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# Scientific Opinion on the assessment of the control measures for category A diseases of Animal Health Law: Foot and Mouth Disease

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

## 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 foot and mouth disease (FMD). 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 zones, and the minimum length of time the measures should be applied in these zones. The general methodology used for this series of opinions has been published elsewhere; nonetheless, the transmission kernels used for the assessment of the minimum radius of the protection zone of 3 km and of the surveillance zone of 10 km are shown. Several scenarios for which these control measures had to be assessed were designed and agreed prior to the start of the assessment. The monitoring period of 21 days was assessed as effective, and it was concluded that the protection and the surveillance zones comprise > 99% of the infections from an affected establishment if transmission occurred. Recommendations, provided for each of the scenarios assessed, aim to support the European Commission in the drafting of further pieces of legislation, as well as for plausible ad hoc requests in relation to FMD.

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**Keywords:** Foot and mouth disease, FMD, foot and mouth diseases virus, FMDV, disease control measures, sampling procedures, monitoring period, protection zone, surveillance zone

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**Correspondence:** alpha@efsa.europa.eu

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

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## Summary

This opinion is part of a series of opinions, in which the three first terms of reference (ToRs) 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.

Specific clinical and laboratory procedures for foot and mouth disease (FMD) for each scenario of ToR 1 have not been found in the EU legislation. Specific sampling procedures for clinical and laboratory examination have been provided for some scenarios.

To answer ToR 2, and to assess the minimum length of time measures should be implemented in the protection and surveillance zones (ToR 3.2), an extensive literature search (ELS) was carried out. This ELS aimed to assess the average, shortest and longest period between the earliest point of infection of cattle with FMD virus and the time of reporting of a suspicion by the competent authority. The average time to the reporting of a suspicion was then used to assess the effectiveness of the length of monitoring periods. For most of the scenarios, the existing length of the monitoring period for FMD (21 days) was considered sufficient. Recommendations were given for some of the relevant scenarios. To assess the effectiveness of the minimum length of time in which 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 of the protection zone (15 days) and the surveillance zone (30 days) that must be in place according to existing legislation were considered effective.

To assess the effectiveness of the minimum radius to be implemented in the protection and surveillance zones (ToR 3.1), transmission kernels were used. These kernels had been built using data from previous outbreaks in the Netherlands, Japan and United Kingdom. These kernels represent the relative risk of transmission to each individual establishment from the affected establishment. For FMD, it was observed that, assuming transmission from an affected establishment occurs, the median probability of transmission beyond the protection zone of 3 km was 4.8%. The median probability of infection of an establishment located beyond 10 km was 0.3%. Nevertheless, transmission to longer distances cannot be excluded if infected animals are moved outside the zones.

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# 1. Introduction

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

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

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

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

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

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

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

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

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

### 1.1.2. ToR 2: Monitoring period

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

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

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

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

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

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

ToR 3.2 Assess the effectiveness to control the spread of the disease of the minimum periods during which the competent authority should apply the restriction measures in the protection and surveillance zones as set out in Annexes 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:

- provide an opinion on the effectiveness of the risk-mitigating treatments for products of animal origin and other materials produced or processed in the restricted zone set out in Annex VII and VIII, and
- if relevant, suggest new treatments or procedures that can be effective to mitigate or to eliminate such risk

## 1.2. Interpretation of the Terms of Reference

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

a) The publication of 14 individual opinions, one per each of the diseases included in the list of Category A diseases for terrestrial animals, with each of these opinions providing the answer to ToRs 1, 2 and 3. The current manuscript is one of the 14 opinions covering ToRs 1, 2 and 3 for foot and mouth disease (FMD).
b) The publication of a unique opinion covering ToR 4 for all diseases listed (i.e. ToR 4 is not covered in this opinion).
c) To address ToR 1 (effectiveness of sampling procedures), EFSA agreed with the European Commission on 21 scenarios based on different articles of the Delegated Regulation (EC) 2020/687 (hereinafter referred to as Delegated Regulation), for which the effectiveness of the sampling procedures will be assessed (Annex B). Although these scenarios will be assessed independently, some of these scenarios may be merged if the assessment processes are the same.
d) To address ToR 2 (effectiveness of the monitoring period), seven scenarios previously agreed with the contractor were defined (Annex D). The assessment of the effectiveness of the monitoring period will be done by assessing its ability to ensure that specific actions can be carried out without posing a risk of disease spread, if the monitoring period is calculated backwards or forwards from a specific date. If the length of the monitoring period estimated by EFSA is longer than the existing monitoring periods, the existing monitoring period will be considered non-effective. If the length of the monitoring period estimated by EFSA is shorter than the existing monitoring period, this existing monitoring period will be considered effective from a disease control point of view. No assessment of the plausible unnecessary economic burden that may be placed on the stakeholders as a result of an excessive length of the monitoring periods will be done by EFSA.
e) The assessment of the minimum duration and the length of the radius of the protection and surveillance zones (ToR 3) will be done independently. The setting of these two zones (protection and surveillance zones) surrounding an affected establishment and the control measures implemented in each one of the zones are based on the general principle that the probability of disease spread is larger the closer the establishment is to an affected establishment. The validity of this statement will not be assessed in this manuscript; nonetheless, the limitations that this assumption may have in the control of certain diseases will, when relevant, be discussed.
f) The following scenarios of the ToR 1 of Annex B are not relevant for the FMD, and therefore not included in the assessment of the current Opinion:

   i) scenario 7 because the minimum radius of the protection zone for FMD is 3 km,
   ii) scenarios 10, 11, 16 and 17 because they are referring to poultry.

g) The duration of the monitoring period for FMD as described in Annex II of the Delegated Regulation is 15 days.
h) The minimum length of the radius of the protection zone and surveillance zone for FMD as described in Annex V of the Delegated regulation are 3 and 10 km, respectively.
i) The minimum duration of the measures in the protection and surveillance zone for FMD as described in Annexes X and XI of the Delegated Regulation is 30 days for both zones.

# 2. Epidemiology and geographical distribution of FMD virus

## 2.1. Epidemiology

### Aetiology

Foot and mouth disease is a highly contagious, usually non-fatal vesicular disease affecting mostly domestic and wild cloven-hoofed animals (of the order of the Artiodactyla). The causative agent is the FMD virus (FMDV), a non-enveloped RNA virus, member of the genus *Aphthovirus* in the family *Picornaviridae*, existing in seven distinct serotypes: O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 (Leforban, 2003; OIE, 2013, 2019; Spickler, 2015).

## Epidemiology

FMDV can infect important livestock species such as pigs, cattle, sheep and goats, water buffalos and yaks. Bactrian camels are susceptible unlike dromedary camels; alpacas and llamas may be infected, but do not play a role in transmission (Fondevila et al., 1995). More than 70 wild ungulate species are susceptible including cervids, bison, European wild boar, warthog, antelopes and gazelles, giraffe and African buffalo, the latter being considered a major reservoir for FMDV in Africa, especially for the SAT serotypes (Leforban, 2003; Spickler, 2015). A few species not belonging to the Artiodactyla order can also be infected (e.g. elephant, hedgehog, bear, kangaroo, capybara, nutrias) (Spickler, 2015). Apart from African buffalo, wildlife does not seem to be able to maintain FMDV infection for long periods (Spickler, 2015; Elnekave et al., 2016), but could play a role in virus transmission to livestock in some circumstances (Elnekave et al., 2016).

Despite the fact that mortality is usually low in adult livestock, FMD is a notifiable disease due to its high contagiousness, high morbidity and impact on animal welfare. It is responsible for severe economic losses in production animals and for disruption in national and international trade due to livestock and livestock products movement restrictions from affected countries or regions to disease-free areas (Leforban, 2003).

The disease can be transmitted directly via the respiratory route (aerosol) or by contact with fluids such as saliva, faeces, urine, milk and semen from infected animals. Indirect transmission can occur through fomites (vehicles, equipment) humans, ingestion of contaminated feed (especially in pigs) or via airborne spread. Pigs are less susceptible to aerosol infection than cattle, yet they excrete far more aerosolised virus than cattle or sheep (Grubman and Baxt, 2004).

Animals that have recovered or have been vaccinated and subsequently exposed can experience persistence of FMDV in the oropharynx for more than 28 days and are defined as virus carriers. The duration of this status depends on the species: usually 6 months or less in cattle (up to 3.5 years), 1–5 months in sheep (up to 12 months), up to 4 months in goats and up to 1 year in water buffalo (Spickler, 2015). It is believed that FMDV carriers do not play a major role in the epidemiology of the disease, except for African buffalo, which can shed the virus for at least 5 years (Leforban, 2003; Grubman and Baxt, 2004; Dekker et al., 2008; Spickler, 2015).

The control of the disease depends on the FMD status and the legislation and policy of the affected countries. In endemic zones, inactivated vaccines are used, but they need to match the circulating strains since there is no cross-protection immunity between serotypes and cross-protection might be limited between different strains of the same serotype. In FMD-free regions such as in the EU, vaccination is not routinely used and is restricted to outbreak control (ring vaccination). In case of re-emergence of the disease, measures such as active and passive surveillance, contact tracing, strict livestock movement restrictions and stamping out (culling) of susceptible animals in infected premises are implemented to avoid the spread of the virus (Leforban, 2003; Grubman and Baxt, 2004; Spickler, 2015).

## Clinical signs and diagnosis

The incubation period is 2–14 days in cattle and small ruminants, and usually shorter in pigs (1–2 days). Typical clinical signs are fever, depression and drop in milk production in dairy animals, followed within 24 h by the eruption of multiple vesicles localised on the feet (interdigital space, hooves), muzzle, mouth (tongue and gums) and udder. Vesicles rapidly rupture and become ulcers causing pain, anorexia, hypersalivation, drooling, lameness and reluctance to walk (Geering and Lubroth, 2002; Leforban, 2003; Grubman and Baxt, 2004; Spickler, 2015). Determining the age of FMD lesions, especially when the disease is first recognised in a herd/flock, is a useful aid to estimate the approximate time of first infection (Geering and Lubroth, 2002).

Morbidity can reach 100% in naive cattle or pigs, especially in highly productive breeds, but depends on the strain virulence and on the affected species, with cattle and pigs being more severely affected than small ruminants, which frequently present mild or asymptomatic forms. Mortality is usually low in adult animals (1–5%), recovering within 1–3 weeks, sometimes with sequelae (chronic lameness, decrease in milk production). In contrast, mortality is higher (up to 50% or more) in very young animals (calves, piglets, lambs), which can die from myocarditis or anorexia (Leforban, 2003; Spickler, 2015).

Detection of FMDV during the acute phase of the disease is routinely performed with antigen capture ELISAs or with RT-PCR (gel based or real time, the latter being highly sensitive), using samples such as vesicular fluids, epithelial tissue from fresh vesicles, blood serum or oesophageal–pharyngeal

(OP) fluids (collected with a probang cup in ruminants or throat swabs in pigs). Commercial lateral flow device tests performed on epithelial suspension are useful at field level to confirm clinical cases once the disease is present in a region (Grubman and Baxt, 2004; Spickler, 2015; OIE, 2019).

Seroconversion occurs 1–2 weeks after infection and can be detected using serological tests such as virus neutralisation tests and ELISAs that detect antibodies to viral structural proteins and are serotype specific but cannot differentiate infected from vaccinated animals (DIVA). In contrast, non-structural proteins (NSP) are only produced during viral replication, inducing antibodies in infected but not in vaccinated animals, which will test negative in NSP ELISAs, provided that the vaccine used is sufficiently purified. DIVA tests allow the use of targeted vaccination to control outbreaks in FMDV-free countries, with the possibility to regain their FMDV-free status without necessarily slaughtering vaccinated but uninfected animals, though this strategy has never been implemented in the EU so far (Leforban, 2003; Grubman and Baxt, 2004; Paton et al., 2014; Spickler, 2015; OIE, 2019).

## 2.2. Geographical distribution of foot and mouth disease

FMD has been eradicated from North America, Australasia, Europe and much of South America (Figure 1) by zoosanitary measures supported in some cases by vaccination campaigns. FMD is still widespread throughout the rest of the world, particularly in Asia, Africa and the Middle East (Figure 2). On average, more than 100 countries are not FMD-free. The FMD situation in Venezuela is of particular concern to South America. Over the last 10 years, new FMD strains belonging to different serotypes have been spread from Indian subcontinent while SAT strains from Southern Africa escaped into North Africa and Middle East (EuFMD, 2020b).

The spread of new FMDV strains in the last decade into Turkey, the Middle East and North Africa raises concerns and warrants increased awareness in Europe mainly for two reasons: i) these new and more frequent introductions show that new pathways (trade, migration) bringing new FMDVs have been established and ii) the vaccines in the EU vaccine bank may have a poor match with these new strains, and therefore, there may be a need to develop new vaccines.

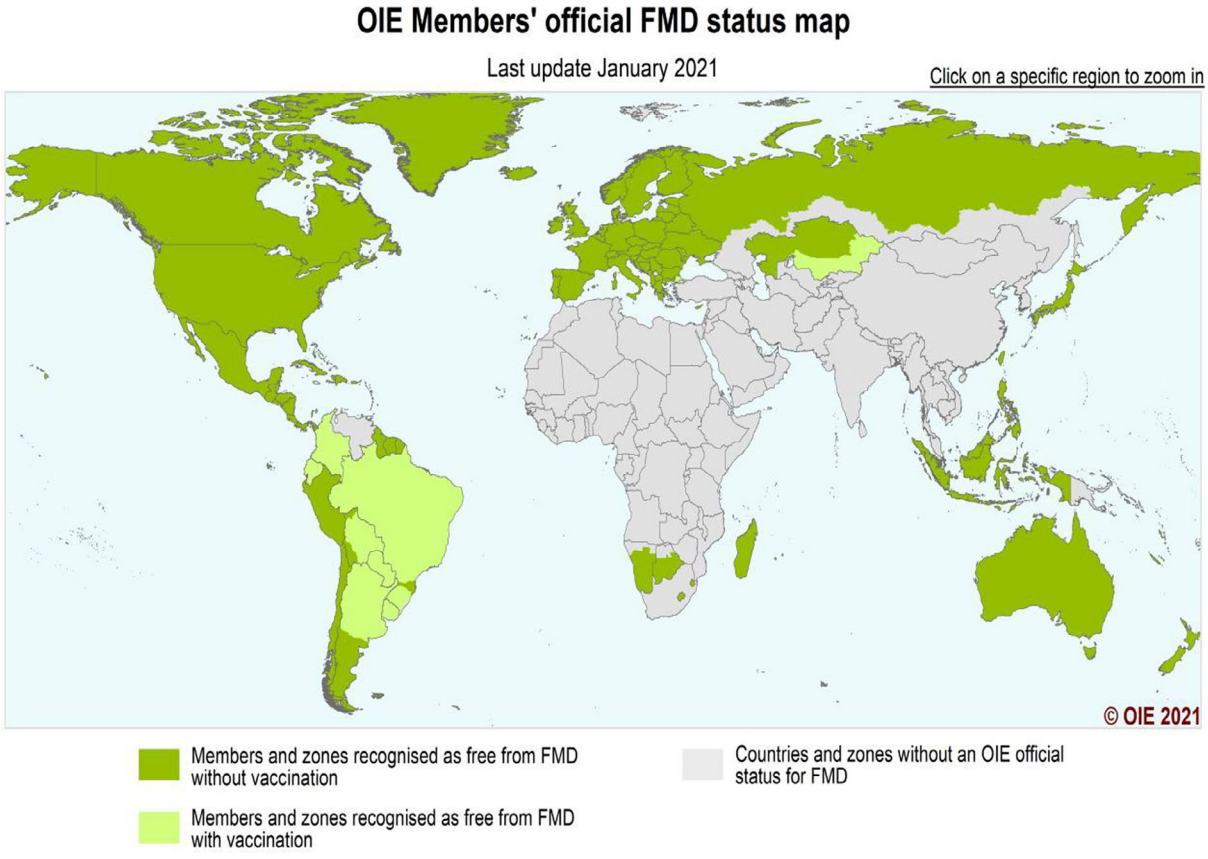


**Figure 1:** Map of countries or zones with the OIE official free status for Foot and mouth disease, 2020 (Source: OIE, © OIE)

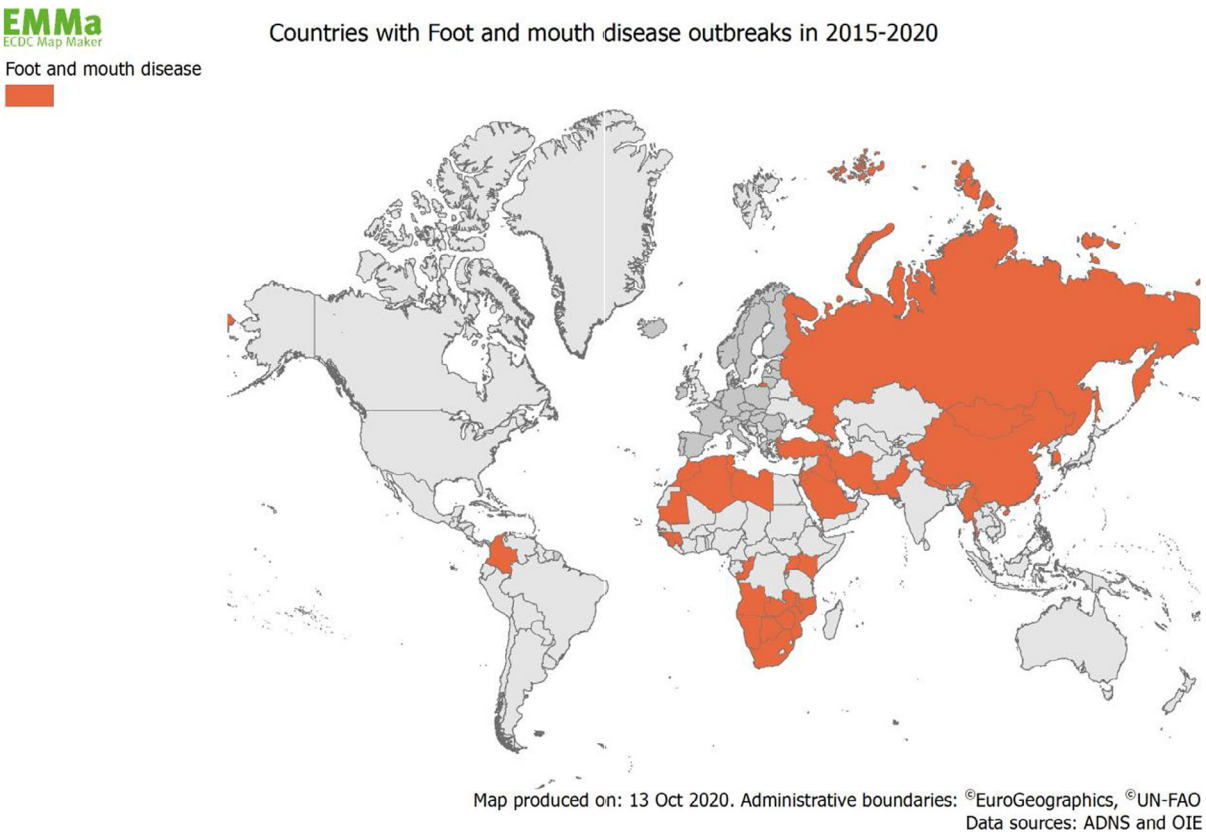


**Figure 2:** Map of countries with notified outbreaks of foot and mouth disease in 2015–2020 (Data sources: ADNS and OIE)

# 3. Data and methodologies

## 3.1. Methodologies

### 3.1.1. Methodology used in ToR 1

A qualitative assessment of the clinical and laboratory procedures was performed to answer ToR 1. Estimation of sample size, when needed, was carried out using the RiBESS+ tool.[1]

To answer the first scenario of ToR 1 in the event of FMD suspicion in an establishment, some additional calculations were needed.

The positive predictive value of the clinical examination ($PPV_{clinical}$, the probability that a selected animal clinically classified as positive is truly FMDV infected) at a certain design prevalence is given by the following equation:

$$PPV_{clinical} = \frac{P(\text{true positive})}{P(\text{true positive}) + P(\text{false positive})} = \frac{Se_{clinical} \cdot DP}{Se_{clinical} \cdot DP + (1 - DP) \cdot (1 - Sp_{clinical})} \quad (1)$$

where $Se_{clinical}$ is the sensitivity of the clinical examination, DP is the design prevalence that needs to be detected and $Sp_{clinical}$ is the specificity of the clinical examination.

The overall probability to detect FMDV by a laboratory test (PCR or Ag ELISA) with a single skin sample would be

$$P_{detect} = PPV_{clinical} \cdot Se_{labtest} \quad (2)$$

where $Se_{labtest}$ is the sensitivity of the laboratory test used.

[1] RiBESS+ tool https://efsa.openanalytics.eu/app/ribess

The probability that at least one truly infected animal is detected is given by the equation:

$$Se_{overall} = 1 - [(1 - P_{detect})]^n \quad (3)$$

Based on the $Se_{overall}$ to be achieved, the n (number of samples needed to be collected) can be calculated

$$n \cong \frac{\ln(1 - Se_{overall})}{\ln(1 - P_{detect})}. \quad (4)$$

### 3.1.2. Methodology used in ToR 2

To answer ToR 2, an extensive literature search (ELS) was outsourced by EFSA (OC/EFSA/ALPHA/2020/02 - LOT 2). 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 FMDV to the time of declaration of a suspicion by the competent authority after the clinical investigation by an official veterinarian) for an outbreak of FMD to be reported. To answer this question, an ELS on case reports, papers describing outbreaks or epidemics of FMD 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 were excluded when the earliest point of infection was determined purely by subtracting a known incubation period from the date of the suspicion of the outbreak. 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 Technical 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**

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

For each kernel, the probability of transmission beyond given distances (if transmission were to occur from an affected establishment) was computed using the estimates and the lower and upper 95% confidence limits for the parameters. In addition, the distances at which a given 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. More details are provided in the Technical report (EFSA, 2020).

**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 4.2.1 were used. Further details can be found in the Technical 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).

# 4. Assessment

## 4.1. Assessment of sampling procedures

### 4.1.1. Assessment of sampling procedures in the event of suspicion or confirmation of Foot and mouth disease (FMD)

#### 4.1.1.1. In the event of a suspicion of FMD in kept animals of listed species in an establishment

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 FMD in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2)). For further details, see Annexes B and C.

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

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

1) It concerns an event of suspicion of FMD in an establishment of kept animals of listed species for FMD;
2) The listed species for FMD as provided in Commission Implemented Regulation 2018/1882 belong to: Artiodactyla and Proboscidea;
3) In the event of a suspicion of FMD, the competent authority shall immediately conduct an investigation to confirm or rule out the presence of the FMD;
4) On the day of the investigation, the official veterinarians must perform clinical examinations and collect samples for laboratory examinations.

**Summary of sampling procedures**

1. Clinical examination: Council Directive 2003/85/EC: Annex III:

'1.1. Holdings must undergo clinical examinations of all animals of susceptible species for signs or symptoms of foot-and-mouth-disease.

1.2. Special emphasis must be laid to animals that may have been exposed to foot-and-mouth disease virus with a high probability, notably transport from holdings at risk or close contact to persons or equipment that had close contact to holdings at risk.

1.3. The clinical examination must take into account the transmission of foot-and-mouth-disease, including the incubation period referred to in Article 2(h) and the way in which animals of susceptible species are kept.

1.4. Relevant records kept on the holding must be examined in detail with particular regard to data required for animal health purposes by Community legislation and, where available, on morbidity, mortality and abortion, clinical observations, changes in productivity and feed intake, purchase or sale of animals, visits of persons likely to be contaminated and other anamnestically important information.

2. Laboratory examination: Council Directive 2003/85/EC: Annex III 2.2.: Sampling on holdings: In holdings where the presence of foot-and-mouth-disease is suspected, but in the absence of clinical signs, sheep and goats, and on recommendation of the epidemiological team other susceptible species, should be examined pursuant to a sampling protocol suitable to detect 5% prevalence with at least 95% level of confidence.'

**Assessment**

In the scenario of a suspicion of FMD in an establishment, the purpose of the clinical examination[2] (including both the initial visual inspection of the herd and the individual examination of the animals) is to identify the cases and collect samples for further laboratory analysis.

[2] Definition of the term 'clinical examination' is provided in the article 3 of the Delegated Regulation: 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.

FMD is of variable severity between species, with dairy cattle and pigs showing obvious signs of illness, whilst infection can be mild or subclinical, especially in small ruminants and partially immune animals (Paton et al., 2014). The clinical signs of FMD are quite specific and suspicion of the disease can be raised when only one animal in a herd is infected (Bouma et al., 2003).

Early detection of FMD in an establishment, when only one or a few animals have been infected, is highly preferred as in such a situation the probability that the infection has spread beyond this affected establishment is still low. Therefore, the minimum number of animals to be examined proposed here allows the detection of the clinical signs' indicative for FMD at the design prevalence of 2% with 95% confidence level.

Regarding the specificity of clinical examination, there are no data from the literature, but it cannot be considered 100% as some clinical signs may be confused with other diseases e.g. mucosal disease and certain manifestations of bluetongue or malignant catarrhal fever in cattle or contagious ecthyma and foot rot in sheep and goats, swine vesicular disease in pigs. Consequently, in a establishment where one of these diseases would be present in addition to FMD, selecting only a single animal for laboratory testing could result in missing the diagnosis of FMD. For the purposes of this opinion, we assume the specificity of clinical examination to detect FMDV infected animals with clinical signs to be 99%. This implies that one in 100 animals not infected with FMD would be considered clinically suspect, because of diseases in the differential diagnosis.

In case of a suspicion of FMD in cattle or a pig herd in naive populations, the sensitivity of the clinical examination to detect animals with clinical signs could be considered high for this scenario. The reason is that the visit is the result of a suspicion raised by the keeper or veterinarian. However, no estimates for the sensitivity of clinical examination in naive populations have been published. In experimental settings, the sensitivity of clinical diagnosis of contact infected animals was 100% (Eble et al., 2006; Orsel et al., 2007). Nevertheless, for this assessment, we assume that it to be lower in practice, because of less ideal circumstances in an establishment compared to a controlled experiment. For that reason, we used a sensitivity of 90% in this assessment. While in populations where the disease is endemic or in sheep and goat herds, the sensitivity is much lower and a sensitivity of 30% was used according to an estimate of Gonzales et al. (2014) in partially immune cattle populations.

PCR and virus isolation can be used to examine epithelium, mouth swabs or oropharyngeal (OP) samples, milk and serum/blood. ELISA, complement fixation (CF) and the lateral flow device are suited to the examination of epithelial suspensions or vesicular fluids but are insufficiently sensitive for the direct examination of OP samples or serum/blood.

Nevertheless, the collection of the OP fluid is not very easy in practice, as it requires a very specific tool (the probang cup), the sample should be stored at −70C$^{o}$ and can be tested only by virus isolation and RT-PCR. Recovery of virus or viral genome can be irregular and one reason for this could be the difficulty in obtaining a consistent sample of mucus and epithelium by means of a probang sampling cup (Parida et al., 2005). Parida et al. (2005) found more samples positive by RT-PCR than by virus isolation, particularly at the later post infection time points. Paton et al. (2006) mentioned that probang sampling followed by RT-PCR had a sensitivity of 50% for detecting FMDV genome in persistently infected animals without clinical signs.

The EFSA Opinion on FMD (EFSA AHAW Panel, 2006) mentions that exact figures for the sensitivity (Se) and specificity (Sp) of the laboratory methods for FMDV are hardly or never found in the literature. The following Table 1 presents the sensitivity and the specificity of some laboratory assays based on the review of several scientific articles.

**Table 1:** Sensitivity and specificity of different laboratory methods, in different sample matrices

| Sample matrices | Method of analysis | Sensitivity (Se) | Specificity (Sp) |
|---|---|---|---|
| **Agent identification** | | | |
| Epithelium | 3D rRT-PCR | 97.7%(1) | Not available |
| | 5′UTR rRT-PCR | 95.4%(1) | 100%(2) |
| | 3D and 5′UTR rRT-PCR* | 99.5%(1) | Not available |
| Serum | 3D rRT-PCR | 98.8% (95% CI: 93.47–99.97)(3) | 100% (95% CI: 94.04–100)(3) |
| | 5′UTR rRT-PCR | 100% (95% CI: 95.65–100)(3) | 100% (95% CI: 94.04–100)(3) |
| Swabs | 3D rRT-PCR | Not available | 100%(4) |

| Sample matrices | Method of analysis | Sensitivity (Se) | Specificity (Sp) |
|---|---|---|---|
| Cell culture after virus isolation from epithelium | Mab Ag-ELISA | 79%[5] | 100%[5] |
| | Polyclonal Ab Ag-ELISA | 72%[5] | 100%[5] |
| Epithelium | Polyclonal Ab Ag-ELISA | 83.3%[6] | Not available |
| **Detection of antibodies** | | | |
| Serum | PrioCHECK™ (Cedi) FMDV NSP-ELISA | 100% Non-vaccinated infected cattle (7–100 dpi)[7] | 99.2%[7] |
| | | 68.1% Vaccinated cattle exposed to infection (28–100 dpi):[7] | 99.2%[7] |
| | SPC-ELISA | 100% 21 dpi or dpv:[8] | Cattle 99.44%; sheep 99.50%; pig 100%[9] |
| | VNT | 95–97%[9],[10] | 99–100%[9],[10] |

rRT-PCR: real-time Reverse Transcription Polymerase Chain Reaction; SPC: Solid-phase competitive.
*: Performing 3D rRT-PCR and 5′UTR rRT-PCR in parallel (Combined PCR).
(1): King et al. (2006).
(2): Reid et al. (2002).
(3): Vandenbussche et al. (2017).
(4): Paixão et al. (2008).
(5): Grazioli et al. (2020).
(6): Roeder and Le Blanc Smith (1987).
(7): Brocchi et al. (2006).
(8): Mackay et al. (2001).
(9): Paiba et al. (2004).
(10): Lefebvre D., Sciensano[3], test validation dossier, personal communication.

## Development of new procedures

### Clinical Examination

The individual clinical examination should focus primarily on those animals identified by the owner/farmer as suspects for FMD or identified by the veterinarians based on clinical signs resembling FMD during the initial visual inspection of the herd (targeted sampling). Animals showing excess salivation, anorexia, lameness, milk drop, reluctance to move and those that are remaining isolated and separated from the herd should be examined carefully for vesicular lesions. Cattle may 'chomb' and grind their teeth. In sheep and goat herds, lameness may be the only symptom at acute stages of the disease.

Appropriate samples for the laboratory analyses must be collected from animals exhibiting clinical signs typical for FMD and sent immediately to the laboratory.

The aim of clinical extermination in this scenario is to detect a sufficient number of animals with clinical signs and collect the minimum number of samples for laboratory examination (see below Table 4). If the number of animals (or more) shown in Table 4 for the laboratory analysis has been already identified, clinical inspection of the whole herd is not needed. On the other hand, if only one or a few suspected animals are identified, further animals should be examined. In that case, the minimum number of animals to be clinically examined to identify animals with clinical signs at a 95% confidence level should be selected according to Table 2 and Figure 3.

From the results in Table 2 and Figure 3, it can be seen that detecting animals with clinical signs with 95% confidence, when assuming only one or a few infected animals (2% in larger populations), cannot be achieved in herd sizes < 100, even if the sensitivity of clinical examination is high (90%); this is the case in naive cattle and pig populations even if all animals are clinically inspected. For herds sizes > 100, the number of animals to be inspected should be as per Table 2. When the herd sensitivity is low (30%), as it is the case in naive sheep and goat populations, vaccinated animals and animals in endemic areas (if such scenario ever arises in the European Union), achieving 95%

[3] European Reference Laboratory for FMD.

confidence is not possible for herds below 500 animals. Even then, most animals in the herd would have to be inspected (Table 2).

In Table 2, the design prevalence had to be adjusted for herd sizes ≤ 50 to reflect the assumption of at least one animal presenting clinical signs. The samples in both examples have been collected from all the various groups of animals present in the establishment.

**Table 2:** Sample size and confidence level (probability to detect animals with clinical signs) achieved in an establishment as a function of the herd size, assuming a target (design) prevalence of animals with clinical signs of 2%, and using two different values of the sensitivity of the clinical examination Se = 90% and Se = 30%

| Herd size (n) | Examples for Sensitivity of the clinical examination Se = 90% (e.g. naive cattle and pig populations) | | | Examples for Sensitivity of the clinical examination Se = 30% (e.g. naive sheep and goat populations, vaccinated animals, animals in endemic areas) | | |
|---|---|---|---|---|---|---|
| | (Design) Prevalence of animals with clinical signs | Sample size | Confidence level | (Design) Prevalence of animals with clinical signs | Sample Size | Confidence level |
| 10 | 10%* | 10 | 89% | 10%* | 10 | – |
| 20 | 5%* | 20 | 89% | 5%* | 20 | – |
| 50 | 2% | 50 | 90% | 2% | 50 | 30% |
| 100 | 2% | 86 | 95% | 2% | 100 | 50% |
| 200 | 2% | 117 | 95% | 2% | 200 | 76% |
| 250 | 2% | 125 | 95% | 2% | 250 | 83% |
| 300 | 2% | 131 | 95% | 2% | 300 | 88% |
| 500 | 2% | 143 | 95% | 2% | 431 | 95% |
| 750 | 2% | 150 | 95% | 2% | 452 | 95% |
| 1000 | 2% | 154 | 95% | 2% | 463 | 95% |

*: The minimum number of animals with clinical signs in a herd is one. Therefore, the values provided here for the design prevalence are the result of the ratio between 1 and the herd size.

In the graphs of Figure 3 and in Table 2, we can see that confidence level of 95% to detect animals with clinical signs, assuming a design prevalence of 2%, cannot be achieved even if all the animals in the herd were sampled, especially when the sensitivity of clinical examination is low. On the other hand, higher values of sensitivity in clinical examination decrease the sample size needed to reach the 95% confidence level with the same design prevalence of 2%.

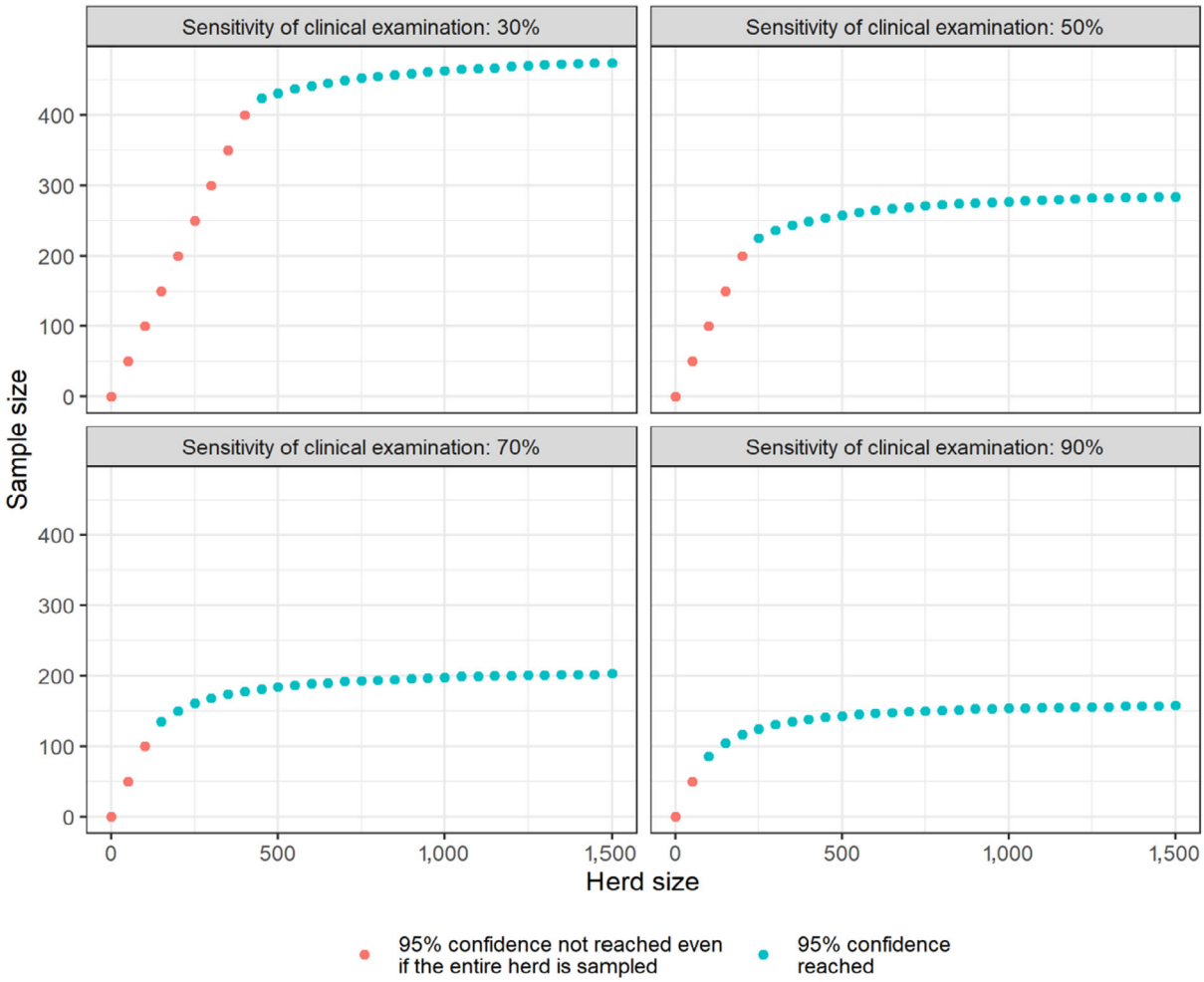


**Figure 3:** Minimum sample size, to detect animals with clinical signs with confidence level of 95%, assuming design prevalence of 2%, using different sensitivity levels for the clinical examination (Se.: 30%, 50%, 70%, 90%)

The confidence to detect animals with clinical signs using clinical examination can be improved by increasing the: i) sensitivity of the clinical examination (e.g. through use of well-trained veterinarians performing a thorough individual clinical examination), and ii) number of animals to be tested (preferably all the animals in the establishment).

Laboratory Examination

For the laboratory diagnosis of FMD, the tissue of choice is epithelium from unruptured or freshly ruptured vesicles or vesicular fluid (Geering and Lubroth, 2002; OIE, 2019). Ideally, at least 1 g or 1–2 $cm^2$ of epithelial tissue should be collected from an unruptured or recently ruptured vesicle, usually from the tongue, buccal mucosa, feet or udder (Geering and Lubroth, 2002; OIE, 2019). Other samples that can be taken to detect the presence of the virus are EDTA-blood, serum and milk (Armson et al., 2018; OIE, 2019). From fatal cases, myocardial tissue or blood can be collected, but epithelium of vesicles are again preferable if present (OIE, 2019).

OIE and several publications proposed that wherever epithelial tissue is not available, for example in advanced or convalescent cases, or where infection is suspected in the absence of clinical signs, samples of mouth swabs can be collected from all species or OP fluid in ruminants, by means of a probang (sputum) cup or in pigs by swabbing the throat, for submission to a laboratory for virus isolation or reverse-transcription polymerase chain reaction (RT-PCR) (OIE, 2019).

The selection of the laboratory test to be used each time depends on the epidemiological situation (e.g. presence of clinical signs, vaccination, endemic areas), the purpose of the sampling, the sample matrices, the results of the previous tests and the capacity of the laboratories (Table 3).

**Table 3:** The preferable sample matrices and the preferable tests for each animal species when clinical signs are present (suspicion) and when clinical signs are not present (preventing killing; testing clinically healthy animals before movement)

| | Cattle/Pigs/Sheep and Goats | |
|---|---|---|
| | **Sample matrices** | **Laboratory tests** |
| **Clinical signs** | Epithelium, Skin lesions, EDTA-blood, Serum, Saliva swabs, Milk | 1st step: 3D rRT-PCR + 5′UTR rRT-PCR; Ag-ELISA against all FMDV serotypes; VI<br>2nd step: In case one of the tests above is positive: FMDV-VP1 sequencing; FMDV serotype specific RT-PCRs<br>3rd step: For more detailed molecular epidemiology: Complete Coding Sequencing or WGS |
| **No clinical signs** | Serum; EDTA-blood; Milk | 1st step: Serum: SP or NSP-Ab-ELISA for non-vaccinated animals; Serum: NSP-Ab-ELISA for vaccinated animals; Milk: 3D rRT-PCR + 5′UTR rRT-PCR<br>2nd step: In case Ab-ELISA is positive: 3D rRT-PCR + 5′UTR rRT-PCR; VI<br>3rd step: In case rRT-PCR or VI is positive: FMDV-VP1 sequencing; FMDV serotype specific RT-PCRs<br>4th step: For more detailed molecular epidemiology: Complete Coding Sequencing or WGS |

VI: virus isolation; Ag: antigen; Ab: antibody; SP: structural proteins; NSP: non-structural proteins; WGS: whole genome sequencing; rRT-PCR: real-time Reverse Transcription Polymerase Chain Reaction; VP1: Viral Protein 1.

Given that for clinically affected animals the sensitivity of the PCR assays in epithelium is higher than 95% and the sensitivity of the Ag-ELISA is 83%, the suggested strategy in the event of suspicion of FMD in an establishment is to take samples from the animals with clinical signs selected as described above.

Samples for the laboratory analysis must be collected first from animals exhibiting prominent FMD lesions, and then from animals showing less specific clinical signs, samples must be sent to the laboratory without delay.

In animals where lesions are detected, and assuming that the specificity of the clinical examination is 100% (no false positive results), and with a sensitivity of the PCR in epithelium from lesions higher than 95%, sampling one animal would be enough to detect FMD with 95% confidence.

However, the specificity of the clinical examination is assumed less than 100% (see Assessment), implying that the confidence would drop, because an FMD false positive animal (animal considered clinically positive for FMD but not infected by FMDV) could be submitted for testing. This is particularly relevant at a low design prevalence (2%).

Assuming i) a specificity of the clinical inspection of 99% (see above) (indicating that 1 in every 100 non-infected animals is considered clinically positive), ii) a design prevalence of 2% and iii) a sensitivity of clinical examination of 90% in naive cattle and pig populations, then the positive predictive value (the probability that a selected animal clinically classified as positive is truly FMD infected) would be 65% (see equation 1 in Section 3.1.1).

The overall probability to detect FMD by PCR with a single sample of epithelium from lesions would be 63% for 3D rRT-PCR and 64% for the combined 3D rRT-PCR and 5′UTR rRT-PCR in parallel (Table 2 and equation 2 in Section 3.1.1). If Ag-ELISA is used instead of PCR, this probability would be 54%.

In order to detect an outbreak with at least 95% confidence, samples from at least four animals need to be sent to the lab for Ag-ELISA, whereas at least three samples would suffice for the single PCR and combined PCR (3D rRT-PCR and 5′UTR rRT-PCR performed in parallel) (Table 4 and equation 4 in Section 3.1.1). For 99% confidence, sample sizes need to be increased at least to 5 for PCR and to 6 for Ag-ELISA (Table 4 and equation 4 in Section 3.1.1).

The sensitivity of the clinical diagnosis is assumed 30% in sheep and goat establishments, animals in endemic areas and vaccinated animals (see Assessment). The overall probability to detect FMD by

PCR with a single sample of epithelium from lesions would be 37% for 3D rRT-PCR and the combined 3D rRT-PCR and 5′UTR rRT-PCR in parallel (Table 1 and equation 2 in Section 3.1.1). If an Ag-ELISA is used instead of PCR, this probability is 32%.

In order to detect an outbreak with at least 95% confidence, samples from at least 7 animals need to be sent to the laboratory if a PCR is used and at least 8 in case Ag-ELISA is used (Table 4 and equation 4 in Section 3.1.1). For 99% confidence, sample sizes need to be increased to 10 for single and combined 3D rRT-PCR and 5′UTR rRT-PCR in parallel and to 13 for Ag-ELISA (Table 4 and equation 4 in Section 3.1.1).

According to Table 1, the test sensitivity of the PCR in serum is not significantly different from that in epithelium and, consequently, the same number of samples can be taken for blood as for epithelium (Table 4).

**Table 4:** Examples of the minimum number of samples needed to be collected from animals with clinical signs using different laboratory methods in several sample matrices, in different populations

| Method (sensitivity) | Sample matrix | Target population | | | |
|---|---|---|---|---|---|
| | | Naive cattle and pig population (clinical examination Se = 90%) | | Naive sheep and goat populations, vaccinated animals and animals in endemic areas (clinical examination Se = 30%) | |
| | | Minimum no. animals 95% confidence | Minimum no. animals 99% confidence | Minimum no. animals 95% confidence | Minimum no. animals 99% confidence |
| 3D rRT-PCR and 5′UTR rRT-PCR in parallel (99.5%) | Epithelium from lesions | 3 | 5 | 7 | 10 |
| 3D rRT-PCR (97.7%) | Epithelium from lesions | 3 | 5 | 7 | 10 |
| Ab Ag ELISA (83.3%) | Epithelium from lesions or blood | 4 | 6 | 8 | 13 |

In summary, to ensure an overall 95% confidence of FMD detection, samples from epithelium from lesions from at least four clinically positive animals are required in previously naive cattle and pig populations. For sheep and goats, this number should increase to 7–8 animals.

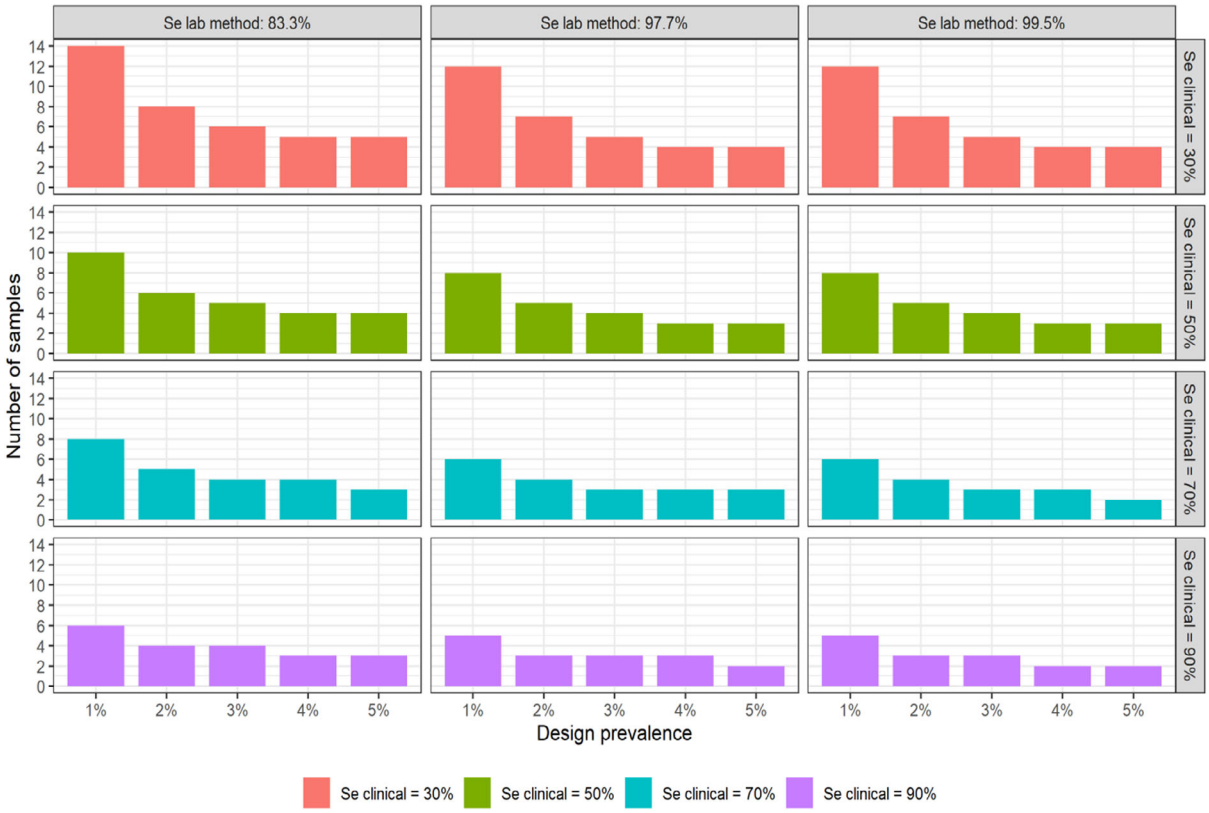


**Figure 4:** Minimum sample size needed to achieve 95% confidence in detecting one infected animal, based to the sensitivity of the clinical examination and the sensitivity of 3 different laboratory tests (Ab Ag ELISA, Se: 83.3%; 3D rRT-PCR, Se: 97.7%; 3D rRT-PCR and 5′UTR rRT-PCR in parallel Se: 99.5%), using different values for Design Prevalence (1%, 2%, 3%, 4%, 5%)

In case no clinical signs are seen, sampling for laboratory examination will follow the procedures described in Section 4.1.1.2 for animals without clinical signs.

Taking different types of samples from each animal (epithelium from different lesions, oral swabs, blood) will increase the confidence to detect or rule out the disease and can prevent technical problems with sampling in the field (e.g. low quality of samples and difficulties to collect samples especially for the epithelium).

In addition, increasing the sensitivity of the clinical examination through well-trained veterinarians performing a thorough individual clinical examination will also increase the level of confidence and decrease the number of samples needed for laboratory examination (Figure 4).

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

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. For further details, see Annexes B and C.

- **2nd scenario of sampling procedures**
- ToR 1.2 in accordance with Article 12(3) and the Art. 7 (4) (Preventive killing) of the Delegated Regulation (EU) 2020/687
- Article 57 of the Regulation (EU) 2016/429

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

1) It concerns an affected establishment officially confirmed
2) Kept animals of listed species found dead or before/when they are killed are sampled
3) The competent authority shall collect samples for laboratory examination
4) The purposes of the sampling are:

   a) to support the epidemiological enquiry:

      i) to identify the likely origin of the disease;
      ii) to calculate the likely length of time that the disease has been present;
      iii) 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; and
      iv) to obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors

   b) to confirm/rule out disease in the event of preventive killing

**Summary of sampling procedures**

Council Directive 2003/85/EC: '2.1.2. To carry out an epidemiological investigation and where sampling is carried out in the framework of disease surveillance after an outbreak, actions shall not commence before at least 21 days have elapsed since the elimination of susceptible animals on the infected holding(s) and the carrying out of preliminary cleansing and disinfection, unless otherwise provided for in this Annex.'

**Assessment**

To support the epidemiological investigation when an affected establishment is officially confirmed, disease-specific sampling procedures based on laboratory examination should be performed.

When FMD has been officially confirmed in an establishment, further sampling procedures will support the needs of the epidemiological enquiry to obtain information on the origin of the disease, the length of time that the disease is present. In addition, in case preventive killing is decided, sampling procedures will confirm or rule out the disease.

**Development of new procedures**

*Estimate the prevalence of animals with clinical signs within the affected establishment*

The prevalence of animals with clinical signs in an affected establishment may help to understand how widespread the infection is within the establishment and for how long it has been present.

For this purpose, when feasible, animals that are still alive or those that are found dead or were culled should be examined to identify clinical signs and lesions compatible with FMD. A brief visual inspection of the mouth and feet of the animals is sufficient for this purpose and in case that is not feasible due to a large population, it is recommended to examine at least 100 animals (allows estimation of 50% prevalence with an accepted error of 10% with 95% confidence). The prevalence of animals with clinical signs and lesions within the establishment can be estimated based on these examinations.

*Estimate the length of time that the disease is present in the establishment*

The estimation of the age of the lesions could contribute to the epidemiological investigation by providing information on the most likely time and pathway of introduction of FMD into the establishment and consequently support the quick tracing of the contacts.

The estimation of the age of the lesions in cattle in the field is generally more accurate when performed up to 5 days after infection by an experienced veterinarian; thereafter the accuracy decreases probably due to secondary bacterial infections of the lesions that may change their

appearance (Alexandersen et al., 2003a; DEFRA, 2005; Ryan et al., 2008; EuFMD, 2020a). There is training material available by EuFMD-FAO[4] that can support veterinarians in the field with this process. The clinical examination should be thorough as the lesion age may vary even in the same animal, with the oldest ones being more important for the epidemiological investigation.

Supplementary information to support the estimation of the time of infection can be provided by the presence or lack of antibodies in animals with clinical signs in an affected establishment. Circulating antibodies against FMDV can be detected by SP-ELISA (e.g. Solid phase competition ELISA) as early as 3–5 days after the first appearance of clinical signs, reaching a high level 2–4 days later (5–9 days after the appearance of clinical signs). The earliest detection of antibodies by virus neutralisation test (VNT) is usually 1–2 days later (Alexandersen et al., 2003b). Following experimental infection, NSP seroconversion takes sometimes 7 days but more often 9–14 days (Paton et al., 2014).

*Collect samples for virus isolation and to identify the likely origin of the disease.*

Since the disease has been confirmed in the establishment and assuming this has not been already done during the investigation of the suspicion, some additional samples may be taken for virus isolation according to the instructions provided by the laboratory. It is particularly important to collect samples, in case clinical signs are present, for those groups of animals in the establishment not sampled previously. The reason is that this may help to identify the origin of the infection or possible onward spread. Material for virus isolation should be collected preferably within the first week of the occurrence of clinical signs, before the development of neutralising antibodies. A range of sample types, including epithelium, OP samples, milk and serum, may be examined by virus isolation.

Sequencing a part of the FMDV genome like VP1 can be enough to determine the origin of the virus (Ularamu et al., 2020). VP1 sequencing also allows the reconstruction of phylogeographic transitions of FMDV strains across and within countries (Bachanek-Bankowska et al., 2018). Using whole genome sequencing (WGS) a higher resolution is obtained and so it is possible to compare FMD viruses from different establishments and to define the virus pathway between establishments during the epidemic (Cottam et al., 2008).

*Confirm the disease in case a preventive killing is decided*

In the Delegated Regulation, preventive killing may be implemented for the animals of listed species for FMD (i.e. members of the orders Artiodactyla, Proboscidea) in three cases: i) in an establishment suspected of FMD, ii) in the establishments in temporary restricted zones and iii) in the establishments of the restricted zone (that is the protection and surveillance zones and further restricted zones).

In case preventive killing is applied, all the animals in the establishment should be subjected to individual clinical examination to identify animals with clinical signs and the whole procedure as described in the first scenario in the event of the suspicion of the disease, should be implemented.

In the absence of clinical signs, the confirmation of FMD will be based on the results of laboratory examinations (Ab-ELISA, RT-PCR) on a sample of animals of the establishment. The samples to be collected are serum, EDTA-blood or milk from a sample of animals. The sample size in the examples is stratified according to the various groups of animals present in the establishment.

Examples for different herd sizes and different sample matrices (EDTA-blood and serum) are presented in Table 5, assuming a design prevalence of FMD (5%). The design prevalence had to be adjusted for herd sizes where $n \leq 10$ to reflect the assumption of at least one animal being infected.

The values for the sensitivity of the laboratory tests used for each type of sampled matrix, are those presented in Table 5.

[4] An online tool with images of FMD lesions in cattle, sheep, goats and pigs in the field is available in EuFMD Lesion Library.

**Table 5:** Sample size to achieve a confidence level of 95% (probability of detecting or ruling out the presence of FMD) in an establishment assuming a design prevalence of 5%, by using PCR on EDTA-blood and NSP-ELISA or SPC-ELISA on serum. The values of the sensitivity of the laboratory tests are those presented in Table 1

| | RT-PCR on EDTA-blood (Se = 98.8%) | | | Ab ELISA on serum | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Non-vaccinated animals NSP-ELISA or SPC-ELISA (Se = 100%) | | | Vaccinated animals NSP-ELISA (Se = 68.1%) | | |
| **Herd size** | **Design prevalence** | **Sample size** | **Confidence** | **Design Prevalence** | **Sample size** | **Confidence** | **Design prevalence** | **Sample size** | **Confidence** |
| **10** | 10%* | 10 | 98% | 10%* | 10 | 100% | 10%* | 10 | 67% |
| **20** | 5% | 20 | 95% | 5% | 19 | 95% | 5% | 20 | 67% |
| **50** | 5% | 39 | 95% | 5% | 39 | 95% | 5% | 50 | 90% |
| **100** | 5% | 45 | 95% | 5% | 45 | 95% | 5% | 66 | 95% |
| **200** | 5% | 52 | 95% | 5% | 51 | 95% | 5% | 75 | 95% |
| **250** | 5% | 55 | 95% | 5% | 55 | 95% | 5% | 80 | 95% |
| **300** | 5% | 54 | 95% | 5% | 54 | 95% | 5% | 80 | 95% |
| **500** | 5% | 56 | 95% | 5% | 56 | 95% | 5% | 82 | 95% |
| **750** | 5% | 57 | 95% | 5% | 56 | 95% | 5% | 83 | 95% |
| **1,000** | 5% | 58 | 95% | 5% | 57 | 95% | 5% | 84 | 95% |

*: The minimum number of animals being infected in a herd is one. Therefore, the values for the design prevalence provided here is the result of the ratio between 1 and the herd size.

FMDV can be detected in milk up to 4 days before the appearance of clinical signs (OIE, 2013). Therefore, milk samples can be collected from individual animals and tested by PCR methods to detect the presence of FMDV at early stage. A study by Armson et al. (2018) shows that collecting milk from bulk storage tanks would allow to detect the presence of FMDV in a dairy cattle herd in an easy and non-invasive way. The findings of this study indicate that it could be possible to identify one acutely-infected milking cow in a typical sized dairy herd (100–1,000 individual) using bulk milk sampling (Armson et al., 2018).

#### 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 FMD affected establishment

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

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the third scenario.

**Assessment**

In an FMD-affected establishment, there might be animals, incubating FMD which have not been detected by laboratory tests carried out. Furthermore, among ruminants, some animals may become 'carriers' following their exposure, and this needs to be taken into consideration when interpreting diagnostic test results. The duration of the carrier state varies with the host species as explained in Section 2.1.

The percentage of animals that become carriers under experimental conditions is variable but is on average around 50% (Alexandersen et al., 2003b); A study by Arnold et al. (2008) showed that the expected prevalence of herds containing carrier-animals after reactive vaccination is likely to be very low, approximately 0.2%; and there will only be a small number of carriers, most likely one, in the infected herds. The virus can be recovered intermittently from such carrier animals from OP samples (Geering and Lubroth, 2002).

**Development of new procedures**

Regular clinical examination should be carried out, preferably every day, to detect early the onset of clinical signs, for a period of at least the existing monitoring period of 21 days calculated forwards from the day of confirmation of the latest case in the establishment.

All the animals intended for derogation from killing should be subjected to thorough individual clinical examination and samples for laboratory examination should be collected from all the animals irrespectively of the presence of clinical signs. This will enable identification of infected animals which have no clinical signs, to estimate the prevalence of FMD in the establishment and to evaluate the risk. Sampling for laboratory examination can be repeated at any time, but the last sampling should be carried out not earlier than 21 days calculated forwards from the day of confirmation of the latest case within the establishment.

Sampling procedures for laboratory examinations in order to detect or rule out the presence of FMD virus should follow the procedures described in Section 4.1.1.2.

#### 4.1.1.4. For the animals of non-listed species kept in an FMD 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. For further details, see Annexes B and C.

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

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

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

## Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the fourth scenario.

Council Directive 2003/85/EC: In the Article 2 (a): *'animal of a susceptible species' means any domestic or wild animal of the suborders Ruminantia, Suina, and Tylopoda of the order Artiodactyla. For specific measures, notably in application of Article 1(2), Article 15 and Article 85(2), other animals, such as for example of the order Rodentia or Proboscidae, may be considered susceptible to foot-and-mouth disease in accordance with scientific evidence.'*

## Assessment

In scientific literature, FMDV natural or experimental infection has been reported in several species other than the Artiodactyla and Proboscidea, although not confirmed by virus isolation or virus detection in all of the cases, and without evidence on their role on the epidemiology (transmission, persistence) of the disease (Thomson et al., 2003; Weaver et al., 2013). The list of orders and species includes Carnivora (bears, minks), Chiroptera (vampire bat), Cingulata (armadilos), Didelphimorphia (possum), Diprotodontia (kangaroos, potoroos, wombats), Eulipotyphla (hedgehogs, moles), Hyracoidea (hyrax), Peramelemorphia (bandicoot), Lagomorpha (rabbits), Rodentia (agoutis, capybara, chinchilla, coypu, gerbils, guinea pigs, mole-rats, porcupine, rats, squirrels, vole) (Weaver et al., 2013).

Some older publications reported suspicion that the European (*Erinaceus europaeus*) and East African (*Atelerix prurei hindu*) hedgehogs were involved in FMD outbreaks in livestock; however, there is no further evidence of their role in FMD spread in recent epidemics in Europe, and there is no report over the last 50 years (Weaver et al., 2013).

Armadillo (*Chaetophractus villosus*) and capybaras (*Hydrochaeris hydrochaeris*) can transmit the FMDV to cattle and pigs under experimental conditions, but there was no evidence of transmission to livestock in the field in Central and South America (Weaver et al., 2013).

FMDV can be experimentally transmitted (usually through intradermal or intradermolingual inoculation) in several species of the orders Rodentia and Lagomorpha that are commonly used as laboratory animals but also in cats, puppies, goldfish and jackdaws (Weaver et al., 2013). Following inoculation of FMDV, the animals developed clinical signs and some of them died (Thomson et al., 2003; EFSA AHAW Panel, 2006; Weaver et al., 2013). Nevertheless, this does not imply that these species can be infected under natural conditions and there is no evidence of their role in the epidemiology of FMD in the field (EFSA AHAW Panel, 2006; Weaver et al., 2013).

## Development of new procedures

Several of the species mentioned above are not natural inhabitants of the European continent, and the role of those that are natural inhabitants of the European continent on FMD transmission is debated. Nevertheless, if they are kept in an establishment affected by FMD, they should be monitored for clinical signs. On the occurrence of clinical signs, samples should be collected for laboratory analysis.

The clinical examination and the sampling for laboratory analysis should be carried out as described in Sections 4.1.1.1 and 4.1.1.2. Nonetheless, the lack of information on the performance of laboratory tests (sensitivity, specificity) in these animal species along with the lack of validation of the diagnostic methods in them will increase the uncertainty on the reliability of the sampling strategy.

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

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

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

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

1) It concerns an affected establishment officially confirmed
2) They may exist wild animals of listed species within the establishment and in the surroundings of the establishment
3) As listed in Commission Implementing Regulation (EU) 2018/1882 for FMD; the wild animals of listed species animals are those of Artiodactyla and Proboscidea
4) The competent authority may establish these sampling procedures in addition to other measures
5) The purpose of the sampling procedures in wild animals of listed species is to ensure the detection of the virus, if the virus is present in these wild species

**Summary of sampling procedures**

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the fifth scenario.

**Assessment**

Based on the review of Weaver et al. (2013), several wild animals of listed species for FMD have been reported to be infected and all seven serotypes of FMDV have been detected.

In most of the cases, infection of wild animals of listed species seems to be secondary, in proximity to outbreaks in domestic livestock and have been reported in endemic areas during/after epidemics in domestic animals. Surveys in wildlife (cervids, wild boar,) conducted in Europe suggested that FMD did not become established in the wildlife and it could not be maintained in the absence of FMD in domestic animals (EFSA AHAW Panel, 2012; Alexandrov et al., 2013; Weaver et al., 2013) (EFSA AHAW Panel, 2012; Weaver et al., 2013). An exception could be for African buffaloes (*Syncerus caffer*) and some species of antelope that are not natural inhabitants of the European Continent.

Genetic and epidemiological data indicated the involvement of African buffaloes in FMDV transmission to cattle populations and their role as reservoir of the three African serotypes (SAT 1,2,3) (Thomson et al., 2003; Weaver et al., 2013).

Occasionally, FMD can cause die-offs of wildlife, as apparently occurred in South Africa in the late 19th century where large numbers of impalas (*Aepyceros melampus*) died, and in Israel where high mortality occurred in mountain gazelles (*Gazella gazella*) (Thomson et al., 2003; Weaver et al., 2013).

There is no evidence to demonstrate the epidemiological involvement of wild animals of listed for FMD species in the spread or maintenance of FMDV. Nonetheless, it is feasible that they could act as spill over hosts and so potentially could be a source of infection for livestock.

**Development of new procedures**

The detection of FMD in wildlife is more complicated than in kept animals not only because of the variation in hosts and virus serotypes but also of the practical difficulties and limitations of surveillance and monitoring activities of wildlife in the natural environment.

The surveillance of wildlife around the affected establishment may include the visual inspection of these animals from a distance and the inspections of any dead animals found, and hunted, trapped animals to identify clinical signs and lesions compatible with FMD.

Samples from dead, hunted or trapped animals should be collected for laboratory analysis, following the procedures of Section 4.1.1.2. Wildlife population health experts would be able to provide additional advice in these circumstances. In addition, it may be possible to collect samples from some wild animal species using non-invasive methods such as salt licks and chewing ropes or baits (Vosloo et al., 2013; Mouchantat et al., 2014).

Nonetheless, the lack of information on the performance of laboratory tests (sensitivity, specificity) in these animal species along with the lack of validation of the diagnostic methods in them will increase the uncertainty of the reliability of the sampling strategy.

#### 4.1.1.6. For non-affected establishments located in a protection zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus if the virus is present in these animals. For further details, see Annexes B and C.

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the sixth scenario.

**Assessment**

For FMD, the minimum radius for the protection zone is 3 km and 10 km for the surveillance zone (Annex V of the Delegated Regulation). An assessment of the effectiveness of the length of the radius of the protection and surveillance zone is presented in Section 4.3.1 and is based on kernels estimations.

According to the assessment for the length of the radius of the protection zone, the median probability of one affected establishment to transmit FMD to another establishment located up to 3 km (within the current protection zone) is 95.2%.

**Development of new procedures**

All establishments located in the protection zone should be visited and all the animals should be clinically examined. Visual inspection of the herd at first place would be very helpful to identify animals with lameness, excessive salvation, reluctance to move and those that are remaining isolated and separated from the herd.

In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible. In this case a minimum sample of animals should be clinically examined, to detect or rule out, at a 95% confidence level or higher, the presence of animals with clinical signs, as described in Section 4.1.1.1.

Since some animals may not show pathognomonic signs in early stages (or in the event of mild cases, as it can occur in sheep and goat herds), the clinical investigation should also focus on some early or more generic signs of the disease such as fever, lethargy, lost appetite, lameness, salivation, nasal discharge, reluctance to move or found separated and isolated from the herd. In addition, it is

necessary to collect further information on the health history of the establishment and the records and documents to be reviewed in order to identify evidence of the presence of the disease such as: morbidity, mortality, clinical observations, changes in productivity and feed intake, purchase or sale of animals, visits of persons likely to be contaminated, transport of animals from holding or areas at risk.

For cattle and pigs, clinical examination would be enough as a typical clinical manifestation of FMD is expected in these species. There is no need for laboratory examination if there are no other reasons based on the national risk assessment to recommend so (e.g. epidemiological link with affected establishment or with affected or high-risk area).

Sheep, goats and animals that have been vaccinated or located in endemic areas (if such scenario ever arises in the European Union) have a less prominent clinical manifestation when infected and therefore laboratory examinations should be considered.

The sampling should be able to detect or rule out the presence of FMD, with a confidence level at least of 95% as described in sampling procedures in Section 4.1.1.2 and in Table 5.

Collecting the number of samples according to Table 5 for detecting antibodies against non-structural proteins (NSP) (indicative for multiplication of the FMDV and therefore the NSP-ELISA can be used as a DIVA test in case of vaccinated animals), depending on the sensitivity of the test allows detection of at least one seropositive animal with 95% confidence if 5% or more are seropositive.

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

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

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the eighth scenario.

**Assessment**

For FMD, the minimum radius for the protection zone is 3 km and 10 km for the surveillance zone (Annex V of the Delegated Regulation). An assessment of the effectiveness of the length of the radius of the protection and surveillance zone is presented in Section 4.3.1 and is based on kernels estimations.

According to the assessment for the length of the radius of the protection and the surveillance zone, the median probability of one affected establishment to transmit FMD to another establishment located beyond 3 km (outside the current protection zone) if transmission occurred, is 4.8%.

In case the surveillance activities do not identify any other affected establishments in the protection zone, the likelihood of FMD having escaped beyond the limits of the protection zone into the surveillance zone is very low.

**Development of new procedures**

For the surveillance zone, it is recommended that the efforts will be allocated to enhance passive surveillance by increasing awareness in all establishments, industry and public. In addition, the

awareness of the veterinarians at the slaughterhouses should be high during the ante-mortem animal inspection and post-mortem inspection of the mouth and the feet in particular for sheep and goats.

Any establishment where more generic signs of the disease such as fever, lethargy, lost appetite, nasal/oral discharge, lameness and even changes in the individual animal behaviour, in the feed intake and productivity are reported should be visited, the animals should be clinically examined and samples should be collected following the procedures described in Sections 4.1.1.1 and 4.1.1.2.

Establishments in the surveillance zone epidemiologically linked to an affected establishment or to any other establishment in the protection zone should be also visited; the animals should be clinically examined, and samples should be collected in case a suspicion is raised following the procedures described in Sections 4.1.1.1 and 4.1.1.2.

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

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

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

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the ninth scenario in EU legislation.

**Assessment**

This scenario includes three different subscenarios: a) the need to transfer animals of listed species for FMD kept in establishments located in the protection zone to a slaughterhouse located within the protection zone; b) the need to transfer animals of listed species for FMD located in the protection zone to a slaughterhouse located within the surveillance zone; and c) the need to transfer animals of listed species for FMD located within the protection zone to slaughterhouse located outside the restricted zone.

During FMD outbreaks, there is risk of undiagnosed infected animals being moved and spreading the disease, and this should be considered when designing animal movement derogations. The highest risk of spread due to movement of undiagnosed animals is associated with subscenario c, then b and finally a. Nevertheless, the fact that the destination of these animals is the slaughterhouse, all biosecurity measures are implemented and given that the animals should be slaughtered within 24 h reduces the risk. In addition, animal slaughtering from the establishments in the protection zone could have beneficial effect encompassing the reduction of the number of potential hosts for the further spread of FMDV.

### Development of new procedures

All the animals in the establishment of origin should be clinically examined before their movement, following the procedures described in Section 4.1.1.1. Visual inspection of the herd would be very helpful to identify animals with lameness, excessive salvation, reluctance to move and those that are remaining isolated and separated from the herd.

In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in this case a minimum sample of animals (including all animals to be moved) should be clinically examined to detect or rule out the presence of animals with clinical signs with at least 95% confidence, as described in Section 4.1.1.1.

In case clinical signs compatible with FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed and any movements should be prohibited.

If animals of listed species for FMD of an establishment located in the protection zone are to be dispatched to slaughterhouses located outside the restricted zone (subscenario c), then in addition to the clinical examination, sampling for laboratory examination should be performed following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals.

#### 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 (Art. 37). For further details, see Annexes B and C.

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

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

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

### Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 12th scenario in EU legislation.

### Assessment

This scenario is very similar to the ninth scenario of Section 4.1.3.1, and therefore, the assessment is the same.

### Development of new procedures

This scenario is very similar to the ninth scenario of Section 4.1.3.1; therefore, the same procedures are suggested.

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

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 13th scenario in EU legislation.

**Assessment**

This scenario includes three different subscenarios: a) the need to transfer animals of listed species for FMD kept in establishments located in the surveillance zone to a slaughterhouse located within the surveillance zone; b) the need to transfer animals of listed species for FMD located in the surveillance zone to slaughterhouse located outside the surveillance zone; and c) the need to transfer animals of listed species for FMD located outside the surveillance zone to slaughterhouse located within the surveillance zone. The highest risk of spread is associated with the subscenario b) where animals move from a higher risk zone to a lower risk zone.

**Development of new procedures**

All the animals in the establishment of origin should be clinically examined before their movement following the procedures described in Section 4.1.1.1. Visual inspection of the herd would be very helpful to identify animals with lameness, excessive salvation, reluctance to move and those that are remaining isolated and separated from the herd.

In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in this case a minimum sample of animals (including all animals to be moved) should be clinically examined to detect or rule out the presence of animals with clinical signs with at least 95% confidence, as described in Section 4.1.1.1.

In case clinical signs compatible with FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed and any movements should be prohibited.

If the dispatch of animals of listed species for FMD located in the surveillance zone is to slaughterhouses located outside the surveillance zone (subscenario b), then in addition to clinical examination, sampling for laboratory examination should be performed following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals.

### 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 the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone. For further details, see Annexes B and C.

- **14th scenario of sampling procedures**
- ToR 1.4 in accordance with article 43(5) and article 45(1) of the Delegated Regulation (EU) 2020/687

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

1) It concerns 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 pastures situated within the surveillance zone
4) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

**Summary of sampling procedures**

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 14th scenario in EU legislation.

Pursuant Art 38 of the Directive 2003/85: '1. Member States shall ensure that animals of susceptible species shall not be removed from holdings within the surveillance zone. 2. By way of derogation, the prohibition provided in paragraph 1 shall not apply to movement of animals for one of the following purposes: (a) for leading them without coming into contact with animals of susceptible species of different holdings, to pasture situated within the surveillance zone not earlier than 15 days after the last outbreak of FMD has been recorded in the protection zone./. . ./ 3. Movements of animals provided for in paragraph 2(a) shall be authorised by the competent authority only after an examination by an official veterinarian of all the animals of susceptible species on the holding, including testing samples taken in accordance with /. . .'

**Assessment**

Animals in a surveillance zone, for which a specific derogation has been granted to be moved to pastures, should be subjected to clinical surveillance, including laboratory examinations.

Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.

Animals of the holding that are negative at the clinical examination and are negative according to procedures described in Section 4.1.1.2 do pose negligible risk of transmission of FMD.

**Development of new procedures**

All the animals in the establishment of origin should be clinically examined before their movement to pastures, following the procedures described in Section 4.1.1.1. Visual inspection of the herd would be very helpful to identify animals with lameness, excessive salvation, reluctance to move and those that are remaining isolated and separated from the herd.

In an establishment where the number of animals is large,, the individual clinical examination of all the animals may not be feasible; in this case a minimum sample of animals (including all animals to be moved) should be clinically examined to detect or rule out the presence of animals with clinical signs with at least 95% confidence, as described in Section 4.1.1.1.

In case clinical signs compatible with FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed and any movements should be prohibited.

In addition to clinical examination, the dispatch of animals of listed species for FMD to pastures situated in the surveillance zone should be done after sampling for laboratory examination, following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals with a confidence level of 95%.

#### 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 them to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone, in order to complete the production cycle before slaughter. For further details, see Annexes B and C.

- **15th scenario of sampling procedures**
- ToR 1.4 in accordance with article 43(5) and article 45(2) of the Delegated Regulation (EU) 2020/687

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

1) It concerns the surveillance zone
2) Grant derogation for movement of kept animals of listed species 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**

No specific guidelines on sampling procedures for clinical or laboratory were found for the 15th scenario in EU legislation.

**Assessment**

Animals in a surveillance zone, for which a specific derogation has been granted to be moved to an establishment of the same supply chain located in or outside the surveillance zone, should be subjected to clinical examination, including laboratory examinations.

Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.

Moving animals from a non-affected establishment found negative at the clinical examination and are negative to laboratory examination, according to procedures described in Sections 4.1.1.1 and 4.1.1.2 minimise the risk of FMDV transmission.

**Development of new procedures**

All the animals in the establishment of origin should be clinically examined before their movement to an establishment belonging to the same supply chain, following the procedures described in Section 4.1.1.1. Visual inspection of the herd would be very helpful to identify animals with lameness, excessive salvation, reluctance to move and those that are remaining isolated and separated from the herd.

In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in this case a minimum sample of animals (including all animals to be moved) should be clinically examined to detect or rule out the presence of animals with clinical signs with at least 95% confidence, as described in Section 4.1.1.1.

In case clinical signs compatible with FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation as described in Section 4.1.1.1 should be followed and any movements should be prohibited.

The dispatch of animals of the listed species for FMD to an establishment belonging to the same supply chain should be done after sampling for laboratory examination, following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals with a confidence level of 95%.

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

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 18th scenario.

**Assessment**

Animals in the restricted zone, for which a specific derogation has been granted for movement within the restricted zone, should be subjected to clinical examination; if they are not immediately slaughtered, they should also be sampled for laboratory examinations.

Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.

Moving animals from non-affected establishments that are negative at the clinical examination and are negative to laboratory examination, according to the procedures described in Sections 4.1.1.1 and 4.1.1.2 minimise the risk of FMDV transmission.

**Development of new procedures**

Sampling procedures should be implemented as described in Sections 4.1.2.1, 4.1.2.3, 4.1.2.4 and 4.1.2.5.

### 4.1.3. Assessment of sampling procedures for repopulation purposes

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

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease. For further details, see Annexes B and C.

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

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

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

**Summary of sampling procedures**

No specific guidelines on sampling procedures for laboratory examination were found for the 19th scenario.

### Assessment

For animals kept for repopulation, clinical examination and sampling should be used as standard procedures to ensure that the animals do not pose a risk of FMD transmission. For animals that are introduced from disease free areas outside the restricted zone, sampling can be omitted because they have not been exposed to virus before entry and, consequently, can only produce a negative test result.

Moving animals from non-affected establishments that are negative at the clinical examination and found negative to laboratory examination, according to the procedures described in Section 4.1.1.1, minimise the risk of FMDV transmission.

### Development of new procedures

Animals intended for repopulation should be subjected to clinical examinations.

In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in this case a minimum sample of animals (including all animals to be moved) should be clinically examined to detect or rule out the presence of animals with clinical signs with at least 95% confidence, as described in Section 4.1.1.1.

In case clinical signs compatible with FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation as described in Section 4.1.1.1 should be followed. The animals intended for the repopulation, even if clinically healthy, should not be dispatched.

If animals are sourced from restricted areas, all the animals in the establishment of origin should be sampled. Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission at a confidence level of 95%. Laboratory examinations should be in accordance to the procedures described in Section 4.1.1.2.

In case the animals originate from establishments located in free areas, there is no need for laboratory examination if there are no other reasons based on the authorities' risk assessment to recommend it (e.g. epidemiological link with an affected establishment or with an affected or high-risk area). Clinical examination as described above would be enough.

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

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

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

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

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

### Summary of sampling procedures

No specific guidelines on sampling procedures for laboratory examination were found for the 20th scenario.

### Assessment

In the case of unusual mortalities or clinical signs compatible with FMD notified during the repopulation, it is important to rule out the presence of the disease.

### Development of new procedures

In the event of animals with clinical signs compatible with FMD, as they have been described in Section 4.1.1.1, being identified in an establishment during the repopulation, the establishment is

considered suspected. The repopulation should be stopped and the procedures for the laboratory confirmation as described in Section 4.1.1.1 should be followed.

In addition, the establishments from where the suspected animals originated from, should be considered as suspected; the procedures described in Section 4.1.1.1 should be followed as well.

#### 4.1.3.3. For animals that have been repopulated

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

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

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

1) It concerns the repopulated establishment
2) Animals that have been used for repopulation
3) The purpose of sampling procedures is to rule out the presence of the disease

**Summary of sampling procedures**

Council Directive 2003/85/EC, Annex V, 1.3: 'Irrespective of the type of farming practised on the holding, re-introduction must conform with the following procedures:

1.3.1 animals must be introduced in all units and buildings of the holding involved;

1.3.2 in the case of a holding consisting of more than one unit or building, re-introduction is not necessary for every unit or building at the same time. However, no animals of species susceptible to foot-and-mouth disease may leave the holding until all the re-introduced animals in all units and buildings have fulfilled all restocking procedures.

1.3.3 animals must be subjected to clinical inspection every three days for the first 14 days following the introduction;

1.3.4 during the period from 15 to 28 days after re-introduction, animals are to be subjected to clinical inspection once every week;

1.3.5 not earlier than 28 days after the last re-introduction, all animals must be clinically examined and samples for testing for the presence of antibody against foot-and-mouth disease virus shall be taken in accordance with the requirements of point 2.2 of Annex III;

1.4 The restocking procedure shall be considered completed when the measures provided for in point 1.3.5 have been completed with negative results.'

**Assessment**

During the repopulation of an establishment previously affected by FMD, there is still a risk of re-introduction of the disease with the new animals being infected either at the establishment of origin or during their transport, and a risk of re-emergence of the disease if the new animals are infected after their arrival at the establishment of destination. The animals that have been used for the repopulation should be submitted to thorough clinical and laboratory examination in order to rule out the presence of the disease.

**Development of new procedures**

Animals must be subjected to clinical inspection at least every three days for the first 14 days following the introduction, and weekly from 15 to at least 21 days (monitoring period as defined in the Delegated Regulation) after re-introduction. The last day of the monitoring period following the latest day of animals' introduction, all the animals should be subjected to thorough clinical examination as described in Section 4.1.1.1 and should be sampled for laboratory examination in accordance to the procedures described in Section 4.1.1.2.

In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in this case a minimum sample of animals (including all animals to be

moved) should be clinically examined, to detect or rule out the presence of animals with clinical signs with at least 95% confidence, as described in Section 4.1.1.1.

If clinical signs are identified, then the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed.

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

The concept of the monitoring period has been introduced as a management tool for the investigation and control of suspected and confirmed outbreaks of Category A diseases in terrestrial animals. This tool aims to standardise the methodology by which relevant authorities respond 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 D in this Opinion describes the seven scenarios, for which an assessment of the length of the monitoring period for FMD had been requested.

For the assessment of this ToR, the methodology described in Section 2.3 of the Technical Report published by EFSA (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 FMDV, 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, as well as the earliest time of antibody detection in blood, with the outputs being discussed with relevant experts.

### 4.2.1. Results

Extensive Literature Search

A search was carried out identifying 2,305 references published after 1/1/2000. Among these references, 15 were selected to be included in the qualitative review. The full selection process is displayed in Figure 5.

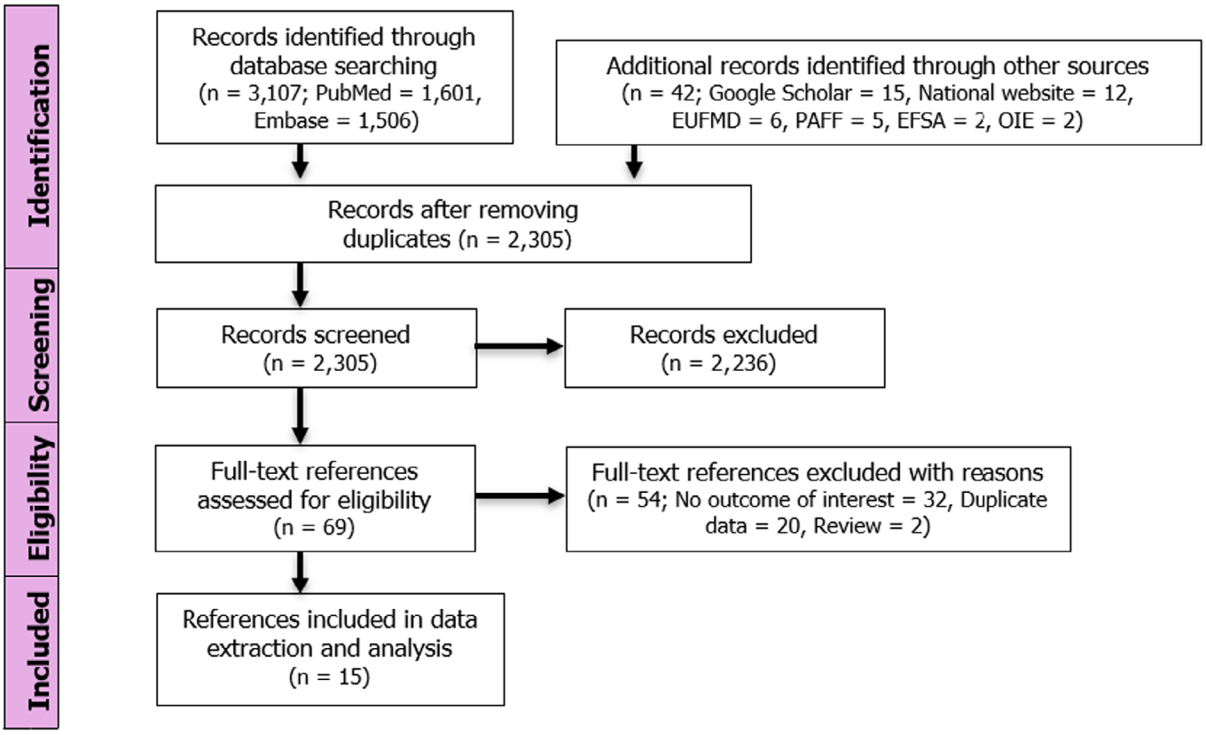


**Figure 5:** PRISMA diagram FMD monitoring period ELS

The majority of the references reported dates instead of periods (10 references out of 15); therefore, the dates were used to calculate the different periods of interest.

Table 6 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.

**Table 6:** Summary of the FMD extraction for the period between earliest point of infection and suspicion report

| Reference | Country | Outbreak year | Species | Period between earliest point of infection and suspicion report (days) |
|---|---|---|---|---|
| Gibbens et al. (2001) | United Kingdom | 2001 | Pig | 21(1) |
| Ferguson et al. (2001) | United Kingdom | 2001 | Cattle<br>Sheep | 8(1)<br>9.51 |
| Alexandersen et al. (2003a) | United Kingdom | 2001 | Cattle | 6–26(2) |
| EuFMD (2001) | France | 2001 | Cattle | 14(3) |
| Bouma et al. (2003) | Netherlands | 2001 | Goat | 19(4) |
| Ryan et al. (2008) | United Kingdom | 2007 | Cattle | 8–13(5) |
| DEFRA (2007b) | United Kingdom | 2007 | Cattle | 7–20(2) |
| DEFRA (2007a) | United Kingdom | 2007 | Cattle | 6–18; 11–23(2) |
| EFSA AHAW Panel (2012) | Bulgaria | 2011 | Cattle | 6–18(2) |
| Rautureau et al. (2012) | France | NA | Cattle, pig, sheep and goat | 6–14(6) |

(1): Average period based on date of first infection determined retrospectively through the examination of lesions.
(2): Most likely window (min-max) based on date of first infection determined retrospectively through the examination of lesions.
(3): The 'shortest' probable period based on the date of slaughter of the UK imported sheep that most likely infected the cattle neighbouring farm.
(4): Suspicion was made on goats, contaminated by imported Irish calves. The period is based on the import date of the calves, which were infected during a stopover in Mayenne (France).
(5): Min–max based on date of first infection determined retrospectively through the examination of lesions and several hypotheses; the most likely source is a release of virus from the Pirbright Institute site.

(6): Median range obtained from simulated epidemics based on an FMD SLIJR transmission model, the general level of actor awareness, passive surveillance network type, FMDV strain virulence and district. The transmission and the detection parameters were estimated using expert opinion and the results of a meta-analysis of data from experimental infections. As this value is not species-specific, it was further excluded from the analysis.

The available information on the main outcome of interest, i.e. the period between the earliest point of infection and the suspicion report, is described in Table 6.

The first outbreak during the 2001 epidemic in the United Kingdom (Ferguson et al., 2001) was suspected following an ante-mortem inspection in an Essex abattoir of pigs presenting signs of acute infection. Three days had occurred between the arrival of the pigs at the abattoir and the suspicion report by the Official Veterinary Surgeon (Gibbens et al., 2001). As a result of backward contact tracing, a veterinary inspection found FMD on a swill-fed pig fattening unit that was identified as the most likely source of the abattoir outbreak. Epidemiological investigations revealed that 21 days had elapsed between the first infection and the confirmation in these premises (Gibbens et al., 2001; Sutmoller et al., 2003; McLaws, 2005).

The longest period retrieved was 26 days and was reported in the context of a cattle outbreak during the 2001 epidemic in the United Kingdom (Alexandersen et al., 2003a). This is the upper value of the time window that was calculated by adding the estimated age (12 days) of the oldest lesion observed at suspicion, to the theoretical incubation period (2–14 days) (OIE, 2013).

Only one extracted value out of fourteen concerned sheep (Ferguson et al., 2001) or goat (Bouma et al., 2003) or pig (Gibbens et al. (2001) outbreaks, and it is therefore difficult to draw conclusions in these species.

Based on the species-specific data presented in Table 6, the average, shortest and longest period was calculated separately for cattle, pigs and small ruminants (i.e. values referring to multiple species were not used):

Cattle (n = 8):

- Average period = 13 days
- Shortest period = 6 days
- Longest period = 26 days

Pigs (n = 1):

- Average/Shortest/Longest period = 21 days

Small ruminants (n = 2):

- Average period = 14 days
- Shortest period = 9.5 days
- Longest period = 19 days

Based on the results presented in Table 6, it can be observed that the current Monitoring Period (21 days) is longer or equal to the average period obtained in the ELS for all species. However, it must be noted that only one reference was available for sheep, goats and pigs. Considering the fact that clinical forms of FMD are generally less severe in small ruminants than in cattle (OIE, 2013), these results might not be representative of sheep or goat outbreaks that are expected to be associated with longer reporting periods compared with cattle outbreaks.

### Seroconversion in animals

Several publications describing experimental infection with FMDV were consulted (Tables 7–9) and the time of seroconversion after infection/inoculation and contact was retrieved from the serological results described. Nevertheless, these studies were not designed to estimate the time between infection and seroconversion (first time when antibodies can be detected) and they can only provide an estimation.

In experimental studies (Table 7), where non-vaccinated naive animals were infected directly with FMDV through inoculation of the virus(es) via intradermal lingual route or inoculation in the coronary band, the latest day of seroconversion was: i) 10 dpi by VNT, ii) 8 dpi by SPCE, iii) 5 dpi by LPBE and iv) 15 dpi by NSP ELISA (Cedi, PrioCHECK).

**Table 7:** Latest day seroconversion started as retrieved from publications describing experimental studies where FMDV was inoculated directly into non-vaccinated naive animals. The antibodies were detected using different laboratory methods

| Laboratory method | Animals | Type of inoculation/ infection | FMDV serotypes used for infection | Latest day when seroconversion started (dpi) | References |
|---|---|---|---|---|---|
| VNT (CO ≥ 1/45) | Cattle | IDL | O | 7 | Paiba et al. (2004) |
| VNT | Cattle | IDL | A, O, SAT | 10 | Parida, personal communication, 2021[5] |
| VNT (CO ≥ 1/45) | Sheep | Coronary band | O | 10 | Paiba et al. (2004) |
| VNT | Sheep | – IDL<br>– Coronary band<br>– IDL + Coronary band | O | 10 | Madhanmohan et al. (2020) |
| | Goats | | | 10 | |
| VNT (titre ≥ 40) | Pigs | IDL | O + ASFV | 11 | Douglas et al. (1993) |
| SPCE (CO ≥ 60 PI) | Cattle | IDL | O | 5 | Paiba et al. (2004) |
| SPCE (CO ≥ 60 PI) | Sheep | Coronary band | O | 8 | Paiba et al. (2004) |
| LPBE (CO ≥ 50% PB at serum dilution 1/10) | Cattle | IDL | A, O, C | 5 | O'Donnell et al. (1996) |
| NSP ELISA (Cedi) (CO ≥ 50 PI) | Cattle | IDL | A, O, SAT | 10–15(1) | Parida, personal communication, 2021[5] |
| NSP ELISA (PrioCHECK (Cedi)) (CO ≥ 50 PI) | Sheep | – IDL<br>– Coronary band | O | 15(2) | Madhanmohan et al. (2020) |
| | Sheep | – IDL +Coronary band | | 10 | |
| | Goats | – IDL<br>– IDL +Coronary band | | 10 | |
| | Goats | – Coronary band | | 15 | |

dpi: days post infection inoculation; IDL: Intradermal lingual route; LPBE: Liquid-phase blocking ELISA; NSP: Non-Structural Protein Antibody Test; SPCE: Solid-phase Competition ELISA; VNT: Virus Neutralisation Test; PI: percentage inhibition; PB: Percentage Blocking; CO: cut-off.
(1): Between 9 and 15 dpi no samples were taken.
(2): Sampling every 5 days.

In experimental studies (Table 8) where non-vaccinated naive animals were infected through direct contact with animals infected with FMDV serotype O, the latest day of seroconversion was: i) 20 dpc by VNT, ii) 20 dpc by SPCE, iii) 14 dpc iELISA, iv) 4 dpc by LPBE, v)16–28 dpc by NSP ELISA commercial tests. It should be mentioned that the time of seroconversion has been calculated forwards from the day when the animals joined the infected animals and not from the day of infection, which is not known. This fact may explain the delay in detection of seroconversion in these experiments.

[5] Personal commutation with Dr Parida Satya from Pirbright Institute (World Reference Laboratory for FMD) on unpublished data.

**Table 8:** The latest day of seroconversion (when the last animal of the study seroconverted) in the experimental studies where non-vaccinated, naive animals were infected through direct contact with infected animals with FMDV serotype O. The antibodies were detected using different laboratory methods

| Laboratory method | Animals | Latest day of seroconversion (dpc) | References |
|---|---|---|---|
| VNT | Cattle | 7 | Cox et al. (2005) |
| VNT (CO ≥ 1/45) | Cattle | 20 | Paiba et al. (2004) |
| VNT (CO ≥ 1/45) | Sheep | 10 | Paiba et al. (2004) |
| SPCE (CO ≥ 60 PI) | Cattle | 12 | Parida et al. (2005) |
| SPCE (CO ≥ 60 PI) | Cattle | 20 | Paiba et al. (2004) |
| SPCE (CO ≥ 60 PI) | Sheep | 8 | Paiba et al. (2004) |
| SPCE (CO ≥ 60 PI) | Pig | 9 | Paiba et al. (2004) |
| Indirect 2B peptide ELISA (CO: OD > 0.67) | Cattle | 14 | Parida et al. (2005) |
| LPBE(CO = 50% PB at serum dilution 1/40) | Pigs | 4 | Alexandersen et al. (2001) |
| NSP ELISA (commercial tests: UBI, Cedi, Bommeli) CO according to the manufacturer | Cattle | Cedi: 28<br>Bommeli: 16<br>UBI: 16 | Parida et al. (2005) |
| NSP ELISA (Cedi) and in house ELISA | Sheep | 15(1) | Madhanmohan et al. (2010) |
| | Goats | 10(1) | |

dpc: days post contact with infected animals; LPBE: Liquid-phase blocking ELISA; NSP: Non-Structural Protein Antibody Test; SPCE: Solid-phase Competition ELISA; VNT: Virus Neutralisation Test; PI: Percentage Inhibition; PB: Percentage Blocking; OD: optical density; CO: cut-off.
(1): Sampling every 5 days.

In experiments where vaccinated animals against FMDV, were infected through direct contact with animals infected with FMDV serotype O, the latest day of seroconversion identified for each laboratory methods is presented in Table 9, and, as can be observed, there is a delay in detecting a seroconversion compared to non-vaccinated animals (Table 8). For these experiments, the effect of the vaccination on the immune system of the animals should be considered in the interpretation of the results. It should be also mentioned that the time of seroconversion has been calculated forwards from the day when the animals joined the infected animals and not from the day of infection, which was not known.

**Table 9:** Latest day of seroconversion (when the last animal of the study seroconverted) in the experimental studies where vaccinated animals were infected through direct contact with infected animals with FMDV serotype O. The antibodies were detected using different laboratory methods

| Laboratory method | Animals | Latest day of seroconversion in the study (dpc) | Animals |
|---|---|---|---|
| VNT (CO ≥ 1/45) | Cattle | 16 | Parida et al. (2005) |
| Indirect 2B peptide ELISA (CO: OD > 0.79) | Cattle | 42 | Parida et al. (2005) |
| NSP ELISA (commercial tests: UBI, Cedi, Bommeli) CO according to the manufacturer | Cattle | Cedi: 42<br>Bommeli: 91<br>UBI: 63 | Parida et al. (2005) |
| NSP ELISA (commercial tests: UBI, Cedi, Bommeli) CO according to the manufacturer | Pigs | Cedi: 42<br>Bommeli: 91<br>UBI: | Parida et al. (2005) |
| NSP ELISA (Cedi) and in house NSP ELISA | Sheep | 28(1) | Madhanmohan et al. (2010) |
| | Goats | 35(2) | |

dpc: days post contact with infected animals; IDL: Intradermal lingual route; LPBE: Liquid-phase blocking ELISA; NSP: Non-structural protein antibody test; SPCE: Solid-phase competition ELISA; VNT: Virus neutralisation test; OD: Optical density.
(1): Previous negative sampling in 21 dpc.
(2): Previous negative sampling in 28 dpc.

In the experimental studies mentioned above, the antibodies remained detectable until the end of the observation period of the trial or until the animals were euthanised. The longest period of antibody detection found in experimental studies was 565 dpi, with LPBE in cattle serum as reported by (O'Donnell et al., 1996).

### 4.2.2. Assessment

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

**Scenarios 1, 2 and 3**

**1st scenario of monitoring period**

- ToR 2 in accordance with article 8 and Annex II of the Delegated Regulation (EU) 2020/687
- Article 57 of the Regulation (EU) 2016/429
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of the notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of a suspicion of a FMD outbreak

**2nd scenario of monitoring period**

- ToR 2 in accordance with article 17(2) and Annex II of the Delegated Regulation (EU) 2020/687
- Article 57 of the Regulation (EU) 2016/429
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of confirmation of a FMD outbreak

**3rd scenario of monitoring period**

- ToR 2 in accordance with article 13(b) and Annex II of the Delegated Regulation (EU) 2020/687
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a FMD outbreak in an epidemiological unit in which the disease has not been confirmed, in order to provide derogations from killing the animals in this unit, if this unit has been completely separated, and handled by different personnel during this monitoring period

For the first three scenarios, the main purpose of the use of the monitoring period is to be able to carry out 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, backward 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 and remains undetected in an establishment, and due to the regular activities carried out in this establishment, could have spread to other epidemiological units.

In the case of scenario 3, if no epidemiological links between the establishment that has been confirmed positive and the other epidemiological units are found during the investigation (and only if other conditions described in the legislation are met), a derogation from killing the animals in the separated non-affected epidemiological units could be granted.

The period of time the disease could have been present and undetected in an establishment equates then to the time period between the entry of FMD into the establishment and the reporting of the suspicion. Once the suspicion has been officially reported, control measures are implemented and further spread should in this way be prevented.

Based on the ELS carried out and presented above, the average length of the time between infection and the suspicion report was estimated as 13 days in cattle, 21 in pigs and 14 days in small ruminants, based on articles where an epidemiological investigation was carried out. Although the monitoring period defined in the Delegated Regulation is longer than the average calculated for all animal species using this methodology, it is important to take into account that when the disease is first introduced in an area, detection may be late. This should be considered when carrying out an epidemiological investigation in the index case (first affected establishments) in an area.

The length of the monitoring period of 21 days as defined in the Delegated Regulation is therefore considered effective, except for the first affected establishments detected in an area, where a monitoring period of 26 days, (the longest period found in the ELS) is recommended.

### Scenario 4

**4th scenario of monitoring period**

- ToR 2 in accordance with article 27(3)c and Annex II of the Delegated Regulation (EU) 2020/687
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of the FMD 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 suspected nor an affected establishment, but restrictions are still in place, for establishments in the protection zone.

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).

As the disease has already been detected in the area, and high awareness is expected, the length of the monitoring period is considered effective in this scenario.

### 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 (EU) 2020/687
- 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 the non-affected establishments (located in a protection or surveillance 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 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 affected establishment that originated the protection zone, and the latest date the semen could have become contaminated would be the date the semen was collected.

FMDV can be detected in high titres in the semen of infected animals (EFSA AHAW Panel, 2006), and the longest reported detection time in frozen semen (–50°C, with or without extenders) is 320 days (Cottral et al., 1968). A more recent study conducted in an affected cattle farm by Sharma et al. (2012) showed that semen from infected bulls was found positive by mPCR 5 months after infection but was negative after 8 months. Based on the EFSA AHAW Panel (2006), the probability of infection through artificial insemination (AI) with contaminated semen is higher than negligible and may be very high.

In this scenario, where the semen might have been contaminated the latest at the date of collection from an infected donor without clinical signs or with mild clinical signs that remained unnoticed, a serological test would indicate if the donor has ever been exposed to FMDV and therefore if the semen could be contaminated.

Based on the results presented in Section 4.2.1 in relation to the seroconversion in non-vaccinated naive animals, the latest date of seroconversion was identified as 15 days post infection by NSP ELISA (Parida, personal communication, 2021[5]; Madhanmohan et al., 2020).

The latest date of seroconversion for non-vaccinated, naive animals infected through contact with already infected animals was identified as 28 days post contact by NSP ELISA as reported by Parida et al. (2005).

Consequently, and based on the results of the publications, sampling the animals at least 28 (21 + 7) days after semen collection as foreseen in the Delegated Regulation is considered effective to detect antibodies with several laboratory methods, given that the infection may have occurred at the latest on the day of semen collection.

### Scenarios 6 and 7

**6th scenario of monitoring period**

- ToR 2 in accordance with article 57 (1) and Annex II of the Delegated Regulation (EU) 2020/687
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated forward from the date of the final cleaning and disinfection in an affected establishment, after which the repopulation of the establishment may be allowed by the competent authority (assuming relevant control of insects and rodents was carried out)

**7th scenario of monitoring period**

- ToR 2 in accordance with article 59 (4) and Annex II of the Delegated Regulation (EU) 2020/687
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated forward from the date the first animal was introduced for the purpose of repopulation, during this monitoring period, all animals of the listed species intended for repopulation should be introduced

In scenarios 6 and 7, the monitoring period is used in the context of repopulation.

In scenario 6, the monitoring period is used to ensure that the repopulation process is not put at risk due to the disease still being present unknowingly in establishments within the surrounding area of the establishment to be repopulated (if an establishment tested positive to FMDV 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 period equal to the monitoring period has elapsed, since the final cleaning, and disinfection of the affected establishment.

In this regard, the number of days of the monitoring period for FMD, counted from the day of the final cleaning and disinfection, must ensure enough time for any potentially affected surrounding establishment to be reported as a suspicion. Considering the results presented in Section 4.1.2, and taking into account that a high level of awareness is expected due to the disease having been present in the area, the EFSA AHAW Panel considers the existing length of the monitoring period (21 days)

effective, as it would allow for the identification of any potentially affected establishment in the surrounding area prior to the repopulation taking place.

In scenario 7, the monitoring period must be counted forwards from the date on 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 all animals have been moved into the repopulated establishment. Although the preferred option is that all animals are introduced into the establishment to be repopulated at the same time, this is not always feasible. The first clinical and laboratory sampling of the repopulated animals takes place once all the animals are in situ. By restricting the period of time during which animals may be introduced into the establishment, the period of time during which the disease could be unknowingly spreading within the establishment is reduced. Assuming that the latest point of infection of an animal introduced into the repopulated establishment is the day when it is moved, and considering that the average length of time to detection is 13 days in cattle, 12 in pigs and 14 days in sheep and goats, it would be likely that some clinical signs would be present in animals if this visit is carried out 21 days after the last introduction. In this scenario, using the average length of time to detection would be justified as a high awareness will exist during the examination of the animals at the first visit. The EFSA AHAW Panel considers the existing length of the monitoring period (21 days) effective, as it would allow for early detection of potentially infected animal at the first visit following re-stocking.

## 4.3. Assessment of the minimum radius and time periods of the protection and surveillance zones set in place subsequent to an FMD outbreak

### 4.3.1. Assessment of the minimum radius

The purpose of this section is to assess the effectiveness to control the spread of FMDV of the minimum radius of the protection and surveillance zones as set out in Annex V of the Delegated Regulation for FMD. According to this regulation, the minimum radius for the protection and surveillance zones for FMD is 3 km and 10 km, respectively (Annex V of the Delegated Regulation).

**Results**

To address this request, transmission kernels (Table 10 and Figure 6) have been used to analyse outbreak data for four epidemics of FMD (Table 10), all of which were caused by strains of FMDV serotype O. Three different functional forms for the kernel were used (Table 10). The fitted kernels are consistent in shape across the epidemics, with a substantial drop off in the kernel beyond 3 km (Figure 6).

**Table 10:** Transmission kernels for FMDV serotype O

| Kernel | Epidemic | Parameters* | | Reference |
|---|---|---|---|---|
| | | $d_0$ (km) | $\alpha$ | |
| $k(r) = 1 + \left(\frac{r}{d_0}\right)^{-\alpha}$ | Japan 2010 | 0.58 | 2.47 | Hayama et al. (2013)† |
| | UK 2001 | 1.32 (1.10, 1.55) | 2.67 (2.56, 2.77) | Chis Ster and Ferguson (2007)† |
| | UK 2001 | 1.38 (1.33, 1.44) | 2.84 (2.77, 2.93) | Chis Ster et al. (2009)† |
| | UK 2001 | 1.92 (1.41, 2.45) | 3.1 (2.8, 3.3) | Chis Ster et al. (2012)† |
| | UK 2001 | 1.84 (1.62, 2.05) | 4.17 (3.93, 4.40) | Schley et al. (2009) |
| $k(r) = \left(1 + \left(\frac{r}{d_0}\right)^{\alpha}\right)^{-1}$ | NL 2001 | 0.9 (0.4, 2.1) | 2.3 (2.0, 2.8) | Backer et al. (2012) |
| | NL 2001 | 1.22 (0.67, 3.35) | 2.8 (2.3, 4.1) | Boender et al. (2010) |
| | UK 2001 | 1 | 3 | Werkman et al. (2016) |
| | UK 2007 | 0.75 | 2 | Jewell et al. (2009) |
| $k(r) = \begin{cases} 1 & r \le d_0 \\ \left(\frac{r}{d_0}\right)^{-\alpha} & r > d_0 \end{cases}$ | UK 2001 | 0.72 (0.44, 1.14) | 1.66 (1.62, 1.70) | Deardon et al. (2010) |

*: 95% confidence or credible intervals are shown in brackets if they were reported in the original reference.
†: These studies considered several kernels or models; kernel parameters for the best-fitting one, are included here.

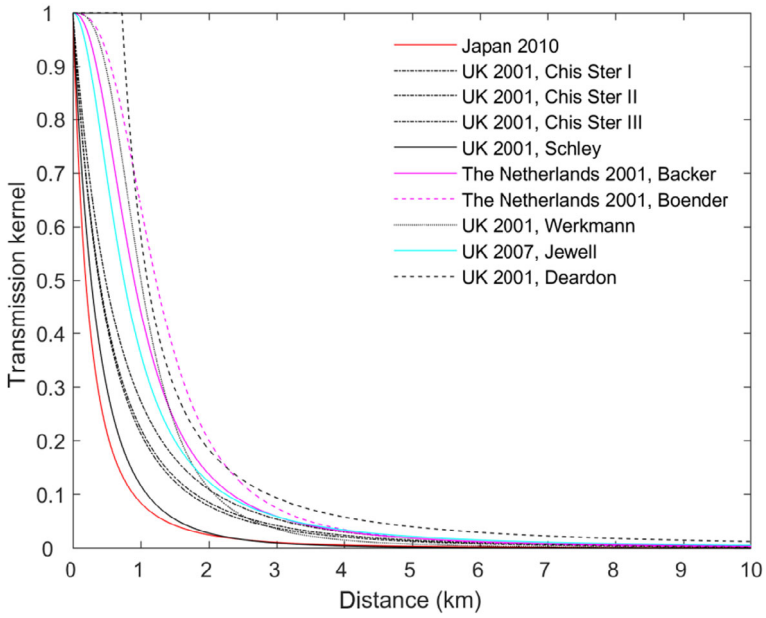


**Figure 6:** Transmission kernels for FMDV

For each kernel in Table 10, the probability of transmission beyond given distances (if transmission was to occur from an affected establishment) was computed using the estimates, lower and upper 95% confidence limits, including distances beyond the proposed radius for the protection and surveillance zones (3 km and 10 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, lower and upper 95% confidence limits (Figure 7). The corresponding values computed using the estimates are summarised in Table 12.

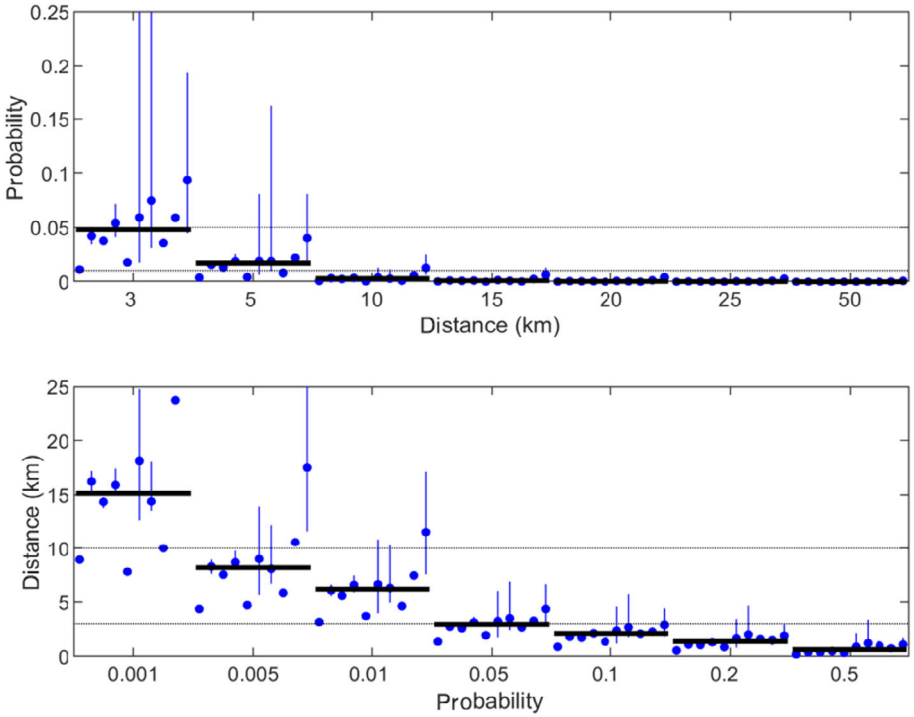


**Figure 7:** Assessment of the radius of the protection and surveillance zone for FMDV. The top panel shows the probability of transmission beyond a given distance (if transmission were to occur from an affected establishment) computed using the estimates (blue circles) and the lower and upper 95% confidence limits (error bars) for each kernel (and in the same order as) in Table 10. The thick black line indicates the median probability for all kernels. The black dotted lines indicate threshold probabilities of 0.05 and 0.01. The bottom panel shows the distances at which a threshold probability of transmission beyond that distance is reached calculated using the estimates (circles) and lower and upper 95% confidence limits (error bars) for each kernel. The thick black line indicates the median distance for all kernels. The black dotted lines indicate distances of 3 and 10 km (i.e. the proposed radius of the protection and surveillance zones, respectively)

**Table 11:** Probability of transmission of foot and mouth disease virus beyond different distances (km) from an affected establishment point based on the kernels of Table 10 and Figure 6

| | **Distance (km) of transmission from affected establishment** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **3** | **5** | **10** | **15** | **20** | **25** | **50** |
| Median | 4.8% | 1.7% | 0.3% | 0.1% | 0.1% | < 0.1% | < 0.1% |
| Minimum | 1.1% | 0.4% | < 0.1% | < 0.1% | < 0.1% | < 0.1% | < 0.1% |
| Maximum | 9.4% | 4.0% | 1.3% | 0.7% | 0.4% | 0.3% | 0.1% |

**Table 12:** Distances (km) at which the probability of transmission of foot and mouth disease virus beyond that distance reaches a threshold level point based on the kernels of Table 10 and Figure 6

| | **Threshold probability of transmission beyond certain distances (km)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0.1%** | **0.5%** | **1%** | **5%** | **10%** | **20%** | **50%** |
| Median | 15.1 | 8.2 | 6.2 | 2.9 | 2.1 | 1.4 | 0.6 |
| Minimum | 7.8 | 4.4 | 3.2 | 1.4 | 0.9 | 0.5 | 0.2 |
| Maximum | 46.2 | 17.5 | 11.5 | 4.4 | 2.9 | 2.0 | 1.2 |

**Assessment**

As seen from Figures 6 and 7, Tables 11 and 12, that the probability of FMD transmission beyond a certain distance from an affected establishment decreases as the distance increases.

The individual transmission event from one affected establishment to another was estimated using different distances and different thresholds of the probability of FMD transmission FMD certain distances.

Table 11 shows that, if transmission occurs, the median probability of transmission of FMD from one affected establishment beyond 3 km (outside the current protection zone) is 4.8%, while the maximum probability is 9.4%. Likewise, the median probability of one affected establishment to transmit FMD to another establishment located beyond 10 km (outside the current surveillance zone) is 0.3% while the maximum probability is 1.3%.

Based on the median relative probability of transmission from an affected establishment beyond given distances if transmission occurs, it is concluded that the minimum radius of the protection zone (3 km) and the surveillance zone (10 km) (as set out in Annex V of the Delegated Regulation for FMD) are effective assuming a threshold of 95% probability as used in several articles of the AHL. For the surveillance zone, the expected effectiveness could be increased up to a 99% probability.

Transmission over longer distances cannot be excluded if infected animals are moved outside the zones.

Based on the estimations of the kernels, the probability of transmission outside the defined zones decreases as the radius of the zones (distance from the affected establishment) increases. Takin into consideration the local epidemiological situation, the density of the establishments and the commercial activities different combinations of radiuses in the protection and the surveillance zones may be selected to further decrease the spread of the disease.

### 4.3.2. Assessment of the minimum period

The purpose of this section is to assess the effectiveness to control the spread of FMD of the minimum periods during which the competent authority should apply the restriction measures in the protection and surveillance zones as set out in Annexes X and XI for the FMD. The minimum period for the protection zone is 15 days, while for the surveillance zone is 30 days

To assess the minimum length of time the protection zone and the surveillance zones should be kept in place, the average (for the protection zones) and the longest (for the surveillance zones) period between the earliest point of infection and the notification of a suspicion will be used (EFSA, 2020).

Based on the results of the ELS as presented in Table 6 in Section 4.2.1, it follows that the average time between infection and notification of the suspicion is 13 days in cattle, 12 in pigs and 14 days in small ruminants. Therefore, the minimum period of 15 days indicated in the Delegated Regulation for the restriction measures in the protection zone is considered effective to detect effected establishments and to prevent the movement of infected animals from the protection zone.

In addition, the maximum period between introduction and suspicion is 26 days in cattle, 21 in pigs and 19 days in small ruminants. Consequently, the minimum period of 30 days indicated in the Delegated Regulation for the restriction measures in the surveillance zone is considered effective to detect affected establishments and to prevent the movement of infected animals from the surveillance zone.

However, it must be noted that only one reference for swine and two for small ruminants (one for sheep and one for goats) were available from the ELS. Considering the fact that clinical forms of FMD are generally less severe in small ruminants than in cattle (OIE, 2013), these results might not be representative of sheep or goat outbreaks that are expected to be associated with longer reporting periods compared with cattle outbreaks.

### 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.

# 5. Conclusions and Recommendations

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
|---|---|---|---|
| **ToR 1: In the event of suspicion or confirmation** | | | |
| **1st scenario** Section 4.1.1.1 In the event of a suspicion of FMD in an establishment where animals of the listed species are kept | 1. Clinical examination: Council Directive 2003/85/EC: Annex III: '1.1. Holdings must undergo clinical examinations of all animals of susceptible species for signs or symptoms of foot and mouth disease.<br>1.2. Special emphasis must be laid on animals which may have been exposed to foot and mouth disease virus with a high probability, notably transport from holdings at risk or close contact to persons or equipment that had close contact to holdings at risk.<br>1.3. The clinical examination must take into account the transmission of foot and mouth disease, including the incubation period referred to in Article 2(h) and the way in which animals of susceptible species are kept.<br>1.4. Relevant records kept on the holding must be examined in detail with particular regard to data required for animal health purposes by Community legislation and, where available, on morbidity, mortality and abortion, clinical observations, changes in productivity and feed intake, purchase or sale of animals, visits of persons likely to be contaminated and other anamnestically important information.<br>2. Laboratory examination: Council Directive 2003/85/EC: Annex III 2.2.: Sampling on holdings: In holdings where the presence of foot and mouth disease is suspected but in the absence of clinical signs, sheep and goats and on recommendation of the epidemiological team other susceptible species, should be examined pursuant to a sampling protocol suitable to detect 5% prevalence with at least 95% level of confidence.' | Foot and mouth disease (FMD) is of variable severity between species, with cattle and pigs showing obvious signs of illness, whilst infection can be mild or subclinical especially in small ruminants and partially immune animals.<br>To detect an outbreak in previously naive cattle and pig herds with 95% confidence, samples of at least 3 animals with typical clinical signs should be submitted to the lab when using the combined PCR test (3D-RT-PCR + 5′UTR-RT-PCR performed in parallel). In case of a single PCR test or antigen ELISA, samples of 4 animals should be submitted.<br>A sample of epithelium of lesions is preferred for laboratory analysis, but if not possible, an EDTA-blood sample can also be used.<br>To detect an outbreak in previously naive sheep or goat flocks with 95% confidence, samples of at least 7 animals with clinical signs should be submitted to the lab when using the PCR test. In case of antigen ELISA, samples of 8 animals should be submitted. | It is recommended to examine as many individual animals in a suspect cattle or pig herd so that at least 3–4 clinically suspect animals have been identified for collecting samples for laboratory analysis that is likely with 95% confidence that the prevalence of such animals is below 2%.<br>In a suspect cattle or pig herd, it is recommended to submit samples from at least 3 animals with clinical signs to a lab where a combined PCR test is used, or from 4 animals in case a single PCR test or Ag ELISA is used.<br>It is recommended to examine as many individual animals in a suspect sheep/goat flock so that at least 7–8 clinically suspect animals have been identified for collecting samples for laboratory analysis, or that it is likely with 95% confidence that the prevalence of such animals is below 2%.<br>In a suspect sheep/goat flock it is recommended to submit samples from at least 7 clinically suspect animals to a lab, where a PCR test is used, or from 9 animals in case Ag ELISA is used.<br>In the absence of clinical signs in a sheep/goat flock, collecting serum samples allowing the detection of a 5% design prevalence with 95% confidence is recommended. |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
|---|---|---|---|
| **2nd scenario** Section 4.1.1.2. For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU) 2016/429 in an FMD officially confirmed establishment | Council Directive 2003/85/EC: 2.1.2. To carry out an epidemiological investigation and where sampling is carried out in the framework of disease surveillance after an outbreak, actions shall not commence before at least 21 days have elapsed since the elimination of susceptible animals on the infected holding(s) and the carrying out of preliminary cleansing and disinfection, unless otherwise provided for in this Annex. | Epidemiological enquiry<br>The epidemiological enquiry in an affected establishment may be supported by sampling procedures for the following purposes:<br>• to estimate the prevalence of clinical signs within the affected establishment to understand how widespread the infection is within the establishment and for how long whenever this is feasible<br>• to estimate the age of the lesions providing information on the most likely time and pathway of introduction of FMD into the establishment and consequently support the quick tracing of the contacts.<br>• to identify the virus and estimate the geographical origin of the disease.<br>Preventive Killing<br>Confirm and rule out the disease in case of preventing killing will be based on clinical and laboratory examination of the animals. | Epidemiological enquiry<br>• Animals that are still alive or those that are found dead or were culled should be examined to identify clinical signs and lesions compatible with FMD. A brief visual inspection of the mouth and feet of the animals is sufficient for this purpose and in case that is not feasible due to a large population, it is recommended to examine at least 100 animals (allows estimation of 50% prevalence with an accepted error of 10% with 95% confidence). Lesions in those animals first infected may allow to estimate the length of time the disease is present in the establishment.<br>• Additional samples for PCR can be collected in a confirmed affected establishment to investigate how widespread the infection is. Sequencing of VP1 can be helpful to determine the origin of the virus.<br>Preventive Killing<br>In case of preventive killing, all animals should be subjected to clinical examination, and if no cases of clinically suspects are observed, blood samples should be collected from a random sample of animals allowing detection of a 5% prevalence to be tested in PCR and indirect antibody ELISA. |
| **3rd scenario** Section 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 | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 3rd scenario. | In an FMD affected establishment, there might be animals, which are in the incubation period without having been detected by laboratory tests carried out. Furthermore, among ruminants some animals may become 'carriers' following their exposure, and this needs to be taken into | Regular clinical examination should be carried out, preferably every day, to early detect the onset of clinical signs, for a period of at least the existing monitoring period of 21 days calculated forwards from the day of confirmation of the latest case.<br>All the animals intended for derogation of killing should be subjected to thorough individual clinical examination to identify those animals with clinical signs in order to take samples for the laboratory examination.<br>Sampling all the animals for laboratory examination, as |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
|---|---|---|---|
| FMD affected establishment | | consideration when interpreting diagnostic test results. | soon as the derogation of killing is applied and irrespectively of the presence of clinical signs, will enable to identify also infected animals without clinical signs, estimate the prevalence of FMD in the establishment and evaluate the risk.<br>Sampling for laboratory examination can be repeated at any time, but the last sampling should be carried out not earlier than 21 days calculated forwards from the day of confirmation of the latest case.<br>For laboratory procedures, see Sections 4.1.1.1 and 4.1.1.2. |
| **4th scenario** Section 4.1.1.4. For the animals of non-listed species kept in an LSD affected establishment. | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 4th scenario.<br>Council Directive 2003/85/EC: In the Article 2 (a): 'animal of a susceptible species' means any domestic or wild animal of the suborders Ruminantia, Suina and Tylopoda of the order Artiodactyla. For specific measures, notably in application of Article 1(2), Article 15 and Article 85(2), other animals, such as for example of the order Rodentia or Proboscidae, may be considered susceptible to foot and mouth disease in accordance with scientific evidence. | FMDV natural or experimental infection has been reported in several species other than the Artiodactyla and Proboscidea, although not confirmed by virus isolation or virus detection in all of the cases, and without evidence of their role on the epidemiology (transmission, persistence) of the disease in the field.<br>In some cases, the species mentioned are not natural inhabitants of the European Continent.<br>The available diagnostic methods for FMD may not be validated for these animals. | If clinical signs occur in animals of non-listed species kept in an affected establishment, samples from these animals should be collected for further laboratory examinations.<br>The lack of information on the performance of laboratory tests (sensitivity, specificity) in these animal species along with the lack of validation of the diagnostic methods in them will increase the uncertainty on the reliability of the sampling strategy.<br>See also 1st scenario in Section 4.1.1.1 and 2nd scenario in Section 4.1.1.2 on sampling procedures. |
| **5th scenario** Section 4.1.1.5. For wild animals of the listed species within the FMD affected establishment and its surroundings. | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 5th scenario. | Several wild animals of listed species for FMD have been reported to be infected in the literature and all seven serotypes of FMDV have been detected. Nonetheless, there is no evidence to demonstrate their epidemiological involvement to the | Surveillance of wildlife in the surroundings of an affected establishment may include the visual inspection of these animals from a distance and the inspection of any dead animal found, hunted and trapped animals to identify clinical signs compatible with FMD.<br>On the occurrence of clinical signs laboratory examination should be followed. |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
|---|---|---|---|
| | | spread or maintenance of FMDV. It is feasible that they could act as spill over hosts and so potentially could be a source of infection for livestock.<br><br>African buffaloes (*Syncerus caffer*) and some species of antelopes that are not natural inhabitants of the European Continent.<br>The available diagnostic methods for FMD may not be validated for these animals. | The lack of information on the performance of laboratory tests (sensitivity, specificity) in these animal species along with the lack of validation of the diagnostic methods in them will increase the uncertainty on the reliability of the sampling strategy.<br><br>See also 1st scenario in Section 4.1.1.1 for clinical examination and sampling for laboratory examination in case clinical signs are present.<br><br>See also 2nd scenario in Section 4.1.1.2 for laboratory sampling in case of absence of clinical signs. |
| **6th scenario**<br>Section 4.1.1.6. For animals of listed species in the non-affected establishments located in a protection zone | No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the 6th Scenario. | Based on kernels estimations (see also Section 4.3.1) the median probability of FMD transmission of one affected establishment to transmit FMD to another establishment located up to 3 km (within the current protection zone) is 95.2%. | All the establishments located in the protection zone should be visited and a minimum sample of animals should be clinically examined with at least 95% confidence level to detect or rule out the presence of animals with clinical signs, as described in Section 4.1.1.1.<br><br>In case of sheep and goat, the sampling should be able to detect or rule out the presence of FMD, with a confidence level at least of 95% as described in sampling procedures in Section 4.1.1.2. |
| **8th scenario**<br>Section 4.1.1.7. For non-affected establishments located in a surveillance zone | No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the 8th scenario. | Based on kernels estimations (see also Section 4.3.1) the median probability of FMD transmission from an affected establishment beyond the borders of a protection zone of 3 km is 4.8% while the probability of escaping beyond the borders of the surveillance zone is 0.3%.<br><br>In case the surveillance activities in the protection zone do not identify other affected establishments, the probability of FMD having escaped beyond the | For the surveillance zone, it is recommended that the efforts will be allocated to enhance passive surveillance by increasing awareness in all establishments, industry and public. In addition, the awareness of the veterinarians at the slaughterhouses should be high during the ante-mortem animal inspection and post-mortem inspection of the mouth and the feet in particular for sheep.<br>Any establishment where more generic signs of the disease such as fever, lethargy, lost appetite, nasal/ophthalmic/oral discharge, oedema of the limbs, lameness and even changes in the individual animal behaviour, in the feed intake and productivity, should be visited, the animals should be clinically examined and samples should be collected following the procedures described in Sections 4.1.1.1 and 4.1.1.2.<br>Establishments in the surveillance zone epidemiologically |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
|---|---|---|---|
| | | limits of the protection zone into the surveillance zone is very low. | linked to an affected establishment or to any other establishment in the protection zone should be also visited; the animals should be clinically examined, and samples should be collected in case a suspicion is raised following the procedures described in Sections 4.1.1.1 and 4.1.1.2. |
| **ToR 1: To grant derogations for animal movements** | | | |
| **9th scenario** Section 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 | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 9th scenario in EU legislation. | This scenario describes three different subscenarios: a) the need to transfer animals of listed species located in the protection zone to slaughterhouse located within the protection zone, b) the need to transfer animals of listed species located in the protection zone to slaughterhouse located within the surveillance zone; and c) the need to transfer animals of listed species located within the protection zone to slaughterhouse located outside the restricted zone. The highest risk of spread due to movement of undiagnosed animals is associated with subscenario c. | All the animals in the establishment of origin should be clinically examined before their movement. Following the procedures described in Section 4.1.1.1.<br>In an establishment where the number of animals is large, and therefore, the individual clinical examination of all the animals is not feasible, a minimum sample of animals (including all animals to be moved) should be clinically examined with at least 95% confidence level to detect or rule out the presence of animals with clinical signs, as described in Section 4.1.1.1.<br>In case clinical signs compatible to FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed and any movements should be prohibited.<br>If animals of listed species for FMD of an establishment located in the protection zone are to be dispatched to slaughterhouses located outside the restricted zone (subscenario c), then in addition to the clinical examination, sampling for laboratory examination should be performed following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals. |
| **12th scenario** Section 4.1.2.2 From non-affected establishments located in the protection zone to a plant approved for | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 12th scenario in EU legislation. | This scenario is very similar to the scenario 9th of Section 4.1.2.1. | This scenario is very similar to the 9th scenario of Section 4.1.2.1; therefore, the same procedures will be followed for this scenario as well. |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
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| processing or disposal of animal by-products in which the animals are immediately killed | | | |
| **13th scenario** Section 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 | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 13th scenario in EU legislation. | This scenario describes three different subscenarios: the need to transfer animals of listed species located in the surveillance to slaughterhouse located within the surveillance zone (a), the need to transfer animals of listed species located in the surveillance zone to slaughterhouse located outside the surveillance zone (b); the need to transfer animals of listed species located outside the surveillance zone to slaughterhouse located within the surveillance zone (c). Overall, it is true what assessed in 4.1.2.1. The highest risk of spread is associated with the subscenario (b) where animals move from a higher risk zone to a lower risk zone. | All the animals in the establishment of origin should be clinically examined before their movement. Following the procedures described in Section 4.1.1.1.<br>If dispatch of animals of listed species located in the surveillance zone is to slaughterhouses located outside the surveillance zone (subscenario b), then in addition to clinical examination, sampling for laboratory examination should be performed following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals.<br>In an establishment where the number of animals is large and therefore the individual clinical examination of all the animals is not feasible, a minimum sample of animals (including all animals to be moved) should be clinically examined with at least 95% confidence level to detect or rule out the presence of animals with clinical signs, as described in Section 4.1.1.1.<br>In case clinical signs compatible to FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed and any movements should be prohibited.<br>If dispatch of animals of listed species located in the protection zone is to slaughterhouses located outside the restricted zone (subscenario b), then sampling should be performed following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals. |
| **14th scenario** Section 4.1.2.4 From an establishment in | Directive 2003/85 article 38: Member States shall ensure that animals of susceptible species shall not be removed from holdings within the surveillance | Animals in surveillance zone for which a specific derogation has been granted to be moved to | All the animals in the establishment of origin should be clinically examined before their movement to pastures following the procedures described in Section 4.1.1.1. |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
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| a surveillance zone to pastures situated within the surveillance zone | zone. However, by way of derogation, this prohibition shall not apply to movement of animals for leading them without coming into contact with animals of susceptible species of different holdings to pasture situated within the surveillance zone not earlier than 15 days after the last outbreak of foot and mouth disease has been recorded in the protection zone. In addition, movements of animals to pastures shall be authorised by the competent authority only after an examination by an official veterinarian of all the animals of susceptible species on the holding, including testing. | pastures should be subjected to clinical surveillance, including laboratory examinations. Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.<br>Animals of the holding that are negative at the clinical examination and are negative to laboratory examinations according to procedures described in Section 4.1.1.2 do pose negligible risk of transmission of FMD. | In case clinical signs compatible to FMD are identified, the establishment is considered suspected, and the procedures for the laboratory confirmation that are described in Section 4.1.1.2 should be followed and any movements should be prohibited.<br>The dispatch of animals of listed species for FMD to pastures situated in the surveillance zone should be done after sampling for laboratory examination, following the procedures described in Section 4.1.1.1, in order to exclude infected but subclinical animals with a confidence level of 95%.<br>In an establishment where the number of animals is large, and therefore, the individual clinical examination of all the animals is not feasible, a minimum sample of animals (including all animals to be moved) should be clinically examined with at least 95% confidence level to detect or rule out the presence of animals with clinical signs, as described in Section 4.1.1.1. |
| **15th scenario** 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 | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 15th scenario in EU legislation. | Animals in surveillance zone for which a specific derogation has been granted to be moved to an establishment of the same supply chain located in or outside the surveillance zone should be subjected to clinical surveillance, including laboratory examinations.<br>Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.<br>Animals of the holding that are negative at the clinical examination and are negative at the laboratory examinations according to | All the animals in the establishment of origin should be clinically examined before their movement to an establishment belonging to the same supply chain, following the procedures described in Section 4.1.1.1.<br>In an establishment where the number of animals is large and therefore the individual clinical examination of all the animals is not feasible, a minimum sample of animals (including all animals to be moved) should be clinically examined with at least 95% confidence level to detect or rule out the presence of animals with clinical signs, as described in Section 4.1.1.1..<br>In case clinical signs compatible to FMD are identified, the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed and any movements should be prohibited. |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
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| | | procedures described in Section 4.1.1.1 do pose negligible risk of transmission of FMD. | The dispatch of animals of listed species for FMD to an establishment belonging to the same supply chain should be done after sampling for laboratory examination, following the procedures described in Section 4.1.1.2, in order to exclude infected but subclinical animals with a confidence level of 95%. |
| **18th scenario** Section 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 | No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 18th scenario. | Animals in the restricted zone, for which a specific derogation has been granted to be moved within the restricted zone, should be subjected to clinical surveillance; if they are not immediately slaughtered, they should also be sampled for laboratory examinations.<br>Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.<br>Animals of the holding that are negative at the clinical examination and are negative according to laboratory procedures described in Section 4.1.1.2 do pose negligible risk of transmission of FMD. | The same sampling procedures, according to different scenarios, should be implemented as those described in Sections 4.1.2.1, 4.1.2.3, 4.1.2.4 and 4.1.2.5 |
| **ToR 1: For repopulation purposes** | | | |
| **19th scenario** Section 4.1.3.1 For the animals that are kept for the repopulation prior to their introduction | No specific guidelines on sampling procedures for laboratory examination were found for the 19th scenario. | Clinical examination and sampling should be used as standard procedures to ensure that the animals do not pose a risk of transmission with a confidence level of 95%.<br>Moving animals from non-affected establishments that are negative at | Animals should be subjected to clinical examination.<br>In an establishment, where the number of animals is large, and therefore, the individual clinical examination of all the animals is not feasible, a minimum sample of animals (including all animals to be moved) should be clinically examined with at least 95% confidence level to detect or rule out the presence of animals with clinical signs, as described in Section 4.1.1.1. |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
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| | | the clinical examination and found negative to laboratory examination, according to the procedures described in Section 4.1.1.1, minimise the risk of FMDV transmission. | If animals are sourced from restricted areas, all the animals in the establishment of origin should be sampled. Sampling procedures for laboratory examination should ensure that the animals do not pose a risk of transmission with a confidence level of 95%. Laboratory examinations should be in accordance to the procedures described in Section 4.1.1.1.<br><br>In case clinical signs compatible to FMD, are identified the establishment is considered suspected and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed. The animals intended for the repopulation even clinically healthy should not be dispatched.<br><br>In case the animals originate from establishments located in free areas, there is no need for laboratory examination if there are no other reasons based on the authorities' risk assessment to recommend it (e.g. epidemiological link with an affected establishment or with an affected or high-risk area). Clinical examination as described above would be enough. |
| **20th scenario** Section 4.1.3.2 In the event of unusual mortalities or clinical signs being notified during the repopulation | No specific guidelines on sampling procedures for laboratory examination were found for the 20th scenario. | In case of unusual mortalities or clinical signs compatible to FMD, notified during the repopulation is important to rule out the presence of the disease. | In the event of animals with clinical signs compatible to FMD, as they have been described in Section 4.1.1.1, notified during the repopulation the establishment is considered suspected. The repopulation should be stopped and the procedures for the laboratory confirmation that are described in Section 4.1.1.1 should be followed.<br>In addition, the establishments from where the suspected animals coming from, should be considered as suspected. The procedures that are described in Section 4.1.1.1 should be followed as well to these establishments of origin. |
| **21st scenario** Section 4.1.3.3 For animals that have been repopulated | Pursuant Annex V of the directive 2003/85 :<br>'1.3 Irrespective of the type of farming practised on the holding, re-introduction must conform with the following procedures:<br>1.3.1. animals must be introduced in all units and | Following restocking animals should be thoroughly examined clinically and by laboratory examinations in order to rule out the presence of the disease. | Animals must be subjected to clinical inspection every three days for the first 14 days following the introduction, and weekly from 15 to 28 days after re-introduction. The last day of the monitoring period following the latest day of animals' introduction, all the animals should be subjected |

| Sampling procedure | Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise | Conclusions | Recommendations |
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| | buildings of the holding involved;<br>1.3.2 in the case of a holding consisting of more than one unit or building, re-introduction is not necessary for every unit or building at the same time; However no animals of species susceptible to foot and mouth disease may leave the holding until all the re-introduced animals in all units and buildings have fulfilled all restocking procedures.<br>1.3.3 animals must be subjected to clinical inspection every three days for the first 14 days following the introduction;<br>1.3.4 during the period from 15 to 28 days after re-introduction, animals are to be subjected to clinical inspection once every week;<br>1.3.5 not earlier than 28 days after the last re-introduction, all animals must be clinically examined and samples for testing for the presence of antibody against foot and mouth disease virus shall be taken in accordance with the requirements of point 2.2 of Annex III;<br>1.4 The restocking procedure shall be considered completed when the measures provided for in point 1.3.5 have been completed with negative results.' | | to thorough clinical examination as described in Section 4.1.1.1 and should be sampled for laboratory examination in accordance to the procedures described in Section 4.1.1.1. |

**ToR 2**

| Description | Conclusions | Recommendations |
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| Section 4.2<br>Assessment of the length of the monitoring period of FMD | Scenarios 1, 2, 3, 4, 6 and 7<br>Based on the results of the ELS as presented in Table 6 in Section 4.2.1:<br>– the longest length of the period between infection and suspicion of FMD is 26 days in cattle, 21 in pigs and 19 days in small ruminants;<br>– the average length was 13 days in cattle, 12 in pigs and 14 days in small ruminants<br>– the shortest length was 6 days in cattle, 3 in pigs and 9.5 days in small ruminants;<br><br>Scenario 5<br>Based on the results of the scientific publications as | Scenarios 1, 2 and 3<br>The length of the monitoring period of 21 days as defined in the Delegate Regulation is considered effective, except for the first affected establishments in an area where 26 days is recommended.<br><br>Scenario 4<br>As the disease has already been detected in the area, and high awareness is expected, the length of the monitoring period is considered effective in this scenario.<br><br>Scenario 5<br>Consequently and based on the results of the publications, sampling the animals at least 28 (21 + 7) days after semen collection as it is foreseen in the Delegated |

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| | presented in Table 7 and Table 8 in Section 4.2.1., the latest date of seroconversion in non-vaccinated, naive animals was identified as 15 days post infection by NSP ELISA while for non-vaccinated, naive animals infected trough contact with already infected animals was identified as 28 days post contact by NSP ELISA.<br>In vaccinated animals, where there is already a level of immunity, the detection of seroconversion after infection or contact may be delayed. | Regulation is considered effective to detect antibodies with several laboratory methods, given that the infection occurred at the latest at the day of semen collection.<br>Scenarios 6 and 7<br>None |
| **ToR 3** | | |
| **Description** | **Conclusions** | **Recommendations** |
| Section 4.3.1<br>Assessment of the minimum radius | The defined minimum radiuses of 3 km and 10 km of the protection and the surveillance zone, respectively, are considered effective to restrain the spread of FMD beyond their borders if it were to occur with a confidence level of 95% and 99%, respectively. | Based on the estimations of the kernels, as long as the radiuses of the zones are increasing the probability of transmission outside the defined zones is decreasing as well. Taken into consideration the local epidemiological situation, the density of the establishments and the commercial activities different combinations of radiuses in the protection and the surveillance zones may be selected to further decrease the spread of the disease. |
| Section 4.3.2<br>Assessment of the minimum period | Based on the results of the ELS as presented in Table 6 in Section 4.2.1 it follows that the average time between introduction and suspicion is 13 days in cattle, 12 in pigs and 14 days in small ruminants. The maximum period between introduction and suspicion is 26 days in cattle, 21 in pigs and 19 days in small ruminants. Consequently, the minimum period of 30 days indicated in the Delegated Regulation for the restriction measures is considered effective to detect affected establishments and to prevent the movement of infected animals from the surveillance zone. | None |

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## Abbreviations

| | |
|---|---|
| ASF | African swine fever |
| AHS | African horse sickness |
| CSF | Classical swine fever |
| CBPP | Contagious bovine pleuropneumonia |
| CCPP | Contagious caprine pleuropneumonia |
| CO | Cut-off (of a diagnostic test) |
| dpi | days post inoculation/infection |
| dpc | days post contact |
| ELISA | enzyme-linked immunosorbent assay |
| ELS | extensive literature search |
| FMD | Foot and mouth disease |
| FMDV | Foot and mouth disease virus |
| HPAI | Highly Pathogenic Avian Influenza |
| LSDV | Lumpy skin disease virus |
| NCDV | Newcastle disease virus |
| OIE | World Organisation for Animal Health |
| PCR | polymerase chain reaction |
| PZ | protection zone |
| RP | rinderpest virus |
| RT-PCR | reverse transcription polymerase chain reaction |
| RVFV | Rift Valley fever virus |
| SPGP | Sheep pox and goat pox |
| SZ | surveillance zone |
| ToR | Terms of Reference |

## Annex A – Definitions in EU legislation

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

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

# Annex B – Scenarios of ToR 1

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

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|---|---|---|---|---|
| ToR 1.1<br>ToR 1.2 | Article 13(3)c of the Delegated Regulation | 3rd scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment, in order to grant a specific derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease. | • affected establishment officially confirmed<br>• kept animals of listed species of specific categories<br>• animal categories based on article 13(2):<br>a) animals kept in a confined establishment<br>b) animals kept for scientific purposes or purposes related to conservation of protected or endangered species<br>c) animals officially registered in advance as rare breeds<br>d) animals with a duly justified high genetic, cultural or educational value<br>• 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<br>• the animals should be subjected to clinical surveillance, including laboratory examinations<br>• sampling procedures should ensure that the animals do not pose a risk of transmission of the category A disease if left alive |
| ToR 1.1<br>ToR 1.2 | Article 14(1) of the Delegated Regulation Art. 57 Reg.2016/429 | 4th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the virus if the virus is present in these species. | • kept animals of non-listed species of epidemiological relevance for the control of the disease<br>• 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<br>• animal species acting purely as mechanical carriers of the virus will not be covered<br>• 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<br>• sampling procedures to ensure detection of the virus in these species |

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|---|---|---|---|---|
| ToR 1.1<br>ToR 1.2 | Article 14(1) of the Delegated Regulation Art. 57 Reg.2016/429 | 5th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species | • affected establishment officially confirmed<br>• wild animals of listed species within the establishment and in the surroundings of the establishment<br>• the competent authority may establish these sampling procedures in addition to other measures<br>• sampling procedures in wild animals of listed species to ensure the detection of the virus, if the virus is present in these wild species |
| ToR 1.1<br>ToR 1.2 | Article 26(2) of the Delegated Regulation | 6th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals. | • protection zone with radius up to 3 km<br>• non-affected establishments with kept animals of listed species<br>• all the non-affected establishments within the protection zone<br>• official veterinarians must visit at least once all the establishments<br>• among others, they must perform a clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination<br>• sampling procedures to confirm or rule out the presence of a category A disease |
| ToR 1.3 | Article 26(5) of the Delegated Regulation point A.3 of Annex I | 7th scenario | To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of establishments located in a protection zone when the radius is larger than 3 km. The purpose of the sampling procedure is to ensure disease detection of the virus if the virus is present in establishments within the protection zone | • protection zone with radius larger than 3 km<br>• non-affected establishments of kept animals of listed species<br>• sample of the non-affected establishments in the protection zone<br>• in a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km<br>• In case of a radius larger than 3 km, official veterinarians may not visit all establishments, but a sample of those. EFSA is requested to assess how many of these establishments should be inspected, in order to ensure the detection of the virus, if the virus is present in animals in these establishments<br>• among others perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination<br>• sampling procedure to ensure the detection of the disease if the disease is present in any of these establishments |

| 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 | • surveillance zone<br>• establishments of kept animals of listed species<br>• sample of the establishments in the surveillance zone<br>• official veterinarians carry out visits to a sample of the establishments<br>• among others perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination<br>• sampling procedure to ensure the detection of the disease if the disease is present in any of the establishments |
| **Derogations to allow animal movements** | | | | |
| ToR 1.4 | Article 28(5) of the Delegated Regulation Article 29 of the Delegated Regulation | 9th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29) | • protection zone<br>• kept animals of listed species<br>• grant derogation for movement from a non-affected establishment in the protection zone<br>• to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 28(5) and Article 30(1) of the Delegated Regulation | 10th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of day-old-chicks located in the protection zone and hatched from eggs originating in the restricted zone or outside the restricted zone. The sampling procedures should ensure that the movement of these day-old-chicks to an establishment located in the same Member State but if possible, outside the restricted zone | • protection zone<br>• grant derogation for movement from a non-affected establishment in the protection zone<br>• day-old-chicks from non-affected establishment located in the protection zone, hatched from eggs originating in or outside the restricted zone<br>• to be moved to an establishment located in the same Member State but if possible, outside the restricted zone<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |

| 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<br>• ready-to-lay poultry<br>• grant derogation for movement from a non-affected establishment in the protection zone<br>• to be moved to an establishment located in the same Member State and if possible, within the restricted zone<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 28(5) and Article 37 of the Delegated Regulation | 12th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37) | • protection zone<br>• kept animals of listed species<br>• grant derogation for movement from a non-affected establishment in the protection zone<br>• to be moved to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed<br>• clinical examinations and laboratory examinations of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 43(5) and Article 44 of the Delegated Regulation | 13th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of listed species in order to grant derogation from prohibitions and allow for these animals to be moved: a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, b)from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone | • surveillance zone<br>• kept animals of listed species<br>• 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<br>• grant derogation for movement from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 43(5) and Article 45(1) of the Delegated Regulation | 14th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone | • surveillance zone<br>• kept ungulates of listed species<br>• grant derogation for movement from an establishment in the surveillance zone<br>• to be moved to pastures situated within the surveillance zone<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|---|---|---|---|---|
| ToR 1.4 | Article 43(5) and Article 45(2) of the Delegated Regulation | 15th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant derogation and allow to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone, in order to complete the production cycle before slaughter | • surveillance zone<br>• kept animals of listed species<br>• grant derogation for movement from the surveillance zone<br>• 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<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 43(5) and Article 46(1) of 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<br>• kept birds of listed species<br>• 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<br>• to be moved to an establishment located in the same Member State<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 43(5) and Article 46(2) of the Delegated Regulation | 17th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same MS. | • surveillance zone<br>• ready-to-lay poultry<br>• to be moved to an establishment located in the same Member State<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |
| ToR 1.4 | Article 56(1)c of the Delegated Regulation | 18th scenario | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI | • restricted zone when restriction measures are maintained beyond the period set out in Annex XI<br>• kept animals of listed species<br>• grant derogation for movement from an establishment within the restricted zone<br>• clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved |

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenario |
|---|---|---|---|---|
| **Repopulation** | | | | |
| ToR 1.5 | Article 59(2),(3) of the Delegated Regulation | 19th scenario | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease. | • repopulation of a previous affected establishment<br>• kept animals of listed species<br>• Animals intended to repopulation shall be sampled prior to their introduction into the establishment of destination<br>• 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)<br>• laboratory examinations<br>• sampling procedures to rule out the presence of the disease |
| ToR 1.5 | Article 59(9) of the Delegated Regulation | 20th scenario | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease. | • repopulated establishment<br>• unusual mortalities or clinical signs during the repopulation<br>• the official veterinarians shall without delay collect samples for laboratory examination<br>• sampling procedures to rule out the presence of the disease |
| ToR 1.5 | Article 59(5) of the Delegated Regulation | 21st scenario | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment. | • repopulated establishment<br>• kept animals of listed species<br>• Animals that have been used for repopulation<br>• Laboratory examinations<br>• Sampling procedures to rule out the presence of the disease |

# Annex C – Existing sampling procedures for FMD

Sampling scenarios for FMD – Based on Council Directive 2003/85/EC 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)). | Annex III<br>1. Clinical examination<br>1.1. Holdings must undergo clinical examinations of all animals of susceptible species for signs or symptoms of foot and mouth disease.<br>1.2. Special emphasis must be laid on animals which may have been exposed to foot and mouth disease virus with a high probability, notably transport from holdings at risk or close contact to persons or equipment that had close contact to holdings at risk.<br>1.3. The clinical examination must take into account the transmission of foot and mouth disease, including the incubation period referred to in Article 2(h) and the way in which animals of susceptible species are kept.<br>1.4. Relevant records kept on the holding must be examined in detail with particular regard to data required for animal health purposes by Community legislation and, where available, on morbidity, mortality<br>and abortion, clinical observations, changes in productivity and feed intake, purchase or sale of animals, visits of persons likely to be contaminated and other anamnestically important information. | Annex III 2.1.1.<br>Serological sampling shall be carried out according to the recommendations of the epidemiological team established within the expert group referred to in Article 78, and<br><br>Annex III 2.2.<br>Sampling on holdings: In holdings where the presence of foot and mouth disease is suspected but in the absence of clinical signs, sheep and goats, and on recommendation of the epidemiological team other susceptible species, should be examined pursuant to a sampling protocol suitable to detect 5% prevalence with at least 95% level of confidence.<br><br>OIE Terrestrial Manual 2018 (p.436-437)<br>For laboratory diagnosis, the tissue of choice is epithelium or vesicular fluid. Ideally, at least 1 g of epithelial tissue should be collected from an unruptured or recently ruptured vesicle, usually from the tongue, buccal mucosa or feet. Where epithelial tissue is not available from ruminant animals, for example in advanced or convalescent cases, or where infection is suspected in the absence of clinical signs, samples of OP fluid can be collected by means of a probang (sputum) cup (or in pigs by swabbing the throat) for submission to a laboratory for virus isolation or reverse-transcription polymerase chain reaction (RT-PCR). Viraemia may also be detected by examining serum samples by means of RT-PCR or virus isolation.<br>• A range of sample types, including epithelium, OP samples, milk and serum, may be examined by virus isolation or RT-PCR. By contrast, ELISA CF and the lateral flow device are suited to the examination of epithelial suspensions, vesicular fluids or cell culture supernatants, but are insufficiently sensitive for the direct examination of OP samples or serum. |

| 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. | No specific guidelines described in legislation. | No specific guidelines are described in this piece of legislation for other subscenarios |
| 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 sampling procedures are described in this piece of legislation describing the sampling procedures in animals of listed species, to grant a derogation from killing these animals |
| 4th | To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the virus if the virus is present in these species. | No specific guidelines described in legislation.<br>Article 2 (a): 'animal of a susceptible species' means any domestic or wild animal of the suborders Ruminantia, Suina and Tylopoda of the order Artiodactyla;<br>For specific measures, notably in application of Article 1(2), Article 15 and Article 85(2), other animals, such as for example of the order Rodentia or Proboscidae, may be considered susceptible to foot and mouth disease in accordance with scientific evidence.<br>There is no concept of listed species in Directive 2003/85 but instead the term *susceptible species* is used.<br>No specific guidelines described in legislation | No specific guidelines described in legislation<br>No specific guidelines described in legislation |

| 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. | Annex III,<br>All holdings with animals of susceptible species shall periodically undergo a veterinary inspection, carried out in such a way as to avoid the spread of foot and mouth disease virus possibly present on the holdings, which shall include in particular the relevant documentation, notably the records referred to in subparagraph (a) and the measures applied to prevent the introduction or escape of foot and mouth disease virus and which may include clinical inspection as described in point 1 of Annex III or taking of samples from animals of susceptible species in accordance to the recommendations of the epidemiological team established within the expert group referred to in Article 78.<br>The clinical inspection should be done as described for scenario 1 | The sampling in protection zone described in Annex III is referring to the freedom of the disease in the area.<br>No specific sampling procedures for the early detection of the virus are described in this piece of legislation. |
| 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 | ANNEX III<br>2.4. *Sampling in surveillance zones*<br>In order to seek the repeal in accordance with Article 44 of the measures provided for in Articles 37 to 43, holdings within the perimeters of the surveillance zone where the presence of foot and mouth disease in the absence of clinical signs must be suspected, notably where sheep and goats are kept, shall be examined. | The sampling in surveillance zone described in Annex III is referring to the freedom of the disease in the area.<br>No specific sampling procedures for the early detection of the virus are described in this piece of legislation. |

| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|---|---|---|---|
| **Derogations to allow animal movements** | | | |
| 9th | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29) | 2. By way of derogation from paragraph 1(c), animals of susceptible species may be transported under official supervision for the purpose of emergency slaughter directly to a slaughterhouse situated inside the same protection zone, or if that zone has no slaughterhouse to a slaughterhouse outside the zone designated by the competent authority in means of transport cleansed and disinfected under official control after each transport operation. The movement referred to in the first subparagraph shall only be authorised if the competent authority is satisfied on the basis of a clinical examination in accordance with point 1 of Annex III by the official veterinarian of all the animals of susceptible species present on the holding and after evaluation of epidemiological circumstances that there is no reason to suspect the presence of infected or contaminated animals on the holding. | Annex III<br>2.3. *Sampling in protection zones*<br>In order to seek the repeal in accordance with Article 36 of the measures provided for in Articles 21 to 35, all holdings within the perimeters of the protection zone where sheep and goats have not been in direct and close contact with bovine animals during a period of at least 21 days prior to taking the samples shall be examined pursuant to a sampling protocol suitable to detect 5% prevalence of disease with at least 95% level of confidence.<br>However, the competent authorities may decide where epidemiological circumstances allow and in particular in application of the measures provided for in Article 36(1)(b), that samples are taken not earlier than 14 days after the elimination of susceptible animals on the infected holding(s) and the carrying out of preliminary cleansing and disinfection, under the condition that the sampling is carried out in accordance with point 2.3 using statistical parameters suitable to detect 2% prevalence of disease within the herd with at least 95% level of confidence. |
| 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 from prohibitions and allow for these animals to be moved: | Article 37<br>2. By way of derogation from the prohibition provided for in Article 22(1)(c) and where there is no or insufficient slaughter capacity available within the surveillance zone, the competent authorities may authorise the removal from | No specific guidelines described in legislation<br>Movements of animals provided for in paragraph 2(a) shall be authorised by the competent authority only after an examination by an official veterinarian of all the animals of susceptible species on the holding, including testing of |

| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|---|---|---|---|
| | a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone,<br>b) from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone | holdings situated in the surveillance zone of animals of susceptible species for transporting them directly and under official supervision for slaughter to a slaughterhouse located outside the surveillance zone, subject to the following conditions:<br>(a) the records referred to in Article 22(1) have been subjected to official control, and the epidemiological situation of the holding does not indicate any suspicion of infection or contamination with the foot and mouth disease virus,<br>(b) all the animals of susceptible species on the holding have been subjected with negative result to an inspection by the official veterinarian,<br>(c) a representative number of animals, taking into account the statistical parameters in point 2.2 of Annex III, has been subjected to thorough clinical examination to rule out the presence or suspicion of clinically infected animals,<br>(d) the slaughterhouse is designated by the competent authority<br>and located as near to the surveillance zone as possible and<br>(e) the meat produced from such animals shall be subjected to the treatment specified in Article 39.<br>No specific guidelines | samples taken in accordance with point 2.2 of Annex III, has ruled out the presence of animals suspected of being infected or animals suspected of being contaminated (2.2 of Annex III- In holdings where the presence of foot and mouth disease is suspected but in the absence of clinical signs, sheep and goats, and on recommendation of the epidemiological team other susceptible species, should be examined pursuant to a sampling protocol suitable to detect 5% prevalence with at least 95% level of confidence.). |
| 14th | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone | No specific guidelines<br>Art 38 of the Directive 2003/85: '1. Member States shall ensure that animals of susceptible species shall not be removed from holdings within the surveillance zone. 2. By way of derogation, the prohibition provided in paragraph 1 shall not apply to movement of animals for one of the following purposes: (a) for leading them without coming into contact with animals of susceptible species of different holdings, to pasture situated within the surveillance zone not earlier than | No specific guidelines described in legislation |

| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|---|---|---|---|
| | | 15 days after the last outbreak of FMD has been recorded in the protection zone./. . ./ 3. Movements of animals provided for in paragraph 2 (a) shall be authorised by the competent authority only after an examination by an official veterinarian of all the animals of susceptible species on the holding, including testing samples taken in accordance with /. . .' | |
| 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 | No specific guidelines described in legislation | No specific guidelines described in legislation |
| 18th | To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI | No specific guidelines described in legislation | No specific guidelines described in legislation |
| **Repopulation** | | | |
| 19th | To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease | ANNEX V<br>1.1. Restocking should not commence until 21 days after completion of the final disinfection of the holding.<br>1.2. *Animals for restocking can only be introduced under the following conditions:*<br>1.2.1. the animals shall not come from areas subject to animal health restrictions in relation to foot and mouth disease; | ANNEX V<br>1.3. Irrespective of the type of farming practised on the holding, re-introduction must conform with the following procedures:<br>1.3.1. animals must be introduced in all units and buildings of the holding involved;<br>1.3.2. in the case of a holding consisting of more than one unit or building, re-introduction is not necessary for every unit or building at the same time; |

| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|---|---|---|---|
| | | 1.2.2. the competent authorities must be satisfied that any possible residual foot and mouth disease virus can be detected in the animals intended for restocking either on the base of clinical signs, in the case of bovine or porcine animals, or though laboratory investigations in the case of other species susceptible to foot and mouth disease, carried out at the end of the observation period specified in paragraph 1.3;<br>1.2.3. in order to ensure an adequate immune response referred to in paragraph 1.2.2 in the animals intend for restocking, the animals must:<br>1.2.3.1. either originate in and come from a holding situated in an area of at least 10 km radius centred on that holding where there was no outbreak of foot and mouth disease for at least 30 days, or<br>1.2.3.2. the animals have been tested with negative results in an assay as described in Annex XIII for the detection of antibodies against the foot and mouth disease virus carried out on samples taken prior to introduction onto the holding.<br>1.3.3. animals must be subjected to clinical inspection every three days for the first 14 days following the introduction;<br>1.3.4. during the period from 15 to 28 days after re-introduction, animals are to be subjected to clinical inspection once every week;<br>1.3.5. not earlier than 28 days after the last re-introduction, all animals must be clinically examined and samples for testing for the presence of antibody against foot and mouth disease virus shall be taken in accordance with the requirements of point 2.2 of Annex III; | However no animals of species susceptible to foot and mouth disease may leave the holding until all the re-introduced animals in all units and buildings have fulfilled all restocking procedures.<br>1.3.3. animals must be subjected to clinical inspection every three days for the first 14 days following the introduction;<br>1.3.4. during the period from 15 to 28 days after re-introduction, animals are to be subjected to clinical inspection once every week;<br>1.3.5. not earlier than 28 days after the last re-introduction, all animals must be clinically examined and samples for testing for the presence of antibody against foot and mouth disease virus shall be taken in accordance with the requirements of point 2.2 of Annex III;<br>1.4. The restocking procedure shall be considered completed when the measures provided for in point 1.3.5 have been completed with negative results. |

| Scenario | Description of the Scenario | Clinical guidelines | Laboratory guidelines |
|---|---|---|---|
| | | 1.4. The restocking procedure shall be considered completed when the measures provided for in point 1.3.5 have been completed with negative results. | |
| 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 legislation for this scenario. | No specific legislation for sampling in this scenario. |
| 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 legislation for this scenario. | No specific legislation for this scenario. |

# 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<br>rticle 57 of 2016/429 Regulation<br>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<br>• in an establishment with kept animals of listed species<br>• time period calculated backwards from the date of the of the notification of the suspicion<br>• time period before the suspicion, during which the pathogenic agent may have been introduced in the establishment and may have spread outside the establishment<br>• the aim of the epidemiological enquire is:<br>a) identify the likely origin of the listed disease in question and the means of its spread<br>b) calculate the likely length of time that the listed disease has been present<br>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<br>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<br>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<br>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<br>• in an establishment with kept animals of listed species<br>• time period calculated backwards from the date of the notification of the suspicion<br>• 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.<br>• 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 Annex II of the Delegated Regulation | 3rd scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a category A disease in an establishment with kept animals of listed species, during which the epidemiological units in which the disease has not been confirmed were kept completely separated and handled by different personnel, in order to provide derogations from killing. | • event of confirmation of a category A disease<br>• in an affected establishment with kept animals of listed species<br>• non-affected epidemiological units kept separated<br>• to provide derogation from killing for animals in non-affected separated epidemiological units<br>• to exclude any possible contact between the affected establishment and the separated epidemiological units as per the epidemiological enquiry<br>• time period calculated backwards from the date of the confirmation<br>• time period before the confirmation, during which the pathogenic agent may have been introduced in the separated non-affected epidemiological units of the affected establishment. |
| ToR 2 | Article 27(3)c of the Delegated Regulation Annex II of the Delegated Regulation | 4th scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of the latest outbreak of a category A disease in the protection zone. Products or other materials likely to spread the disease, must had been obtained or produced, before this time period in order to be exempted from prohibitions of movements. | • protection zone<br>• non-affected establishments<br>• 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<br>• time period calculated backwards from the date of suspicion of the latest outbreak in the protection zone<br>• time period before the notification of the suspicion, during which the products and materials produced in the non-affected establishments of a protection zone may have been contaminated by the pathogenic agent of the disease. |

| ToRs | Legislation | Scenario | Description of the Scenario | Elements of the Scenarios |
|---|---|---|---|---|
| ToR 2 | Article 32(c) of the Delegated Regulation<br>Article 48(c) of the Delegated Regulation<br>Annex II of the Delegated Regulation | 5th scenario | To assess the effectiveness of the length of the Monitoring Period, as the time period calculated forwards from the date of semen collection from animals of listed species kept in approved germinal product establishments in the protection or in the surveillance zone, to prove that the donor animal has tested favourable on a sample taken not earlier than 7 days after the monitoring period. | • protection or surveillance zone<br>• non-affected approved germinal establishments<br>• semen from kept animals (donor) of listed species<br>• 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)<br>• to take samples from the donor for laboratory analysis at least 7 days after the end of the monitoring period<br>• to authorise movements of semen from approved germinal product establishments located in the protection or surveillance zones in case of favourable laboratory results<br>• time period calculated forwards from the date of semen collection<br>• time period after the semen collection, during which the animal donor if infected could be detected by the relevant diagnostic test. |
| ToR 2 | Article 57(1)b of the Delegated Regulation<br>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<br>• kept animals of listed species<br>• to allow the repopulation of an affected establishment<br>• time period calculated forwards from the date of the final cleaning and disinfection of the establishment<br>• 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<br>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<br>• kept animals of listed species to be repopulated<br>• the animals may not be introduced at the same time<br>• time period calculated forwards from the date when the first animal was introduced<br>• 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 day | 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 |
|---|---|---|---|
| ToR 1 | 1 | There is limited data on the performance of the diagnostic tests considered in the assessment, particularly regarding the sensitivity and specificity of clinical examination, in the different species. | The effectiveness of the sampling strategies could be over or underestimated. |
| ToR 2 and ToR3 | 2 | Information on the period elapsed between the earliest point of infection and the suspicion report could only be retrieved from one reference for swine, and two references for small ruminants (one for sheep and one for goats). | The effectiveness of the proposed monitoring period based on the limited available evidence, could be overestimated if infection affected species in which clinical signs are less evident (sheep, goats). |
| | 3 | Most references retrieved presented data from outbreaks occurring in 2001 and 2007, and in one country (UK), and therefore, data may not be representative for other regions/periods due to differences in production systems affecting the effectiveness of surveillance systems. | The effectiveness of the proposed monitoring period could be over or underestimated. |
| | 4 | Kernels are based on analyses of epidemics involving strains of serotype O. The efficiency of transmission routes may differ for other serotypes/strains (e.g. airborne spread; survival on fomites) changing the shape of the kernel. | The effectiveness of the proposed zone size could be over or underestimated. |
| | 5 | Kernels used in this assessment are based on the analyses of four epidemics in northern Europe (UK and NL) and one in Japan. The efficiency of different transmission routes is likely to vary with environmental conditions (e.g. temperature and relative humidity) and with farming practices, which may influence the shape of the kernel. | The effectiveness of the proposed zone size could be over or underestimated. |