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Assessment of the control measures of the category A diseases of Animal Health Law: Rift Valley Fever

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Abstract

EFSA received a mandate from the European Commission to assess the effectiveness of some of the control measures against diseases included in the Category A list according to Regulation (EU) 2016/429 on transmissible animal diseases ('Animal Health Law'). This opinion belongs to a series of opinions where these control measures were assessed for several diseases, with this opinion covering the assessment of control measures for Rift Valley Fever (RVF). In this opinion, EFSA and the AHAW Panel of experts review the effectiveness of: (i) clinical and laboratory sampling procedures, (ii) monitoring period and (iii) the minimum radius of the protection and surveillance zone and the minimum length of time the measures should be applied in these zones. The general methodology used for this series of opinions has been published elsewhere; nonetheless, the transmission kernels used for the assessment of the minimum radius of the protection and surveillance zones are shown. Several scenarios for which these control measures had to be assessed were designed and agreed prior to the start of the assessment. Different risk-based sampling procedures based on clinical visits and laboratory testing are assessed in case of outbreak suspicion, granting animal movements and for repopulation purposes. The length of monitoring period and minimum duration of measures to be implemented in the restricted zones as defined in the Delegated Regulation (30 days) are considered effective for the investigation and control of suspected and confirmed RVF outbreaks, as well as the size of protection and surveillance zone of 20 and 50 km, respectively, which are assessed as sufficient to contain disease transmission with at least 95% probability.

Keywords: Disease control measures, Rift Valley Fever, RVF, sampling procedures, monitoring period, protection zone, surveillance zone

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Summary

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

- the clinical and laboratory examination in their capacity to detect disease (or estimate the disease prevalence within an establishment), either in suspect or confirmed animals in a single establishment, or in establishments within restriction zones (ToR 1);
- the effectiveness of the duration of the monitoring period (for different scenarios) in the control of suspected and confirmed outbreaks (ToR 2);
- the size (ToR 3.1) and duration (ToR 3.2) of the restriction zones, in their capacity for mitigating disease spread.

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 Rift Valley Fever (RVF) for each scenario of ToR 1 have been assessed. For assessing the effectiveness of detecting RVF in a flock, a model to study the within-herd transmission of RVF was designed. This allowed the calculation of infection and seroprevalence at different points in time from virus introduction in a herd, so to calculate the sample size needed for early detection of suspected animals in an infected herd.

In the event of the first introduction of RVF into a previously free territory like the EU, the first suspicion would be based most likely on the observation of the most evident clinical signs, i.e. abortion and death in young animals. Samples from few of these suspect clinically affected animals or aborted fetuses (e.g. five animals or fetuses) would be enough to confirm the presence of RVFV in the herd by polymerase chain reaction (PCR). In case of the absence of clinical signs and if the suspicion is based on other factors (e.g. animals originating from infected areas), they should all be sampled and tested. In case of disease introduction, the public health surveillance on human cases of RVF may also help in early detection, since passive surveillance in animals may not be as effective as needed. When RVF is officially confirmed in an establishment, all the animals in the involved herd should be sampled in order to have a complete picture of the infection prevalence in the herd. Given that the infection can be fully inapparent in adults, apart from abortion in pregnant females, serological tests may be used for the identification of already infected animals, by repeating the test on two blood samples collected 1 week apart.

In the scenarios to grant derogation for animal movement, due to the zoonotic significance and the fact that RVF is a vector-borne disease it is not recommended to move animals from restricted zone towards a zone at lower risk, unless they are moved to be immediately slaughtered; in this case, biosecurity measures should be applied and clinical examination of animals to be moved before movement should be conducted. If animals are moved within a zone with same risk level and remain alive (e.g. movement to pasture or repopulation), they should be subjected to clinical surveillance, including laboratory examinations according to what foreseen in case of suspicion, and the sampling (blood samples for PCR test) should ensure that the animals do not pose a risk of transmission with a confidence level of 95%.

To answer ToR 2, the assessment of the length of the monitoring period, and to assess the minimum duration of measures to be implemented in the protection and surveillance zones (ToR 3.2), an extensive literature search was carried out. This aimed to assess the average, shortest and longest period between the earliest point of infection of animals with RVF virus and the time to report a suspicion by the competent authority. Based on the data available in the literature, the current monitoring period for RVF as defined in the Delegated Regulation (30 days) is considered effective for all scenarios mentioned in ToR2.

To assess the effectiveness of the minimum radius to be implemented in the protection and surveillance zones (ToR 3.1), transmission kernels were used. The estimated probability of transmission beyond a protection zone with 20 km radius, if transmission occurred, is 5.7% (CI: 0.7–23.2%), and 0.1% (0.1–2.6%) for a zone with 50 km radius, which is considered sufficient to contain disease transmission with at least 95% probability.

The minimum period of 30 days indicated in the Delegated Regulation for the restriction measures in the protection zone is considered effective to detect infected establishments and to prevent the

movement of infected animals from the protection zone, since the maximum time between earliest point of infection and notification of the suspicion for RVF retrieved in the literature is 20 days. In addition, the maximum period between the earliest point of infection and the suspicion report has been reconstructed as 25 days, consequently, the minimum period of 45 days (30 plus additional 15 days) indicated in the Delegated Regulation for the restriction measures in the surveillance zone is considered sufficient to detect infected establishments and to prevent the movement of infected animals from the surveillance zone.

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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 establishment for repopulation, in accordance with Article 59(2), (3) and (9) of the Delegated Regulation.

1.1.2. ToR 2: Monitoring period

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

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

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

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

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

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

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

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

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

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

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

1.2. Interpretation of the Terms of Reference

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

a) The publication of 14 individual opinions, one per each of the diseases included in the list of category A diseases for terrestrial animals, with each of these opinions providing the answer to ToRs 1, 2 and 3. The current manuscript is one of the 14 opinions covering ToRs 1, 2 and 3 for Rift Valley Fever (RVF).
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 C). 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 ToR1 of Annex B are not relevant for RVF, and therefore not included in the assessment of the current Opinion:
    i) Scenario 6 because the minimum radius of the protection zone for RVF is 20 km,
    ii) Scenarios 10, 11, 16 and 17, because they are referring to poultry.
g) The duration of the monitoring period for RVF as described in Annex II of the Delegated Regulation is 15 days.
h) The minimum length of the radius of the protection zone (PZ) and surveillance zone (SZ) for RVF as described in Annex V of the Delegated regulation are 20 and 50 km, respectively.
i) The minimum duration of the measures in the PZ and SZ for RVF as described in Annexes X and XI of the Delegated Regulation is 28 and 45 days, respectively.
j) Vaccination against RVF has not been taken into consideration in the assessment of ToRs 2 and 3 as agreed with the requestor. For ToR 1, some relevant aspects related to vaccination were discussed (a deep review was not requested or maybe needed).

2. Disease characterisation and geographical distribution of Rift Valley Fever

2.1. Aetiology

Rift Valley fever (RVF) is a mosquito-borne zoonotic viral disease affecting mainly sheep, goat, cattle and other domestic ruminants along with some wild species and humans. The causative agent is the RVF virus (RVFV), a single-stranded RNA virus of the order Bunyavirales, family Phenuiviridae, genus *Phlebovirus*, existing as only one serotype (CFSPH, 2015; Paweska, 2015; OIE, 2018, 2019).

2.2. Epidemiology

RVF is responsible for severe epidemics in sheep, goats and cattle and to a lesser extent in water buffalo and camels, causing abortion in adults and high mortality in newborn animals. Many African wild species can seroconvert such as African buffalo (*Syncerus caffer*), several species of gazelles and antelopes, giraffes, elephants, rhinoceros, zebras, warthogs and some monkeys and bats. Several domestic and wild rodents are also susceptible to the disease as well as newborn domestic carnivores. Wildlife hosts, mainly ruminants, may act as a reservoir during the inter-epidemic periods (sylvatic cycle), although their significance remains insufficiently known and is likely to vary depending on the composition of the regional host community (Gortázar et al., 2021). Horses can seroconvert, but pigs, guinea pigs, rabbits and birds are refractory to the infection.

RVFV is transmitted to susceptible animals by infected mosquitoes belonging to several genera, mainly *Aedes*, *Culex* and *Anopheles*. Other arthropods such as biting midges or ticks can act as mechanical vectors. Vertical transmission occurs in *Aedes* species so that the virus can survive long periods in the environment in dormant eggs. During outbreaks, livestock can also be infected by contact with infected placenta or aborted fetus. Humans can be infected by mosquito bites, but mostly through direct contact with animal secretions (nasal, ocular, vaginal) and tissues (aborted fetus, placenta) or manipulation of meat from infected livestock. Ingestion of raw milk is also suspected as a source of infection. RVFV has also been found in bull's semen 1 week after vaccination with the live-attenuated RVF vaccine, and it was detected till 3 weeks post-vaccination (Kamal, 2011).

In endemic areas, epidemics in livestock and humans are cyclical, occurring every 5–15 years, usually after heavy rainfall following a dry period and after floods, causing the pullulation of mosquitoes (FAO, 2003; Lefèvre, 2003; AFSSA, 2008; CFSPH, 2015; Paweska, 2015).

Initially described in East Africa (1931), RVF is currently endemic in eastern, western and southern Africa (including Madagascar and several Indian Ocean islands). Severe animal and human outbreaks have occurred in Egypt and the Arabic peninsula (CFSPH, 2015; OIE, 2019; EFSA AHAW Panel, 2020). Seropositive animals have been found in several countries considered as RVF-free including Turkey (EFSA AHAW Panel, 2020b). The risk of spreading of RVFV to free regions or countries is increased by climate change and intense livestock trade, but also by changes in the agro-ecological systems such as large dam constructions (AFSSA, 2008; Paweska, 2015).

In endemic regions, RVF outbreaks can be predicted by early-warning systems based on rainfall data records and satellite image of vegetation cover. The control of mosquito populations is usually unpractical. Regional movement bans of livestock may limit the spread of the disease to uninfected areas. Live-attenuated vaccines are available for livestock and confer long immunity after a single dose (3 years), but can provoke side effects such as abortions and fetal abnormalities. Inactivated vaccines are safer, but need yearly boosters. Immunity after infection is protective in livestock, causing outbreaks to decline after 2–4 months (FAO, 2003; CFSPH, 2015; Paweska, 2015; OIE, 2019).

2.3. Clinical signs and diagnosis

In an endemic zone, high mortality (> 90%) in lambs, kids and to a lesser extent in calves (70%) younger than 1–2 weeks, associated with high abortion rates (15–100%) in pregnant sheep, goats, cattle and camels are characteristic of an RVF animal outbreak. Adult sheep are more susceptible (case fatality 20–70%) than adult cattle and goats (case fatality < 10%) (FAO, 2003; Lefèvre et al., 2003; CFSPH, 2015; OIE, 2018, 2019).

The incubation period is 1–6 days (12–36 h in lambs). In a review of experimental infections, the median incubation period was 4 days and the minimum was 1 day in sheep (Dorea et al., 2022). The severity of the disease is linked to the age of the animals, with peracute forms in newborns, acute forms in young animals older than 2 weeks, and often subacute or asymptomatic forms in adults especially in indigenous African cattle breeds and camels. Clinical signs in the peracute and acute forms are: persisting high fever (40–42°C), anorexia, weakness, lymphadenopathy, haemorrhagic and fetid diarrhoea, melena, regurgitation, colic, bloodstained mucopurulent nasal discharge, elevated respiratory rate and sometimes icterus. Death occurs within 24 h in newborn lambs and kids and within 3–10 days in older animals. Abortion, sometimes several weeks after infection, and decrease in milk production may be the only signs in adult cattle. Recovering animals may remain weak for weeks with jaundice and photosensitisation as possible sequela due to liver damage (Davies and Martin, 2003; Lefèvre et al., 2003; CFSPH, 2015; OIE, 2019).

In humans, RVF usually causes an asymptomatic form or a mild flu-like syndrome. A small minority develop a severe form characterised by ocular and neurological signs, acute hepatitis with associated jaundice, renal failure; in 1% of cases, a severe and often fatal haemorrhagic syndrome can occur (AFFSA, 2008; Adam et al., 2010; CFSPH, 2015; Paweska, 2015).

Histopathological examination of inactivated liver sample conserved in formalin shows lesions pathognomonic of RVF and can be confirmed by immunostaining allowing identification of RVF viral antigen. Detection of RVFV or of its antigens can be made from serum, plasma and unclotted blood taken during the febrile phase in sick animals or in tissue samples from dead animals (liver, spleen, brain, lymph nodes) or from aborted fetus. Biosecurity measures should be taken at collection, transport and treatment of samples due to the zoonotic risk. Virus isolation is possible, but is not routinely performed due to hazard for laboratory personnel. Usual tests are gel-based and real-time RT-PCR, RT-LAMP (loop-mediated isothermal amplification technique), lateral flow tests and antigen capture ELISA. Commonly used serological tests include antibody ELISA and virus neutralisation test (VNT); however, they cannot discriminate vaccinated from infected animals. VNT is very specific but requires live virus and hence needs biocontainment facilities. Commercial IgG and IgM antibody ELISA are available and can be used with inactivated samples, the latter being able to confirm a recent infection (CFSPH, 2015; Paweska, 2015; OIE, 2018, 2019). Details on different diagnostic tests for RVF are provided in EFSA AHAW Panel (2020b).

Diagnostic test for surveillance purpose

Different laboratory diagnostic tests can be used for RVF early detection under different scenarios. In case of investigations on clinical suspicions, direct tests, such as RT-PCR or virus isolation, performed on blood and other tissues taken from animals showing clinical signs suggestive of RVFV infection are the best option, above all in free countries where the notification of a new case of RVF should be based on virus detection and not only on detection of antibodies. The choice of PCR compared to serological tests is also justified in vaccinated population where the focus should be on the detection of active infection. In the framework of a random active surveillance programme based on blood sampling, RT-PCR might not be the best choice, given the limited time window on which infected animals are viraemic or with detectable RVFV's genome in blood (Pepin et al., 2010; Paweska, 2015) and the cost of a survey based on this molecular technique, especially when a large number of animals must be tested. In this context, the test of choice in a population free from infection (unvaccinated animals) is the one identifying the immune response, which is, in case of RVF, the ELISA detecting IgM and IgG towards RVFV (Williams et al., 2011). The laboratory diagnostic tests for RVF have been reviewed in Nielsen et al. (2020). The commercially available ELISA, e.g. ID Screen® Rift Valley Fever Competition Multi-species, set for bovine, ovine, caprine, horses, dogs, cats, humans (ID Vet), has a reported diagnostic sensitivity and specificity estimated at 100% (CI 95%: 91.24–100%; n = 40) and 100% (CI 95%: 99.58–100%; n = 839), respectively (Comtet et al., 2010). A EU ring trial estimated a sensitivity of 98% and specificity of 100% (Kortekaas et al., 2013). In the following assessment, the lower CI (91.24%) for Se will be considered.

2.4. Vaccine and vaccination

At present, no vaccines have been authorised for use in the EU by the European Medicine Agency (EMA, online). Their use for emergency vaccination should be on an ad hoc basis and authorised following the proper EU procedure.

Inactivated and live-attenuated RVF vaccines (LAV) represent the most developed and tested vaccines currently available for livestock immunisation. Both the inactivated and the live-attenuated vaccines (Smithburn and MP-12 strains) have been obtained from virulent RVFV isolates using conventional technologies, and represent the most sustainable strategy to mitigate the impact of RVF on livestock agriculture.

The live-modified Smithburn vaccine can readily be produced in large quantities at low cost, and induces a durable immunity lasting at least 18 months following vaccination in sheep and cattle after a single inoculation (Coackley et al., 1967), although in a proportion of pregnant female animals, it may cause abortions or fetal teratology (Botros et al., 2006; Kamal, 2009). Genetic reassortment between RVFV field strains and the Smithburn strain has been described in mammals (Grobbelaar et al., 2011) and mosquitoes (Turell et al., 2011).

In contrast to live-attenuated vaccines, inactivated vaccines are described as safer, specifically for use in pregnant animals, though they are expensive to produce and require the administration of

booster doses 3–4 weeks after first initial vaccination (in addition to yearly booster) to ensure adequate long-term protection (up to 38 weeks) (Lagerqvist et al., 2012).

Inactivated vaccines are normally used in non-endemic RVFV countries (CFSPH, 2015; DISCONTOOLS, 2016). Although both types of vaccines have contributed significantly to the control of RVF in endemic countries of Africa, the requirement of repeated immunisations (for inactivated vaccines) and risk of inducing teratogenic effects, abortion and potential reassortment/reversion due to residual neuro-invasiveness and neurovirulence (for the LAV vaccines) highlight the need for a new generation of vaccines with a higher safety profile.

A critical advance over currently existing livestock vaccines would be the ability to discriminate naturally infected from vaccinated animals (DIVA). A DIVA approach (vaccine and accompanying diagnostic tests) is an essential requirement for vaccines to be used in both endemic and non-endemic countries allowing compliance with mandatory international trade restrictions during active RVF outbreaks.

Preventive mass vaccination is the most effective means to control RVF circulation when climatic, environmental and epidemiological evaluations suggest a high probability of RVF outbreaks.

Details on different types of vaccines are provided in EFSA AHAW Panel (2020b).

2.5. Geographical distribution of RVF

RVF has been reported in Africa and Arabian Peninsula. In Figure 1, a map shows countries where RVF is endemic and where it has been occasionally reported, while Figure 2 shows the countries with reported outbreaks of RVF between 2015 and 2021.

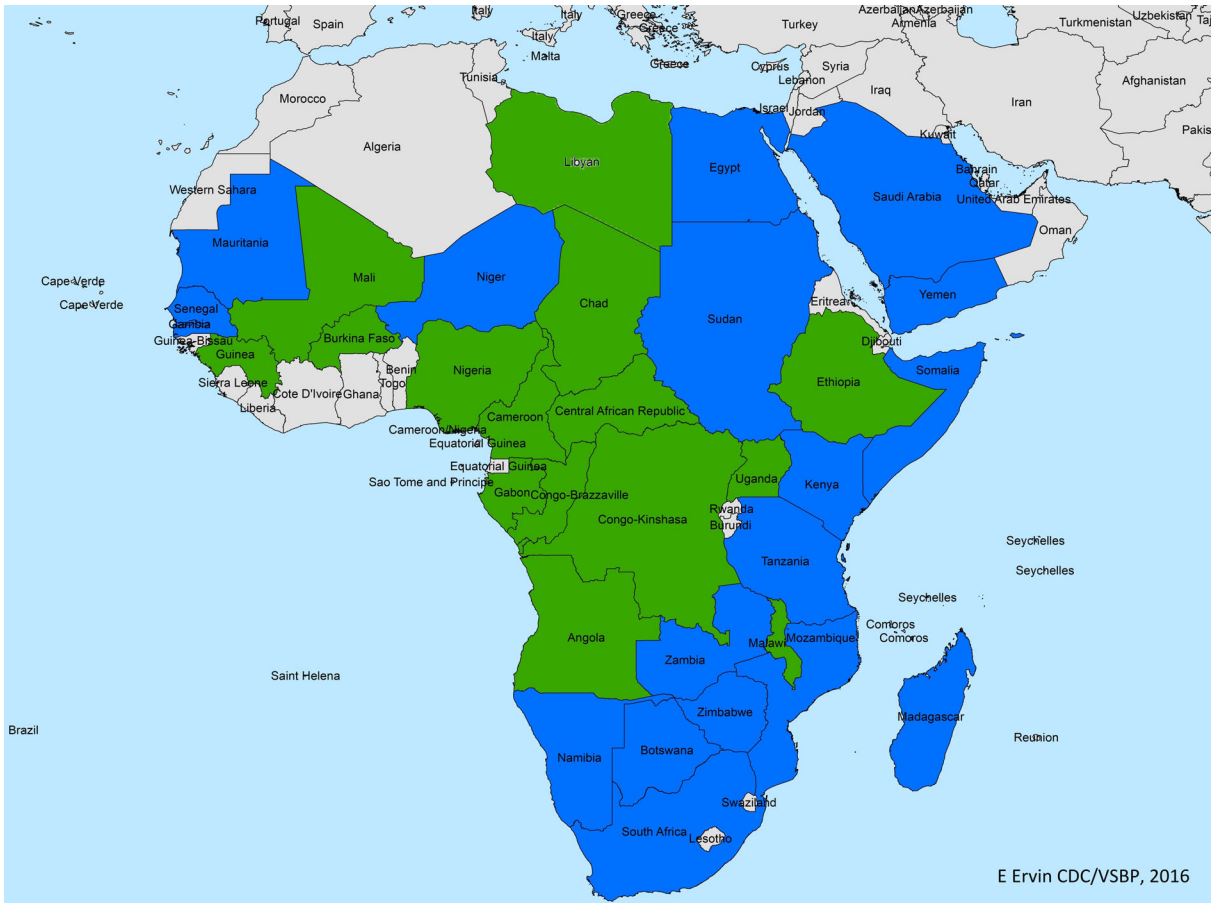


Figure 1: Amended map of CDC classification of the countries where RVF has been confirmed: (i) blue: countries reporting endemic disease and substantial outbreaks of RVF; (ii) green: countries reporting few cases, periodic isolation of virus, or serological evidence of RVF; and (iii) grey: RVF status unknown or not reported (modified from E. Ervin CDC/VSBP 2016[1])

[1] Downloaded on 7/10/2019; https://www.cdc.gov/vhf/rvf/outbreaks/distribution-map.html

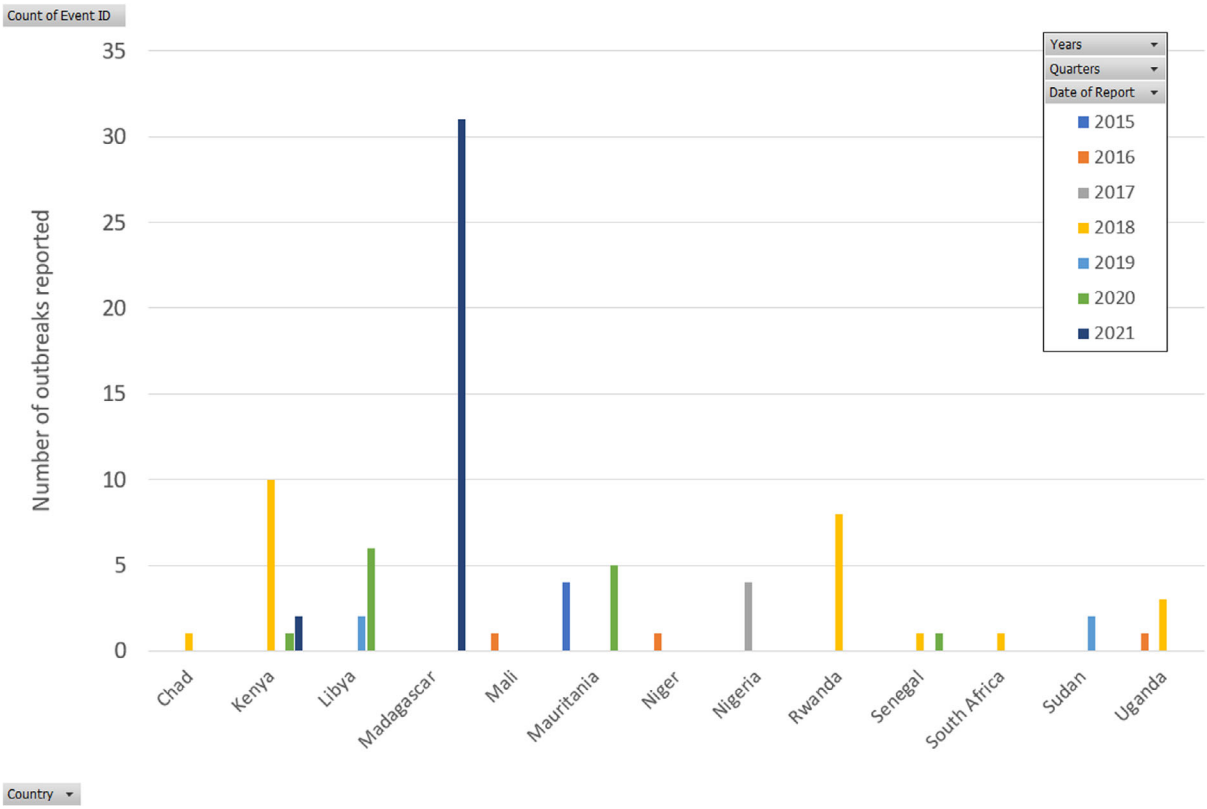


Figure 2: Countries with reported outbreaks of RVF between 2015 and August 2021 (Data sources: OIE)

3. Data and methodologies

3.1. Methodology used in ToR 1

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

3.1.1. Mathematical model and transmission scenarios considered

3.1.1.1. Within-herd dynamics of RVF

The dynamics of RVFV within a season were described using a stochastic compartmental model that includes a single ruminant host (i.e. cattle, sheep and goats are assumed to be equivalent, this irrespective of the age structure, which would substantially complicate the model and any higher mortality in younger animals can be averaged out in the population as a whole) and a single mosquito vector.

The host population is assumed to be constant (H), except for disease-associated mortality, and is subdivided into the number of susceptible (i.e. uninfected), latent (i.e. infected, but not yet infectious), infectious animals and recovered animals, denoted by S, E, I and R, respectively. To allow for a more general gamma distribution for the latent and infectious periods, the latent and infectious host populations, E and I, are subdivided into a number of stages. If the time spent in each stage follows an exponential distribution with mean μ_j/k_j (j = E, I), the total length of time spent in the k_j stages follows a gamma distribution, with mean μ_j and variance μ_j^2/k_j (Anderson and Watson, 1980).

The vector population (N) is subdivided into the number of adult female mosquitoes that are susceptible (i.e. uninfected), latent (i.e. infected, but not infectious) and infectious, denoted by X, Y and Z, respectively. To allow for a more general gamma distribution for the extrinsic incubation (i.e. latent) period (EIP), the latent class is subdivided into a number of stages in a similar approach to that

described above. Vector mortality occurs at the same rate in all classes and is balanced by the recruitment of susceptible vectors, so that the total vector population remains constant.

The force of infection for the host, λ_H, is given by,

$$\lambda_H(t) = bam\frac{Z(t)}{N},$$

where b is the probability of transmission from an infected vector to a host, a is the reciprocal of the time interval between blood meals for the vector (assumed to be equal to the biting rate), m (= N/H) is the vector-to-host ratio and Z/N is the proportion of bites which are from infectious vectors. The force of infection for vectors, λ_V, is

$$\lambda_V(t) = \beta\alpha\frac{I(t)}{H},$$

where β is the probability of transmission from an infected host to a vector and I is the total number of infectious animals.

Parameters in the model are summarised in Table 1; their values were extracted from the published literature. For temperature-dependent parameters (biting rate, vector mortality rate and reciprocal of the mean extrinsic incubation period), values were computed assuming a daily mean temperature of 20, 25 or 30°C. The herd size was assumed to be 100 or 1,000 animals (for smaller herd size, e.g. < 50 animals, the whole herd should be sampled). Virus was assumed to be introduced to a herd by a single infectious vector.

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

Table 1: Parameters in the model for the transmission of Rift Valley fever virus

Description		Symbol	Estimate or function	Comments and references
Probability of transmission from vector to host		b	1	Turell et al. (2008)
Probability of transmission from host to vector (for *Culex* spp.)		β	0.1	Brustolin et al. (2017), Vloet et al. (2017), Lumley et al. (2018)
Vector to host ratio		m	40	Gachohi et al. (2016)
Reciprocal of the time interval between blood meals		a	a(T) = 0.0173(T-9.6)	Depends on temperature (Madder et al., 1983)
Latent period	Mean (days)	μ_E	2	Pepin et al. (2010), Vloet et al. (2017)
	No. stages	k_E	3	
Infectious period	Mean (days)	μ_I	6	Bird et al. (2009), Pepin et al. (2010)
	No. stages	k_I	3	
Disease-associated mortality rate (per capita death rate due to disease, which gives a case fatality of 5–30%)		d	0.03	Assumes 5–30% of livestock die of disease (Bird et al., 2009)
Extrinsic incubation period (EIP) (estimated for *Aedes* spp.)	Mean (days)	1/v	v(T) = 0.0071(T-14.6)	Depends on temperature (Turell et al., 1985; Barker et al., 2013)
	No. stages	k	3	
Vector mortality rate		μ	μ(T) = 1/(69.1-2.14T)	Depends on temperature (Fischer et al., 2013)
Vector recruitment rate		ρ	–	Assumed equal to the mortality rate
Vector population size		N	–	For simplicity, assumed to be constant; given by N = mH

In Figure 3, the within-herd dynamics of Rift Valley fever virus in ruminants are shown.

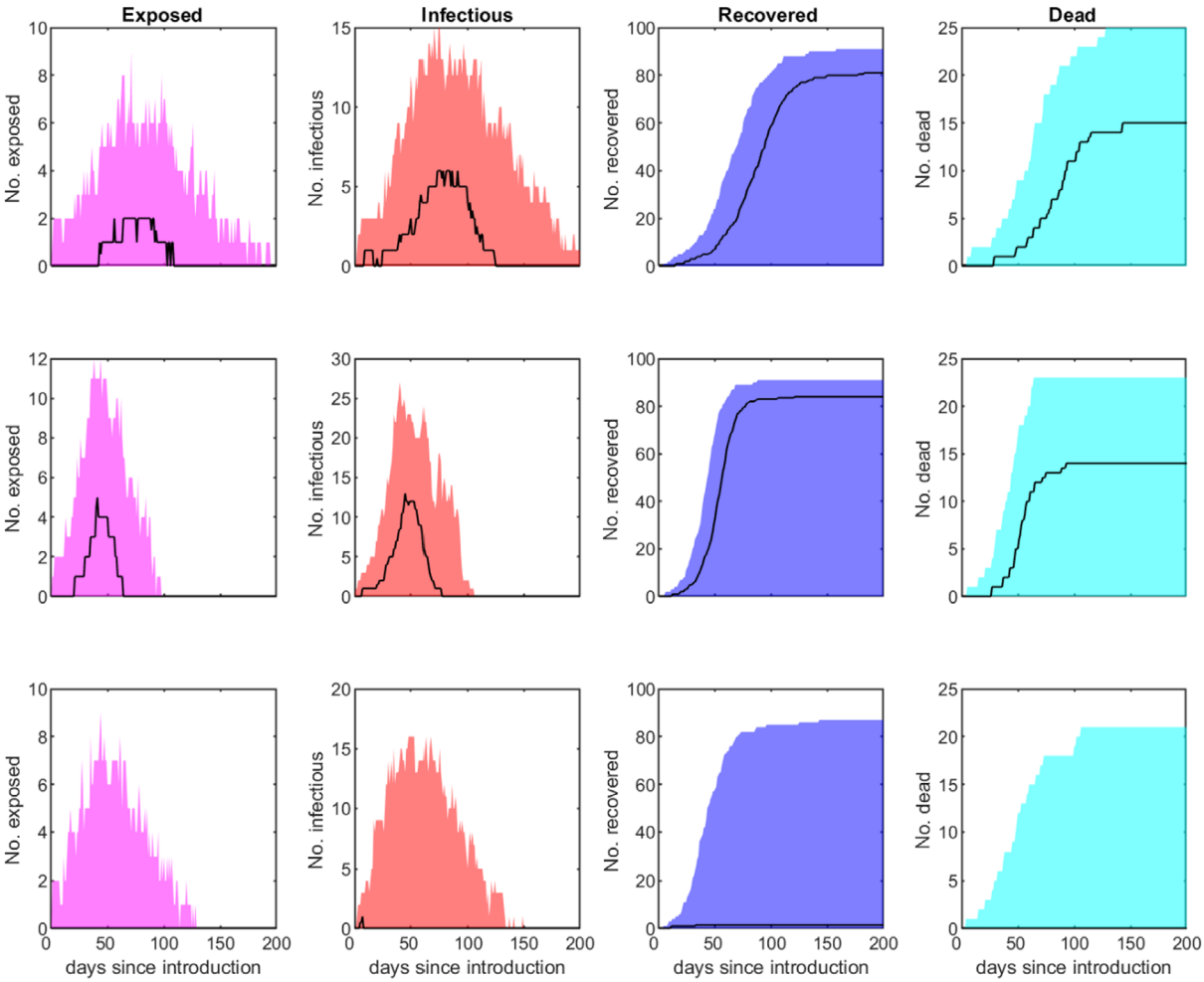


Figure 3: Within-herd dynamics of RVFV in ruminants. The plots show the median (solid line) and 95% prediction interval (shading) for the number of exposed animals (left-most column), infectious (second column), recovered (third column) and cumulative number of dead animals (right-most column) for the three daily mean temperatures: 20°C (top row); 25°C (middle row); and 30°C (bottom row)

3.1.1.2. Detection of Rift Valley fever virus in ruminants

The prevalence of virus-positive animals was assumed to correspond to the prevalence of infectious animals. Seroprevalence was assumed to correspond to the prevalence of recovered animals.

The infection prevalence and seroprevalence are the proportion of live animals infected or seropositive, respectively. So, the denominator in the calculations is the initial herd size minus the cumulative number of animals that have died of RVFV.

In Tables 2, 3 and 4, the prediction intervals for the infection prevalence at 14, 21, 28 days after introduction into the herd are shown, respectively, while in Tables 5, 6, 7 prediction intervals for the seroprevalence of Rift Valley fever virus in ruminants at same time intervals are displayed.

Table 2: Median (M), lower (L) and upper (U) 95% prediction intervals for the infection prevalence of RVFV in ruminants at 14 days post introduction to a herd

Scenario	Herd size					
	100			1,000		
	M	L	U	M	L	U
20°C	1.0	0	3.0	0	0	0.3
25°C	1.0	0	5.0	0.1	0	0.4
30°C	0	0	4.0	0	0	0.6

Table 3: Median (M), lower (L) and upper (U) 95% prediction intervals for the infection prevalence of RVFV in ruminants at 21 days post introduction to a herd

Scenario	Herd size					
	100			1,000		
	M	L	U	M	L	U
20°C	0	0	4.0	0	0	0.4
25°C	1.5	0	9.1	0.2	0	0.9
30°C	0	0	9.2	0	0	0.8

Table 4: Median (M), lower (L) and upper (U) 95% prediction intervals for the infection prevalence of RVFV in ruminants at 28 days post introduction to a herd

Scenario	Herd size					
	100			1,000		
	M	L	U	M	L	U
20°C	1.0	0	4.1	0.1	0	0.4
25°C	4.0	0	13.4	0.4	0	2.0
30°C	0	0	12.1	0	0	1.5

Table 5: Median (M), lower (L) and upper (U) 95% prediction intervals for the seroprevalence of RVFV in ruminants at 14 days post introduction to a herd

Scenario	Herd size					
	100			1,000		
	M	L	U	M	L	U
20°C	0	0	4.0	0.1	0	0.4
25°C	1.0	0	4.0	0.1	0	0.4
30°C	1.0	0	4.0	0.1	0	0.4

Table 6: Median (M), lower (L) and upper (U) 95% prediction intervals for the seroprevalence of RVFV in ruminants at 21 days post introduction to a herd

scenario	Herd size					
	100			1,000		
	M	L	U	M	L	U
20°C	1.0	0	5.0	0.1	0	0.6
25°C	2.0	0	7.2	0.2	0	0.9
30°C	1.0	0	9.2	0.1	0	0.9

Table 7: Median (M), lower (L) and upper (U) 95% prediction intervals for the seroprevalence of RVFV in ruminants at 28 days post introduction to a herd

Scenario	Herd size					
	100			1,000		
	M	L	U	M	L	U
20°C	3.0	0	8.2	0.2	0	0.9
25°C	5.0	0	16.3	0.6	0	1.9
30°C	1.0	0	16.2	0.2	0	1.6

3.2. Methodology used in ToR 2

3.2.1. Time lag between infection and reporting

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

3.2.2. Seroconversion period

Considering Scenario 5 of ToR 2, the earliest day of the seroconversion after the infection, as well as the time interval between the earliest day of antibodies detection and the latest day of antibodies detection, detected by different serological methods in different animal species needs to be identified for each disease of concern. Thus, a scientific literature review on the earliest day of seroconversion and the latest day of antibodies detection after infection for Rift Valley Fever (RVF) and the relevant target population (*listed species)* was considered appropriate to successfully address this scenario.

The objectives of the literature review were to identify:

- the earliest day/or range of days of seroconversion (earliest day when antibodies have been detected) after infection/inoculation for each serological test used, for different animal species,
- the duration of seroconversion (interval between the earliest day when antibodies have been detected) after infection/inoculation for each serological test used, per each animal species,
- the target population (*listed species)* for the disease

The methodology described in Section 2.3 of the EFSA AHAW Panel (2020) and in scientific opinions about RVF (EFSA AHAW Panel, 2020a,b,c) was followed for the assessment of Scenario 5 of ToR 2. Details about the review protocol are in Annex H.

3.3. Methodology used in ToR 3

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

The assessment of radius size of restricted zones (ToR 3), to prevent further disease spread at a given probability, was performed by using disease transmission kernels (EFSA AHAW Panel, 2020c).

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

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

3.4. Uncertainty

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

4. Assessment

4.1. Assessment of sampling procedures (ToR 1)

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

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

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

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

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination in the event of a suspicion of RVFV are available in the EU legislation.

Assessment

In the event of the first introduction of RVF into a previously free territory like the EU, it is highly probable that the first suspicion would be based on the observation of the most evident clinical signs, which are abortion and death in young animals. However, it is important to highlight that early detection of RVF based on passive surveillance may not be as effective as needed, depending on various circumstances. In fact, it is reasonable to assume that the farmer's awareness has arisen only when a certain number of animals in the herd are affected, and this may occur when multiple cycles of virus transmission have been already established in the vector population. In addition to clear and noticeable clinical signs, a certain number of susceptible pregnant females and newborn animals must be available, and depending on the period of the year of RVFV introduction, this may not be the case for species characterised by seasonal calving, like sheep and goats.

For these reasons, it should not be excluded that the first signs of RVF disease may be observed in humans rather than animals.

When abortions are observed, the whole aborted fetuses should be collected as well as dead lambs or calves in case of mortality in newborn animals. When the whole carcass cannot be dispatched to the laboratory, liver, spleen and lymph nodes can be taken from dead animals and organs, as well as brain from aborted fetuses. Personal protection equipment and proper procedures for sample manipulation must be followed to avoid any possible exposure of personnel to RVFV.

When clinical signs are observable in adult animals, blood samples with EDTA for RT-PCR should be taken during the febrile phase of the disease. The samples should be kept on ice during transport. If transport lasts more than 24 h, samples should be stored in glycerol-saline. Samples are tested by

RT-PCR for the detection of viral genome. Virus isolation can be performed only by reference laboratories, properly equipped for the manipulation of this viral agent (in laboratories with appropriate BSL 3 facilities or higher). Whole blood samples should also be taken for serology.

When the disease has to be ruled out even in the absence of clinical signs, for other reasons (e.g. in case of introduction of animals which are suspected to be infected as a result of epidemiological investigations, e.g. uncontrolled/illegal import from infected areas/establishments), blood samples (with and without EDTA) should be taken from the suspect animals, which should be kept indoors, in stables with vector protection to reduce exposure to mosquitoes.

Development of new procedures

Considering the high sensitivity of the RT-PCR in specific organs (RVFV persistence is long in e.g. spleen, so this is a preferable sample matrix) when fresh and properly stored or blood samples are provided (EFSA AHAW Panel, 2020b), samples from few suspect clinically affected animals or aborted fetuses (e.g. 5 animals or fetuses) would be enough to confirm the presence of RVFV in the herd.

In case of the absence of clinical signs and if the suspicion is based on other factors (e.g. animals originating from infected areas), all animals should be sampled and tested.

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 RVF

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

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

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

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

    a) supporting the epidemiological enquiry to:

        i) identify the likely origin of the disease;
        ii) calculate the likely length of time that the disease is present;
        iii) identify establishments where the animals could have contracted the disease and movements from the affected establishment that could have led to the spread of the disease; and
        iv) obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors

    b) confirming/ruling out disease in the event of preventive killing.

Summary of sampling procedures

No specific guidelines on sampling procedures for laboratory examination for RVFV in the event of preventive killing or to support the epidemiological investigation are available in the EU legislation.

Assessment

Given that the infection can be fully inapparent in adults, apart from abortion in pregnant females, clinical examination alone is insufficient to detect all infected animals. In addition, due to the short course of viraemia, RT-PCR assay alone cannot be used as reference test for this purpose.

In an unvaccinated population, serological tests may be used for the identification of already infected animals. Virus neutralisation test (VNT) and ELISA are available; the latter is easier to perform and does not require handling live virus. Commercial ELISA kits are available for IgG and IgM antibodies detection. IgM-capture ELISA allows diagnosis of recent infections, since usually IgM are not more detectable after 2–4 months from the infection. The majority of available ELISA kits have good diagnostic performances (EFSA AHAW Panel, 2020b), but they may fail to detect antibodies against RVFV in the first 3–7 days after the infection, before a detectable level of immune response is established in the infected animal. To reduce the probability of not detecting those animals infected for a few days, the testing of blood samples with EDTA by RT-PCR can be coupled or a twofold sampling scheme can be applied, repeating a second blood sampling collection at least 1 week after the first one. In the case of preventive killing, a combination of serology and PCR can also be used for confirmation purpose.

In some cases, the laboratory examinations on animals should be complemented by entomological investigations in order to identify the possible vectors of infection, to assess their abundance and to provide more information about the possibility of further disease spread. It is fundamental to clearly and precisely define the objectives of the entomological investigations in order to choose the most appropriate trapping systems (for diurnal or nocturnal species, to caught only flying adults, aquatic stages or new emerging generation, etc.) and the laboratory tests to be applied. Mosquito pools can also be tested by RT-PCR for the presence of the viral genome. However, while the virological testing of mosquito pools can be useful for identifying the vectors and for the estimation of the infectious rate in the vector population, its results are greatly affected by the dilution effect of the infection over billions of mosquitoes. Often, a high trapping pressure (both in terms of number and frequency of trapping sessions) is needed to detect the virus in the vector population.

Development of new procedures

Ideally, all the animals in the involved herd should be sampled in order to have a complete picture of the infection prevalence in the herd. When the date of RVFV introduction in the herd is unknown, it is difficult to estimate the prevalence. A random sampling approach in this context would easily lead to the necessity of testing a large part of the herd (see low level of estimated infection and serological prevalence in Section 3.1.1).

However, when the number of involved herds to be tested makes it unfeasible to test all the animals, a random sampling approach could be used considering the all involved animals from these herds. In fact, since vector-borne diseases do not tend to cluster within the herds (unless particularly favourable vector conditions are present in specific herds), the whole population of animals in the herds belonging to RVF susceptible species can be considered as the target population of a representative sampling scheme. Following this approach, a sample size for prevalence estimation, at 95% confidence level and with unknown expected prevalence (thus set to 50%), varies according to the precision required for the estimation (e.g. 2,400 samples are required for a ±2% precision, whereas around 9,600 are needed for ±1%).

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

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

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

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

1) It concerns an affected establishment where infection is officially confirmed;
2) In the establishment where 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 examinations in order to grant a specific derogation from killing animals in RVFV-affected establishments are available in the EU legislation.

Assessment

In the EU context, which is historically free from this disease, and due to the zoonotic characteristics of RVF, specific derogations from killing animals infected by RVFV should be very carefully assessed.

Adult (males and non-pregnant females) not exhibiting clinical signs and pre-pubertal animals may not pose a major risk for humans, particularly after the end of the viraemic period and the establishment of a solid immunity. During the possible viraemic period, however, the animals must be isolated and protected from mosquito bites.

The viraemic and immune status of the animals may be assessed through the collection of blood samples with EDTA for RT-PCR testing and whole blood samples for serology, which should include not only IgM and IgG ELISA testing.

Development of new procedures

Each single animal for which the derogation claimed must be sampled and tested.

4.1.1.4. For the animals of non-listed species kept in an RVF-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 Annex B.

- **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 during 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 examinations of the animals of non-listed species kept in an affected establishment are available in the EU legislation.

Assessment

Apart from domesticated or wild ruminants, in Africa, RVFV has been found in few species (e.g. some species of rodents and bats and even non-human primates), whereas serological positive findings have been detected in several animal species. However, the significance of these findings and the possible role of these species in virus transmission are fully unknown. In the EU Regulation 1882/2018, the listed species for RVF are Perissodactyla, Antilocapridae, Bovidae, Camelidae, Cervidae, Giraffidae, Hippopotamidae, Moschidae, Proboscidea.

In addition, the performances of the diagnostic tests, which have been validated for domestic ruminant species, are unknown in terms of their application to other species.

Development of new procedures

For the reasons reported above, the application of any clinical or laboratory-based sampling procedure for non-listed species is not recommended, apart for purely research purposes.

4.1.1.5. For wild animals of the listed species within an RVF-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 Annex B.

- **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 were taken into consideration for the assessment:

1) It concerns a RVF affected establishment (officially confirmed)
2) It refers to wild animals of listed species within the establishment and in the surroundings of the establishment

As listed in Commission Implementing Regulation (EU) 2018/1882 for RVF; the wild animals of listed species animals are those belonging to the families of *Perissodactyla*, *Antilocapridae*, *Bovidae*, *Camelidae*, *Cervidae*, *Giraffidae*, *Hippopotamidae*, *Moschidae*, *Proboscidea*. species.

1) The competent authority may establish these sampling procedures in addition to other measures.
2) The purpose of the sampling procedures in wild animals of listed species is to ensure the detection of the virus, if the virus is present in these wild animals.

Summary of sampling procedures

There are no sampling procedures defined for wild animals of the listed species within the RVFV-affected establishment and its surroundings.

Assessment

In the scenario where wild ruminants (buffaloes, antelopes, camels) are kept or living in the surrounding area of the affected establishment, these may acquire the infection by direct or indirect contact with affected animals, if no or low biosecurity measures are in place to keep animal species separated and protected from the mosquito biting.

Development of new procedures

The surveillance of wildlife around the affected establishment may include the visual inspection of these animals from a distance and the testing of fallen stock and hunted animals both by RT-PCR and serology (although the latter are not validated in wild species). Unexpected mortality events in susceptible wildlife should be investigated.

Samples from animals with clinical signs from dead or hunted animals should be collected for laboratory analysis, following the procedures of Section 4.1.1.1. Wildlife population health experts would be able to provide additional advice in these circumstances.

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

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. 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. For RVF, the size of the protection zone foreseen in the legislation is 20 km; therefore, the next scenario in Section 4.1.1.7 applies.

4.1.1.7. For non-affected establishments located in a protection zone with a radius larger than 3 km

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 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. For further details, see Annex B.

- **7th scenario of sampling procedures**
- ToR 1.3 in accordance with Article 26(5) of the Delegated Regulation (EU) 2020/687

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

1) It concerns a protection zone with radius larger than 3 km
2) Sampling of the non-affected establishments of kept animals of listed species in the protection zone
3) In a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km
4) In case of a radius larger than 3 km (the case for RVF), official veterinarians may not visit all establishments, but a sample of those
5) EFSA is requested to assess how many of these establishments should be inspected, in order to ensure the detection of the virus, if the virus is present in animals in these establishments
6) The purpose of sampling procedure is to ensure the detection of the disease if the disease is present in any of these establishments

Summary of sampling procedures

There are no sampling procedures defined for non-affected establishments located in a protection zone for RVF larger than 3 km.

Assessment

A strict surveillance should be applied in the protection zone with a radius up to 20 km. Periodical herd visits with clinical examination of animals within the herds should be applied. In case of any clinical finding, the procedures of Section 4.1.1.1 must be followed.

During the vector season and depending on the climatic conditions (temperatures, wind dispersal trajectories), an enhanced surveillance based on laboratory examinations could be appropriate. Both blood samples with EDTA for RT-PCR testing and whole blood samples for serology should be collected. Considering the extent of the protection zone in this case (up to 20 km of radius), it could be difficult to apply an active surveillance approach all over the zone. After an assessment of the geographical distribution of herds, the active surveillance approach as described in Section 4.1.1.2 could be limited to the most internal part of the zone, up to 3 km from the infected zone.

For the rest of the protection zone, it could be more effective to apply a clinical investigation programme of herds with clinical examination of the animals, coupled with an awareness campaign targeting farmers in order to enhance the capacity of detecting any possible sign of the disease.

Development of new procedures

For the active surveillance, a random sampling approach across all the animal population in the protection zone can be followed. For the detection of infection within the zone, with 95% of confidence, and considering the outcomes of the model for RVF transmission (see Section 3.1.1.2), a number of samples varying from around 160 (for 1.9% of expected prevalence) to 750 (for 0.4% expected prevalence) animals should be periodically tested, from 14 to 28 days, respectively, as indicated in Section 3.1.1.2.

In case of any clinical finding, the procedures of Section 4.1.1.1 must be followed.

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

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

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

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

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

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species in the non-affected establishments located in a protection zone for RVF.

Assessment

It is unlikely that establishments in this zone, not epidemiologically linked to an outbreak, will become infected with RVFV without having additional outbreaks in the protection zone.

Consequently, 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.

Development of new procedures

Any establishment where generic signs of disease are reported should be visited, the animals should be clinically examined and samples should be collected following the procedures described in Section 4.1.1.1.

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 Section 4.1.1.1.

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

As a general consideration, in case of RVF but also for other vector-borne diseases, in relation to animal movements, the key condition is the sanitary situation of the population where the animals to be moved are coming from, not only the condition of the single animal.

All aspects such as level of transmission, incidence, vaccination status, vector season, etc. should be considered, in relation to the risk that animals to be moved may be viraemic at the moment of dispatch.

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 (Art. 29). For further details, see Annex B.

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

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

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

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species in the non-affected establishments located in a protection zone to slaughterhouses located within the protection zone or in the surveillance zone or outside the restricted zone.

Assessment

Due to the zoonotic significance and the fact that RVF is a vector-borne disease, it is not recommended to move animals from the protection zone. However, since the destination of these animals is the slaughterhouse, if derogation is granted, all biosecurity measures should be implemented and given that the animals should be slaughtered within 24 h this would reduce further the risk. In addition, animal slaughtering from the establishments in the protection zone could have a beneficial effect of reducing the number of potential hosts for the further spread of RVFV.

Development of new procedures

All the animals in the establishment of origin should be examined before their movement, following the procedures described in Section 4.1.1.1. In an establishment where the number of animals is large, the 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 examined to rule out the presence of RVFV with at least 95% confidence, as described in Section 4.1.1.1.

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

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

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

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

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

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species in the non-affected establishments located in a protection zone.

Assessment

This scenario is very similar to the ninth scenario (Section 4.1.2.1); therefore, the assessment is the same.

Development of new procedures

This scenario is very similar to the ninth scenario (Section 4.1.2.1); 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 Mandate
- Article 43(5) and article 44 of the Delegated Regulation (EU) 2020/687

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

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

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species.

Assessment

This scenario includes two different subscenarios: (a) the need to transfer animals of listed species kept in establishments located in the surveillance zone to a slaughterhouse located within or 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. Of those, the first subscenario represents a risk for the spread of RVFV from infected animals, especially if the slaughterhouse is located outside the surveillance zone. Considering the vector-borne component of the disease, the movement of animals of listed species from establishments located in surveillance zone has a similar level of risk than that of ninth scenario (Section 4.1.2.1). Conversely, the movement of animals of listed species from establishments located outside the surveillance zone to a slaughterhouse located within or outside the surveillance zone poses a negligible risk to spread RVFV.

Development of new procedures

The movement of animals of listed species from establishments located in surveillance zone to a slaughterhouse located within or outside the restricted zone should be allowed after the procedure proposed in ninth scenario (Section 4.1.2.1) is followed.

When there is the need to move animals of listed species located outside the surveillance zone to slaughterhouse located within the surveillance zone, no restrictions are foreseen.

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

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone. For further details, see Annex B.

- **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 were taken into consideration for the assessment:

1) It concerns kept animals of listed species from establishments located in the surveillance
2) zone
3) To grant derogation for movement from the surveillance zone
4) To be moved to pastures situated within the surveillance zone
5) Clinical examinations and laboratory examination of animals kept in the establishment

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species.

Assessment

Animals in a surveillance zone, for which a specific derogation has been granted to be moved to pastures, remain alive therefore they should be subjected to clinical surveillance, including laboratory examinations according to what described in Section 4.1.1.1. 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 according to procedures described in Section 4.1.1.1 do pose a negligible risk of transmission of RVF.

Development of new procedures

This scenario is very similar to the ninth scenario (Section 4.1.2.1); therefore, the same procedures are suggested.

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

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant derogation and allow to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone, in order to complete the production cycle before slaughter. For further details, see Annex B.

- **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 were taken into consideration for the assessment:

1) It concerns the surveillance zone
2) Grant derogation for movement of kept animals of listed species
3) from the surveillance zone
4) 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
5) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species.

Assessment

Animals in a surveillance zone, for which a specific derogation has been granted to be moved 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, remain alive therefore they should be subjected to clinical surveillance, including laboratory examinations according to what described in Section 4.1.1.1. 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 according to procedures described in Section 4.1.1.1 do pose a negligible risk of transmission of RVF.

Development of new procedures

This scenario is very similar to the ninth scenario (Section 4.1.2.1); therefore, the same procedures are suggested.

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 Annex B.

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

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

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

Summary of sampling procedures

There are no sampling procedures defined for animals of listed species for RVF.

Assessment

Animals in a restricted zone, for which a specific derogation has been granted to be moved within the restricted zone when restriction measures are maintained beyond the period set out in Annex XI of the Delegated Regulation, remain alive; therefore, they should be subjected to clinical surveillance, including laboratory examinations according to what described in Section 4.1.1.1. 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 according to procedures described in Section 4.1.1.1 pose a very low risk of transmission of RVF.

Development of new procedures

Animal movement is allowed only from an establishment located in surveillance zone within the restricted zone or from an establishment located in the protection zone within the protection zone. When animal movement is allowed, the procedure suggested as per Section 4.1.2.1 should be followed.

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 Annex B.

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

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

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

Summary of sampling procedures as described in the diagnostic manual

There are no specific procedures for sampling of animals prior to repopulation.

Assessment

If the animals intended for repopulation originate from an establishment located outside the surveillance zone (disease-free area), there are no requirements for pre-movement testing of the animals and general regulations in place for moving animals should be applied.

If the animals intended for repopulation originate from an establishment located in the surveillance zone, the procedures in place for the movement of animals from such holdings should apply (Section 4.1.2.1).

In areas where and when biological vectors are present, a vector monitoring and control strategy should be in place before introducing new animals. Aspects about the life cycle of vectors under local circumstances should be considered before moving animals (e.g. presence of breeding sites, season, etc.).

Development of new procedures

As per Section 4.1.1.1.

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 Annex B.

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

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

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

Summary of sampling procedures as described in the diagnostic manual

In the EU legislation, there is no indication of specific procedures for sampling of animals during repopulation after RVF outbreaks.

Assessment

The animals to be used for repopulation should be tested for the presence of RVFV and antibodies; therefore, the procedure can be considered effective for early detection in the repopulated animals.

Development of new procedures

As per Section 4.1.1.1

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 Annex B.

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

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

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

Summary of sampling procedures as described in the diagnostic manual

No specific sampling procedures are available.

Assessment

As a vector-borne disease, if the reintroduction takes place during the vector season, testing of the animal should be carried out (as in Section 4.1.1.1) after the monitoring period has elapsed, bearing in mind that all suspect cases will be investigated irrespective if there is vector activity or not.

If reintroduction occurs during the confirmed vector-free period, continuous monitoring of vector activity should be undertaken. If the vector activity starts before the end of the monitoring period, the animals should be tested at the end of new monitoring period.

Development of new procedures

Negative tests should be obtained at the end of a monitoring period. The monitoring period on vector activity monitoring should include the vector active season.

4.2. Assessment of the length of the monitoring period

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

The length of the monitoring period for each disease is set out in Annex II of the Commission Delegated Regulation (EU) 2020/687 supplementing the rules laid down in Part III of Regulation (EU) 2016/429 (Animal Health Law).

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

4.2.1. Period between the earliest point of infection and the suspicion report

The details of the review protocol are in Annex G.

Database search was carried out on 12/4/2021, identifying 636 unique references. As no reference was available for outbreak data from the EU/EEA, the search was extended to simulation data and to data from the rest of the world. Among the 636 references, six were selected to be included in the qualitative review. The full selection process is displayed in Figure 4.

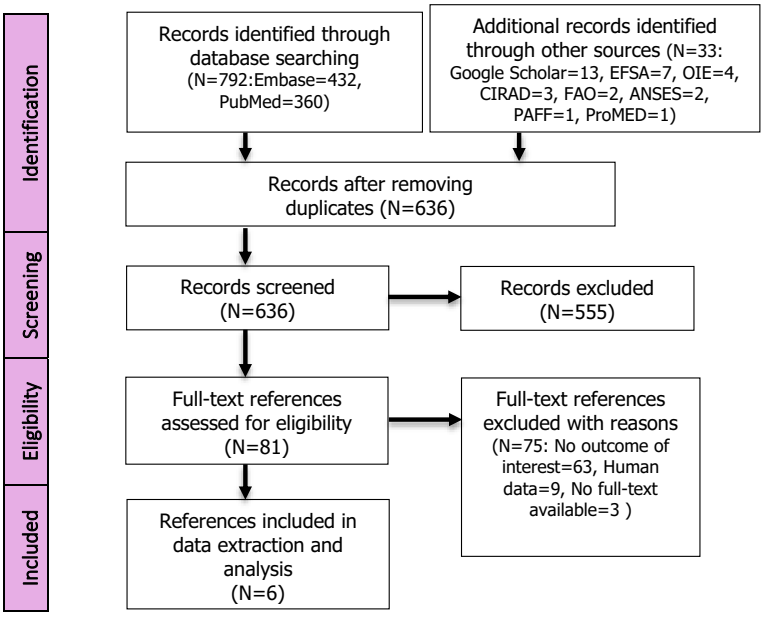


Figure 4: PRISMA diagram RVF Monitoring period ELR

4.2.1.1. Extracted data

The selected studies are shown in Annex G. Three references (out of six) reported dates instead of periods; therefore, the dates were used to calculate the different periods of interest (as described in Section 4.2.1).

Tables 8 and 9 provide an overview of the data that were extracted for the main outcome of interest, i.e. the period between the earliest point of infection and the suspicion report, for which two references were retrieved.

Table 8: Summary of the RVF extraction for the period between earliest point of infection and suspicion report: Outbreak data

Reference	Country	Year	Host animal/Farm type	Period (days)
Mapaco et al. (2012b)	South Africa	2008	Cattle/dairy	18(1)

(1): Primary outbreak; Based on the diagnostic laboratory data.

Table 9: Summary of the RVF extraction for the period between earliest point of infection and suspicion report: simulation data

Reference	Country	Year	Species/farm type	Period (days)
(EFSA AHAW Panel et al., 2020b)	EU (Netherlands)	NA	NA	20(1)

NA: not applicable.

(1): Assumption used in a model that was used to simulate the spread of RVF after introduction into the EU, and to assess the effectiveness of surveillance and control measures. The assessment was made using data from the Netherlands.

4.2.1.2. Discussion and conclusions

As described in Table 8 (outbreak data), the shortest period between the earliest point of infection and the suspicion report was 18 days. It was found in the context of a primary outbreak that occurred in 2008 on a dairy farm of the Bela-Bela district in South Africa (Map aco et al., 2012a). Seven calves died on this farm where no vaccination was practiced. No apparent disease was reported in the other 300 cattle and 200 sheep on the farm. There was no history of newly introduced animal. The date of first infection was estimated based on the results of the laboratory data.

The longest period found in the selected references (Table 9), 20 days, was assumed in a model used to simulate the spread of RVF after introduction into the EU, and to assess the effectiveness of surveillance and control measures. The 'mean time to reporting' was selected considering that the

incubation period for clinical signs is around 4–7 days, and considering the time for the development of serious signs and to have more animals affected, to get the farmer's attention (EFSA AHAW Panel, 2020c). In addition, in the same report, it is also mentioned that during the 2018–2019 RVF in Mayotte, the outbreaks in animals were usually confirmed about 10–15 days after the onset of clinical signs. Moreover, it is noteworthy that the epidemiological investigation conducted in the context of the 2018–2019 epidemic in Mayotte revealed that RVF had probably circulated for 3–4 months in the livestock population before it was detected (EFSA AHAW Panel, 2020a).

Based on the extracted values from Tables 8 and 9, the following set of values for the period between the earliest point of infection and the suspicion report is proposed, i.e. range between 18 and 20 days.

Based on the very scarce data that were available in the literature, we would conclude that the current monitoring period for RVF (30 days) is long enough to capture the period between the earliest point of infection and the suspicion report. Nevertheless, due to the very limited amount of evidence from published literature, this conclusion is affected by a wide margin of uncertainty.

4.2.2. Seroconversion in animals

Results regarding the time to seroconversion after infection and the latest detected day of antibody presence in listed species of RVF after experimental infection with the RVFV are presented in Table 10.

4.2.2.1. Sheep and goats

According to the available scientific literature reviewed, when detection of serum RVFV specific antibodies was performed with the use of IgG-ELISA, the range of days for seroconversion for sheep that were directly challenged sub-cutaneously (SC) or intravenously (IV) was 4–10 days post infection (dpi) (Paweska et al., 2003a,b; Fafetine et al., 2007; Busquets et al., 2010; Bird et al., 2011; Faburay et al., 2016) and the latest day of antibody detection was 77 dpi (Fafetine et al., 2007).

When detection of serum RVFV-specific antibodies was performed with IgM-ELISA, the range of start of seroconversion for sheep that were directly challenged SC or IV was 3–9 dpi (Fafetine et al., 2007; Bird et al., 2011) and the latest day of antibody detection was 70 dpi (Fafetine et al., 2007).

When detection of serum RVFV-specific antibodies was performed with the use of virus neutralisation tests (VNT/PRNT: plaque reduction neutralisation test), the range of start of seroconversion for sheep that were directly challenged SC or intra-muscularly (IM), was 2–14 dpi (Paweska et al., 2003a,b; Fafetine et al., 2007; Busquets et al., 2010, 2014; Dungu et al., 2010; Kortekaas et al., 2012; Antonis et al., 2013; Weingartl et al., 2014; Faburay et al., 2016; Lorenzo et al., 2018), and the latest day of antibody detection was 72 dpi (Paweska et al., 2003a,b). However, two large ring trials that used PRNT estimated day 6 post infection as the earliest day of seroconversion (Kortekaas et al., 2013; Upreti et al., 2018).

One major difference with all previously mentioned studies was that of Antonis et al. (2013), who reported that 4 out of 11 ewes, that were pregnant at first, second or third trimester, and challenged intra-peritoneally, did not developed detectable viraemia, nor clinical signs and did not seroconvert for IgM or IgG (using IgM-ELISA, C-ELISA and VNT). These ewes contained viral RNA in maternal and fetal organs. Unfortunately, seroconversion data for the remaining seven ewes were not demonstrated in the article.

In studies with goats as experimental animals, seroconversion was apparent between 5 and 7 dpi, using hemagglutination-inhibition (HI) or PRNT serological tests.

4.2.2.2. Cattle

In studies with cattle as experimental animals, seroconversion was apparent between 5 and 7 dpi, using N-based-IgG-ELISA, C-ELISA or PRNT serological tests (Wilson et al., 2016; Upreti et al., 2018). However, when Gn-based-IgG-ELISA was used, seroconversion was apparent between 10 and 14 dpi (Wilson et al., 2016).

4.2.2.3. Other listed species

The literature search for RVF in American antelope (*Antilocapra americana*), African buffalo (*Syncerus caffer*), camel (*Camelus dromedarius* and *C. bactrianus)*, red deer (*Cervus elaphus*), giraffe (*Giraffa camelopardalis*), hippopotamus (*Hippopotamus amphibius*) and elephant (*Loxodonta africana*) has not yielded any result for seroconversion. Only one study with RVFV challenge to white-tailed American deer (*Odocoileus virginianus*) was found, yet no serological tests were performed (Wilson et al., 2018).

Table 10: Range of days for seroconversion and latest detected day of antibody presence in ruminants (sheep, goats and cattle) after experimental inoculation with RVFV

Animals in the study	Laboratory method	Infection	Range of days for seroconversion (dpi[(d)])		Latest day of antibody detection/end of experiment	Total number of references	Reference
			Earliest day of seroconversion	Latest day of seroconversion			
Sheep	IgG-ELISA	SC	4	10[(a)]	77[(b)]	6	Paweska et al. (2003a,b), Fafetine et al. (2007), Bird et al. (2011), Busquets et al. (2014), Faburay et al. (2016)
		IV	5	NS	NS	1	Bird et al. (2011)
	IgM-ELISA	SC	3	9	70	1	Fafetine et al. (2007)
		IV	3	NS	NS	1	Bird et al. (2011)
	C-ELISA[(c)]	IP	7 (all animals seroconverted on the same day)		18	1	Kortekaas et al. (2012)
		SC	5	9	16	1	Busquets et al. (2014)
		Ring trial	5	NS	28	2	Kortekaas et al. (2013), Upreti et al. (2018)
	VNT/PRNT	SC, IM	2	14	72	11	Paweska et al. (2003a,b), Fafetine et al. (2007), Dungu et al. (2010), Bird et al. (2011), Kortekaas et al. (2012), Antonis et al. (2013), Busquets et al. (2014), Weingartl et al. (2014), Faburay et al. (2016), Lorenzo et al. (2018)
		Ring trial	6	NS	28	2	Kortekaas et al. (2013), Upreti et al. (2018)
	HI	SC	5	7	72	3	Paweska et al. (2003a), Fafetine et al. (2007)
Goats	VNT/PRNT	SC	5	NS	30	1	Nfon et al. (2012)
Cattle	IgG-ELISA[(d)]	SC	10	14	21	1	Wilson et al. (2016)
	IgG-ELISA[(b)]	SC	5	7	21	1	Wilson et al. (2016)
	C-ELISA[(c)]	Ring trial	5	NS	38	1	Upreti et al. (2018)
	VNT/PRNT	SC	5	6	38	2	Wilson et al. (2016), Upreti et al. (2018)

VNT: virus neutralisation test; PRNT: plaque reduction neutralisation test; NS: not specified; HI: hemagglutination-inhibition; IP: intraperitoneally; IM: intramuscularly; IV: intravenously; SC: subcutaneously.
(a): Gn-specific protein IgG-ELISA.
(b): N-based IgG-ELISA.
(c): No differentiation between IgM-IgG antibodies.
(d): Downloaded on 7/10/2019; https://www.cdc.gov/vhf/rvf/outbreaks/distribution-map.html

4.2.3. Assessment

Considering the results presented above, an assessment of the effectiveness of the current monitoring period for RVF, depending on the purpose of that period in the different scenarios shown in Annex C, was carried out. For RVF, the length of the monitoring period as defined in Annex II of the Delegated Regulation is 30 days.

Scenarios 1, 2, and 3 of monitoring period for RVF

- 1st scenario of monitoring period
- ToR 2 in accordance with article 8 and Annex II of the Delegated Regulation (EU) 2020/687
- Article 57 of the Regulation (EU) 2016/429
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of the notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of a suspicion of an RVF 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 an RVF 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 an RVF outbreak in an epidemiological unit in which the disease has not been confirmed, in order to provide derogations from killing the animals in this unit, if this unit has been completely separated, and handled by different personnel during this monitoring period

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

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

Based on the ELS carried out and presented above (Section 4.2.1), the maximum length of the time between the earliest point of infection and the suspicion report, reconstructed as described above, was estimated as 20 days (minimum 18 day). The monitoring period as defined in Annex II of the Delegated Regulation of 30 days widely covers this range and could therefore be considered effective, even considering the uncertainty around the estimation.

Scenario 4 of monitoring period for RVF

- 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 RVF outbreak in the protection zone. Products or other materials likely to spread the disease, must had been obtained or produced, before this time period in order to be exempted from prohibitions of movements

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

Considering this assumption and given that monitoring period for RVF is 30 days, products or other materials likely to spread RVF (raw meat or milk), obtained or produced before this time period from the date of notification of RVF suspicion in the protection zone could be exempted from prohibitions of movements.

Scenario 5 of monitoring period for RVF

- 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 a non-affected establishment (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.

In the case of an RVF outbreak, based on the existing legislation, the animals would have to be tested not earlier than the time in days of the monitoring period plus 7 days ($30 + 7 = 37$ days) counted after the semen was taken.

RVFV can be excreted also in semen of affected bulls or rams and can be found up to 3 weeks (see Section 2.2). For import of semen from countries/areas not free of RVF, OIE recommends the animals should not show clinical signs of RVF within the period from 14 days prior to and 14 days following collection of the semen or embryos; and either being vaccinated against RVF at least 14 days prior to collection; or to be seropositive on the day of collection; or testing of paired samples has demonstrated that seroconversion did not occur between the time of semen or embryo collection and 14 days after.

A negative serological test, if carried out at the right time, would indicate that the animal has never been exposed to the agent, and therefore, it will indicate that the semen is free of the agent too.

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 14 days post infection.

Consequently, and based on the results of the publications, sampling the animals at least 37 days after semen collection for antibody testing, as it is foreseen in the Delegated Regulation, and with negative results, is considered effective to ensure that semen is safe to be moved without posing a risk of disease spread.

Scenarios 6 and 7 of monitoring for RVF

- 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 RVF virus within a distance equal or lower to the radius of the surveillance zone, the repopulation process could not take place). Repopulation can only take place after a number of days equal to the monitoring period has elapsed since the final cleaning, disinfection and disinfestation of the affected establishment.

In this regard, the number of days of the monitoring period for RVF, counted from the day of the final cleaning and disinfection must ensure enough time for any potentially infected surrounding establishment to be reported as a suspicion. Considering the results presented above (Section 4.1.1), the monitoring period as defined in Annex II of the Delegated Regulation of 30 days is considered effective for this scenario.

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

The aim of the monitoring period in this scenario is to ensure the early detection of any potentially recently infected animal intended for repopulation once they have been moved into the repopulated establishment. Although the preferred option is that all animals are introduced into the establishment 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 during which animals may be introduced into the establishment, the period during which the disease could be unknowingly spreading within the establishment is reduced. Assuming that the latest point of infection of the first animal or batch of animals introduced into the repopulated establishment is the day when the animals are moved, clinically ill animals would be observed at the first visit, if this visit is carried out after a number of days equal to the incubation period. For RVF, the incubation period is typically 1 week (even shorter in fully susceptible young animals, the situation as it would be in the EU). The EFSA AHAW Panel thus considers the existing length of the monitoring period as defined in Annex II of the Delegated Regulation (30 days) effective, as it would allow for early detection of potentially infected animals 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 a disease outbreak

4.3.1. Assessment of the minimum radius

The purpose of this section is to assess the effectiveness to control the spread of RVF by implementing a protection and surveillance zones of a minimum radius, as set out in Annex V of the Delegated Regulation, surrounding the establishment where the disease has been confirmed. Based on this regulation, the minimum radius of the protection and surveillance zone for RVF should be of 20 and 50 km, respectively (see Annex E).

Transmission kernels have been used to previously to assess potential spread and control of RVF in Europe (EFSA AHAW Panel, 2020c). A fat-tailed kernel was estimated from outbreak data from Tanzania in 2007, while two exponential kernels were constructed based on the mean dispersal distance of mosquito vectors, with a value assumed to be up to 7 km (EFSA AHAW Panel, 2020b) (Table 11). No other better fitting outbreak data are available for this purpose, this approach and related assumptions were already discussed in a previous opinion on RVF (EFSA AHAW Panel, 2020c) (Figure 5).

Table 11: Transmission kernels for Rift Valley fever virus

Kernel	Source	d_0 (km)
Fat-tailed: $k(r) = \left(1 + \left(\frac{r}{d_0}\right)^2\right)^{-1}$	Outbreak data, Tanzania 2007	1.66 (0.88, 2.77)*
Exponential: $k(r) = \exp\left(-\frac{r}{d_0}\right)$	Mean mosquito dispersal distance 7 km	7.0
	Mean mosquito dispersal distance 13.7 km	13.7

*: 95% credible interval is shown in brackets.

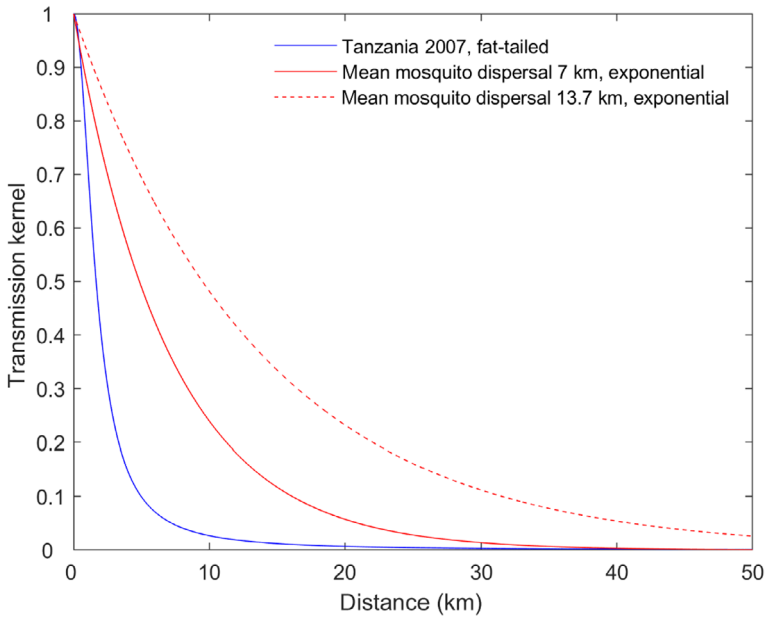
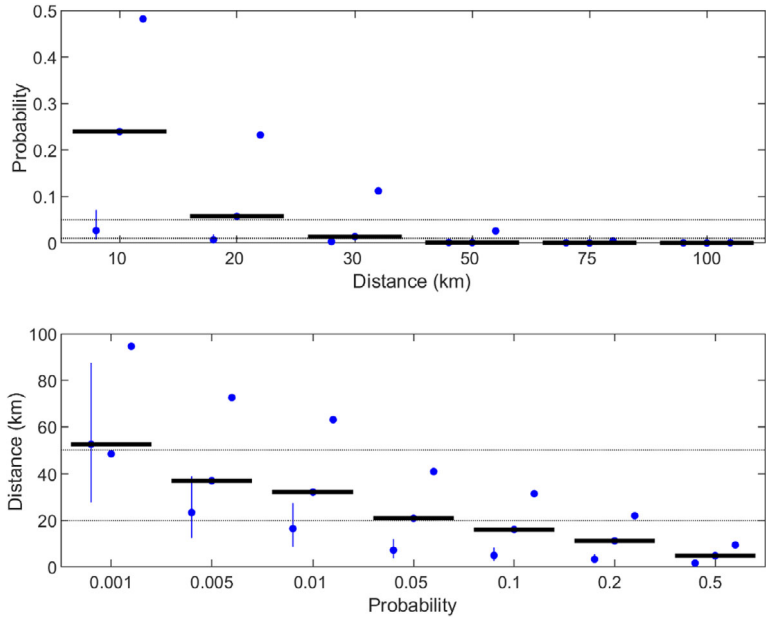


Figure 5: Transmission kernels for Rift Valley fever virus

For each kernel in Table 11, the probability of transmission beyond given distances (if transmission were to occur from an infected establishment) was computed using the estimates, lower 95% confidence limits and upper 95% confidence limits, including beyond the proposed radius for the protection and surveillance zones (20 km and 50 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 95% confidence limits and upper 95% confidence limits (Figure 6). The corresponding values computed using the estimates are summarised in Tables 12 and 13.



The top panel shows the probability of transmission beyond a given distance (if transmission were to occur from an infected establishment) computed using the estimates (blue circles) and the lower and upper 95% confidence limits (error bars) for each kernel (and in the same order as) in Table 13. 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 20 and 50 km (i.e. the proposed radius of the protection and surveillance zones, respectively).

Figure 6: Assessment of the radius of the protection and surveillance zone for Rift Valley fever virus

Table 12: Probability of transmission of Rift Valley fever virus beyond different distances

	Distance (km)					
	10	**20**	**30**	**50**	**75**	**100**
Median	0.239	0.057	0.014	0.001	0.001	< 0.001
Minimum	0.027	0.007	0.003	0.001	< 0.001	< 0.001
Maximum	0.482	0.232	0.112	0.026	0.004	0.001

Table 13: Distances (km) at which the probability of transmission of Rift Valley fever virus beyond that distance reaches a threshold level

	Threshold probability of transmission						
	0.001	**0.005**	**0.01**	**0.05**	**0.1**	**0.2**	**0.5**
Median	52.5	37.1	32.2	21.0	16.1	11.3	4.9
Minimum	48.4	23.4	16.5	7.2	5.0	3.3	1.7
Maximum	94.6	72.6	63.1	41.0	31.5	22.0	9.5

From Table 13, the estimated probability of transmission beyond a protection zone with 20 km radius, if transmission occurred, is 5.7% (CI: 0.7–23.2%), and 0.1% (0.1–2.6%) for a zone with 50 km radius, which is considered sufficient to contain disease transmission with at least 95% probability.

The results obtained here about the spread distance of RVFV differ from those obtained in previous EFSA opinion on RVF (EFSA, 2020), since the approach used is different. The methods used previously cannot be applied using the kernel estimated using outbreak data from Tanzania and, for the mosquito dispersal-based kernels, they require additional assumptions to be made about the density of farms (assumed to be constant) and the between-herd basic reproduction number, both of which will be context specific.

4.3.2. Assessment of the minimum period

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

The length of the minimum period of the protection zone and surveillance zone are 30 and additional 15 days, respectively (see Annex E). In the protection zone, all farms are visited for a clinical inspection. This aims to quickly identify infected farms where infection has started before control measures were implemented. The movement control applies for 30 days, ensuring that possibly infected animals in both protection and surveillance zones are not moved to uninfected farms.

To assess the minimum length of time the protection 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 AHAW Panel, 2020).

Based on the results of the ELS as presented in Section 4.2.1, it follows that the maximum time between earliest point of infection and notification of the suspicion for RVF is 20 days. This is shorter than the minimum period of 30 days indicated in the Delegated Regulation for the restriction measures in the protection zone; therefore, the latter is considered effective to detect infected establishments and to prevent the movement of infected animals from the protection zone.

In addition, the maximum period between the earliest point of infection and the suspicion report has been reconstructed as 25 days, by adding the maximum incubation period (7 days) to the maximum period between first suspicion and suspicion report (18 days). Consequently, the minimum period of 45 (30 + 15) days indicated in the Delegated Regulation for the restriction measures in the surveillance zone is considered largely sufficient to detect infected establishments and to prevent the movement of infected animals from the surveillance zone.

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 could not be quantified. In particular, the uncertainty of the assessment is linked to the main following aspects:

- the model choice for estimation of radius as possible source of uncertainty should be discussed
- vectors: the specific vector transmission situation should be considered, this may change largely the epidemiological pattern;
- genetic characteristics of the host population in EU compared to autochthonous breeds in endemic areas (e.g. Africa)

5. Conclusions and recommendations

Sampling procedure	Conclusions	Recommendations
1st scenario 4.1.1.1 Sampling procedures in the event of a suspicion of RVF in an establishment where animals of the listed species are kept	In the event of the first introduction of RVF into a previously free territory like the EU, it is highly probable that the first suspicion is based on the observation of the most evident clinical signs, which are abortion and death in young animals. Nevertheless, the first signs of RVF disease may be observed in humans rather than animals. Considering the high sensitivity of the RT-PCR in specific organs, samples from few (e.g. 5) suspected clinically sick animals or aborted fetuses would be enough to confirm the presence of RVFV in the herd	When abortions are observed, the whole aborted fetuses should be collected as well as dead lambs or calves in case of mortality in newborn animals. When the whole carcass cannot be dispatched to the laboratory, liver, spleen and lymph nodes can be taken from dead animals and organs, brain from aborted fetuses. When clinical signs are observable in adult animals, blood samples with EDTA for PCR should be taken during the febrile phase of the disease. When the disease has to be ruled out even in absence of clinical signs, blood samples (with and without EDTA) should be taken from the suspected animals, which should be kept indoors, in stables with vector protection to reduce exposure to mosquitoes. In case of the absence of clinical signs and if the suspicion is based on other factors (e.g. animals originating from infected areas), all animals should be sampled and tested.
2nd scenario 4.1.1.2. Sampling procedures in the event of suspicion or confirmation of RVF for the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU) 2016/429 in an RVF officially confirmed establishment	In order to support epidemiological investigation and to detect all infected animals in an establishment officially confirmed infected with RVF, clinical examination alone is insufficient since the infection can be fully inapparent in adult animals, and due to the short course of viraemia, RT-PCR assay alone cannot be used as reference test for this purpose. In this case, serological tests may be used for the identification of already infected animals. ELISA kits are available for IgG and IgM antibodies detection.	Ideally, all the animals in the involved herd should be sampled in order to have a complete picture of the infection prevalence in the herd. However, when it is unfeasible to test all the animals or herds, a random sampling approach could be used. Since vector-borne diseases do not tend to cluster within the herds, the whole population of animals in the herds belonging to RVF susceptible species can be considered as the target population of a representative sampling schema. A. sample size for prevalence estimation, at 95% confidence level and with unknown expected prevalence (thus set to 50%), varies according to the precision required for the estimation (e.g. 2,400 samples are required for a $\pm$ 2% precision, whereas around 9,600 are needed for $\pm$ 1%), which would mean testing all animals in smaller herds The laboratory examinations on animals should be complemented by entomological investigations in order to identify the possible vectors of infection, to assess their abundance and to provide more information about the possibility of further disease spread.
3rd scenario Sampling procedures for granting a specific derogation from killing animals of the categories of article 13.2 of the Delegated Regulation in an RVF affected establishment	The viraemic and immune status of the animals may be assessed through the collection of blood samples with EDTA for RT-PCR testing and whole blood samples for serology, which should include not only IgM and IgG ELISA testing, but also VN with the quantification of antibody titre.	In the EU context, historically free from RVF and due to its zoonotic characteristics of RVF, derogations from killing animals infected by RVFV should be very carefully assessed. Therefore, each single animal for which the derogation is claimed should be sampled and tested.

Sampling procedure	Conclusions	Recommendations
4th scenario Sampling procedures for the animals of non-listed species kept in an RVF affected establishment.	Apart from domesticated or wild ruminants, in Africa where RVF is endemic. RVFV has been found in few species, e.g. some species of rodents and bats and even non-human primates, whereas serological positive findings have been detected in several animal species. However, the significance of these findings and the possible role of these species in virus transmission are fully unknown	The application of any clinical or laboratory-based sampling procedure for non-listed species is not recommended, apart for purely research purposes
5th scenario Sampling procedures for wild animals of the listed species within the RVF affected establishment and its surroundings.	Where wild ruminants (buffaloes, antelopes, camels) are kept or living in the surrounding area of the affected establishment, these may acquire the infection by direct or indirect contact with affected animals, if no or low biosecurity measures are in place to keep animal species separated and protected from the mosquito biting	The surveillance of wildlife around the affected establishment may include the visual inspection of these animals from a distance and the testing of fallen stock and hunted animals by both RT-PCR and serology (although the latter are not validated in wild species). Unexpected mortality events in susceptible wildlife should be investigated. Samples from animals with clinical signs from dead or hunted animals should be collected for laboratory analysis, following the procedures of the 1st scenario.
6th scenario Sampling procedures for animals of listed species in the non-affected establishments located in a protection zone	For RVF the size of the protection zone foreseen in the legislation is 20 km, therefore the 7th scenario applies	
7th scenario Sampling procedures for non-affected establishments located in a protection zone with a radius larger than 3 km	During the vector season and depending on the climatic conditions (temperatures, wind dispersal trajectories), an enhanced surveillance based on laboratory examinations could be appropriate. After an assessment of the geographical distribution of herds, the active surveillance approach could be limited to the most internal part of the zone, up to 3 km from the infected zone. For the rest of the protection zone, it could be more effective to apply a clinical investigation programme of herds with clinical examination of the animal, coupled with an awareness campaign targeting farmers in order to enhance the capacity of detecting any possible sign of the disease.	A strict surveillance should be applied in the protection zone with a radius up to 20 km. Periodical herd visits with clinical examination of animals within the herds should be applied. In case of any clinical finding, the procedures of the 1st scenario must be followed
8th scenario Sampling procedures for non-affected establishments located in a surveillance zone	It is unlikely that establishments in this zone, not epidemiologically linked to an outbreak, will become infected with RVFV without having additional outbreaks in the protection zone.	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.

Sampling procedure	Conclusions	Recommendations
9th scenario Sampling procedures to grant derogations for animal movements 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	Due to the zoonotic significance and the fact that RVF is a vector-borne disease, it is not recommended to move animals from the protection zone. However, since the destination of these animals is the slaughterhouse, if derogation is granted, all biosecurity measures should be implemented and given that the animals should be slaughtered within 24 h this would reduce further the risk	All the animals in the establishment of origin should be examined before their movement, following the procedures described in scenario 1. In an establishment where the number of animals is large, the 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 examined to rule out the presence of RVFV with at least 95% confidence, as described in scenario 1.
12th scenario Sampling procedures to grant derogations for animal movements 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	This scenario is very similar to the ninth scenario; thus, the same indications apply.	
13th scenario Sampling procedures to grant derogations for animal movements 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	This scenario includes two different subscenarios: (a) the need to transfer animals of listed species kept in establishments located in the surveillance zone to a slaughterhouse located within or 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. The first subscenario represents a risk for the spread of RVFV from infected animals, especially if the slaughterhouse is located outside the surveillance zone, while the second subscenario poses a negligible risk to spread RVFV.	The movement of animals of listed species from establishments located in surveillance zone to a slaughterhouse located within or outside the restricted zone should be allow after the procedure proposed in 9th scenario is followed. When there is the need to move animals of listed species located outside the surveillance zone to slaughterhouse located within the surveillance zone, no restrictions are needed.
14th scenario Sampling procedures to grant derogations for animal movements from an establishment in a surveillance zone to pastures situated within the surveillance zone	Animals in a surveillance zone, for which a specific derogation has been granted to be moved to pastures, remain alive therefore they should be subjected to clinical surveillance, including laboratory examinations according to what described in 1st scenario. 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 do pose very low risk of transmission of RVF.	This scenario is very similar to the ninth scenario; therefore, the same procedures are recommended.

Sampling procedure	Conclusions	Recommendations
15th scenario Sampling procedures to grant derogations for animal movements from an establishment in a surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone	Same conclusions as in 14th scenario apply	This scenario is very similar to the ninth scenario; therefore, the same procedures are recommended
18th scenario Sampling procedures to grant derogations for animal movements 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	Same conclusions as in 14th scenario apply	Animal movement should be allowed only from an establishment located in surveillance zone within the restricted zone or from an establishment located in the protection zone within the protection zone. When animal movement is allowed, the procedure suggested as in 2nd scenario should be followed.
19th scenario Sampling procedures for repopulation purposes for the animals that are kept for the repopulation prior to their introduction	If the animals intended for repopulation originate from an establishment located outside the surveillance zone (which is disease free area), there are no requirements for pre-movement testing of the animals.	If the animals intended for repopulation originate from an establishment located in the surveillance zone, the procedures in place for the movement of animals from such holdings should apply (see scenario 1). In areas where and when biological vectors are present, a vector monitoring and control strategy should be in place before introducing new animals. Aspects about the life cycle of vectors under local circumstances should be considered before moving animals (e.g. presence of breeding sites, season, etc.).
20th scenario Sampling procedures for repopulation purposes in the event of unusual mortalities or clinical signs being notified during the repopulation.		The animals to be used for repopulation should be tested for the presence of RVFV and antibodies; therefore, the procedure can be considered effective for early detection in the repopulated animals
21st scenario Sampling procedures for repopulation purposes for animals that have been repopulated	As a vector-borne disease, if the reintroduction takes place during the vector season, testing of the animal should be carried out (as in scenario 1) after the monitoring period has elapsed, bearing in mind that all suspect cases will be investigated irrespective if there is vector activity or not.	Negative tests should be obtained at the end of a monitoring period. The monitoring period on vector activity monitoring should include the vector active season.

Sampling procedure	Conclusions	Recommendations
	If reintroduction occurs during the confirmed vector-free period, continuous monitoring of vector activity should be undertaken. If the vector activity starts before the end of the monitoring period, the animals should be tested at the end of new monitoring period.	

ToR 2

Description	Conclusions	Recommendations
Assessment of the length of the monitoring period of RVF	The current monitoring period for RVF of 30 days proposed in the Delegated Regulation is considered adequate, since it is long enough to capture the assessed period between the earliest point of infection and the suspicion report. Nevertheless, due to the very limited amount of evidence from published literature, this conclusion is affected by wide margin of uncertainty.	

ToR 3

Description	Conclusions	Recommendations
Assessment of the minimum radius for protection and surveillance zone	The estimated probability of RVF transmission beyond a protection zone with 20 km radius, if transmission occurred, is 5.7% (CI: 0.7–23.2%), and 0.1% (0.1–2.6%) for a zone with 50 km radius.	The size of the protection and surveillance zones for RVF of 20 and 50 km, respectively, is recommended as sufficient to contain disease transmission with at least 95% probability.
Assessment of the minimum period for the restriction measures	The minimum period of 30 days indicated in the Delegated Regulation for the restriction measures in the protection zone is assessed as effective to detect infected establishments and to prevent the movement of infected animals from the protection zone. In addition, the minimum period of 45 days (30 plus additional 15 days) indicated in the Delegated Regulation for the restriction measures in the surveillance zone is assessed as largely sufficient to detect infected establishments and to prevent the movement of infected animals from the surveillance zone.	

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Abbreviations

CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
C-ELISA	Complement-Enzyme Linked Immunosorbent Assay
DIVA	Differentiating Infected From Vaccinated Animals
dpi	days post inoculation
ELISA	enzyme-linked immunosorbent assay
ELS	extensive literature search
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
HPAI	Highly Pathogenic Avian Influenza
RT-LAMP	Loop mediated isothermal amplification technique
VNT	virus neutralisation test
LSD	Lumpy skin disease virus
NCD	Newcastle disease virus
OIE	World Organization for Animal Health
PCR	polymerase chain reaction
PRNT	Plaque Reduction Neutralisation Tests

PZ	protection zone
RP	rinderpest virus
RT-PCR	reverse transcription polymerase chain reaction
RVF	Rift valley Fever
RVFV	Rift Valley fever virus
SPGP	Sheep pox and goat pox
SZ	surveillance zone
ToR	Terms of Reference

Annex A – Definitions in EU legislation

Terms	Definitions
Clinical examination	The clinical examination comprises: (i) an initial general evaluation of the animal health status of the establishment which comprises all the animals of listed species kept in the establishment; and (ii) an individual examination of the animals included in the sample referred to in point (a). The sampling of animals for clinical examination is carried out in accordance with point A.1 of Annex I for terrestrial animals (Delegated Regulation article 3)
Confined establishment	Means any permanent, geographically limited establishment, created on a voluntary basis and approved for the purpose of movements, where the animals are: (a) kept or bred for the purposes of exhibitions, education, the conservation of species or research; (b) confined and separated from the surrounding environment; and (c) subject to animal health surveillance and biosecurity measures; (AHL: Regulation 2016/429 article 4(48))
Epidemiological unit	Means a group of animals with the same likelihood of exposure to a disease agent; (AHL: Regulation 2016/429 article 4(39))
Establishment	Means any premises, structure, or, in the case of open-air farming, any environment or place, where animals or germinal products are kept, on a temporary or permanent basis, except for: (a) households where pet animals are kept; (b) veterinary practices or clinics; (AHL: Regulation 2016/429 article 4(27))
Health status	Means the disease status as regards the listed diseases relevant for a particular listed species with respect to: (a) an animal; (b) animals within: (i) an epidemiological unit; (ii) an establishment; (iii) a zone; (iv) a compartment; (v) a Member State; (vi) a third country or territory; (AHL: Regulation 2016/429 article 4(34))
Infected zone	Means a zone in which restrictions on the movements of kept and wild animals or products and other disease control and biosecurity measures may be applied with the view to preventing the spread of a category A disease in the event of official confirmation of the disease in wild animals. (Delegated Regulation article 2(15))
Kept animals	Means animals which are kept by humans, including, in the case of aquatic animals, aquaculture animals; (AHL: Regulation 2016/429 article 4(5))
Outbreak	Means the officially confirmed occurrence of a listed disease or an emerging disease in one or more animals in an establishment or other place where animals are kept or located; (AHL: Regulation 2016/429 article 4 (40)
Protection zone	Means a zone around and including the location of an outbreak, where disease control measures are applied in order to prevent the spread of the disease from that zone (AHL: Regulation 2016/429 article 4(42))
Listed diseases	Means diseases listed in accordance with Article 5(1); (AHL: Regulation 2016/429 article 4 (18)) List of the diseases (AHL: Regulation 2016/429, Annex II)
Listed species	Means an animal species or group of animal species listed in accordance with Article 8(2), or, in the case of emerging diseases, an animal species or group of animal species which meets the criteria for listed species laid down in Article 8(2); (AHL: Regulation 2016/429 article 4(20)) List of species and groups of species (Commission Implemented Regulation 2018/1882)
Monitoring periods	It is appropriate to follow a single approach for the measures to apply in the event of a category A disease. However, the epidemiology of diseases should be taken into account to establish the appropriate moment for the competent authority to apply control measures and to carry out investigations if there is suspicion or confirmation of those diseases. Therefore 'monitoring periods' should be provided, as reference time frames for each category A disease affecting terrestrial animals based on incubation periods and other relevant elements that may affect the spread of the disease. (Delegated Regulation whereas 10).

Terms	Definitions
Restricted zone	Means a zone in which restrictions on the movements of certain animals or products and other disease control measures are applied, with a view to preventing the spread of a particular disease into areas where no restrictions are applied; a restricted zone may, when relevant, include protection and surveillance zones (AHL: Regulation 2016/429 article 4(41))
Surveillance zone	Means a zone which is established around the protection zone, and where disease control measures are applied in order to prevent the spread of the disease from the protection zone (AHL: Regulation 2016/429 article 4(43))
Wild animals	Means animals which are not kept animals (AHL: Regulation 2016/429 article 4(8))
Zone	Means: (a) for terrestrial animals, an area of a Member State, third country or territory with a precise geographical delimitation, containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases subject to appropriate surveillance, disease control and biosecurity measures (AHL: Regulation 2016/429 article 4 (35))

Annex B – Scenarios of ToR 1

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

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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
				• sampling procedures to ensure detection of the virus in these species
ToR 1.1 ToR 1.2	Article 14(1) of the Delegated Regulation Art. 57 Reg.2016/429	5th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species	• affected establishment officially confirmed • wild animals of listed species within the establishment and in the surroundings of the establishment • the competent authority may establish these sampling procedures in addition to other measures • sampling procedures in wild animals of listed species to ensure the detection of the virus, if the virus is present in these wild species
ToR 1.1 ToR 1.2	Article 26(2) of the Delegated Regulation	6th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals.	• protection zone with radius up to 3km • non-affected establishments with kept animals of listed species • all the non-affected establishments within the protection zone • official veterinarians must visit at least once all the establishments • among others, they must perform a clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination • sampling procedures to confirm or rule out the presence of a category A disease
ToR 1.3	Article 26(5) of the Delegated Regulation point A.3 of Annex I	7th scenario	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of establishments located in a protection zone when the radius is larger than 3 km. The purpose of the sampling procedure is to ensure disease detection of the virus if the virus is present in establishments within the protection zone	• protection zone with radius larger than 3 km • non-affected establishments of kept animals of listed species • sample of the non-affected establishments in the protection zone • in a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
				• In case of a radius larger than 3 km, official veterinarians may not visit all establishments, but a sample of those. EFSA is requested to assess how many of these establishments should be inspected, in order to ensure the detection of the virus, if the virus is present in animals in these establishments • among others perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination • sampling procedure to ensure the detection of the disease if the disease is present in any of these establishments
ToR 1.3	Article 41 of the Delegated Regulation	8th scenario	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the virus is present in establishments within the surveillance zone	• surveillance zone • establishments of kept animals of listed species • sample of the establishments in the surveillance zone • official veterinarians carry out visits to a sample of the establishments • among others perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination • sampling procedure to ensure the detection of the disease if the disease is present in any of the establishments
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	• protection zone • kept animals of listed species • grant derogation for movement from a non-affected establishment in the protection zone • to be moved to a slaughterhouse located within the protection zone or in the

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			within the protection zone or in the surveillance zone or outside the restricted zone (Art29)	surveillance zone or outside the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 30(1) of the Delegated Regulation	10th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of day-old-chicks located in the protection zone and hatched from eggs originating in the restricted zone or outside the restricted zone. The sampling procedures should ensure that the movement of these day-old-chicks to an establishment located in the same Member State but if possible, outside the restricted zone	• protection zone • grant derogation for movement from a non-affected establishment in the protection zone • day-old-chicks from non-affected establishment located in the protection zone, hatched from eggs originating in or outside the restricted zone • to be moved to an establishment located in the same Member State but if possible, outside the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 30(2) of the Delegated Regulation	11th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone to establishments located in the same MS and if possible within the restricted zone.	• protection zone • ready-to-lay poultry • grant derogation for movement from a non-affected establishment in the protection zone • to be moved to an establishment located in the same Member State and if possible, within the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 37 of the Delegated Regulation	12th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from	• protection zone • kept animals of listed species

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

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 • kept animals of listed species • grant derogation for movement from the surveillance zone • to be moved to an establishment belonging to the same supply chain, located in or outside the surveillance zone, to complete the production cycle before slaughter • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 46(1) of the Delegated Regulation	16th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations to grant derogation of movements of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating within the surveillance zone and eggs originating outside the restricted zone, to an establishment located in the same Member State where they were hatched	• surveillance zone • kept birds of listed species • grant derogation for movement of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating from establishment within the surveillance zone or eggs originating from outside the restricted zone • to be moved to an establishment located in the same Member State • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 46(2) of the Delegated Regulation	17th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same MS.	• surveillance zone • ready-to-lay poultry • to be moved to an establishment located in the same Member State • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.4	Article 56(1)c of the Delegated Regulation	18th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI	• restricted zone when restriction measures are maintained beyond the period set out in Annex XI • kept animals of listed species • grant derogation for movement from an establishment within the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
Repopulation				
ToR 1.5	Article 59(2),(3) of the Delegated Regulation	19th scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease.	• repopulation of a previous affected establishment • kept animals of listed species • Animals intended to repopulation shall be sampled prior to their introduction into the establishment of destination • samples shall be collected from a representative number of animals to be introduced of each consignment from each establishment or from a representative number of animals of each consignment (if animals are all to be introduced at different times or from different establishments of origin) • laboratory examinations • sampling procedures to rule out the presence of the disease
ToR 1.5	Article 59(9) of the Delegated Regulation	20th scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease.	• repopulated establishment • unusual mortalities or clinical signs during the repopulation • the official veterinarians shall without delay collect samples for laboratory examination • sampling procedures to rule out the presence of the disease

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.5	Article 59(5) of the Delegated Regulation	21st scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment.	• repopulated establishment • kept animals of listed species • Animals that have been used for repopulation • Laboratory examinations • Sampling procedures to rule out the presence of the disease

Annex C – Existing sampling procedures for RVF

Sampling scenarios for RVF – 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 (TOR1.1) and laboratory examination (TOR1.2), in their ability to detect a category A disease in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2))**.	***Article 4:*** 1) When animals on a holding are suspected of being infected or contaminated with RVF, Member States shall ensure that the official veterinarian immediately activates official investigation arrangements to confirm or rule out the presence of the disease in question. A 2. As soon as the suspected presence of the disease is notified, the competent authority shall have the holding placed under official surveillance and shall in particular require that: a) a census be made of all categories of animals of susceptible species and that, in respect of each of these categories, the number of animals already dead, infected or liable to be infected or contaminated be recorded; the census must be kept up to date to take account of animals born or dying during the period of suspicion; the information in the census must be kept up to date and produced on request and may be checked at each visit. ***The Foreign Animal Disease Preparedness and Response Plan (USDA-APHIS-VS, 2016):*** Animals on Suspect, and Monitored Premises may be monitored daily for clinical signs compatible with RVF; serum and tissue samples may undergo diagnostic testing for the presence of RVFV. ***Note: Scientific opinion (EFSA, 2005):*** Infection with RVF virus may present in domestic animals with moderate or serious clinical disease. RVF most commonly presents with abortions in 15–30% of the pregnant animals (sheep, goats or cattle). However, considerable RVF virus activity may occur in ruminants, cattle sheep or goats, without any obvious clinical signs. Brief periods of viraemia with or without a febrile response would not be detected.	***Article 4:*** 1) When animals on a holding are suspected of being infected or contaminated with RVF, Member States shall ensure that the official veterinarian immediately activates official investigation arrangements to confirm or rule out the presence of the disease in question and, in particular, must take or have taken the samples necessary for laboratory examination. To that end the animals in question may be transported to the laboratories under the supervision of the competent authority, which shall take appropriate steps to prevent the disease from spreading. ***Scientific opinion (EFSA AHAW Panel, 2020b):*** The most appropriate matrix to isolate or detect RVFV is either whole blood or serum samples collected during the acute (febrile) stage of the disease or different organs collected post-mortem from fresh carcasses or aborted fetuses such as brain, liver and spleen. ***OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2018):*** Laboratory confirmation of clinical cases should require a combination of at least two positive results from two different diagnostic test methods: either positive for virus or viral RNA and antibodies or positive for IgM and IgG with demonstration of rising titres between paired sera samples collected 2–4 weeks apart. Depending of the stage of the disease, virus or antibodies will be detected. RVFV may be isolated from serum but preferentially from plasma or blood collected with anticoagulant during the febrile stage of the disease in live animals, or from liver, spleen and brain of animals that have died, or from aborted fetuses. Primary isolation is usually

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		Note: Scientific opinion (EFSA AHAW Panel, 2020b): The introduction of RVFV into a fully susceptible ruminant population, such as in the Europe, would result in a significant number of clinical cases, with high mortality in young animals and abortion storms. For this reason, passive surveillance can be considered the most effective for the early detection of the infection under these circumstances. ***Note: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2018):*** The susceptibility of different species and breeds to RVF may vary considerably. Some animals may have inapparent infections, while others have severe clinical disease with mortality and abortion. Susceptible, older non-pregnant animals often do not show signs of disease. Signs of the disease tend to be nonspecific, rendering it difficult to recognise individual cases during epidemics.	performed in cell cultures of various types or by intracerebral inoculation of sucking mice. A rapid diagnosis can also be made by detection of viral RNA using validated conventional or real-time RT-PCR. These techniques should be followed by sequencing of selected samples. ***FAO Animal Health Manual: Recognising rift valley fever (FAO, 2003):*** At an outbreak site, where sheep, cattle or camels are aborting and there are deaths in neonates, it is suggested that the following samples be collected: • at least 10–20 serum samples from animals that have recently aborted • 10–20 samples from animals that have not aborted • blood in anticoagulant from any animals with a fever of 40.5–42°C • liver and spleen from any freshly dead animals, on ice, in glycerol buffered saline and/or in buffered formalin • liver, spleen and brain from fresh fetuses ***Factsheet Rift Valley Fever (CFSPH, Iowa State University, 2015):*** Rift Valley fever can be diagnosed by detecting the virus in the blood of febrile animals, or the tissues of dead animals and aborted fetuses. Some recommended tissues for sampling include the liver (the major site of replication), spleen and brain, with some sources also recommending kidney and lymph nodes; however, the virus may also be found at other sites. ***Disease Strategy (Animal Health Australia, 2016):*** Whole blood, liver, lymph nodes and spleen are the tissues of choice for isolation of the virus. Blood samples (about 20 mL each) should be collected from febrile animals into EDTA or heparin. Duplicate samples

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			of liver and spleen should be collected aseptically, and placed in sterile containers for virus isolation, and in neutral buffered formalin for histopathology. Such samples should be taken from both freshly dead animals at post-mortem examination and aborted fetuses (if available). ***Emergency animal diseases: A field guide for Australian veterinarians (Breed et al., 2019):*** Characteristic histopathology findings (such as acute hepatocellular necrosis with eosinophilic intranuclear inclusion bodies) may increase suspicion of RVF. However, definitive diagnosis requires identification of the virus through one or a combination of the following tests: PCR, virus isolation, immunofluorescence, ELISA or virus neutralisation.
2nd	**To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, virus identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry is described in Article 57 of Regulation (EU)2016/429**.	NA	No specific guidelines described in legislation ***Article 8:*** 1) The epizootiological enquiry shall deal with: a) the length of time during which the disease may have existed on the holding before being notified or suspected; b) the possible origin of the disease on the holding and the identification of other holdings on which there are animals of susceptible species which may have become infected or contaminated; c) the movement of persons, animals, carcasses, vehicles, equipment or any other substances likely to have carried the agent of the disease to or from the holdings in question; 2) A crisis unit shall be established in order to provide full coordination of all measures necessary to ensure eradication of the disease as quickly as possible and for the purpose of carrying out the epizootiological enquiry.

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
3rd	**To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR1.1) and laboratory (ToR1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment, in order to grant a specific derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease**.	NA ***Article 5:*** 1) Once it has been officially confirmed that RVF is present on a holding, Member States shall ensure that, in addition to the measures laid down in Article 4 (2), the competent authority requires application of the following measures: a) all animals of susceptible species on the holding shall be killed on the spot, without delay. The animals which have died or been killed shall either be burnt or buried on the spot, if possible, or destroyed in a carcase disposal plant.	NA
4th	**To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the virus if the virus is present in these species**.	No specific guidelines described in legislation	No specific guidelines described in legislation
5th	**To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species**.	No specific guidelines described in legislation ***Article 6:*** Where animals living in the wild are infected or suspected of being infected, Member States shall ensure that appropriate action is taken. ***Disease Strategy (Animal Health Australia, 2016):*** Surveillance will need to be undertaken on animals, including wild animals, on and around the IPs and dangerous contact premises, to determine the sizes of the transmission/restricted/control areas. Surveillance in these areas will also be required if zoning is introduced. Epidemiological investigations to determine the distribution and abundance of wild animals (especially goats, camels and buffalo) will need to be undertaken	No specific guidelines described in legislation ***Disease Strategy (Animal Health Australia, 2016):*** If wild animals pose a threat, the presence and extent of antibody or virus in the various populations will be determined. This may involve intensive trapping, baiting and shooting operations. If serological or virological evidence of RVF is found in wild animals, more extensive and systematic epidemiological studies will be undertaken to monitor the extent and spread of the disease in the wild animal populations. If a large wild animal population is found to be infected, the disease would be considered endemic, and wild animal controls would not be implemented.

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		early in the outbreak to assess which (if any) wild animals are likely to be in contact with domestic stock and/or insect vectors, and the likely role of these animals in the outbreak. Initially, these investigations will focus on the IP, followed by sites throughout the transmission area (TA) (> 50 km radius area around IPs). ***The Foreign Animal Disease Preparedness and Response Plan (USDA-APHIS-VS, 2013):*** Wildlife management involves identifying susceptible wildlife species, determining how many species may be infected and preventing the spread by implementing appropriate control measures. ***Note: Scientific opinion (EFSA, 2020a):*** No data are available about the susceptibility of European wild ruminant species to RVFV, or the capacity of the virus of causing a detectable viraemia in these animals. The sole indication outside the African continent is related to white-tailed deer (Odocoileus virginianus) in North America (Wilson et al., 2018). The density of wild ruminants species in Europe is much lower than domestic ruminants; however, the possible involvement of some European wild ruminant species on RVFV transmission cannot be excluded, especially for those species and geographical areas with highest density, such as Central Europe	
6th	**To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR1.1) and laboratory (ToR1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals**.	***Article 11:*** 1) Member States shall ensure that the following measures are applied in the protection zone: a) all holdings within the zone having animals of susceptible species shall be identified; b) there shall be periodic visits to holdings having animals of susceptible species, a clinical examination of those animals; a record of visits and findings must be kept, with the frequency of visits being proportional to the seriousness of the epizootic on those holdings at greatest risk.	No specific guidelines described in legislation ***Article 11:*** 1) Member States shall ensure that the following measures are applied in the protection zone: a) all holdings within the zone having animals of susceptible species shall be identified; b) there shall be periodic visits to holdings having animals of susceptible species, a clinical examination of those animals including, if necessary, the collection of samples for laboratory examination; a record of visits and findings must be kept, with the frequency of visits

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		Disease Strategy (Animal Health Australia, 2016): Livestock in the transmission area (TA) (> 50 km radius area around IPs) and restricted areas (RA) (> 100 km from TA boundary) will be observed daily, where feasible, for clinical signs of disease.	being proportional to the seriousness of the epizootic on those holdings at greatest risk. ***Disease Strategy (Animal Health Australia, 2016):*** Blood will be taken at weekly intervals from a statistically valid sample of unvaccinated animals and tested for antibodies to RVF virus. This testing will commence following the index case and continue for 30 days following the last confirmed case. Serological monitoring will then be continued at monthly intervals for the next 12 months, and then quarterly for a further 4 years.
7th	**To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) examinations of the animals of listed species, for the sampling of establishments located in a protection zone when the radius is larger than 3 km. The purpose of the sampling procedure is to ensure disease detection of the virus if the virus is present in establishments within the protection zone.**	***Article 10:*** 1) Once the diagnosis of one of the diseases in question has been officially confirmed, Member States shall ensure that the competent authority establishes around the infected holding a protection zone with a minimum radius of three kilometres, itself contained in a surveillance zone with a minimum radius of 10 kilometres. The establishment of the zones must take account of geographical, administrative, ecological and epizootiological factors relating to the disease in question, and of monitoring facilities. → See 6th scenario	→ See 6th scenario
8th	**To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the virus is present in establishments within the surveillance zone.**	No specific guidelines described in legislation ***Article 12:*** 1) Member States shall ensure that the following measures are applied in the surveillance zone: a) all holdings having animals of susceptible species shall be identified; b) the movement of animals of susceptible species on public roads shall be prohibited except for the purpose of leading them to pasture or animal buildings; the competent authority may, however, grant a derogation from that prohibition for the transit of animals by road or rail without unloading or stopping;	No specific guidelines described in legislation ***Disease Strategy (Animal Health Australia, 2016):*** Livestock in the transmission area (TA) (> 50 km radius area around IPs) and restricted areas (RA) (> 100 km from TA boundary) will be observed daily, where feasible, for clinical signs of disease. Blood will be taken at weekly intervals from a statistically valid sample of unvaccinated animals and tested for antibodies to RVF virus. This testing will commence following the index case and continue for 30 days following the last confirmed case. Serological monitoring will then be

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		c) the transport of animals of susceptible species within the surveillance zone shall be subject to authorisation by the competent authority; d) animals of susceptible species must remain inside the surveillance zone for a maximum incubation period after the most recent recorded case of disease.	continued at monthly intervals for the next 12 months, and then quarterly for a further 4 years.
Derogations to allow animal movements			
9th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29)**.	***Article 11:*** 1) Member States shall ensure that the following measures are applied in the protection zone: d) animals of susceptible species must remain on the holding on which they are being kept, except to be transported under official supervision directly to a slaughterhouse located in that zone for emergency slaughter or, if that zone has no slaughterhouse under veterinary supervision, to a slaughterhouse in the surveillance zone designated by the competent authority. Such transport may be authorised by the competent authority only after the official veterinarian has carried out an examination of all the animals of susceptible species on the holding and confirmed that none of the animals is suspected of being infected. The competent authority responsible for the slaughterhouse shall be informed of the intention to send animals to it.	No specific guidelines described in legislation
10th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of 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**.	NA	NA

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
11th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone, to establishments located in the same Member State and if possible within the restricted zone**.	NA	NA
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 : a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, b)from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone**.	***Article 12:*** M1. Member States shall ensure that the following measures are applied in the surveillance zone: (d) animals of susceptible species must remain inside the surveillance zone for a maximum incubation period after the most recent recorded case of disease. Thereafter, animals may be removed from that zone to be transported under official supervision directly to a slaughterhouse designated by the competent authority for emergency slaughter. Such transport may be authorised by the competent authority only after the official veterinarian has carried out an examination of all the animals of the susceptible species on the holding and confirmed that none of the animals is suspected of being infected. The competent authority responsible for the slaughterhouse shall be informed of the intention to send animals to it.	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
14th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone.**	NA ***Article 12:*** 1) Member States shall ensure that the following measures are applied in the surveillance zone: b) the movement of animals of susceptible species on public roads shall be prohibited except for the purpose of leading them to pasture or animal buildings.	NA
15th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant derogation and allow for them to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone, in order to complete the production cycle before slaughter.**	NA ***Article 12:*** 1) Member States shall ensure that the following measures are applied in the surveillance zone: b) the movement of animals of susceptible species on public roads shall be prohibited except for the purpose of leading them to pasture or animal buildings.	NA
16th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations to grant derogation of movements of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating within the surveillance zone and eggs originating outside the restricted zone, to an establishment located in the same Member State where they were hatched.**	NA	NA

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
17th	**To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same Member State**.	NA	NA
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**.	***Article 13:*** Where the prohibitions provided for in Articles 11 (1) (d) and 12 (1) (d) are maintained beyond 45 days because of the occurrence of further cases of the disease and as a result problems arise in keeping the animals, the competent authority may, following an application by the owner explaining the rounds for such application, by the owner explaining the grounds for such applications authorise the removal of the animals from a holding within the protection zone or the surveillance zone, provided that: a) the official veterinarian has verified the facts; b) an inspection of all animals on the holding has been carried out; c) the animals to be transported have undergone a clinical examination, with negative result; d) each animal has been marked by ear marking or has been identified by any other approved method; e) the holding of destination is located either in the protection zone or within the surveillance zone.	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**.	NA	No specific guidelines described in legislation ***Article 5:*** The restocking of the holding shall be authorised by the competent authority, following the satisfactory inspection by the official veterinarian of the cleaning and disinfection operations carried out in accordance with Article 16.

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
20th	**To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease**.	NA	No specific guidelines described in legislation
21st	**To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment**.	NA	No specific guidelines described in legislation

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Annex D – Scenarios of ToR 2

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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
			of the epidemiological enquiry in the event of confirmation of the disease.	• time period before the suspicion, during which the pathogenic agent was introduced in the establishment and during which it could have spread outside the establishment. • The aim of the epidemiological enquire is the same as above.
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 • in an affected establishment with kept animals of listed species • non-affected epidemiological units kept separated • to provide derogation from killing for animals in non-affected separated epidemiological units • to exclude any possible contact between the affected establishment and the separated epidemiological units as per the epidemiological enquiry • time period calculated backwards from the date of the confirmation • time period before the confirmation, during which the pathogenic agent may have been introduced in the separated non-affected epidemiological units of the affected establishment.
ToR 2	Article 27(3)c of the Delegated Regulation Annex II of the Delegated Regulation	4th scenario	To assess the effectiveness of the length of the monitoring period, as the time period calculated backwards from the date of notification of the suspicion of the latest outbreak of a category A disease in the protection zone. Products or other materials likely to spread the disease, must had been obtained or produced, before this time period in order to be exempted from prohibitions of movements.	• protection zone • non-affected establishments • Products or other materials likely to spread the disease, obtained or produced, before the start of the monitoring period of the affected establishment that originated the protection zone • time period calculated backwards from the date of suspicion of the latest outbreak in the protection zone • time period before the notification of the suspicion, during which the products and materials produced in the non-affected establishments of a protection zone may have

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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	Article 59(4)b of the Delegated Regulation Annex II of the Delegated Regulation	7th scenario	To assess the effectiveness of the length of the monitoring period, as the appropriate time period calculated forwards the date when the first animal was introduced, during which all the animals of listed species intended for repopulation should be introduced.	• repopulation of a previous affected establishment • kept animals of listed species to be repopulated • the animals may not be introduced at the same time • time period calculated forwards from the date when the first animal was introduced • time period during which animals intended for repopulation, should be introduced and the process of repopulation be completed.

Annex E – Minimum radius and minimum period of duration of protection and surveillance zones

Category A diseases	Minimum radius of Protection zone Annex V	Minimum radius of Surveillance zone Annex V	Minimum period of duration of measures in the protection zone (Article 39(1)) Annex X	Additional period of duration of surveillance measures in the protection zone (Article 39(3)) Annex X	Minimum period of duration of measures in the surveillance zone (as referred to in Articles 55 and 56 of this Regulation) Annex XI
Foot and mouth disease (FMD)	3 km	10 km	15 days	15 days	30 days
Infection with rinderpest virus (RP)	3 km	10 km	21 days	9 days	30 days
Infection with Rift Valley fever virus (RVFV)	20 km	50 km	30 days	15 days	45 days
Infection with lumpy skin disease virus (LSD)	20 km	50 km	28 days	17 days	45 days
Infection with *Mycoplasma mycoides* subsp. *mycoides* SC (Contagious bovine pleuropneumonia) (CBPP)	Establishment	3 km	45 days	Not applicable	45 days
Sheep pox and goat pox (SPGP)	3 km	10 km	21 days	9 days	30 days
Infection with peste des petits ruminant virus (PPR)	3 km	10 km	21 days	9 days	30 days
Contagious caprine pleuropneumonia (CCPP)	Establishment	3 km	45 days	Not applicable	45 days
African horse sickness (AHS)	100 km	150 km	12 months	Not applicable	12 months
Infection with *Burkholderia mallei* (Glanders)	Establishment	Establishment	6 months	Not applicable	Not applicable
Classical swine fever (RVF)	3 km	10 km	15 days	15 days	30 days
African swine fever (RVF)	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	Parameters in the transmission model were estimated from the published literature and each is based on a small number of studies. Estimates for some parameters are available for European vector species, but others are based on African vector species.	The effectiveness of the sampling strategies could be over- or underestimated
	2	The transmission model only considers a single vector species (assumed to be *Culex* mosquitoes), but other mosquito species may be involved in transmission of RVFV in Europe.	The effectiveness of the sampling strategies could be over- or underestimated
	3	Genetic characteristics possibly affecting clinical features and/or pathogenicity of RVF in the host population in Europe compared to endemic areas	The effectiveness of the sampling strategies could be over- or underestimated
ToR 2	4	Very limited amount of evidence from published literature	The effectiveness of the proposed length of monitoring period could be over- or underestimated
	5	One kernel is based on analysis of a single epidemic in Africa (Tanzania 2007) and may not be representative of transmission in Europe due to differences in, e.g. environmental conditions, vector species involved, farm density and management practices.	The effectiveness of the proposed length of monitoring period could be over or underestimated
ToR 3	6	The other two kernels are based on assumed dispersal distances of mosquitoes and may not be representative of spread in a region due to differences in, e.g. environmental conditions, vector species involved or farm density.	The effectiveness of the proposed zone size could be over- or underestimated

Annex G – Monitoring Period: Extensive Literature Review Protocol

Rationale

The EFSA has been requested to provide scientific opinions to support the European Commission in the production of amending and implementing acts related to Regulation 2016/429 (the 'Animal Health Law' (AHL)) which lays down rules for the prevention and control of transmissible animal diseases. One of these scientific opinions will consist in assessing the effectiveness of several control measures for Category A (listed) diseases such as the length of the monitoring periods set out in Annex II of the Delegated Regulation (Mandate ToRs 2) and the duration of the control measures in restricted zones set out in Annex X-XI for each category A disease of terrestrial animals (ToR 3b).

As part of this, EFSA has asked P95 (within FWC OC/EFSA/ALPHA/2020/02 LOT 2) to carry out an extensive literature review (ELR) on the epidemiological parameter 'time for an outbreak to be reported' for the following diseases: Peste des Petits Ruminants (PPR), Classical Swine Fever (RVF), Newcastle Disease (ND), Sheep Pox and Goat Pox (SPGP), Rift Valley Fever (RVF), Glanders, contagious caprine pleuropneumoniae (CCPP), contagious bovine pleuropneumoniae (CBPP) and Rinderpest.

The current protocol describes the methodology that will be used by P95 to conduct the ELR.

Review question

The specific objective of this review will be to answer the epidemiological question of: 'what is the average, shortest and longest period of time (measured as the number of days from the earliest point of infection with the agent, to the time of reporting of a suspicion by the competent authority after the clinical investigation by an official veterinarian) for an outbreak of each of the 9 diseases of concern to be reported'.

Extensive literature review

Criteria for including studies

Starting with the objectives of the review stated above, the study inclusion criteria are based on the PICOS strategy:

Population	**Domestic animal species**
Intervention	PPR, RVF, ND, SPGP, RVF, Glanders, CCPP, CBPP, Rinderpest
Comparison	Not applicable
Outcome	• **Number of days between the earliest point of infection and the suspicion report** • Number of days between the earliest point of infection and the first suspicion(1) • Number of days between the earliest point of infection and the confirmation report • Number of days between the first suspicion and the suspicion report • Number of days between the first suspicion and the confirmation report • Number of days between the suspicion report and the confirmation report
Study design	Outbreak investigation, case report, surveillance data, modelling studies

(1): The suspicion based on the first observed clinical signs.

Exclusion criteria

The references will be excluded from the ELR if they meet one or more of following criteria:

i) References in another language than English, Spanish, German, Dutch, Portuguese and French.
ii) Review papers. However, original studies included in the review papers complying with the inclusion/exclusion criteria will be included.
iii) References published before 1/1/2000.
iv) References pertaining exclusively to diagnostics/vaccine development, entomology, in vitro/vivo studies.
v) References where the earliest point of infection is determined only by subtracting a known incubation period from the date of the suspicion of the outbreak. However, after discussion with EFSA and comment from experts, outbreaks investigations that do not determine the

true date of infection but report about the time between the onset of clinical signs and date of suspicion of the disease could be included.

vi) References presenting simulation exercises. However, if none or very few articles are retrieved (less or equal to 5) in the first search, these studies may be included, but their data should be presented in a separate table in the report together with a description of the methodology.

vii) References from outside the EU/EEA countries. However, If none or very few articles are retrieved (less or equal to 5) in the first search, the search should be extended to the rest of the world.

viii) References related to outbreaks that took place in a slaughterhouse. Nonetheless, references referring to outbreaks that occurred elsewhere, and are detected in a slaughterhouse may be included if all other conditions for inclusion are met.

Information sources

Electronic databases

We will conduct a literature search in MEDLINE (via PubMed) and EMBASE to obtain peer-reviewed, scientific publications related to the ELR.

Reference checking and hand searching

The reference list of relevant studies retrieved from the electronic database search will be hand searched to identify additional studies.

Grey literature selection

Data in the public domain pertaining to the objective of this study, outbreak investigation reports or surveillance data will be obtained via PAFF, OIE, EFSA, FAO, EuFMD websites, Google scholar and websites of EU veterinary reference laboratories for to the nine investigated diseases as well as websites of national veterinary/animal health institutes, reference veterinary laboratories or ministry of livestock from EU countries that previously experienced outbreaks of any of the five investigated diseases.

Search strategy

The following search strategy will be used in PubMed:

#	Search string	# of results
1	(((((((((((((((((("first infection"[All Fields] OR "index case"[All Fields]) OR ("introduction"[All Fields] OR "introductions"[All Fields])) OR "source of infection"[All Fields]) OR "clinical signs"[All Fields]) OR "clinical symptoms"[All Fields]) OR "case studies"[All Fields]) OR ("suspicion"[All Fields] OR "suspicions"[All Fields])) OR ((("suspect"[All Fields] OR "suspected"[All Fields]) OR "suspecting"[All Fields]) OR "suspects"[All Fields])) OR (((((("confirm"[All Fields] OR "confirmation"[All Fields]) OR "confirmations"[All Fields]) OR "confirmative"[All Fields]) OR "confirmed"[All Fields]) OR "confirming"[All Fields]) OR "confirms"[All Fields])) OR (((((("confirm"[All Fields] OR "confirmation"[All Fields]) OR "confirmations"[All Fields]) OR "confirmative"[All Fields]) OR "confirmed"[All Fields]) OR "confirming"[All Fields]) OR "confirms"[All Fields])) OR (((((((("reportable"[All Fields] OR "reporting"[All Fields]) OR "reportings"[All Fields]) OR "research report"[MeSH Terms]) OR ("research"[All Fields] AND "report"[All Fields])) OR "research report"[All Fields]) OR "report"[All Fields]) OR "reported"[All Fields]) OR "reports"[All Fields])) OR (((((((("reportable"[All Fields] OR "reporting"[All Fields]) OR "reportings"[All Fields]) OR "research report"[MeSH Terms]) OR ("research"[All Fields] AND "report"[All Fields])) OR "research report"[All Fields]) OR "report"[All Fields]) OR "reported"[All Fields]) OR "reports"[All Fields])) OR (((((((("reportable"[All Fields] OR "reporting"[All Fields]) OR "reportings"[All Fields]) OR "research report"[MeSH Terms]) OR ("research"[All Fields] AND "report"[All Fields])) OR "research report"[All Fields]) OR "report"[All Fields]) OR "reported"[All Fields]) OR "reports"[All Fields])) OR ("notification"[All Fields] OR "notifications"[All Fields])) OR (((((("notifiable"[All Fields] OR "notified"[All Fields]) OR "notifier"[All Fields]) OR "notifiers"[All Fields]) OR "notifies"[All Fields]) OR "notify"[All Fields]) OR "notifying"[All Fields])) OR (((((("declaration"[All Fields] OR "declaration s"[All Fields]) OR "declarations"[All Fields]) OR "declare"[All Fields]) OR "declared"[All Fields]) OR "declares"[All Fields]) OR "declaring"[All Fields])) OR (((((("declaration"[All Fields] OR "declaration s"[All Fields]) OR "declarations"[All Fields])	11,433,545

#	Search string	# of results
	OR "declare"[All Fields]) OR "declared"[All Fields]) OR "declares"[All Fields]) OR "declaring"[All Fields])) OR ((((((((((("detect"[All Fields] OR "detectabilities"[All Fields]) OR "detectability"[All Fields]) OR "detectable"[All Fields]) OR "detectables"[All Fields]) OR "detectably"[All Fields]) OR "detected"[All Fields]) OR "detectible"[All Fields]) OR "detecting"[All Fields]) OR "detection"[All Fields]) OR "detections"[All Fields]) OR "detects"[All Fields])) OR ((((("trace"[All Fields] OR "traced"[All Fields]) OR "traces"[All Fields]) OR "tracing"[All Fields]) OR "tracings"[All Fields])) OR ((((((((((("investigated"[All Fields] OR "investigates"[All Fields]) OR "investigating"[All Fields]) OR "investigation"[All Fields]) OR "investigations"[All Fields]) OR "investigative"[All Fields]) OR "investigator s"[All Fields]) OR "research personnel"[MeSH Terms]) OR ("research"[All Fields] AND "personnel"[All Fields])) OR "research personnel"[All Fields]) OR "investigator"[All Fields]) OR "investigators"[All Fields])	
2	#1 AND "time"[MeSH Terms] OR "time"[All Fields] OR "delay"[All Fields] OR "delayed"[All Fields] OR "delaying"[All Fields] OR "delays"[All Fields] OR "length"[All Fields] OR "lengths"[All Fields] OR "period"[All Fields] OR "periodic"[All Fields] OR "periodical"[All Fields] OR "periodically"[All Fields] OR "periodicals"[All Fields] OR "periodicity"[MeSH Terms] OR "periodicity"[All Fields] OR "periodicities"[All Fields] OR "periods"[All Fields] OR "duration"[All Fields] OR "durations"[All Fields] OR "days"[All Fields] OR "date"[All Fields] OR "timelier"[All Fields] OR "timeliness"[All Fields] OR "timelier"[All Fields] OR "timeliness"[All Fields] OR "timely"[All Fields] OR "timing"[All Fields] OR "timings"[All Fields]	3,497,425
3a	#2 AND ("peste des petits ruminants") Filters: from 2000 to 2021	**168**
3b	#2 AND ("classical swine fever") Filters: from 2000 to 2021	**530**
3c	#2 AND ("newcastle disease") Filters: from 2000 to 2021	**821**
3d	#2 AND ("sheep pox and goat pox") Filters: from 2000 to 2021	**31**
3e	#2 AND ("rift valley fever") Filters: from 2000 to 2021	**360**
3f	#2 AND ("glanders") Filters: from 2000 to 2021	**57**
3g	#2 AND ("contagious caprine pleuropneumonia") Filters: from 2000 to 2021	**18**
3h	#2 AND ("contagious bovine pleuropneumonia") Filters: from 2000 to 2021	**47**
3i	#2 AND ("rinderpest") Filters: from 2000 to 2021	**66**

Review methods

Selection of the studies

The list of studies identified from the different databases will be appended into a single file using Endnote and de-duplicated. The resulting list will be exported to Rayyan[2] to proceed with the title, abstract and keywords screening and study selection.

To decrease the risk of selection bias, two P95 reviewers will independently review the list of references obtained by screening keywords in title/abstract to identify studies that fulfil the above-mentioned selection criteria. Discrepancies will be discussed, and if not resolved, a third reviewer will take the final decision.

All identified reviews and studies conducted outside EU/EEA will be classified in specific folders for subsequent use.

Data extraction

In the second phase, full papers will be assessed for eligibility by a single reviewer. Data from the eligible full-text papers identified will be extracted by two reviewers using a standardised extraction form in MS Excel (see Annex) to ensure that all relevant data are collected systematically. In addition, the section of the pdf manuscript from where data will be collected will be noted and/or highlighted.

The complete selection process will be documented in an Endnote file, containing folders that reflect the selection criteria.

Analysis and reporting

During the selection process, the results of the literature search will be imported into Endnote where a clear track of the selection process will be maintained, and the flow of publications will be noted. Based on these numbers, a flowchart of the studies selected in accordance with the PRISMA guidelines will be prepared (Figure 1) for use in the subsequent reports.

If needed, extracted dates of interest will be combined together in order to calculate the periods of interest. Extracted data on age of the lesions can also be used to estimate the earliest point of infection. All these calculations will be described in an assigned column of the extraction form (See Annex).

Using the data collected, a qualitative data synthesis of results will be performed for each specific disease and parameter in terms of average, shortest and longest period of time. The different findings will be synthesised using tables providing sufficient details on the methodology used in the references.

Data extraction table template

ID
Reference
Source
Author
Disease
Objective
Country
Region
Year
Species
Farm type
Level
Sample size
Parameter_type
Parameter_unit
Parameter_value
Comment
Calculation_description
Calculation_output

[2] https://rayyan.qcri.org

The selected studies are

1) EFSA, 2020a. 'Rift Valley Fever–assessment of effectiveness of surveillance and control measures in the EU', EFSA Journal 2020;18: e06292.
2) EFSA, 2020b. Rift Valley Fever: risk of persistence, spread and impact in Mayotte (France). EFSA Journal 2020;18: e06093.
3) Elfadil AA, Hasab-Allah KA, Dafa-Allah OM and Elmanea AA, 2006. The persistence of rift valley fever in the Jazan region of Saudi Arabia. Revue scientifique et technique (International Office of Epizootics), 25, 1131–1136.
4) Jost CC, Nzietchueng S, Kihu S, Bett B, Njogu G, Swai ES and Mariner JC, 2010. Epidemiological assessment of the Rift Valley fever outbreak in Kenya and Tanzania in 2006 and 2007. American Journal of Tropical Medicine and Hygiene, 83, 66–72.
5) Mapaco LP, Coetzer JA, Paweska JT and Venter EH, 2012. 'An investigation into an outbreak of Rift Valley fever on a cattle farm in Bela-Bela, South Africa, in 2008. Journal of the South African Veterinary Association, 83.
6) Federica M, Pinoni C, Cosseddu GM, Khaiseb S, Calistri P, Molini U, Bishi A, Conte A, Scacchia M and Lelli R, 2013. Rift valley fever in Namibia, 2010. Emerging Infectious Diseases, 19, 2025.
7) ProMED, 2010. "Rift Valley fever, animal - Namibia: OIE" In. Available online: https://promedmail.org/promed-posts/

Annex H – Review protocol for seroconversion period

Framework of methodology

Framework to meet the objectives is depicted in Table H.1.

Table H.1: Framework of methodology

Years	From 2000 onwards
	Comments/Explanation: This will depend on the availability of the bibliographic databases
Language	Only studies written in English will be reviewed
	Comments/Explanation:
Publication type	Only primary research studies will be reviewed
	Comments/Explanation: • Reviews (i.e. secondary research studies) will not be included in the review, but their reference lists will be screened as sources of studies • Book chapters, theses and unpublished data will not be included • Letters and editorials will be excluded as normally these do not include any primary research studies • Patents will be excluded • No geographical limits
Population	*Perissodactyla, Antilocapridae, Bovidae, Camelidae, Cervidae, Giraffidae, Hippopotamidae, Moschidae, Proboscidea*
	Comments/Explanation:
Intervention	Serological diagnostic tests for Rift Valley Fever
	Comments/Explanation:
Target	Rift Valley Fever virus (family *Phenuiviridae*, genus *Phlebovirus*) will be the targeted pathogens
	Comments/Explanation:

Information sources

Search strategies included the use of electronic search engines for bibliographic databases. Two databases were searched:

- PubMed, and
- Mendeley
- Due to the specificity of the objective and time constrains only clinical trials and randomised controlled trials were included. Moreover, recent Oie diagnostic manual and relevant previous EFSA scientific opinions were also included. **Reviews were not included, but their reference lists were screened as sources of studies**. Book chapters, theses and informally reported or unpublished data were not collated.

Search strategy

The following search strategy was followed:

- Population: sheep* or goat* or cattle or calf or calves or American antelope or Antelope or Buffalo or Camel* or Deer or Giraffe or Hippopotamus or Elephant or *Perissodactyla or Antilocapridae or Bovidae or Camelidae or Cervidae or Giraffidae or Hippopotamidae or Moschidae or Proboscidea*
- Serological Tests: "diagnostic test" or serolog* or antibod* or "virus neutralisation" or *ELISA or VNT or PRNT
- Target: "Rift Valley Fever" or RVF or *Phenuiviridae* **or** *Phlebovirus*

A scoping search identified:

- 2,116 papers in PubMed, and
- 3,317 papers in Mendeley

A database of the electronic search results was created with Mendeley software. Duplicate citations were deleted automatically or manually when appropriated (n = 322).

Study selection

Study selection was based on the following predefined inclusion criteria (questions):

1.	**Is the paper in English?**		**Yes**	**Unclear**	**No**
2.	Is the paper an original clinical trial or a randomised controlled trial for the targeted listed species?	Yes	Unclear	No	
3.	Is the paper describing a serological diagnostic test for RVF?		Yes	Unclear	No
4.	Is the paper describing **the earliest day of seroconversion and the latest day of antibodies detection after infection**	Yes	Unclear	No	
Final decision:	Include	Review			Exclude

All papers with a 'no' or 'unclear' for any question were excluded based on title/abstract.

All papers with a 'yes' for each question were reviewed based on title/abstract.

Screening of titles and abstracts after the application of the above inclusion criteria was conducted for 79 papers.