dawn and dusk; therefore, vector proof transport should only happen during the day. Provided animals are not destined for slaughter, pour on repellents/insecticides can be used. Avoid pastures with high vector abundance or with high density of other livestock which may attract vectors.

4.1.2.5. From an establishment in a surveillance zone to an establishment belonging to the same supply chain, located in or outside 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 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.

Summary of sampling procedures

Council Regulation 92/35/EEC did not allow such a movement. No other specific guidelines for this type of movement have been found.

Assessment

The SZ is a large area, and therefore, there is potential that this type of movement is a considerable distance from the index premises and the risk of introducing infection to a new region is greater. Any movements should not take place until the monitoring period has elapsed at the index establishment and all visits, examinations and sampling of Equidae in the establishments in the 3 km have taken place (and negative results received).

For movements to the outside of the restriction zones, each animal should test negative prior to the movement and there must be vector control in place for the transport and the pre-movement accommodation. The animals should be in vector proof accommodation for the duration of the period since disease was suspected on the index case establishment.

Development of new procedures

Test every animal on entering and leaving vector proof accommodation. Move the animals in vector proof transport to the new establishment. When moving to an approved slaughterhouse the transport should be under official supervision.
4.1.2.6. 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.

Summary of sampling procedures

This move is not allowed in the Council Regulation 92/35/EEC. No other specific guidelines for this type of movement have been found.

Assessment

The Seasonal Vector Free period (SVFP) should be established, as laid out in Annex II of the bluetongue Regulation as amended by Commission Regulation (EC) No 456/2012 and is based on those in the OIE Terrestrial Animal Health Code (2011). In general, animals may move during the seasonal vector free period. The SVFP should be determined for each region knowing the Culicoides species composition, seasonal dynamics and environmental drivers such as temperature.

At temperature extremes transmission is reduced because either the vector does not survive beyond the shortened EIP (at high temperatures) or the biting rate is lower than the lengthened EIP (at low temperatures). The highest rate of transmission occurs at a temperature when midges survive, they have a high biting rate and a concurrent short EIP (Wittmann et al., 2002). It is important to note that when the Extrinsic Incubation Period is longer than the expected life span of the midge, virus transmission will cease. Therefore, in terms of the length of the restrictions in the PZ (12 months) this could also be reduced, based on the life cycle of the Culicoides vector and the environmental temperatures. Modelling suggests that at an average daily temperature of 20°C, 99% of vectors will die within approximately 25–30 days, as the average Extrinsic Incubation Period is between 10 and 12 days, transmission will continue; at 15°C average daily temperature the EIP is only marginally longer than the expected survival of 99% of the vector population; therefore, there may be virus still present in midges even though the transmission rate will be lower; at 10°C, 99% of the midges will survive for at least 100 days and the EIP is estimated much longer than 100 days; therefore, transmission will cease (Figure 4).

The model also shows that, in terms of vector transmission, the duration of an outbreak in a stable host population size can be more than 100 days when the average daily temperature is 15°C; at 20°C, it could be between 19 and 99 days, and at 25°C, it may be as short as 10–92 days. That is, there is little difference in the duration when average daily temperatures are above 15°C.

Therefore, depending on the average daily temperatures for the duration of the monitoring period a derogation to move Equidae from non-affected establishments in a zone may be possible.
Development of new procedures

Provided the seasonal vector free period is established and providing there are no surviving Equidae acting as a virus reservoir, the risk manager should make an assessment of the temperature and vector population to allow any movements to happen.

Animals should receive negative test results for the presence of the virus prior to movement. As above for testing to give 95% confidence of less than 5% prevalence.

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.
Summary of the sampling procedures

No specific guidelines have been found.

Assessment

If animals are sourced from the free area there is no need for testing, provided the animals do not show clinical signs on the day of the movement and there have been no reports of increased mortality or suspect clinical signs in the herd during the monitoring period.

Development of new procedures

Animals with clinical signs or from herds where animals had clinical signs, including unexplained mortality, should not be used for repopulation.

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.

Summary of sampling procedures

No specific guidelines have been found.

Assessment

As AHS is a vector borne disease, it is not known whether virus could overwinter in the vector population as it is temperature dependent (see Section 4.1.2.6). Vector control in open air farming establishments will be difficult to implement and the monitoring period may not be long enough if there is over-wintering. Therefore, this is not possible to assess in general and an epidemiological survey of the vector population, which should include as assessment for each region of the Culicoides...
species composition, seasonal dynamics and environmental drivers such as temperature, should be undertaken.

As for Section 4.1.2.6, the risk manager should require an assessment of whether infected vectors could be present at the establishment before repopulation is considered.

As in previous sections, if animals show clinical signs or there are any mortalities suggestive of infection, the animals should be tested.

**Development of new procedures**

As for the confirmation of disease on a suspect establishment, laboratory samples should be taken. Clinical signs (or the absence of) alone are not effective to rule out disease. Monitoring should continue for at least 40 days depending on the vector activity at the establishment.

See Section 4.1.1.1 for details about confirming disease in a new suspect establishment.

### 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.

**Summary of sampling procedures**

No specific guidelines have been found.

**Assessment**

Surveillance of fallen stock should be used during the monitoring period, considering the season, environmental conditions and vector abundance.

**Development of new procedures**

As for Section 4.1.1.1.

### 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).

Annex E in this manuscript describes the seven scenarios for which an assessment of the length of the monitoring period for AHS had been requested.
For the assessment of this ToR, the methodology described in section 2.2 of the Technical Report (EFSA, 2020) was followed. In essence, in order to assess the length of the monitoring period, the purpose of this monitoring period for each of the scenarios was ascertained.

To answer all scenarios except scenario 5, an extensive literature search (ELS) on the average, shortest and longest period of time between the earliest point of infection of an animal with an AHS virus, and the time of reporting of a suspicion by the competent authority, was carried out. The time period between reporting of a suspicion and the notification of the disease was also assessed. Several outcomes were designed for the ELS as shown in the protocol, and the results are presented below.

To answer scenario 5, a literature search was conducted by EFSA on the seroconversion period in horses, as well as the time when antibodies are not anymore detectable in blood, with the outputs being discussed with relevant experts.

4.2.1. Results

A search was carried out identifying 323 references published after 01/01/1987 (because of the small number of references retrieved for European countries (n = 3), the selection was extended to the rest of the world). Among the 323 references, 12 were selected to be included in the qualitative review. The full selection process is displayed in Figure 5.

The majority of the references reported dates instead of periods (nine references out of 12); these dates were used to calculate the periods of interest. Table 1 provides 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.

**Figure 5:** PRISMA diagram AHS Monitoring period ELS

The majority of the references reported dates instead of periods (nine references out of 12); these dates were used to calculate the periods of interest. Table 1 provides 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.
Information on the main outcome of interest – the period between the earliest point of infection and the suspicion report – was retrieved from three references. The article from Mellor et al. reports an observed period of 26 days between the introduction of an AHS-infected animal (undetected index zebra case) and the suspicion report in susceptible horses (Mellor, 1993). This period includes both the extrinsic incubation period of the vector (Culicoides) (Wittmann et al., 2002; Carpenter et al., 2011) and the incubation period of the horses (OIE, 2013).

In contrast, the South African papers of Meiswinkel (1998) and OIE (1999) report the time between the (probable) first infection and the suspicion report in the same animal, with values of 8 and 10 days, respectively.

The observed differences in these values might be related to several factors: (i) the level of awareness in the outbreak country (Spain being an AHS-free country versus South Africa having a controlled zone) and, (ii) the duration of the viraemia, the morbidity and the mortality among the index case species (there is a longer viraemia and lower morbidity/mortality in zebra compared to horses) (OIE, 2020c). No information was retrieved for other domestic equine species such as donkeys or mules.

Based on extracted data, the median/mean period estimated was 10/15 days, with a shortest period of 8 days, and a longest period of 26 days.

As very few references were obtained, data were extracted for other periods such as the period from suspicion to the suspicion report, period from suspicion to confirmation and period from suspicion report to confirmation, with the aim to reconstruct the period of interest (period between the earliest point of infection and the suspicion report) using these partial periods (Table 2).

### Table 1: Summary of the AHS extraction for the ‘period between earliest point of infection and suspicion report’

| Reference | Country     | Outbreak year | Period between earliest point of infection and suspicion report (days) |
|-----------|-------------|---------------|---------------------------------------------------------------------|
| Mellor (1993) | Spain       | 1987          | 26                                                                   |
| Meiswinkel (1998) | South Africa | 1996          | 8*                                                                  |
| OIE (1999) | South Africa | 1999          | 10                                                                  |

*: Although this is not very clear from the description, the date of first death seems to correspond to the date of suspicion report. Moreover, in the South African outbreak reported to the OIE in 1999, there was no period between the first death and the suspicion report.

Information on the main outcome of interest – the period between the earliest point of infection and the suspicion report – was retrieved from three references. The article from Mellor et al. reports an observed period of 26 days between the introduction of an AHS-infected animal (undetected index zebra case) and the suspicion report in susceptible horses (Mellor, 1993). This period includes both the extrinsic incubation period of the vector (Culicoides) (Wittmann et al., 2002; Carpenter et al., 2011) and the incubation period of the horses (OIE, 2013).

In contrast, the South African papers of Meiswinkel (1998) and OIE (1999) report the time between the (probable) first infection and the suspicion report in the same animal, with values of 8 and 10 days, respectively.

The observed differences in these values might be related to several factors: (i) the level of awareness in the outbreak country (Spain being an AHS-free country versus South Africa having a controlled zone) and, (ii) the duration of the viraemia, the morbidity and the mortality among the index case species (there is a longer viraemia and lower morbidity/mortality in zebra compared to horses) (OIE, 2020c). No information was retrieved for other domestic equine species such as donkeys or mules.

Based on extracted data, the median/mean period estimated was 10/15 days, with a shortest period of 8 days, and a longest period of 26 days.

As very few references were obtained, data were extracted for other periods such as the period from suspicion to the suspicion report, period from suspicion to confirmation and period from suspicion report to confirmation, with the aim to reconstruct the period of interest (period between the earliest point of infection and the suspicion report) using these partial periods (Table 2).

### Table 2: Summary of the AHS extraction for the periods in the outbreak reporting process

| Period (days) | Ref. | n | Median | Mean | Min | Max |
|--------------|------|---|--------|------|-----|-----|
| First suspicion* and suspicion report | Portas et al. (1999), OIE (2012), Weyer et al. (2012), Grewar et al. (2013), OIE (2020a,c) | 5 | 0 | 9 | 0 | 30 |
| First suspicion* and confirmation | Rodriguez et al. (1992) | 1 | 11 | 11 | 11 | 11 |
| Suspicion report and confirmation | Portas et al. (1999), OIE (2012), Weyer et al. (2012), Grewar et al. (2013), OIE (2020a,b,c) | 6 | 3 | 4 | 2 | 7 |

*: Based on the first observed clinical signs of AHS or first death.

Six references provided information on the period between the first suspicion (based on first observed clinical signs or death) and the suspicion report (Portas et al., 1999; OIE, 2012; Weyer et al., 2012; Grewar et al., 2013; Weyer, 2017; OIE, 2020a,c), and from these references 5 values were extracted (Table 2). In the outbreaks in South Africa and Thailand (OIE, 2012; Grewar et al., 2013; OIE, 2020b), the trigger for suspicion was described as unexpected horse deaths. During the 2011 South African outbreak in the controlled area, the suspicion report occurred the day the first case died. In Thailand, a previously AHS-free country, 20 days occurred before the first AHS-related death and the suspicion by the private veterinary, and 10 days between this suspicion and the suspicion report to the disease control and veterinary services (i.e. total of 30 days). On the other hand, the Portas et al. (1999) study indicated that the suspicion report occurred 2 days before the first case died. It is important to note that the level of awareness and preparedness in Portugal was very high at that time.
because of the outbreak in neighbouring Spain. In addition, the OIE report of the Malaysian outbreak (OIE, 2020a) indicates a period of 14 days between the suspicion by the horse owner and the date of suspicion report to the veterinary authorities.

Our findings pertaining to the period between the first clinical signs and the suspicion reports indicate that the onset of AHS clinical signs among the index case is rarely the trigger for outbreak suspicion, especially in an AHS-free setting. Indeed, in several cases such as the emergence of AHS in Thailand, suspicion took place after (several) abnormal equine deaths occurred.

Finally, eight references reported the period between suspicion and confirmation report; the reported period varied between 2 days (OIE, 2012; Weyer, 2017; OIE, 2020b), and 7 days (OIE, 2020a).

Based on data from Table 2, we could reconstruct the period of interest as:

- Average period = 1 incubation period (5 days\textsuperscript{3} (OIE, 2013; Weyer, 2017), plus the period between clinical signs onset and death (7 days (OIE, 2013, 2020c), plus the median/mean period between first death and suspicion report (0/9 days) = 12/21 days
- Shortest period = 1 incubation period = 5 days (OIE, 2013)
- Longest period = 1 incubation period, plus period between clinical signs onset and death, plus maximal period between first death and suspicion report (30 days) = 42 days

It is important to note, that in the first methodology, the values are based on a limited number of data entries (n = 3), while in the second methodology, the variability and uncertainty are increased as we add together three parameters.

Another important consideration is that, as highlighted by the discrepancies in the ELS findings, these figures obtained in the literature highly depend on the epidemiological context and the level of awareness and preparedness in the outbreak area. Indeed, the period between first clinical/death and suspicion report may vary from zero in the case of a high preparedness situation such as the South African controlled area, to 30 days in Thailand where AHS had never occurred previously.

4.2.2. Assessment

Considering the results presented above, an assessment of the effectiveness of the current monitoring period for AHS, depending on the purpose of that period in the different scenarios shown in Annex E, was carried out. For AHS, the length of the existing monitoring period is 14 days.

**Scenarios 1, 2 and 3**

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

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

\textsuperscript{3} The overall incubation period of AHS is on average of 7 days, but fully susceptible horses usually suffer from the pulmonary form which is characterised by an incubation period between 3 and 5 days.
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. However, in the case of AHS, where spread may have occurred through the movement of infected vectors, as assessment of vector dispersal should be made, in terms of likely daily movement, environmental conditions and geographic barriers.

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 AHS virus into the establishment, either through the movement of an infected equid or the movement of infected midge vectors, and the reporting of the suspicion. Once the suspicion has been officially reported, control measures are implemented (including vector controls when plausible), and further spread is in this way prevented.

Based on the ELS carried out and presented above, the period between the earliest point of infection and the suspicion was 10/15 days (median/mean) based on three relevant papers, and 21 days when this period was reconstructed based on three other periods (incubation period + period between death and suspicion + period between suspicion and the suspicion report).

These two averages should be cautiously interpreted; the first average resulted from a very small number of papers (only three publications were found), while with the second methodology the uncertainty and variability increase due to the adding up of three different parameters.

In terms of the effectiveness of the length of the existing monitoring period for outbreaks of AHS in horses and taking into consideration the average length obtained by both methodologies, the existing length of the monitoring period (14 days) is considered effective only once there is a good awareness of the possibility of having the disease in the specific Member State. Using the first methodology, a maximum length of the monitoring period of 26 days was estimated reflecting both the lack of awareness, and the introduction occurring via zebras (for which a lower mortality compared to horses is expected); in contrast, the other two articles from which the information was extracted (Meiswinkel, 1998; OIE, 1999) showing shorter periods referred to outbreaks in a protection zone in South Africa. This refers to the South African ‘AHS Protection Zone’ which is the large region surrounding the South African ‘AHS Free Zone’ for export to the EU and where vaccination is not allowed and there are movement checks for Equidae entering from the other parts of South Africa. Therefore, there is a high level of awareness already. The data obtained from the second methodology (mean of 21 days and a maximum of 42 days) also support the argument of the existing monitoring period not being sufficient for the detection of an index case (in a region, or country, or Europe as a whole) due to the lack of awareness (30 days from the first AHS death-related case to the submission report were required in Thailand, being an AHS-free country at the time.

Considering the purpose of the monitoring period in these three scenarios, and particularly for scenario 3 where a derogation from killing the animals in a separated non-affected establishment may be granted, the recommendation would be to increase the length of the monitoring period from 14 days (current period) to 21 days, unless the earliest point of infection can be determined precisely by the epidemiological investigation (especially when dealing with the first suspicion in an area). This

### 3rd Scenario of monitoring period
- ToR 2 in accordance with article 13(b) and Annex II of the Delegated Regulation
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a AHS outbreak in an epidemiological unit in which the disease has not been confirmed, in order to provide derogations from killing the animals in these unit, if this unit has been completely separated, and handled by different personnel during this monitoring period.
would help to ensure that tracings back and forward encompass the full period where the disease maybe have been present in the establishment.

It is important to note that with existing EU Import legislation, the importation of zebras from Non-EU or non-EEA countries is not allowed unless the country appears on 2009/156/EC⁴ and animals are originating from an approved confined establishment. If an illegal importation occurs, the longer viraemia and lower morbidity/mortality in zebra compared to horses (among other considerations such as plausible contacts between species) would certainly influence the length of time between introduction and the raising of a suspicion.

Further, a note of caution should be made about the fact that no information was retrieved for other domestic equine species such as donkeys.

Also, this monitoring period does not take into account the spread off the index premises from the dispersal of infected vectors.

Scenarios 4

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

The main purpose of the monitoring period in scenario 4 is to ensure that certain products or materials, likely to spread the disease, that have been produced in a non-affected establishment located in the protection zone of an affected establishment, can be moved safely and without posing a risk of disease spread. In this scenario, and in contrast with the previous three scenarios, the establishment of concern is neither a suspect establishment nor an affected establishment, but one where infection has not been detected at that point in time. For the assessment of this scenario, we assume that the earliest plausible point of infection of these products or materials in the establishment of concern would be the earliest plausible point of infection of the establishment that originated the protection zone. If these products have been obtained or produced before the earliest point of infection of the affected establishment, then they could be exempted from prohibitions to be moved, as long as other conditions specified in the legislation are met (e.g. the products must have been clearly separated during the production process, storage and transport, from products not eligible for dispatch outside the restricted zone).

Considering the average length of time between the earliest point of entry and suspicion in establishments with horses as described in the control zone of South Africa (8 and 10 days), the existing length of the monitoring period (14 days) is considered effective. Precise information on the earliest point of infection of the farm that originated the protection zone might be available subsequent to the epidemiological investigation carried out in this establishment.

Scenario 5

- **5th Scenario of monitoring period**
- ToR 2 in accordance with article 32 (c), article 48(c) and Annex II of the Delegated Regulation
- The purpose of this section is 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.

The aim of the monitoring period is to ensure that semen from animals in a non-affected establishment (located in a protection zone) that has been collected and frozen after the earliest time of infection of the affected establishment that originated the protection zone, is safe to be moved without posing a risk of disease spread. In this scenario, EFSA is requested to assess the length of

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⁴ Council Directive 2009/156/EC of 30 November 2009 on animal health conditions governing the movement and importation from third countries of equidae.
time, after the semen was taken, when the animal should be tested in order to allow that semen to be moved. Here, it is assumed that the earliest point of infection of the animal would be on, or after the earliest point of infection of the index case around which the protection zone is established, and the latest date the semen could have become contaminated would be the date the semen was collected.

In order to assess the infection status of the animal at the time the semen was taken (indicating whether the semen was infected or not), a test to detect antibodies in the animal should be used. A negative serological test, if carried out at the right time, would indicate that the animal has never been exposed to the agent, and therefore, it will indicate that the semen is free of the agent too, provided the sample is taken at a time when antibody production is detectable and before antibody levels drop below the level of detection of the test used.

In the case of AHS, based on the existing legislation, the horse would have to be tested not earlier than the time in days of the monitoring period plus 7 days (14 + 7 = 21 days) counted after the semen was taken.

In AHS experimentally infected horses, the earliest detection of antibodies was found on day 11 after inoculation (Hamblin et al., 1992). In this experiment, antibodies were found until the day the only surviving horse was put down (without showing any clinical signs), which was 38 days after inoculation (based on Burrage and Laegreid (1994) immunity after disease may be lifelong).

Based on this unique piece of literature, the 21 days reflected in the current legislation as the minimum time for sampling the horse subsequent to the semen collection, would be considered effective. Furthermore, the length of the existing monitoring period, without the addition of the 7 days would still be considered appropriate for sampling the horses subsequent to the semen collection.

Nonetheless, the lack of available literature in terms of the serological response observed subsequent to infection with AHS virus in non-endemic countries must be highlighted. Only one paper was retrieved where this information was available for horses and there is no information or other Equidae.

**Scenarios 6 and 7**

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

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

In Scenarios 6 and 7, the monitoring period is used in the context of repopulation. In Scenario 6, the monitoring period is used to ensure that any repopulated equid 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 AHS virus within a distance equal or lower to the radius of the surveillance zone, the repopulation process could not take place). Repopulation can only take place after a number of days equal to the monitoring period has elapsed since the final culling of infected Equidae and disinsection of the affected establishment.

In this regard, the number of days of the monitoring period for AHS, counted from the day of the culling infected Equidae and disinsection, must ensure enough time for any potentially infected surrounding establishment to be reported as a suspicion. Modelling of vector survival has been covered in Section 4.1.2.6 and risk managers should also take into account potential for survival of infected vectors and the presence or absence of vectors in the field. Considering the results presented above, and taking into account that a good level of awareness is expected due to the disease having been present in the locality, the existing length of the monitoring period (14 days) is considered effective,
depending on the presence of vectors and average daily temperatures, as it would allow for the identification of any potentially infected establishment in the surrounding area prior to the repopulation taking place. In the event of lower daily average temperatures, the monitoring period should be extended to 21 days.

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 are introduced into the establishment 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 horse or batch of horses introduced into the repopulated establishment is the day when the animals are moved, clinically ill horses would be observed at the first visit, if this visit is carried out a number of days equal to the incubation period. The existing length of the monitoring period (14 days) is considered effective, as it would allow for early detection at the first visit following re-stocking of potentially infected horses.

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 AHS by implementing a protection and surveillance zones of a minimum radius, as set out in Annex V of the Delegated Regulation, surrounding the establishment where the disease has been confirmed. Based on this regulation, the minimum radius of the protection and surveillance zones for AHS should be of 100 and 150 km, respectively.

Transmission kernels have not been estimated for AHSV. However, two studies have estimated kernels for the transmission of bluetongue virus (BTV), a closely related virus, via Culicoides dispersal (Sedda et al., 2012; Sumner et al., 2017) and these were used for the assessment instead.

Sedda et al. (2012) used a detailed model of Culicoides dispersal to infer the distance between donor and recipient farms for the 2006 BTV epidemic in north-western Europe. These distances were used to construct the transmission kernel in Table 3.

Sumner et al. (2017) developed a model for the transmission of BTV between farms in which spread via animal movements and via vector dispersal were described separately. The authors fitted four models, which used different transmission kernels to describe spread between farms via vector dispersal (Table 3; Figure 6). The fits of the different models were not formally compared. However, the fat-tailed kernel best reflects distances over which Culicoides are likely to disperse (Hendrickx et al., 2008; Sedda et al., 2012) and this was used in the zone size assessment.

Table 3: Kernels for the transmission of BTV via Culicoides dispersal used for African horse sickness virus

| Epidemic      | Kernel     | Function                                      | Parameters* |               |
|---------------|------------|-----------------------------------------------|-------------|---------------|
|               |            | $k(r) = \left(1 + \frac{r}{d_0}\right)^{-\alpha}$ | $d_0$ (km)  | $\alpha$      |
| NW Europe 2006| SWOTS      | $k(r) = \left(1 + \frac{r}{47.0}\right)^{-5.8}$ | (33.2, 71.1)| (4.3, 8.4)    |
| GB 2007       | Fat-tailed | $k(r) = \left(1 + \left(\frac{r}{13.4}\right)^{-1}\right)$ | (5.8, 21.8) | (1.1, 3.0)    |

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For the SWOTS (spatiotemporal wind-outbreak trajectory simulation) and the fat-tailed kernel in Table 3, the probability of transmission beyond given distances (if transmission were to occur from an infected establishment) was computed using the estimates, and the lower and upper 95% confidence limits, including beyond the proposed radius for the protection and surveillance zones (100 and 150 km, respectively) (Figure 6). In addition, the distances at which a threshold probability of transmission beyond that distance is reached were also calculated for each kernel using the estimates, and lower and upper 95% confidence limits (Figure 7). The corresponding values computed using the estimates are summarised in Tables 4 and 5.

As the GB kernels use the same data but are analysed by different groups, the working group agreed that only the fat-tailed model should be used as they are based on vector dispersal.

| Epidemic | Kernel | Function | Parameters* |
|----------|--------|----------|-------------|
|          |        |          | $d_0$ (km)  | $\alpha$    |
| Stepped  | k$(r) = \begin{cases} 1 & r \leq d_0 \\ \left(\frac{r}{d_0}\right)^{-3} & r \geq d_0 \end{cases}$ | 18.7 \hspace{1em} (9.8, 28.3) | 3.0 \hspace{1em} (1.2, 4.7) |
| Exponential | $k(r) = \exp\left(-\frac{r}{d_0}\right)$ | 71.4 \hspace{1em} (30.3, 833.3) | – |
| Gaussian | $k(r) = \exp\left(-\frac{r}{2d_0}\right)^2$ | 57.7 \hspace{1em} (36.3, 223.6) | – |

*: Posterior median (95% credible interval); $d_0$ is a distance scaling and $\alpha$ controls the rate at which the kernel decays with distance.

Figure 6: Kernels for the transmission of AHSV via Culicoides dispersal. Parameters were estimated by fitting models to data from bluetongue virus epidemics in north-western Europe in 2006 (Sedda et al., 2012) or in Great Britain in 2007 (Sumner et al., 2017).

For the SWOTS (spatiotemporal wind-outbreak trajectory simulation) and the fat-tailed kernel in Table 3, the probability of transmission beyond given distances (if transmission were to occur from an infected establishment) was computed using the estimates, and the lower and upper 95% confidence limits, including beyond the proposed radius for the protection and surveillance zones (100 and 150 km, respectively) (Figure 6). In addition, the distances at which a threshold probability of transmission beyond that distance is reached were also calculated for each kernel using the estimates, and lower and upper 95% confidence limits (Figure 7). The corresponding values computed using the estimates are summarised in Tables 4 and 5.

As the GB kernels use the same data but are analysed by different groups, the working group agreed that only the fat-tailed model should be used as they are based on vector dispersal.
Figure 7: Assessment of the radius of the protection and surveillance zone for AHSV. The top panel shows the probability of transmission beyond a given distance (if transmission were to occur from an infected establishment) computed using the estimates (blue circles) and the lower and upper 95% confidence limits (error bars) for the SWOTS and fat-tailed kernels in Table 3. The thick black line indicates the median probability at each distance and 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 the two kernels (SWOT and fat tailed). The thick black line indicates the median distance for each probability and the black dotted lines indicate distances of 100 km and 150 km (i.e. the proposed radius of the protection and surveillance zones, respectively).

Table 4: Probability of transmission of AHSV beyond different distances

| Distance (km) | 25 | 50 | 75 | 100 | 125 | 150 | 200 |
|--------------|----|----|----|-----|-----|-----|-----|
| SWOTS        | 0.08 | 0.01 | 0.004 | 0.001 | < 0.001 | < 0.001 |     |
| Fat-tailed   | 0.25 | 0.09 | 0.05 | 0.03 | 0.02 | 0.02 | 0.009 |
Table 4 therefore demonstrates that for each of the two kernels, using a distance of 100 km would capture at least 95% of the risk of spread from the PZ (between 97% and 99.9%), while a smaller PZ (e.g. 50 km) would capture at least 90% of the risk of spread (between 90% and 99.9%). The 95% of the risk of spread is captured at distances of 31.6 and 73.2 km for the SWOT and fat tailed kernels, respectively.

As the decision for the risk managers is to decide the level of testing to be carried out in the PZ and to allow derogations for movements within and out of the 100 km zone, they should take account of the balance of impact of restrictions in a very wide zone against preventing disease spread, given the equid population density, environmental conditions and vector population. Table 5 therefore can be used for such decision, whereby the probability of transmission at 95% or 90% could be captured in an inner PZ of reduced size (e.g. 20 km), within which targeted surveillance and no derogations would be applied. The establishments in the outer PZ may have derogations applied.

As the zones are so large, it is quite possible that a restriction zone may cover areas in another Member State. Where this is the case, the risk manager should consider any geographical barriers to vector dispersal as well as movement over water.

### 4.3.2. Assessment of the minimum period

The purpose of this section is to assess the effectiveness to control the spread of disease of the minimum periods during which the competent authority should apply the restriction measures in the protection and surveillance zones as set out in Annex X and XI for AHS.

According to the Delegated Regulation 2020/687, for AHS, the minimum period for the PZ restriction is set at 1 year; this was based on the Council Directive 93/25 where vaccination was also allowed. The TOR did not include considering the use of vaccination. During this period, movement restrictions remain in place although derogations are allowed for certain activities (see section 4.1.2).

Modelling AHS using the kernels for *Culicoides* dispersal based on BT show that a small number of infected midges may survive at low average temperatures (10°C) and while the EIP for AHSV increases at cold temperatures, the likelihood of overwintering is uncertain given the small number of effective vectors. Nevertheless, the virus is more likely to continue to circulate (and overwinter) in regions where the average daily temperature is higher (15°C or more).

The continuous circulation of AHS and potential for overwintering will depend on environmental conditions in each country and freedom could be applied for earlier, if the evidence is sound.

### 4.3.3. Uncertainty analysis

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

| Threshold probability of transmission | 0.001 | 0.005 | 0.01 | 0.05 | 0.1 | 0.2 | 0.5 |
|--------------------------------------|-------|-------|------|------|-----|-----|-----|
| SWOTS                                | 106.9 | 69.7  | 56.6 | 31.6 | 22.8| 15.0| 5.9 |
| Fat-tailed                           | > 200 | > 200 | 190.1| 73.2 | 47.5| 29.8| 13.4|

Table 5: Distances (km) at which the probability of transmission of AHSV beyond that distance reaches a threshold level.
### 5. Conclusions and Recommendations

| Sampling procedure | Conclusions | Recommendations |
|--------------------|-------------|-----------------|
| 4.1.1.1 In the event of a suspicion of AHS in an establishment where animals of the listed species are kept | Clinical examination alone is not sufficient to confirm AHS in a susceptible population. In a susceptible population, an almost 100% case fatality rate (pulmonary form) is expected in horses (case fatality rate of zebras and donkeys can be much lower). | Laboratory examination should always be used to diagnose AHS in a susceptible population. All fallen stock of susceptible species showing pathognomonic clinical signs should be tested for presence of the virus by PCR and/or virus isolation. Serology could be of use where the chronic or per-acute infection is suspected, or in populations of Equidae which are less susceptible to acute infection. Confirmation of the presence, the species composition and seasonality of *Culicoides* spp. around the establishment is recommended for assessing vector control methods and to define period of low or nil virus transmission. |
| 4.1.1.2 For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU) 2016/429 in an AHS officially confirmed establishment | Sequence analysis of the virus strain may also contribute to identify a source or likely geographic origin. | To identify the origin of infection, not only should all epidemiological links be identified, but sequence analysis of the virus may be used. For establishments with less than 60 horses, all animals present should be sampled in order to detect a 5% prevalence with 95% confidence. For establishments with more than 60 horses the statistical sample to detect 5% prevalence with 95% confidence should be calculated. Sampling should focus on those with the highest risk contact through potential vector transmission. For disease confirmation, recommendations from Section 4.1.1.1 should be followed. |
| 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 AHS affected establishment | Not all animals will become infected on an affected farm as it will depend on vector abundance, vector competence and capacity, activity and whether other animals can be protected from vector blood feeding. Data from South Africa outbreaks suggest between 10% and 20%. It is important to gather entomological information on suitable vectors, period of vector activity, vector proofing conditions in the establishment etc. | Provided all infected animals are culled as quickly as possible, and provided effective vector control is implemented, it is possible that not all animals will become infected and therefore these animals can be kept alive. Testing should be carried out every three to seven days as the minimum time required to capture the minimum incubation period after which an infected animal may test positive. Recommendations for vector control as described in Section 4.1.1.1 should be followed. |
### Sampling procedure

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

| Conclusions | Recommendations |
|-------------|-----------------|
| Dogs can be infected by eating meat from infected horse carcases or through the bites of infected midges, but it is unclear whether they play a role in the epidemiology of disease. | None |

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

| Conclusions | Recommendations |
|-------------|-----------------|
| Wild Equidae in the restricted zones must be considered. There are no data on the susceptibility of rare native breeds of equids. | For captive Equidae, as they are less likely to show clinical signs, they should be sampled (EDTA blood and clotted blood) for laboratory tests by both real time RT-PCR for viral genetic material, and by ELISA for specific antibodies. For the wild or feral Equidae, as these various breeds are expected to, but may not show severe clinical signs and high case fatality rates, a monitoring programme for fallen stock of wild populations should be undertaken. In the event of suspicion of infection in these populations, samples taken for laboratory tests (same as per Section 4.1.1.1). |

#### 4.1.1.6
For animals of listed species in the non-affected establishments located in a protection zone, and for non-affected establishments located in a protection zone with a radius larger than 3 km.

| Conclusions | Recommendations |
|-------------|-----------------|
| Horse establishments are not very large in general; as there may be few Equidae on each establishment, all animals of listed species should be tested (for establishments with more than 60 animals see Section 4.1.1.1). It might not be feasible to clinically examine all horses within a 100 km PZ. The probability of an establishment to be infected beyond a radius of 75 km is 5%, (versus a probability of 95% inside that radius). Culicoides may fly or be blown several kilometres a day (depending on the weather conditions and geographic barriers). | Where the establishments have fewer than 60 animals, all animals should be sampled. Where there are 60 animals or more, in a single epidemiological group, a statistical sample to give 95% confidence there is less than 5% prevalence should be taken. Vector surveillance must be carried out to detect breeding sites, parous or blood-fed Culicoides females in and around establishments with listed species present. All listed species should be housed and protected from vectors, particularly during vector active periods. The total radius of the protection zone could be reduced to 75 km. Expert opinion, and meteorological monitoring, combined with locations of the listed species should be used to inform the sampling strategy and vector monitoring. |

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

| Conclusions | Recommendations |
|-------------|-----------------|
| Given the very wide size of the zone, periodic visits and clinical examination may not be feasible within the monitoring period. Laboratory testing of all the horses is not necessary as the case fatality rate is expected to be 95%. Fallen stock should always be tested. | A census of the establishments in the SZ should be taken. All keepers of listed species must immediately report any sudden deaths, clinical suspicion to the competent authority when a visit and laboratory examination of all listed species should be undertaken. Zebras, African donkeys and asses in kept establishments, should be targeted for laboratory examination if they are not vector protected. |
| Sampling procedure                                                                 | Conclusions                                                                                                                                   | Recommendations                                                                                                               |
|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| 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 | Clinical examination of listed species is not sensitive enough to confirm disease but is considered useful a priori to determine whether laboratory tests are needed. Official designation of slaughterhouses makes certain that records are kept, biosecurity is maintained, animals inspected ante- and post-mortem and meat would be stamped. The midge vectors are most active during dawn and dusk. | Real-time RT–PCR on EDTA blood should be taken and give negative results for the animals before they move. Testing to provide confidence of 95% of less than 5% prevalence in the establishment means taking laboratory samples from 60 animals for virus identification tests. Vector treatment of the transport and disposal/disinfection of the bedding, feed and water in the vehicle should be carried out. Transport should only happen during the day. |
| 4.1.2.2 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 | As per Section 4.1.2.2                                                                                                                     | Testing to provide confidence of 95% of less than 5% prevalence in the establishment means taking laboratory samples from 60 animals for virus identification tests. |
| 4.1.2.3 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 | It is concluded that provided all necessary precautions to prevent vector feeding activity during transport and slaughter, and laboratory testing is carried out, the derogation can be applied. For movement of listed species from the free zone to a slaughterhouse in the Restriction Zones provided animals are killed immediately and a clinical examination is carried out (and no animals have clinical signs) there is no requirement for testing. | Transport should only happen during the day. |
| 4.1.2.4 From an establishment in a surveillance zone to pastures situated within the surveillance zone | It is concluded that if the Equidae are in vector proof accommodation prior to the move and have tested negative after the monitoring period, they could be moved in transport under official supervision with little risk of introducing disease. | The recommendation is that any animals to be moved permanently and not just for exercise should receive negative test results for presence of the virus prior to movement. |
### Sampling procedure

| Section |
|---------|
| 4.1.2.5 | From an establishment in a surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone |
| 4.2.1.6 | 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 |
| 4.1.3.1 | For the animals that are kept for the repopulation prior to their introduction |
| 4.1.3.2 | In the event of unusual mortalities or clinical signs being notified during the repopulation |

#### Conclusions

| Section |
|---------|
| 4.1.2.5 |
| 4.2.1.6 |
| 4.1.3.1 |
| 4.1.3.2 |

- It is concluded that any movements should not take place until the monitoring period has elapsed at the index establishment and all visits, examinations and sampling of Equidae in the establishments in the 3 km have taken place (and negative results received).
- For movements to the outside of the restriction zones, each animal should test negative prior to the movement and there must be vector control in place for the transport and the premovement accommodation. The animals should be in vector proof accommodation for the duration of the period since disease was suspected on the index case establishment.
- It is concluded that the SVFP should be determined for each region knowing the *Culicoides* species composition, seasonal dynamics, and environmental drivers such as temperature.
- Depending on the average daily temperatures for the duration of the monitoring period a derogation to move Equidae from non-affected establishments in a zone may be possible.
- It is concluded that if animals are sourced from the free area or the SZ, there is no need for testing, provided the animals do not show clinical signs on the day of the movement and there have been no reports of increased mortality or suspect clinical signs in the herd during the monitoring period.
- It is concluded that the risk manager should make an assessment of the temperature and vector population to allow any movements to happen, Provided the seasonal vector free period is established and providing there are no surviving Equidae acting as a virus reservoir.
- It is concluded that the risk manager should require an assessment of whether infected vectors could be present at the establishment before repopulation is

#### Recommendations

| Section |
|---------|
| 4.1.2.5 |
| 4.2.1.6 |
| 4.1.3.1 |
| 4.1.3.2 |

- It is recommended to test every animal on entering and leaving vector proof accommodation; to move the animals in vector proof transport to the new establishment; when moving to an approved slaughterhouse the transport should be under official supervision.
- It is recommended that the risk manager should make an assessment of the temperature and vector population to allow any movements to happen, Provided the seasonal vector free period is established and providing there are no surviving Equidae acting as a virus reservoir.
- The recommendation is that animals with clinical signs or from herds where animals had clinical signs, including unexplained mortality, should not be used for repopulation.
- It is recommended that laboratory samples should be taken for the confirmation of the disease. Monitoring should continue for at least 40 days depending on the vector activity at the establishment.
### Sampling procedure

| Conclusions | Recommendations |
|-------------|-----------------|
| For animals that have been repopulated | considered, and if animals show clinical signs or there are any mortalities suggestive of infection, the animals should be tested. |
| ToR 2 | The recommendation is to increase the length of the monitoring period from 14 days (current period) to 21 days, unless the earliest point of infection can be determined precisely by the epidemiological investigation (especially when dealing with the first suspicion in an area). |

### Tor 2

#### 4.2 Assessment of the length of the monitoring period of AHS

It is concluded that the existing length of the monitoring period (14 days) is considered effective only once there is a good awareness of the possibility of having the disease in the specific Member State. The 21 days reflected in the current legislation as the minimum time for sampling the horse subsequent to the semen collection, would be considered effective. Furthermore, the length of the existing monitoring period, without the addition of the 7 days would still be considered appropriate for sampling the horses subsequent to the semen collection.

### Tor 3

#### 4.3.1 Assessment of the minimum radius

It is concluded that using a distance of 100 km would capture at least 95% of the risk of spread from the PZ (between 97 and 99.9%), while a smaller PZ (e.g. 50 km) would capture at least 90% of the risk of spread (between 90 and 99.9%). The 95% of the risk of spread is captured at distances of 31.6 and 73.2 km for the SWOT and fat tailed kernels, respectively.

It is recommended that when risk managers need to decide the level of testing to be carried out in the PZ and to allow derogations from movements within and out of the 100 km zone, they should take account of the balance of impact of restrictions in a very wide zone against preventing disease spread, given the equid population density, environmental conditions and vector population. Also, the risk manager should consider any geographical barriers to vector dispersal as well as movement over water.

#### 4.3.2 Assessment of the minimum period

It is concluded that although infected midges may survive at low average temperatures (10°C), according to certain models, the EIP for AHSV is expected to be longer than the lifespan of the vector when temperatures are low over the winter. Therefore, the virus is more likely to continue to circulate in regions where the average daily temperature is higher (15°C or more).

It is recommended that the duration of restriction zones should be based on a risk assessment which considers the vector population, EIP and daily average temperatures. The continuous circulation of AHS and potential for overwintering will depend on environmental conditions in each country and freedom could be applied for earlier, if the evidence is sound.
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**Abbreviations**

| Acronym | Description |
|---------|-------------|
| Ab | Antibody |
| AHS | African horse sickness |
| AHSV | African horse sickness virus |
| ASF | African swine fever |
| BTV | Bluetongue virus |
CBPP    Contagious bovine pleuropneumonia
CCPP    Contagious caprine pleuropneumonia
CSF     Classical swine fever
DIVA    Differentiation of Infected from Vaccinated Animals
EDTA    Ethylenediamine tetraacetic acid
EIP     extrinsic incubation period
ELISA   enzyme-linked immunosorbent assay
ELS     extensive literature search
EURL    European Union Reference Laboratory
FMD     Foot and mouth disease
HPAI    Highly Pathogenic Avian Influenza
LSD     Lumpy skin disease virus
MSs     Member States
NCD     Newcastle disease virus
OIE     World Organization for Animal Health
PCR     polymerase chain reaction
PPR     Peste des petites ruminant
PZ      protection zone
RNA     ribonucleic acid
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
| 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                                                                                                                                                                                                 |
|-----------|--------------------------------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ToR 1.1  | 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)). | • 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) and Art. 7 (4) of the Delegated Regulation, and Art. 57 of the Reg.2016/429 | 2nd Scenario | To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (TOR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, virus identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU)2016/429. | • affected establishment officially confirmed  
• kept animals of listed species found dead or before/when they are killed  
• competent authority collects samples for laboratory examination for the purposes of:  
  a) supporting the epidemiological enquiry:  
  - to identify the likely origin of the disease  
  - to calculate the likely length of time that the disease is present  
  - to identify establishments where the animals could have contracted the disease and movements from the affected establishment that could have led to the spread of the disease  
  - to obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors  
  b) confirming/ruling out disease in the event of preventive killing |
| ToR 1.1  | 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 ensuring that they do not pose a risk for the transmission of the disease. | • affected establishment officially confirmed  
• kept animals of listed species of specific categories  
• animal categories based on article 13(2):  
  (a) animals kept in a confined establishment  
  (b) animals kept for scientific purposes or purposes related to conservation of protected or endangered species  
  (c) animals officially registered in advance as rare breeds  
  (d) animals with a duly justified high genetic, cultural or educational value  
• the competent authority may grant specific derogation from killing all the animals of listed species belonging to any of the above categories in an affected establishment, provided that specific conditions are fulfilled  
• the animals should be subjected to clinical surveillance, including laboratory examinations |
| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|------|-------------|----------|------------------------------|--------------------------|
| ToR 1.1 | 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. | • sampling procedures should ensure that the animals do not pose a risk of transmission of the category A disease if left alive |
| 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. | • kept animals of non-listed species of epidemiological relevance for the control of the disease |
| ToR 1.1 | Article 26(2) of the Delegated Regulation | 6th Scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals. | • affected establishment officially confirmed |
| ToR 1.2 | 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 | • protection zone with radius up to 3 km |
| | | | | • non-affected establishments with kept animals of listed species |
| | | | | • all the non-affected establishments with kept animals of listed species located in the protection zone |
| | | | | • official veterinarians must visit at least once all the establishments |
| | | | | • on these establishments, 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 |
| | | | | • sampling procedures to ensure detection of the virus in these species |
| | | | | • sampling procedures to ensure detection of the virus, if the virus is present in these wild species |
| | | | | • protection zone with radius larger than 3 km |
| | | | | • non-affected establishments of kept animals of listed species |
| | | | | • sample of the non-affected establishments of kept animals of listed species in the protection zone |
| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|------|-------------|----------|----------------------------|--------------------------|
|      |             |          |                             |                          |
|      |             | 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. | • in a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km radius. | • in case of a radius larger than 3 km, official veterinarians may not visit all establishments, only 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. |
|      |             |          |                             |                          |
|      |             |          |                             |                          |
|      |             | ToR 1.4  | Article 28(5) of the Delegated Regulation |                          |
|      |             |          | 9th Scenario               |                          |
|      |             |          | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29) | • protection zone | • kept animals of listed species |
|      |             |          |                             |                          |
|      |             |          |                             |                          |
|      |             | 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- | • protection zone | • grant derogation for movement from a non-affected establishment in the protection zone |
|      |             |          | animals.                  |                          |

**Derogations to allow animal movements**

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|------|-------------|----------|----------------------------|--------------------------|
|      |             |          |                             |                          |
|      |             | ToR 1.4  | Article 28(5) of the Delegated Regulation |                          |
|      |             |          | 9th Scenario               |                          |
|      |             |          | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29) | • protection zone | • kept animals of listed species |
|      |             |          |                             |                          |
|      |             |          |                             |                          |
|      |             | 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- | • protection zone | • grant derogation for movement from a non-affected establishment in the protection zone |
|      |             |          | animals.                  |                          |
| ToRs   | Legislation                                      | Scenario                                                                 | Description of the Scenario                                                                                                                                                                                                 | Elements of the Scenario                                                                                                                                                                                                 |
|--------|-------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ToR 1.4 | Article 28(5) and Article 30(2) of the Delegated Regulation | 11th Scenario                                                           | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone to establishments located in the same MS and if possible within the restricted zone. | • protection zone  
• ready-to-lay poultry  
• grant derogation for movement from a non-affected establishment in the protection zone  
• to be moved to an establishment located in the same Member State and if possible, within the restricted zone  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95%                                                                                     |
| ToR 1.4 | Article 28(5) and Article 37 of the Delegated Regulation | 12th Scenario                                                           | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37) | • protection zone  
• kept animals of listed species  
• grant derogation for movement from a non-affected establishment in the protection zone  
• to be moved to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed  
• clinical examinations and laboratory examinations of animals kept in the establishment, including those animals to be moved  
• sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95%                                                                                     |
| 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 restricted zone  | • surveillance zone  
• kept animals of listed species  
• grant derogation for movement from an establishment in the surveillance zone to be moved to a slaughterhouse within the restricted zone or outside the restricted zone  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95%                                                                                     |
| ToRs   | Legislation                                                                 | Scenario                                      | Description of the Scenario                                                                 | Elements of the Scenario                                                                 |
|--------|------------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| ToR 1.4 | Article 43(5) and Article 45(1) of the Delegated Regulation                  | 14th Scenario                                 | establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone | • a) sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95%  
• b) sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95% |
| 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 a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone | • surveillance zone  
• kept ungulates of listed species  
• grant derogation for movement from an establishment in the surveillance zone  
• to be moved to pastures situated within the surveillance zone  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95% |
| ToR 1.4 | Article 43(5) and Article 46(1) of the Delegated Regulation                  | 16th Scenario                                 | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations to grant derogation of movements of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating within the surveillance zone and eggs originating outside the restricted zone, to an establishment located in the same Member State where they were hatched | • surveillance zone  
• kept birds of listed species  
• grant derogation for movement of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating from establishment within the surveillance zone or eggs originating from outside the restricted zone  
• to be moved to an establishment located in the same Member State  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• sampling procedures to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95% |
| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|------|-------------|----------|----------------------------|--------------------------|
| ToR 1.4 | Article 43(5) and Article 46(2) of the Delegated Regulation | 17th Scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same MS. | • surveillance zone  
• ready-to-lay poultry  
• to be moved to an establishment located in the same Member State  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• sampling procedure to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95% |
| ToR 1.4 | Article 56(1)c of the Delegated Regulation | 18th Scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI | • restricted zone when restriction measures are maintained beyond the period set out in Annex XI  
• kept animals of listed species  
• grant derogation for movement from an establishment within the restricted zone  
• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved  
• sampling procedures to ensure that movements do not pose a risk of spreading the disease with a confidence level of at least 95% |
| Repopulation | | 19th Scenario | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease. | • repopulation of a previous affected establishment  
• kept animals of listed species  
• animals intended to repopulation shall be sampled prior to their introduction into the establishment of destination  
• samples shall be collected from a representative number of animals to be introduced of each consignment from each establishment or from a representative number of animals of each consignment (if animals are all to be introduced at different times or from different establishments of origin)  
• laboratory examinations  
• sampling procedures to rule out the presence of the disease |
| ToR 1.5 | Article 59(2),(3) of the Delegated Regulation | 20th Scenario | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease. | • repopulated establishment  
• unusual mortalities or clinical signs during the repopulation  
• the official veterinarians shall without delay collect samples for laboratory examination  
• sampling procedures to rule out the presence of the disease |
| ToRs   | Legislation                                      | Scenario   | Description of the Scenario                                                                                                                                                                                                 | Elements of the Scenario                                                                                   |
|--------|--------------------------------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| ToR 1.5 | Article 59(5) of the Delegated Regulation        | 21st Scenario | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment. | • repopulated establishment  
• kept animals of listed species  
• animals that have been used for repopulation  
• laboratory examinations  
• sampling procedures to rule out the presence of the disease |
### Annex C – Sampling procedures for AHS

Sampling scenarios for AHS – Based on Commission Directive 92/35/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 (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)). | Article 4  
1) Where a holding contains one or more equidae suspected of being infected with African horse sickness, Member States shall ensure that the official veterinarian immediately sets in motion official means of investigation to confirm or rule out the presence of the said sickness.  
2) From the moment when the suspected infection is notified, the official veterinarian shall:  
a) have the suspect holding(s) placed under official surveillance;  
b) initiate:  
i) an official census of the species of equidae, stating in the case of each species the number of equidae already dead, infected or liable to be infected, and the updating of that census to take account of equidae born or dying during the period of suspicion; the information in the census must be produced on request and may be checked at each inspection;  
ii) a census of places likely to facilitate the survival of the vector or to accommodate it and the use of appropriate means of eradicating insects in such places;  
iii) an epizootiological inquiry in accordance with Article 7;  
c) regularly visit the holding(s), when he shall:  
i) examine each equid kept there;  
ii) carry out a detailed clinical examination or an autopsy on the suspect or dead animals and take the samples necessary for laboratory examinations;  
d) ensure that:  
i) all equidae on the holding(s) are kept in their living quarters or in other places protected against the vector;  
ii) all movement of equidae to or from the holding(s) is prohibited;  
iii) appropriate means of eradicating insects are employed in and around the buildings housing the equidae;  
iv) the carcases of equidae which have died on the holding are destroyed, disposed of, burnt or buried in accordance to national law;  
| No specific guidelines described in legislation  
OIE Technical disease Card: African Horse Sickness  
OIE Terrestrial Manual 2017 (p.4, 9)  
Samples:  
- **Virus isolation**  
  - Unclotted whole blood collected in an appropriate anticoagulant at the early febrile stage and sent at 4°C/39°F to the laboratory  
  - Spleen, lung and lymph node samples collected from freshly dead animals are placed in appropriate transport media and sent at 4°C/39°F to the laboratory; do not freeze  
- **Serology**  
  - Preferably paired serum samples should be taken 21-days apart and kept frozen at –20°C/-4°F  
EURL Guidelines  
PCR Methodology [https://www.mapa.gob.es/en/ganaderia/temas/laboratorios/guidelinesrtrt-pcrcrreded1jan2018_tcm38-441092.pdf](https://www.mapa.gob.es/en/ganaderia/temas/laboratorios/guidelinesrtrt-pcrcrreded1jan2018_tcm38-441092.pdf)  
Serology Methodology [https://www.mapa.gob.es/en/ganaderia/temas/laboratorios/guidelineselisaahs-ingezimcompacplused1june2017_tcm38-441095.pdf](https://www.mapa.gob.es/en/ganaderia/temas/laboratorios/guidelineselisaahs-ingezimcompacplused1june2017_tcm38-441095.pdf)  
Directive 90/426 Annex D has methodology for diagnosis with the c-ELISA, a blocking ELISA and an i-ELISA for Ab detection. These are trade tests but are not for immediate diagnosis of active infection (no PCR or virus isolation).
| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|----------|-----------------------------|---------------------|----------------------|
| 2nd      | To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, virus identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU) 2016/429. | Article 7  
1) The epizootiological inquiry shall cover:  
o the length of time during which African horse sickness may have existed on the holding,  
o the possible origin of the African horse sickness on the holding and the identification of other holdings on which there are equidae which may have become infected or contaminated from the same source,  
o the presence and distribution of disease vectors,  
o the movement of equidae to or from the holdings concerned or any carcases of equidae removed from them.  
2) In order to provide full coordination of all measures necessary to ensure eradication of African horse sickness as quickly as possible and for the purpose of carrying out the epizootiological inquiry, a crisis unit shall be established. | No specific guidelines described in legislation |
| 3rd      | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment, in order to grant a specific derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease. | No specific guidelines described in legislation | No specific guidelines described in legislation |
| 4th      | To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure | No specific guidelines described in legislation | No specific guidelines described in legislation |

with Council Directive 90/667/EEC of 27 November 1990 laying down the veterinary rules for the disposal and processing of animal waste, for its placing on the market and for the prevention of pathogens in feedstuffs of animal or fish origin and amending Directive 90/425/EEC (1).
| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|----------|----------------------------|---------------------|----------------------|
| 5th      | To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species. | No specific guidelines described in legislation | No specific guidelines described in legislation |
| 6th      | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals. | No specific guidelines described in legislation | No specific guidelines described in legislation |
| 7th      | To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of establishments located in a protection zone when the radius is larger than 3 km. The purpose of the sampling procedure is to ensure disease detection of the virus if the virus is present in establishments within the protection zone. | Article 8 2. (a) The protection zone shall consist of a part of Community territory with a radius of at least 100 km around the entire infected holding. 3. At the duly substantiated request of a Member State a decision may be taken in accordance with the procedure laid down in Article 19, with a view to amending the demarcation of the zones defined in paragraph 2, taking into account: o their geographical situation and ecological factors, o the meteorological conditions, o the presence and distribution of the vector, o the results of the epizootiological studies carried out in accordance with Article 7, o the results of the laboratory examinations, | No specific guidelines described in legislation |
| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|----------|----------------------------|---------------------|----------------------|
| 8th      | To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the virus is present in establishments within the surveillance zone. | o the application of the control measures, in particular the insect o eradication measures. Article 9 1) Member States shall ensure that the following measures are applied in the protection zone: a) all holdings containing equidae within the zone are identified; b) the official veterinarian conducts: o periodic visits to all holdings containing equidae, o a clinical examination of the said equidae including, if necessary, the collection of samples for laboratory examination; o a record of visits and findings must be kept; c) equidae leave the holding on which they are kept only for transport directly under official supervision for emergency slaughter to a slaughterhouse located in that zone or, if that zone has no slaughterhouse, to a slaughterhouse in the surveillance zone designated by the competent authority. 2) In addition to the measures provided for in paragraph 1, a decision to carry out systematic vaccination of equidae against African horse sickness and to identify them in the protection zone may be taken under the procedure laid down in Article 19. | No specific guidelines described in legislation |
| 8th      | Article 10 Member States shall ensure that: 1) the measures provided for in Article 9 (1) apply in the surveillance zone. However, if the surveillance zone has no slaughterhouse, the equidae may be slaughtered in the protection zone in a slaughterhouse designated by the competent authority. | | |
| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|----------|----------------------------|---------------------|-----------------------|
| **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 1) Member States shall ensure that the following measures are applied in the protection zone: (c) equidae leave the holding on which they are kept only for transport directly under official supervision for emergency slaughter to a slaughterhouse located in that zone or, if that zone has no slaughterhouse, to a slaughterhouse in the surveillance zone designated by the competent authority. | No specific guidelines described in legislation |
| **12th** | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37) | No specific guidelines described in legislation | No specific guidelines described in legislation |
| **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 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 | Article 10 1) the measures provided for in Article 9 (1) apply in the surveillance zone. However, if the surveillance zone has no slaughterhouse, the equidae may be slaughtered in the protection zone in a slaughterhouse designated by the competent authority. | No specific guidelines described in legislation |
| **14th** | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of | Article 6 1) Where the presence of African horse sickness is officially confirmed, the official veterinarian: | No specific guidelines described in legislation |
### Scenario Description of the Scenario
- **Scenario 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 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.

### Clinical guidelines
- a) shall proceed immediately with the killing under official control of any equidae on the infected holding which are infected with or present clinical symptoms of African horse sickness;

**Article 11**
- However, notwithstanding Articles 9 (1) (c) and 10 (1):
  - a) equidae from the protection zone and from the surveillance zone may be transported under official supervision and under the conditions laid down in Article 5 (3) of Directive 90/426/EEC to the quarantine station referred to in Article 5 (3) (d) of that Directive;
  - b) movements of equidae within zones of the same status shall be subject to authorisation from the competent authorities on the basis of the following rules:
    1. equidae shall:
       - undergo a prior official check,
       - require identification, and
       - be accompanied by an official document;

- **Scenario 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.

### Laboratory guidelines
- No specific guidelines described in legislation

### Table

| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|----------|-----------------------------|---------------------|----------------------|
| 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 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. | See 14th scenario | 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 | No specific guidelines described in legislation |
| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|----------|----------------------------|---------------------|----------------------|
| Repopulation |
| 19th | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease. | No specific guidelines described in legislation | No specific guidelines described in legislation |
| 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. | No specific guidelines described in legislation | 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. | No specific guidelines described in legislation | No specific guidelines described in legislation |
## Annex D – Scenarios of ToR 2

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenarios |
|------|-------------|----------|------------------------------|--------------------------|
| ToR 2 | Article 8 of the Delegated Regulation  
Article 57 of 2016/429 Regulation  
Annex II of the Delegated Regulation | 1st Scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of the notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of a suspicion. | • event of suspicion of a category A disease  
• in an establishment with kept animals of listed species  
• time period calculated backwards from the date of the of the notification of the suspicion  
• time period before the suspicion, during which the pathogenic agent may have been introduced in the establishment and may have spread outside the establishment.  
• the aim of the epidemiological enquire is:  
  a) identify the likely origin of the listed disease in question and the means of its spread  
  b) calculate the likely length of time that the listed disease has been present  
  c) identify establishments and epidemiological units therein, food and feed businesses or animal by-products establishments, or other locations, where animals of listed species for the suspected listed disease may have become infected, infested or contaminated  
  d) obtain information on the movements of kept animals, persons, products, vehicles, any material or other means by which the disease agent could have been spread during the relevant period preceding the notification of the suspicion or confirmation of the listed disease  
  e) obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors |
| ToR 2 | Article 17(2) and Article 57 of 2016/429 Regulation  
Annex II of the Delegated Regulation | 2nd Scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of confirmation of the disease. | • Event of confirmation of a category A disease  
• in an establishment with kept animals of listed species  
• time period calculated backwards from the date of the notification of the suspicion  
• time period before the suspicion, during which the pathogenic agent was introduced in the establishment and during which it could have spread outside the establishment.  
• The aim of the epidemiological enquire is the same as above. |
| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenarios |
|------|-------------|----------|-----------------------------|--------------------------|
| ToR 2 | Article 13(b) of the Delegated Regulation | 3rd Scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a category A disease in an establishment with kept animals of listed species, during which the epidemiological units in which the disease has not been confirmed were kept completely separated and handled by different personnel, in order to provide derogations from killing. | - Event of confirmation of a category A disease  
- in an affected establishment with kept animals of listed species  
- non-affected epidemiological units kept separated  
- to provide derogation from killing for animals in non-affected separated epidemiological units  
- to exclude any possible contact between the affected establishment and the separated epidemiological units as per the epidemiological enquiry  
- time period calculated backwards from the date of the confirmation  
- time period before the confirmation, during which the pathogenic agent may have been introduced in the separated non-affected epidemiological units of the affected establishment. |
| ToR 2 | Article 27(3)c 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. | - 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. |
| ToR 2 | Article 32(c) of the Delegated Regulation | 5th Scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated forwards from the date of semen collection from animals of listed species kept in approved germinal product establishments in the protection or in the surveillance zone, to prove that the donor animal has tested favourable on a sample taken not earlier than 7 days after the monitoring period. | - Protection or surveillance zone  
- non-affected approved germinal establishments  
- semen from kept animals (donor) of listed species  
- semen collected after the estimated date of the earliest infection of the earliest affected establishment that originated the protection zone/surveillance zone (if belonging to more than one protection or surveillance zones).  
- to take samples from the donor for laboratory analysis at least 7 days after the end of the monitoring period  
- to authorise movements of semen from approved germinal product establishments located in the protection or surveillance zones in case of favourable laboratory results.  
- time period calculated forwards from the date of semen collection |
| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenarios |
|------|-------------|----------|-----------------------------|--------------------------|
| ToR 2 | Article 57(1)b of the Delegated Regulation Annex II of the Delegated Regulation | 6th Scenario | To assess the effectiveness of the length of the Monitoring Period, as the appropriate time period calculated forwards from the date after the final cleaning and disinfection and when relevant control of insects and rodents was carried out in an affected establishment, after which the repopulation of the establishment may be allowed by the competent authority. | • Repopulation of a previous affected establishment  
• kept animals of listed species  
• to allow the repopulation of an affected establishment  
• time period calculated forwards from the date of the final cleaning and disinfection of the establishment  
• time period to ensure that the repopulation exercise is not put at risk due to the disease being unknowingly present in an establishment in the surrounding area. |
| ToR 2 | Article 59(4)b of the Delegated Regulation Annex II of the Delegated Regulation | 7th Scenario | To assess the effectiveness of the length of the Monitoring Period, as the appropriate time period calculated forwards the date when the first animal was introduced, during which all the animals of listed species intended for repopulation should be introduced. | • Repopulation of a previous affected establishment  
• kept animals of listed species to be repopulated  
• the animals may not be introduced at the same time  
• time period calculated forwards from the date when the first animal was introduced  
• time period during which animals intended for repopulation, should be introduced and the process of repopulation be completed. |
## Annex E – Minimum radius and minimum period of duration of protection and surveillance zones

| Category A diseases | Minimum radius of Protection zone Annex V | Minimum radius of Surveillance zone Annex V | Minimum period of duration of measures in the protection zone (Article 39(1)) Annex X | Additional period of duration of surveillance measures in the protection zone (Article 39(3)) Annex X | Minimum period of duration of measures in the surveillance zone (as referred to in Articles 55 and 56 of this Regulation) Annex XI |
|---------------------|------------------------------------------|--------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Foot and mouth disease (FMD) | 3 km | 10 km | 15 days | 15 days | 30 days |
| Infection with rinderpest virus (RP) | 3 km | 10 km | 21 days | 9 days | 30 days |
| Infection with Rift Valley fever virus (RVFV) | 20 km | 50 km | 30 days | 15 days | 45 days |
| Infection with lumpy skin disease virus (LSD) | 20 km | 50 km | 28 days | 17 days | 45 days |
| Infection with Mycoplasma mycoides subsp. mycoides SC (Contagious bovine pleuropneumonia) (CBPP) | Establishment | 3 km | 45 days | Not applicable | 45 days |
| Sheep pox and goat pox (SPGP) | 3 km | 10 km | 21 days | 9 days | 30 days |
| Infection with peste des petits ruminant virus (PPR) | 3 km | 10 km | 21 days | 9 days | 30 days |
| Contagious caprine pleuropneumonia (CCPP) | Establishment | 3 km | 45 days | Not applicable | 45 days |
| African horse sickness (AHS) | 100 km | 150 km | 12 months | Not applicable | 12 months |
| Infection with Burkholderia mallei (Glanders) | Establishment | Establishment | 6 months | Not applicable | Not applicable |
| Classical swine fever (CSF) | 3 km | 10 km | 15 days | 15 days | 30 days |
| African swine fever (ASF) | 3 km | 10 km | 15 days | 15 days | 30 days |
| Highly pathogenic avian influenza (HPAI) | 3 km | 10 km | 21 days | 9 days | 30 days |
| Infection with Newcastle disease virus (NCD) | 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                                                                                                                                                                                                 |
|--------------------------------------|-----|--------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **ToR1**                             | 1   | The transmission model assumes 100% mortality, which is adequate for horses but represents an overestimation for other susceptible species, such as donkeys. | The effect of this uncertainty on the assessment is unclear, however, as dead animals are targeted for laboratory testing rather than clinical cases, it was determined using the RIBESS tool, that the lower mortality expected for donkeys would not impact on ability to detect disease. |
|                                      | 2   | There is no reliable data on the establishment size for horses, and this was assumed to be a maximum of 300 and median of fewer than 10 based on reports of equine diseases in the EU Animal Disease Notification System in the last ten years. | The effect of this uncertainty on the assessment is unclear. However, as the recommendation to target testing on dead animals, given the likely all-cause mortality rates, the size of the establishment has little bearing on the sampling protocol. |
|                                      | 3   | Only limited data are available on temperature-dependent parameters (biting rate, extrinsic incubation period and vector mortality rate) for European vector species; those used in the model are based instead on *Culicoides sonorensis*, a north American vector species. | The effect of this uncertainty on the assessment is unclear. Flexibility in determining seasonal vector activity period should always use midge trap data to inform any decisions. |
|                                      | 4   | The impact on viral transmission of mammalian species (primarily cattle and sheep) which will be fed on by *Culicoides* biting midges, but which are not susceptible to AHSV is unclear. | The effect of this uncertainty is unclear but will depend on local densities of horses and other mammalian species and vector feeding preferences. |
|                                      | 5   | The probability of AHSV transmission from vector to host is not known. An estimate based on BTV transmission from vector sheep was used instead. | If transmission of AHSV is less efficient than transmission of BTV, the rate of spread in an establishment will overestimated. |
| **ToR2**                             | 6   | Out of the 12 references used for the assessment, nine were referring to outbreaks occurring outside the EU. The only information from the EU was from an outbreak affecting Spain and Portugal that occurred more than 30 years ago. Therefore, both factors related to the host-vector populations (production systems, animal and vector density, vector species etc.) and to the sensitivity of the surveillance systems (awareness of clinical presentation of the disease, preparedness, presence of other diseases with similar clinical presentations etc.) may differ between the population evaluated in the references retrieved and in the animal population present currently in the EU. | The effectiveness of the proposed monitoring period could be over or underestimated. |
|                                      | 7   | Information on the period elapsed between the earliest point of infection and the suspicion report could only be retrieved from three references, and was reconstructed based on an assumed average incubation period on the remaining references (that provided | The effectiveness of the proposed monitoring period could be over or underestimated. |
| Source or location of the uncertainty | # | Nature or cause of uncertainty as described by the experts | Impact of the uncertainty on the assessment |
|--------------------------------------|---|--------------------------------------------------------|------------------------------------------|
|                                       | 8 | No information from certain species (e.g. donkeys) affected by AHS was available in the scientific literature. | The effectiveness of the proposed monitoring period could be overestimated if the estimated period from infection to suspicion report was longer in donkeys. |
|                                       | 9 | Evidence on the dynamics on antibody detection in AHS-infected animals was restricted to one single paper based on experimental infection. | The effectiveness of the proposed monitoring period could be over or underestimated. |
| ToR 3                                 | 10| No kernels are available for AHSV. Kernels based on transmission of bluetongue virus (BTV) via dispersal of Culicoides vectors used instead. Ruminants are kept at higher densities than equids, which may influence the distance between donor and recipient establishments. This will also affect the transmission frequencies, particularly if Culicoides have a strong feeding preference for ruminants over equids (or vice versa). | The effectiveness of the proposed zone size could be over or underestimated. |