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Assessment of the control measures for category A diseases of Animal Health Law: Contagious Bovine Pleuropneumonia

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Abstract

EFSA received a mandate from the European Commission to assess the effectiveness of some of the control measures against diseases included in the Category A list according to Regulation (EU) 2016/429 on transmissible animal diseases ('Animal Health Law'). This opinion belongs to a series of opinions where these control measures will be assessed, with this opinion covering the assessment of control measures for Contagious Bovine Pleuropneumonia (CBPP). In this opinion, EFSA and the AHAW Panel of experts review the effectiveness of: (i) clinical and laboratory sampling procedures, (ii) monitoring period, (iii) the minimum radius of the protection and surveillance zones, and (iv) the minimum length of time the measures should be applied in these zones. The general methodology used for this series of opinions has been published elsewhere. Several scenarios for which these control measures had to be assessed were designed and agreed prior to the start of the assessment. Different clinical and laboratory sampling procedures are proposed depending on the scenarios considered. The monitoring period of 45 days was assessed as not effective and at least 90 days (3 months) is recommended in affected areas where high awareness is expected; when the index case occurs in an area where the awareness is low the monitoring period should be at least 180 days (6 months). Since transmission kernels do not exist and data to estimate transmission kernels are not available, the effectiveness of surveillance and protection zones for CBPP was based on expert knowledge. A surveillance zone of 3 km was considered effective, while a protection zone including establishments adjacent to affected ones is recommended. Recommendations, provided for each of the scenarios assessed, aim to support the European Commission in the drafting of further pieces of legislation, as well as for plausible ad hoc requests in relation to CBPP.

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Summary

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

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

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

Specific clinical and laboratory procedures for Contagious Bovine Pleuropneumonia (CBPP) for each scenario of ToR 1 have not been found in the EU legislation. Specific sampling procedures for clinical and laboratory examination have been provided for some scenarios.

To answer ToR 2, and to assess the minimum length of time measures should be implemented in the protection and surveillance zones (ToR 3.2), an extensive literature search (ELS) was carried out. This ELS aimed to assess the average, shortest, and longest period between the earliest point of infection of cattle with *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*) and the time of reporting of a suspicion by the competent authority. The average time to the reporting of a suspicion was then used to assess the effectiveness of the length of monitoring periods. For most of the scenarios, the existing length of the monitoring period for CBPP (45 days) was not considered sufficient and a monitoring period of at least 90 days (3 months) was proposed since it was concluded (certainty ranging between 66% and 100% depending on the scenario) it would be effective. Recommendations were given for some of the relevant scenarios. To assess the effectiveness of the minimum length of time in which the measures should be applied in the protection and surveillance zones, the average and the longest time assessed via the ELS were used, respectively. In this regard, the minimum length of time of the protection zone (45 days) and the surveillance zone (45 days) that must be in place according to existing legislation, were not considered effective.

To assess the effectiveness of the minimum radius to be implemented in the protection and surveillance zones (ToR 3.1), transmission kernels could not be used because they do not exist in the literature and data to develop them are not available. Taken into consideration that *Mmm* is mainly transmitted by direct contact between animals and airborne transmission is not expected beyond 200 m, the protection zone should include at least all the adjacent (contiguous) premises to the affected establishment, in which case it is considered effective for preventing transmission beyond 95% or more of all protection zones with a 90–100% certainty. The length of the radius of 3 km for the surveillance zone is considered effective for preventing transmission in 95% or more of all surveillance zones with a 95–100% certainty. Nevertheless, transmission over longer distances cannot be excluded if infected animals are moved outside the zones.

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1. Introduction

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

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

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

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

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

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

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

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

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

1.1.2. ToR 2: Monitoring period

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

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

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

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

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

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

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

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

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

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

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

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

1.2. Interpretation of the Terms of Reference

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

- The publication of fourteen 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 CBPP.
- The publication of a separate opinion covering ToR 4 for all diseases listed (i.e. ToR 4 is not covered in this opinion).
- To address ToR 1 (effectiveness of sampling procedures), EFSA agreed with the EC 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.
- To address ToR 2 (effectiveness of the monitoring period), seven scenarios previously agreed with the contractor were defined (Annex D). The assessment of the effectiveness of the monitoring period will be done by assessing its ability to ensure that specific actions can be carried out without posing a risk of disease spread, if the monitoring period is calculated backwards or forwards from a specific date. If the length of the monitoring period estimated by EFSA is longer than the existing monitoring periods, the existing monitoring period will be considered not 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.
- 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.
- The following scenarios of the ToR 1 of Annex B are not relevant for the CBPP, and therefore not included in the assessment of the current Opinion:
 - Scenario 7 because the length of the radius of the protection zone for CBPP is not greater than 3 km radius
 - Scenarios 10, 11, 16 and 17 because they are referring to poultry.
- The duration of the monitoring period for CBPP as described in Annex II of the Delegated Regulation is 45 days.
- The minimum radius of the protection zone and surveillance zone for CBPP as described in Annex V of the Delegated regulation are at the level of infected establishment and 3 km, respectively.
- The minimum duration of the measures in the protection and surveillance zone for CBPP as described in Annexes X and XI of the Delegated Regulation is 45 days for both zones.

2. Epidemiology and geographical distribution of CBPP

2.1. Aetiology

Contagious bovine pleuropneumonia (CBPP) is a severe respiratory disease mainly affecting domestic species of Bovidae. The causative agent is a wall-less bacterium (Mollicutes), *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*), a member of the family Mycoplasmataceae. It belongs to a cluster of genetically

closely related mycoplasma ('mycoides cluster') including also *Mycoplasma capricolum* subsp. *capricolum* (Mcc), *Mycoplasma leachii* (Ml), *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) and *Mycoplasma mycoides* subsp. *capri* (Mmc) (Spickler, 2015; EFSA AHAW Panel, 2017; OIE, 2018, 2020).

2.2. Epidemiology

CBPP is a contagious respiratory disease of cattle (*Bos taurus* and *Bos indicus*), water buffalo (*Bubalus bubalis*), and yaks (*Bos grunniens*). Sheep and goats can be occasionally infected but are not thought to transmit the disease to cattle nor to play an important epidemiological role. Only experimental cases have been reported in wild ruminants (e.g. *Syncerus caffer*) and these species do not play an epidemiological role. CBPP is not a zoonotic disease (Lefevre et al., 2010; Spickler, 2015; EFSA AHAW Panel, 2017; OIE, 2018, 2020).

Transmission of CBPP occurs through inhalation of aerosol and by direct contact with infected animals excreting *Mmm* in droplets when coughing, nasal discharge, saliva, fetal membranes and uterine discharge. Chronically infected animals recovered from acute infection may also act as carriers, because they can harbour mycoplasmas for several months in encapsulated lung lesions ('sequestra'). Airborne transmission, up to 200 m, may occur; indirect transmission through fomites is not significant (Spickler, 2015; EFSA AHAW Panel, 2017; OIE, 2020).

CBPP had been described since the 18th century in Europe where it has been eradicated. The latest European outbreaks took place at the end of the 1990s following a resurgence of the disease in the 1980s in several southern countries. The disease was probably introduced from Europe into Africa by colonial settlers and is now endemic in most Sub-Saharan African countries. The situation in Asia and in the Middle East is unclear due to lack of surveillance, apart from China where it has been eradicated. It was also eradicated from the USA and Australia, and has never been reported in South America (Lefèvre et al., 2003; Dupuy et al., 2012; Spickler, 2015; EFSA AHAW Panel, 2017; OIE, 2018, 2020).

The main control measures in the event of an outbreak include stamping out of the animals in the affected establishments, restrictions of movements of animals and products, backward and forward traceability and surveillance activities within and outside the restricted zones. In endemic areas, antibiotics (e.g. tetracyclines, macrolides or quinolones) are effective to treat clinically infected cattle. However, recovered animals may remain carriers.

In Africa, vaccination has been extensively used for years to control the disease using different types of vaccines and various strains. Currently, two strains are recommended by OIE for the production of live attenuated vaccines: T1sr and T1/44 that provide a 6-month or one-year protection, respectively (OIE, 2020). CBPP vaccine strain T1/44 is widely used by vaccine manufacturers in most parts of Africa with the exception of a few countries that still use the T1/SR (FAO, 2016; Mwirigi et al., 2016; Thiaucourt et al., 2021).

The African Union Pan African Veterinary Vaccine Centre (AU-PANVAC) performs quality control of the veterinary vaccines produced or imported into Africa and publishes the list with the certified vaccine batches on its website¹ (FAO, 2016; Thiaucourt et al., 2021). Vaccination is used in endemic areas but is prohibited in officially free countries and in the EU (Spickler, 2015; EFSA AHAW Panel, 2017; OIE, 2018, 2020).

Vaccination using these attenuated vaccines is effective at the condition it is targeting the entire susceptible population. The expected protection rate is around 60% after an initial dose but the herd immunity will rise following annual revaccinations; after the third vaccination, herds are considered completely immune (Wesonga and Thiaucourt, 2000). CBPP can be controlled with vaccination but there are no examples that vaccination alone without other measures can lead to the eradication. The experience from Australia showed that eradication can be achieved by stamping out once the prevalence has been sufficiently reduced by vaccination (Newton and Norris, 2000).

Some research has been conducted to develop new DIVA vaccines allowing a combined strategy with stamping out of infected herds and vaccination of the others. These new types of vaccines are not yet authorised.

2.3. Clinical signs and diagnosis

The morbidity and mortality of CBPP and its clinical manifestation depend on several factors such as the cattle breed, age, immune status, season and individual factors. The disease is more severe in

¹ AU/PANVAC website: <https://aupanvac.org/about-us/> and list of certified batches: <https://aupanvac.org/brizy-4163/certified-vaccines-batches/>.

adult cattle with a typical pulmonary form than in young stock in which polyarthritis is the major sign. Morbidity can reach 80–90%, but usually less than 50% of animals will show acute clinical signs. The case-fatality rate is highly variable (0–70% in experimental infections). Subclinical forms are frequent, and many diseased animals can become chronically infected. While mortality rates can reach 10–70% during epidemics in Africa, the latest outbreaks in Europe were characterised by low morbidity and no or low mortality (2–3%); this was possibly due to the common practice of early treatment of the respiratory diseases with antimicrobials, good livestock management practices, improved surveillance and immediate culling of confirmed cases, and a lower virulence of circulating strains (Regalla et al., 1996; European Commission, 2001; Lefèvre et al., 2003; EFSA AHAW Panel, 2017).

The incubation period is usually 3–8 weeks but can last up to 6 months. In the acute form in adult animals, the first signs are fever (40°C), depression, anorexia, and drop in milk production, followed by respiratory signs (coughing, polypnoea and nasal discharge). In more severe cases, these signs will be more pronounced with dyspnoea, and a typical position with an arched back, head and neck extended and forelimbs apart. Auscultation and percussion of the thoracic area is painful and reveals pneumonia and pleurisy, often unilateral. Without treatment, death can occur within 2–3 weeks in 50% of acute cases. Some animals survive with a chronic form, but often remain emaciated and weak, showing a mild cough exacerbated by physical exercise, associated with encapsulated pulmonary lesions ('sequestra'). Others recover clinically after several weeks or months, although many remain carriers and maintain the disease in the herd. In young cattle (less than 6 months old), the main signs are pain when walking and swelling of the carpal and tarsal joints due to polyarthritis, sometimes associated with weakness due to cardiac lesions (endocarditis and myocarditis) (Lefèvre et al., 2003; Spickler, 2015; OIE, 2020).

Typical lesions at post-mortem examination are yellow sero-fibrinous exudate in the thoracic cavity, 'sequestra' in the lungs and marbled aspect of the lungs. Identification of *Mmm* can be performed on samples from sick animals such as nasal swabs, bronchoalveolar washing and punctured pleural fluid or on samples taken from lesions at necropsy (lung tissue, pleural or synovial fluid, lymph nodes). Culture and isolation of *Mmm* is performed on mycoplasma media, which are notably enriched in horse serum (Bonnefois et al., 2016). Culture in liquid medium can be observed after 2 days; observing the mycoplasma colonies can take 4 days on solid media but can be unsuccessful after antibiotic treatment. PCR is the method of choice for confirmation from an isolated strain or directly from a pathological sample. Serological tests, performed on blood samples, are valid only at herd level due to reduced sensitivity in the early stage of the disease and in subacute or chronic forms. The routinely used assays are the complement fixation test (CFT), immunoblotting (as confirmatory test), slide agglutination test and latex agglutination test (LAT) (pen-side tests that can be used in the field) and a competitive enzyme linked immunosorbent assay (c-ELISA). Cross-reactions with other members of the *Mycoplasma mycoides* cluster are observed, except for the c-ELISA (Lefèvre et al., 2003; Spickler, 2015; OIE, 2018, 2020).

2.4. Geographical distribution of Contagious Bovine Pleuropneumonia

The exact origin of CBPP is not clear. According to ter Laak (1992) it seems to have occurred for the first time in 1713 in Germany and Switzerland and then introduced and spread into other European countries (ter Laak, 1992). However, Dupuy et al. (2012) consider that CBPP was described for the first time in Switzerland in 1773 by Albrecht von Haller. Based on the application of next generation sequencing technologies on the genome of *Mmm* strains, CBPP was estimated to have emerged around 1700 AD and most probably in Europe from where it was exported to other continents through animal trade in former colonies, apart from South America (Dupuy et al., 2012). In the 19th century, CBPP was distributed worldwide.

CBPP was eradicated from many countries at the beginning of the 20th century, mostly through stamping-out strategies (Europe, USA) or by vaccination campaigns followed by stamping-out strategies (Australia) (OIE, 2020). Nevertheless, CBPP sporadic outbreaks occurred in 1935, 1956 and in 1967 in the Iberian Peninsula and at the French-Spanish border in the Pyrenees (Dupuy et al., 2012). The disease re-emerged in Europe in the 1980s and 1990s in Spain, Portugal, France, and Italy. The study by Dupuy et al. (2012) showed that all strains isolated after 1980 derived from a common ancestor showing that a single strain may have spread in Southern Europe (France, Spain, Portugal and Italy) between 1980 and 1993. In Europe the last CBPP cases were observed in Portugal in 1999 (OIE, 2018, 2020).

The USA is free from the disease since 1892, and Australia is free since 1973 (Campbell, 2015).

Today, CBPP remains endemic in many Sub-Saharan African countries (see Figures 1 and 2). In Asia, India and China are currently officially free from CBPP, but for the rest of the countries the situation is unclear due to a lack of effective CBPP surveillance (OIE, 2018, 2020). The presence of CBPP in Pakistan was reported in two publications in 2019 and 2020 but there were no official notifications to the OIE (Anjum et al., 2019, 2020).

CBPP is one of the diseases for which the OIE has established an official procedure for the recognition of disease-free areas within a country or at national level.² The OIE Terrestrial Animal Health Code specifies the steps a country must follow to be characterised as officially free of CBPP. Figure 1 presents the countries and zones with recognised by OIE official free status. In Europe, France, Portugal and Switzerland applied and granted free status.

OIE Members' official CBPP status map

Last update May 2020

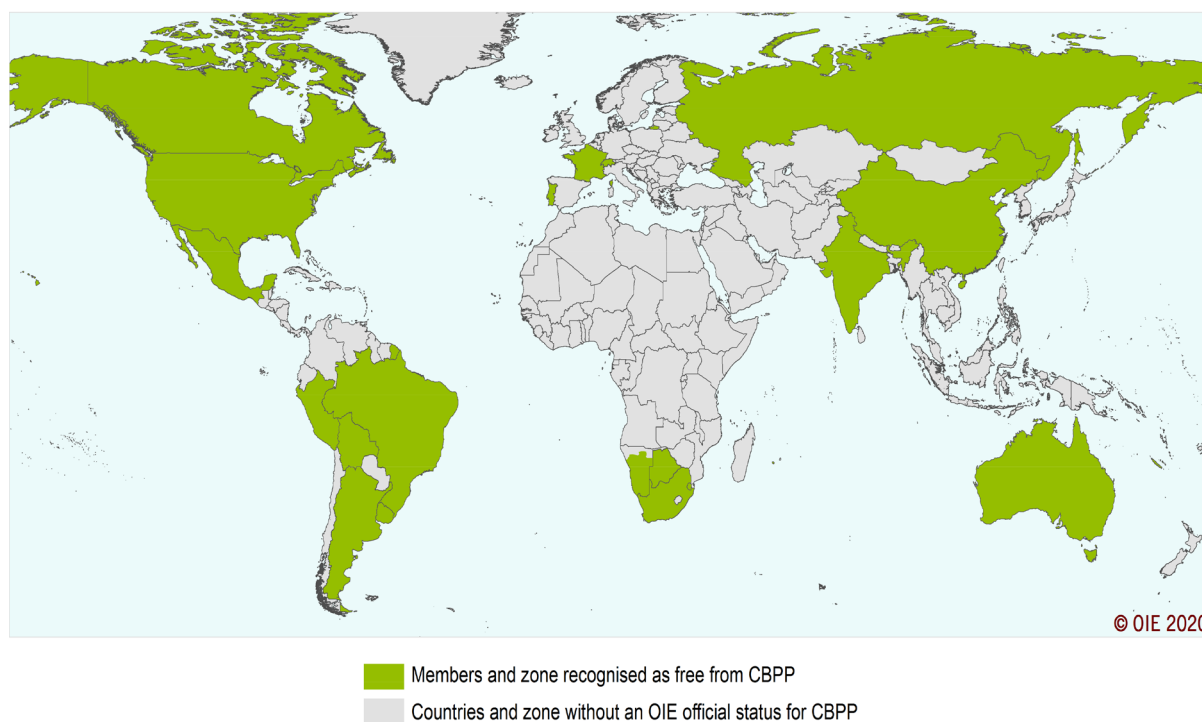


Figure 1: Map of countries or zones of countries with the OIE official free status for Contagious Bovine Pleuropneumonia, 2020 (Source: OIE, © OIE)

² For more information, visit the status portal on the OIE: <https://www.oie.int/en/what-we-do/animal-health-and-welfare/official-disease-status/>, <https://www.oie.int/en/disease/contagious-bovine-pleuropneumonia/>, <https://www.oie.int/en/disease/contagious-bovine-pleuropneumonia/#ui-id-2>

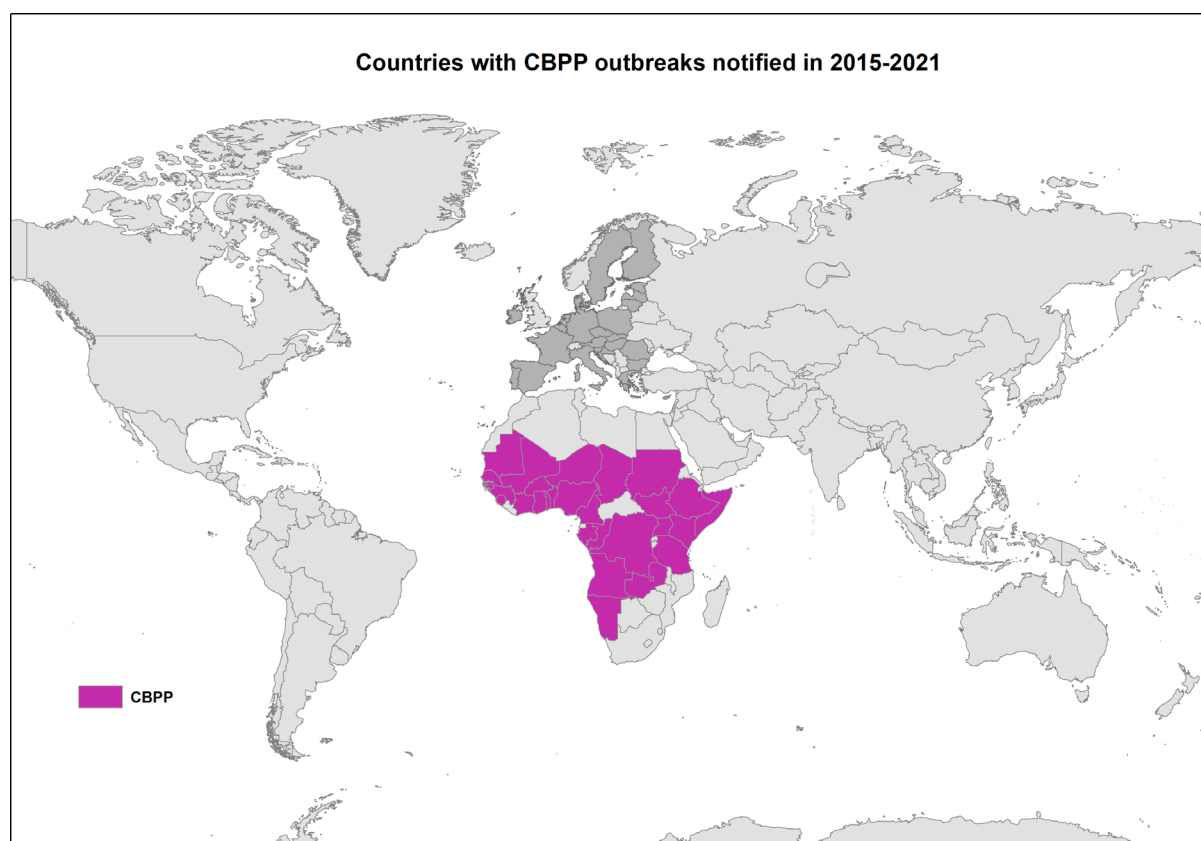


Figure 2: Map of countries with notified-to-OIE outbreaks of Contagious Bovine Pleuropneumonia in 2015–2021 (Data sources: ADIS-EC and WAHIS-OIE). The southern part of Namibia has the free status according to OIE, the outbreaks have been notified in the northern part of the Country

3. Data and methodologies

3.1. Methodologies

3.1.1. Methodology used in ToR 1

A qualitative assessment of the clinical and laboratory procedures was performed to answer ToR 1. Estimation of sample size, when needed, was carried out using the RiBESS+ tool.³

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

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

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

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

The overall probability to detect *Mmm* or antibodies by a laboratory test (PCR or c-ELISA) with a single sample from an animal with clinical signs would be

³ RiBESS + tool <https://efsa.openanalytics.eu/app/riBESS>

$$P_{\text{detect}} = \text{PPV}_{\text{clinical}} \cdot \text{Se}_{\text{labtest}}, \quad (2)$$

where $\text{Se}_{\text{labtest}}$ is the sensitivity of the laboratory test used.

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

$$\text{Se}_{\text{overall}} = 1 - [(1 - P_{\text{detect}})]^n. \quad (3)$$

Based on the $\text{Se}_{\text{overall}}$ to be achieved, the n (number of samples needed to be collected) can be calculated as follows:

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

3.1.2. Methodology used in ToR 2

To answer ToR 2, an extensive literature search (ELS) was outsourced by EFSA to a contractor (OC/EFSA/ALPHA/2020/02 – LOT 2). The aim of this ELS was to answer the epidemiological question: ‘what is the average, shortest and longest period of time (measured as the number of days from the earliest point of infection with *Mmm* to the time of declaration of a suspicion by the competent authority after the clinical investigation by an official veterinarian) for an outbreak of CBPP to be reported’. To answer this question, an ELS on case reports, papers describing outbreaks or epidemics of CBPP, and any other relevant grey literature or data, was carried out. For the inclusion in the ELS, the earliest point of infection had to have been estimated following an epidemiological investigation. Papers and other sources of data were excluded when the earliest point of infection was determined purely by subtracting a known incubation period from the date of the suspicion of the outbreak. The ELS was restricted to studies conducted in Europe or describing results obtained in Europe. If none or very few articles were retrieved (less or equal to 5) in the first search, the search was extended to the rest of the world. The general protocol used for the ELS is shown in Annex 5 of the Technical report on Methodology (EFSA, 2020). To answer Scenario 5 of ToR 2 in relation to semen, an ELS was performed, to determine the time to seroconversion as it can be identified by different laboratory methods. This work was outsourced by EFSA to an expert (EOI/EFSA/SCIENCE/2020/01 – CT 02 ALPHA).

3.1.3. Methodology used in ToR 3

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

Two studies were identified which considered transmission of *Mmm* between farms in Europe: Portugal 1985–1995 (Regalla et al., 1996) and Italy 1990–1993 (Regalla et al., 1996; Giovannini et al., 2001). However, neither of these studies estimated transmission kernels or provided data that could be used to estimate a kernel. Furthermore, no suitable data from epidemics outside of Europe were identified that could be used to estimate a transmission kernel nor were kernels available for other diseases with similar transmission routes to *Mmm*. Accordingly, expert knowledge was used to assess the zone sizes for CBPP.

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

To estimate the duration of measures in the protection and surveillance zones, the outputs obtained from the ELS described in Section 4.2 were used. Further details can be found in the Technical report (EFSA, 2020).

3.1.4. Uncertainty

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

For Scenario 1 in ToR 1, which aims to assess the effectiveness of existing or proposed sampling procedures to detect or rule out the presence of CBPP in kept animals in a suspected establishment based on clinical and laboratory examinations, it was agreed that a sampling strategy would be considered effective if it would allow the detection of the disease in at least 95% of the cattle establishments in which it was applied. Two quantities of interest (QoI) were defined based on the reason triggering the suspicion (occurrence of clinical disease and CBPP-related mortality or other reasons in the absence of clinical disease and mortality, e.g. contact tracing with a previously infected holding) and the sampling and diagnostic approach proposed:

- QoI 1a: probability that, in 95% (or more) of all **cattle establishments suspected due to the occurrence of clinical disease and mortality with signs/lesions resembling to CBPP**, the presence of the disease would be detected based on **laboratory tests (PCR/culture) performed on dead animals with characteristic lesions if present, or clinical inspection involving testing in LAT at least 20 animals with clinical signs and the slaughter of at least five positive reactors for post-mortem inspection and PCR**,
- QoI 1b: probability that, in 95% (or more) of all cattle establishments suspected (and eventually confirmed) but in which no CBPP-compatible clinical signs/lesions have been found (e.g., suspected due to contact tracing), the presence of the disease would be detected based on c-ELISA performed on all animals in the establishment (for establishments with < 255 animals) or between 255 and 370 animals (including those with unspecific signs if present) depending on establishment size (see Table 4) up to two times separated by three months in case no positive animals are detected in the first herd test.

For ToR 2, which aims to assess the effectiveness of the length of the monitoring period under different scenarios, a given length was considered effective if it would serve its scenario-specific purpose in at least 95% of the cases in which it was implemented. In this case, four QoI were defined based on the scenarios among those listed in Annex D and whether the suspected establishment was the first case in a region or not:

- QoI 2a (Scenarios 1, 2 and 4): probability that, in 95% (or more) of **all cattle establishments suspected (and eventually confirmed) in a previously unaffected region or country**, the initial infection would have occurred within the 90 days (proposed length for the monitoring period) before the date of notification of the suspicion.
- QoI 2b (Scenarios 1, 2 and 4): probability that, in 95% (or more) of **all cattle establishments suspected (and eventually confirmed) in a region or country where CBPP cases have been already reported**, the initial infection would have occurred within the 90 days (proposed length for the monitoring period) before the date of notification of the suspicion.
- QoI 2c (Scenario 3): probability that, in 95% (or more) of the **independent epidemiological units within CBPP affected cattle establishments that eventually become infected**, infection would have occurred within the 90 days (proposed length for the monitoring period) before the date of confirmation of infection in the establishment.
- QoI 2d (Scenario 6): probability that, in 95% (or more) of all **repopulated CBPP-affected cattle establishments that become reinfected**, reinfection takes place in the 90 days (proposed length for the monitoring period) following the introduction of the animals.

For ToR 3, which aims at the assessment of the effectiveness of the minimum radii established in the protection and surveillance zones, a given radius was assumed to be effective if it would prevent transmission to outside of the zone in the 90 days (proposed length for the monitoring period in both the protection and surveillance zones) following the setting up of these zones. In this case, two QoI were defined:

- QoI 3a: Probability that, in 95% or more of all protection zones established around an affected establishment, there is no transmission to outside the protection zone in the 90 days following their establishment.
- QoI 3b: Probability that, in 95% or more of all surveillance zones established around an affected establishment, there is no transmission to outside the surveillance zone in the 90 days following their establishment.

Members of the WG provided their judgements individually for each of the QoI, along with the rationale supporting them, using the probability scale of Table 1 proposed in the EFSA uncertainty guidance (EFSA Scientific Committee, 2018).

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

Table 1: Approximate probability scale used for quantification of the uncertainty in the assessment

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

4. Assessment

4.1. Assessment of sampling procedures

4.1.1. Assessment of sampling procedures in the event of suspicion or confirmation of Contagious Bovine Pleuropneumonia (CBPP)

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

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

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

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

- 1) It concerns an event of suspicion of CBPP in an establishment of kept animals of listed species for CBPP;
- 2) The listed species for CBPP as provided in Commission Implemented Regulation 2018/1882 are those of *Bison ssp.*, *Bos ssp.*, *Bubalus ssp.*, *Syncerus caffer*;
- 3) In the event of a suspicion of CBPP, the competent authority shall immediately conduct an investigation to confirm or rule out the presence of the CBPP;
- 4) On the day of the investigation, the official veterinarians must perform clinical examinations and collect samples for laboratory examinations.

Summary of sampling procedures

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

Information on clinical examination and laboratory methods have been described in the OIE Terrestrial Manual (OIE, 2018) and in the EC Report of 2001 on the Diagnostics tests for CBPP (European Commission, 2001) and in EFSA Scientific Opinion on CBPP (EFSA AHAW Panel, 2017).

Clinical examination is considered of low diagnostic value for CBPP diagnosis since the clinical signs are not disease specific and can appear in any respiratory disease. The lesions in the lungs and the pleural cavity are pathognomonic, with unilateral pleuropneumonia and enlargement of interlobular septa.

Samples to be collected from live animals are nasal swabs or nasal discharge, broncho-alveolar lavage or transtracheal washing, pleural fluid collected aseptically by puncture made in the lower part of the thoracic cavity between the seventh and eighth ribs and blood samples.

Samples taken at necropsy are lungs with lesions, pleural fluid, lymph nodes of the broncho-pulmonary tract, and synovial fluid from those animals with arthritis. The lung samples should be collected from lesions at the interface between diseased and normal tissue.

In vitro culture and isolation are implemented to identify the agent, with PCR based methods having become the method of choice for rapid, easy and specific identification of *Mmm*.

The serological tests that are available: CFT, c-ELISA, immunoblotting test (IBT) and rapid agglutination tests. Results of serological tests should not be interpreted on animal but on herd level, because false negative results may occur in individual animals. Tests on single animals can be misleading, either because the animal is in the early stage of disease, which may last for several months before specific antibodies are produced and be detectable, or it may be in the chronic stage of the disease when very few animals are still seropositive (OIE, 2018).

Assessment

Clinical examination and Inspection of Lesions

In the scenario of a suspicion of CBPP in an establishment, the purpose of the clinical examination⁴ (including both the initial visual inspection of the herd and the individual examination of the animals) is to identify suspect cases and collect samples for further laboratory analysis. This will either happen when a suspicion has been raised at the slaughterhouse based on post-mortem inspection and the laboratory results of the samples of a slaughtered animal, or when an establishment is in contact with an establishment that was found infected previously.

No data on the sensitivity and specificity of clinical examination exist in the literature. Nevertheless, the specificity cannot be considered high since the clinical signs are not pathognomonic to CBPP but are similar to those of very common respiratory diseases (e.g. pneumonic Pasteurellosis, respiratory parasitosis) in bovine establishments. Moreover, when the animals are in the chronic phase, the clinical signs are very mild and cannot be easily detected, so the sensitivity of clinical examination decreases. The affected animals become weak, sometimes called '*lungers*'.

On the other hand, the lesions identified in lungs and in thoracic cavity in carcasses of dead or culled animals or identified during the post-mortem inspection in slaughterhouses are considered pathognomonic and can play a crucial role in the diagnosis since they contain a large amount of *Mmm* organisms.

In the acute phase of the disease, the main pathognomonic lesions (see Figure 3) are characterised by:

- i) unilateral pleuropneumonia with a '*marbled*' appearance of the affected lungs,
- ii) enlargement of interlobular septa,
- iii) presence of straw-coloured exudate in the thoracic cavity,
- iv) costal pleura covered with a fibrinous deposit,
- v) when the lesions are more ancient, multiple fibrous tissues connecting the costal pleura to the lung (which explain the painful and difficult breathing of the surviving animals in the chronic form of the disease),
- vi) renal infarct (resulting from mycoplasma polysaccharide complexed with immunoglobulin M (IgM)),
- vii) regional lymph nodes enlarged and filled with exudate.

⁴ Definition of the term 'clinical examination' is provided in the article 3 of the Delegated Regulation: the clinical examination comprises: (i) an initial general evaluation of the animal health status of the establishment which comprises all the animals of listed species kept in the establishment; and (ii) an individual examination of the animals included in the sample referred to in point (a). The sampling of animals for clinical examination is carried out in accordance with point A.1 of Annex I for terrestrial animals.



Figure 3: Acute lung lesions of CBPP in cattle: the whole lung is affected, the interlobular septa are enlarged and filled with fibrin deriving from exudate. Copyright of the picture: François Thiaucourt and Yaya Aboubakar

In the chronic phase, the characteristic lesions called '*sequestra*' can be identified (see Figure 4). They are formed by a portion of affected lung, which is surrounded by fibrous tissue that separates these lesions from the normal lung. The size of these '*sequestra*' varies tremendously from 'pea size' to 'rugby ball size'. At the beginning, the lung structure is still recognisable but, in the end, there may be a complete necrosis and the sequesterum is filled with liquefied necrotic material.

In case of antibiotic treatment, typical lesions may not be observed, and the results of the laboratory examinations may be affected.

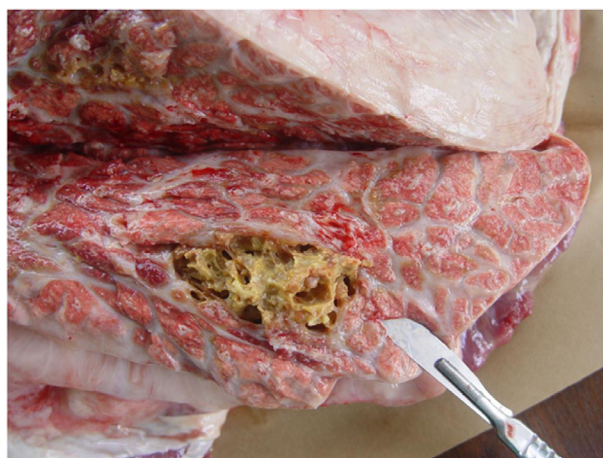


Figure 4: Chronic lung lesions of CBPP in cattle: sequestrum in an affected lung where its content is starting to necrotise. Copyright of the picture: François Thiaucourt and Yaya Aboubakar

Consequently, in non-affected areas or in areas away from the affected ones and let alone in free from the disease areas, clinical signs alone most likely will not trigger the suspicion of CBPP; other more common respiratory diseases will be suspected and probably treated with antimicrobials. The suspicion will be usually triggered at the slaughterhouse during post-mortem inspection of the lungs and the thoracic cavity or during necropsy of dead animals submitted to post-mortem examination.

On the contrary, clinical signs may raise a suspicion of CBPP in an establishment located in an affected area or close to an affected area where the awareness is higher or in establishment epidemiological linked with an affected one.

Laboratory Examination

Pleural fluid is one of the best matrices for laboratory examination, because it contains a huge amount of the *Mmm* ($> 10^9$ colony forming units (CFU) per mL) and is the preferable matrix for the

sequencing (personal communication with François Thiaucourt). Therefore, the sensitivity of the laboratory method to be implemented is not an issue as there is ample material in the matrix.

Although collection from live animals of pleural fluid and other matrices like nasal swabs or nasal discharges, broncho-alveolar lavage or transtracheal washing is proposed by the OIE and by several publications, it is technically very difficult to collect them from live animals under field conditions. Furthermore, pleural fluid is not always present especially in less acute cases. Samples from lungs with lesions is the next most appropriate sample followed by the pulmonary lymph nodes that directly drain the lungs, followed by the more distant mediastinal lymph nodes (Schnee et al., 2011).

In vitro culture and isolation (followed by species identification tests) is quite easy if the quality of samples is adequate (no bacterial contamination, no antimicrobial residues, well preserved) and if the laboratory has experience in mycoplasma isolation and identification. The samples of choice are pleural fluid (in acute cases from live animals or carcasses), pieces of affected lung (*'sequestra'* in chronic cases) and regional lymph nodes.

Several PCR methods are available and can be implemented to the extracted DNA coming either directly from the samples (pleural fluid, pieces of lungs, lymph nodes) or after culture and isolation (Bashiruddin et al., 1994; Dedieu et al., 1994; Miserez et al., 1997; Lorenzon et al., 2008; Schnee et al., 2011). The specificity of the PCR methods is estimated at 100%, while the sensitivity may vary. Most studies estimated the relative analytical sensitivity in comparison with previous published methods (nested PCR by Miserez et al. (1997), $Se = 10^4$ – 10^5 higher than that of conventional single PCR, and real-time PCR by Lorenzon et al. (2008), $Se = 2$ – 3 log increased compared to established conventional PCR). According to the study by Schnee et al. (2011), the diagnostic sensitivity of the real-time PCR method that they have developed is 94.7% in lung tissue, 31% in mediastinal lymph nodes and 25% in pleural fluid. If the extracted DNA is from isolation and not from a sample, the sensitivity is up to 100% as huge amounts of DNA can be obtained from a pure *Mmm* culture (OIE, 2018).

Serological tests such as c-ELISA (IDEXX⁵) developed by Le Goff and Thiaucourt (1998) can be used to detect the presence of antibodies and to confirm the presence of CBPP in an establishment. This method is highly specific ($Sp = 99.8\%$ according to OIE (2018) and $Sp > 99.5\%$ according to EFSA AHAW Panel (2017)). c-ELISA is able to detect antibodies about 15 days after the onset of clinical signs and possibly for more than 6–12 months post-infection (Yaya et al., 1999). Its sensitivity is lower than the CFT (see below) at an early stage after infection (roughly one month after, as CFT detects mostly IgMs (Amanfu et al., 1998; Tardy et al., 2011; Muuka et al., 2013; Sery et al., 2015).

For acute cases and in recent infected establishments, the LAT (BoviLAT)⁶ is able to detect IgMs about 8 days after the onset of clinical signs and during the first 2 months post-infection. It can be implemented in the field using sera or whole blood and give results in less than two minutes. The sensitivity of this test is comparable to CFT (see below). The lack of absolute specificity of this test may lead to false positive results; therefore, it cannot be used for the confirmation of the disease. It is a useful test that can be performed at the establishment while waiting the results of other tests. The main concern is that it has so far not been subject to proper validation studies in CBPP free countries.

CFT is another serological test available with a sensitivity of 63.8% (OIE, 2018) or 70% (Le Goff and Thiaucourt, 1998) and a specificity of 98% (OIE, 2018). Since CFT detects mostly IgMs that develop shortly after the onset of lesions and symptoms (and decline thereafter), it can identify nearly all sick animals with acute lesions, but a rather smaller proportion of animals in the incubation period or at the very early stage of the disease or of animals with chronic lesions (EFSA AHAW Panel, 2017). Nevertheless, there are very few European laboratories, which run it and in addition, it does not have any advantage in terms of sensitivity compared to c-ELISA and is less specific.

IBT can be used to confirm doubtful CFT and c-ELISA results. Additionally, it can be used to rule out false positive results since it is more specific than CFT.

For most of the laboratory methods used for the diagnosis of CBPP, quantitative information for the test performance and the quality parameters is not available in scientific literature. Available test quality information is shown in Table 2.

⁵ c-ELISA by IDEXX is the only one commercially available.

⁶ BoviLAT is the only commercially available latex agglutination test.

Table 2: Sensitivity and specificity of different laboratory methods, in different sample matrices

	Sample matrices	Analysis method	Sensitivity (Se)	Specificity (Sp)
Agent identification				
Acute and chronic cases	Pieces of lungs (with lesions or 'sequestra' in chronic cases) Lymph nodes from carcasses (slaughterhouse or necropsy at establishment from animals without antibiotic therapy)	Culture	+++	+++
		PCR methods	+++	++++
Acute cases	Pleural fluid from live animals or carcasses (acute cases from animals without antibiotic therapy)	Culture	+++ ⁽¹⁾	+++
		PCR methods	+++ ⁽¹⁾	++++
		Sequencing	–	++++
Detection of antibodies				
Acute cases or recently affected establishments	Serum	Latex agglutination test (BoviLAT)	Will detect IgM about 8 days after clinical signs and during the first 2 months	++
Chronic cases or old affected establishments		c-ELISA (IDEXX)	Will detect mostly IgG about 15 days after clinical signs and possibly for more than 6–12 months (in fact the antibody persistence is not known and it may depend mostly on the extension of lesions) 70% (Le Goff and Thiaucourt, 1998)	++++ ⁽²⁾ 99.8% (OIE, 2018)
		CFT	63.8% OIE (2018) 70% (Le Goff and Thiaucourt, 1998)	98% (OIE, 2018)

(1): Since the pleural fluid contains more than 10⁹ CFU/mL of mycoplasmas, the sensitivity of the laboratory method is not an issue.

(2): This high specificity of the c-ELISA can be obtained only in laboratories which are run under quality assurance.

The major concern with c-ELISA and LAT is that there are no proper validation studies to estimate the performance of these methods (sensitivity, specificity) in conditions similar to EU Countries (free from the disease), where the prevalence of CBPP in the establishments is expected zero or very low (in case of occurrence). From the studies conducted in affected countries, the sensitivity of the serological methods has been reported in the range 60–70% and varies based on the stage of the disease in the herd (acute, chronic). It may also be affected by previous antimicrobial therapy. Therefore, some infected animals may remain undetected even if all the animals in the establishment are tested (see Table 4 and Figure 7).

In addition, the quality of the samples, especially the tissues, and the conditions of their transport to the laboratory may affect the final diagnosis.

Development of new procedures

The sampling procedures for CBPP detection are related to the epidemiological conditions that may trigger the suspicion and in case of EU Countries the suspicion may be raised:

- i) at the slaughterhouses during post-mortem inspection of the lungs and the thoracic cavity,
- ii) when animals with clinical signs are detected at an establishment located in an affected area or close to it or at an establishment epidemiologically linked with an affected establishment or area and
- iii) at an establishment without clinical signs that is epidemiologically linked with an affected establishment or area.

In each case, the findings during the visit at the establishment from the clinical examination and the health history will imply the sampling procedures to be implemented (see Figure 5).

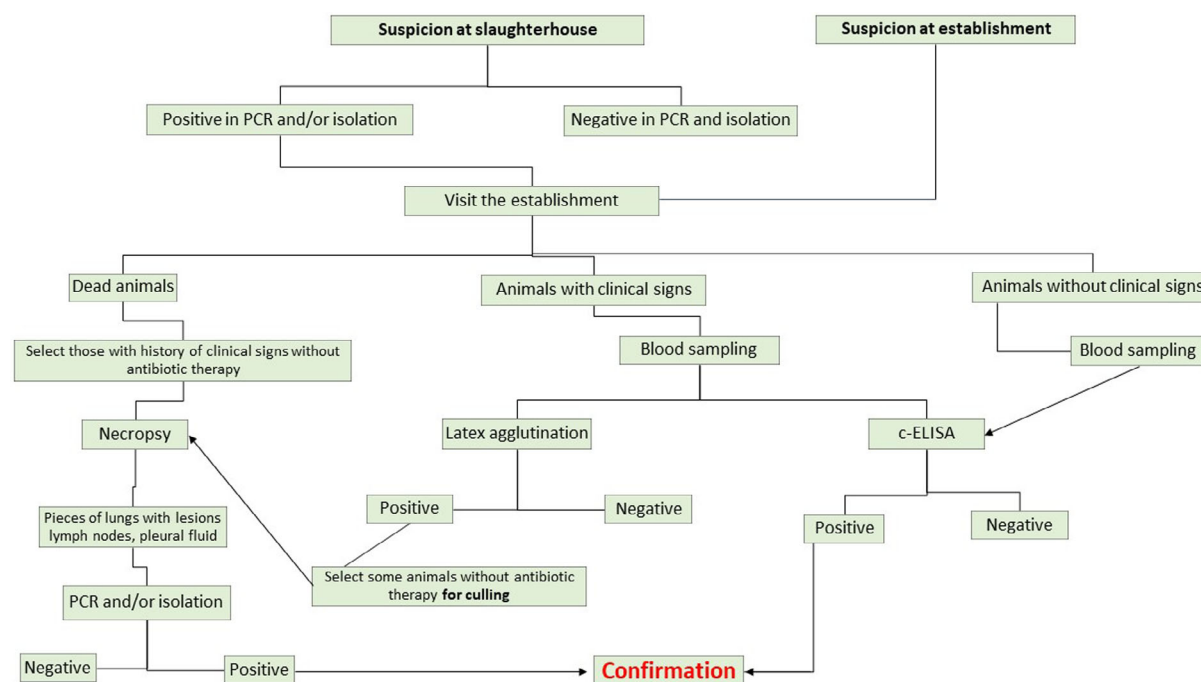


Figure 5: A schematic description of the sampling procedures in case a suspicion of CBPP is raised either at the slaughterhouse or at an establishment

Clinical Examination and Inspection of lesions

Although clinical examination has marked limitations for the diagnosis and confirmation of CBPP, it is an important tool to identify the animals with clinical signs or history of clinical signs to be sampled for further laboratory analyses.

The lesions identified in lungs and pleural cavity even if pathognomonic, alone are insufficient to confirm CBPP and laboratory analyses are necessary to confirm the disease. Therefore, in some suspect establishments it is necessary to kill some animals to collect samples from the lesions. If such lesions are observed at the slaughterhouse, visit and clinical examination at the establishment of origin is the next step.

The individual clinical examination should focus primarily on those animals identified by the owner as suspects for CBPP or identified by the veterinarians based on clinical signs resembling CBPP during the initial visual inspection of the herd (targeted sampling). The health history of the herd at least 90 days backwards from the day of the suspicion, and the subsequent visit by the veterinarian, should be investigated during the interview with the farmers and the documents inspection. Any evidence of respiratory symptoms, deaths or contacts with affected establishments and the use of antimicrobials as treatment for respiratory symptoms should be thoroughly investigated and recorded, and the involved animals prioritised for clinical examination and sampling.

Laboratory Analysis

i) Suspicion at slaughterhouse: When CBPP suspicion is raised at slaughterhouse either because the characteristic lesions have been identified in carcasses or because *Mmm* has been identified in a sample taken at the slaughterhouse under investigation of other diseases, e.g. *Mycobacterium bovis*, the establishment of origin should be visited for further investigation.

The sampling for laboratory analyses should start from those animals that are found dead and preferably those with a history of respiratory disease without receiving antimicrobial treatment. Thorough inspection of the lungs and the pleural cavity should be performed to identify the characteristic lesions that have been described above. Samples of lungs with lesions, regional lymph nodes and pleural fluid when available (acute cases) should be collected from the dead animals to be cultured and tested with PCR methods.

In addition to dead animals or if dead animals are not available for sampling, animals with clinical signs associated with CBPP should be killed for necropsy to identify the pathognomonic lesions and to collect samples to be tested by PCR.

The clinical examination is not specific for CBPP diagnosis, and its positive predictive value (PPV_{clinical}) which indicates the probability that a selected animal clinically classified as CBPP positive is truly *Mmm* infected, is expected low. Nevertheless, in combination with LAT it will increase the likelihood that an *Mmm* infected animal will be detected by laboratory tests and will reduce the number of animals to be tested (see Table 3 and Figure 6).

To justify the procedure proposed, the following assumptions were made:

- i) a specificity of clinical examination of 80% to take into account the existence of other respiratory disease in the establishment;
- ii) a design prevalence in an establishment, where CBPP is suspected, assumed to be 10% as the infection is already present for several months at least; and
- iii) a sensitivity of clinical examination of 90% in naïve cattle populations, because veterinarians will normally identify respiratory distress in an animal.

Based on the above assumptions, the positive predictive value of clinical examination (PPV_{clinical}) would be 33%. Consequently, the prevalence of animals infected by CBPP in each selected group of animals with clinical signs is 33%, which requires that at least 8 animals should be killed, necropsied and tested by PCR alone (without LAT) to achieve a confidence level of 95% (see Table 3 and Figure 6).

To reduce the number of animals to be killed and sampled for PCR, an additional step can be introduced; the LAT is implemented in the field to blood samples collected from at least 20 animals classified as CBPP suspected based on clinical examination. The LAT is proposed here only to support the selection of animals to be killed for necropsy and not for the confirmation of CBPP. The positive predictive value to identify CBPP of an animal that is both clinically suspect and LAT positive ($PPV_{\text{clinical/LAT}}$) is higher than for an animal that is just clinically suspect, so fewer animals must be submitted to necropsy.

For the LAT, the sensitivity is considered 70% and the specificity is 70% because of possible cross reactions with other *Mycoplasma mycoides* subspecies (assumption based on the expert knowledge). With a design prevalence of 33% (positive predictive value of the clinical examination), the positive predictive value following LAT increases to 54% (see equation (1) in Section 3.1.1).

The overall probability to detect CBPP by PCR, with a sensitivity of 94%, with a single sample from lesions, from a culled animal with clinical signs that tested positive to LAT, would be 51% (equation (2) in Section 3.1.1). In this case, at least five animals should be killed, to detect the CBPP by PCR in lesions with confidence level of 95% (see Table 3 and Figure 6). Consequently, to detect an outbreak with at least 95% confidence, and taken into consideration the assumptions and the uncertainty, it is recommended to collect blood samples from up to 20 animals with clinical signs and test them firstly with LAT in the field. From those animals that tested positive to LAT, five should be killed and necropsied and samples from the lesions should be tested by PCR. The 20 blood samples tested by LAT should be afterwards complementary tested by c-ELISA for which a sensitivity of 70% is assumed (Table 3).

In the event that all 20 samples are negative by LAT, it can be considered that the animals are in the later stages of infection and no more IgM is present. In that case there will be some c-ELISA positives. If both the LAT and c-ELISA are negative, it is very, if not extremely unlikely that the clinical signs are caused by *Mmm*. To further increase the certainty of absence of CBPP a random sample of animals (according to Table 4) could be tested by c-ELISA (which will detect 'older infections'). Restrictions on the establishment are maintained until negative testing with c-ELISA and further clinical examination of the animals can resolve the suspicion. The visit to the establishment and the sampling procedures should be repeated after a period of 90 days to have a very high certainty of the absence of the infection.

Based on the available evidence and considering the existing uncertainty regarding the performance of the diagnostic tests, **it was concluded with a 90–100% certainty that the proposed sampling strategy** (post-mortem examination and testing on dead animals with lesions if present or

LAT testing of at least 20 animals with signs and slaughter of at least five LAT-reactors for post-mortem inspection and PCR) **would be able to detect the infection in 95% or more of all CBPP affected establishments in which suspicion was triggered due to the occurrence of clinical signs (given that there is an epidemiological link with affected establishment or area) or due to findings at slaughterhouse or dead animals resembling CBPP.** The 90–100% certainty range was due to the potential for increased difficulty detecting infection in the case of smaller herds in which sample size would be necessarily more reduced, and therefore, the limited sensitivity of the diagnostic tests could lead to increased uncertainty regarding the detection of the infection. Importantly, this judgement assumes that the diagnostic techniques are already set up and optimised in laboratories conducting the testing.

Table 3: Minimum number of animals with clinical signs needed: (i) to be killed in order to collect samples for PCR without and with LAT testing and (ii) to be sampled and tested with c-ELISA, in order to detect or rule out the CBPP in the establishment with a confidence level of 95% and 99%, assuming a design prevalence in the establishment of 10%

Target Population	Sample matrices	Laboratory method	Minimum number of animals needed for sampling		
			95% confidence	99% confidence	
Animals found positive in clinical examination Design prevalence DP = 10% Clinical examination Se = 90%					
Animals to be killed for necropsy	Lung lesions, pleural fluid, lymph nodes	PCR (Se = 94%)	8	13	
Animals found positive to LAT to be killed for necropsy	Lung lesions, pleural fluid, lymph nodes from killed animals with clinical signs and positive to LAT (BoviLAT) ⁶	LAT (Se = 70%, Sp = 70%) and PCR (Se = 94%) to those animals found positive to LAT	5	7	
Live animals	Blood from live animals with clinical signs	c-ELISA (Se = 70%)	12	18	

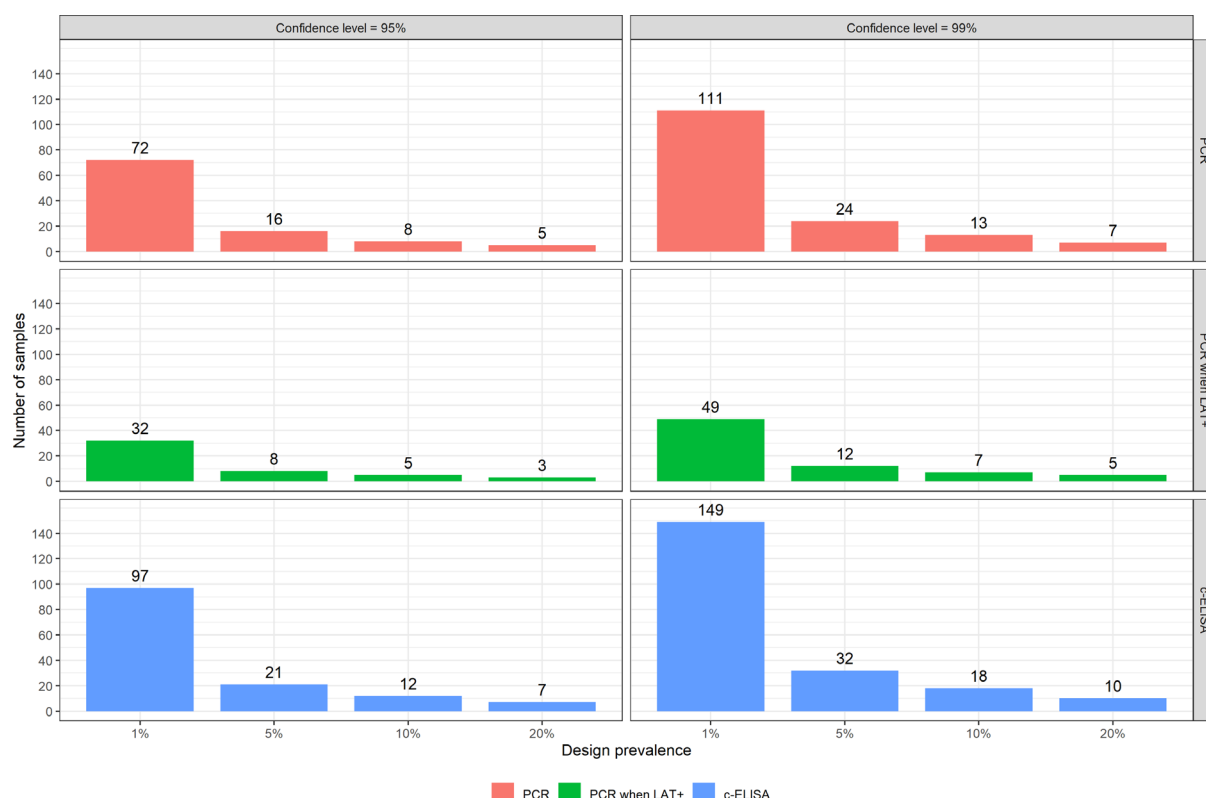


Figure 6: Minimum number of animals needed to be sampled or to be killed for the purpose of sampling, to achieve 95% and 99% confidence level accordingly, in detecting one infected animal with clinical signs, assuming different values for design prevalence (1%, 5%, 10% and 20%) by using: (i) directly PCR to samples from killed animals, (ii) PCR to samples from killed animals that have been previously positive to LAT and (iii) c-ELISA to samples from live animals

ii) Suspicion at an establishment with animals with clinical signs: In case the establishment is in an affected area, or close to an affected area where high awareness is in place or it is epidemiologically linked to an affected establishment or area, animals with clinical respiratory signs may raise the suspicion. The laboratory procedures will be the same as described above, and the certainty regarding the effectiveness of the proposed sampling strategy would also be the same.

iii) Suspicion at an establishment with animals without clinical signs: In case the establishment is epidemiologically linked with an affected establishment or area, may raise the suspicion even without the occurrence of clinical signs. In the absence of animals with clinical signs or dead animals, blood samples are collected from live animals according to Table 4 for the laboratory to be examined by c-ELISA assuming a prevalence of 1% in the establishment. In addition, a low design prevalence is assumed as there are no clinical signs and if the infection is present, then the prevalence will probably be low. c-ELISA can detect IgG antibodies from infected and recovered animals even if they have been treated by antimicrobials. The IgG antibodies can be detected for several months although the exact duration cannot be ascertained with confidence.

Here the LAT is not recommended, because of the low sensitivity in later stages of infection and the low specificity. As we can see from the Table 4 and Figure 7, using a test as c-ELISA with a sensitivity 70% in establishments where a low (1%) prevalence is expected, it is not possible to detect or rule out the CBPP with a confidence level at least 95%, when the size of the establishment is $n < 255$ animals, even if all the animals are tested.

Table 4: Minimum sample size for 95% confidence level (probability to detect CBPP infected animals) achieved in an establishment as a function of the herd size, assuming a target (design) prevalence of 1% and 10%, and using two different values of the sensitivity of the c-ELISA (Se = 70%)

Herd size	c-ELISA (Se = 70%)					
	Examples of sampling calculations by using different design prevalence of 1% and 10%					
	DP = 1%			DP = 10%		
	Design prevalence	Sample size	Confidence	Design prevalence	Sample Size	Confidence
10	10%*	10	69%	10%*	10	69%
20	5%*	20	69.5%	10%	20	91.4%
50	2%*	50	69.8%	10%	32	95%
70	2%*	70	70%	10%	34	95%
100	1%	100	70%	10%	36	95%
200	1%	200	91%	10%	39	95%
250	1%	250	91%	10%	40	95%
255	1%	230	95%	10%	39	95%
300	1%	271	95%	10%	40	95%
350	1%	315	95%	10%	40	95%
500	1%	322	95%	10%	41	95%
750	1%	334	95%	10%	41	95%
1,000	1%	369	95%	10%	41	95%

*: The minimum number of animals with clinical signs in a herd is one it cannot be lower. Therefore, the values provided here for the design prevalence are the result of the ratio between 1 and the herd size rounded to an integer.

Values in red: the confidence level of 95% cannot be reached even if all the animals in the establishment are tested.

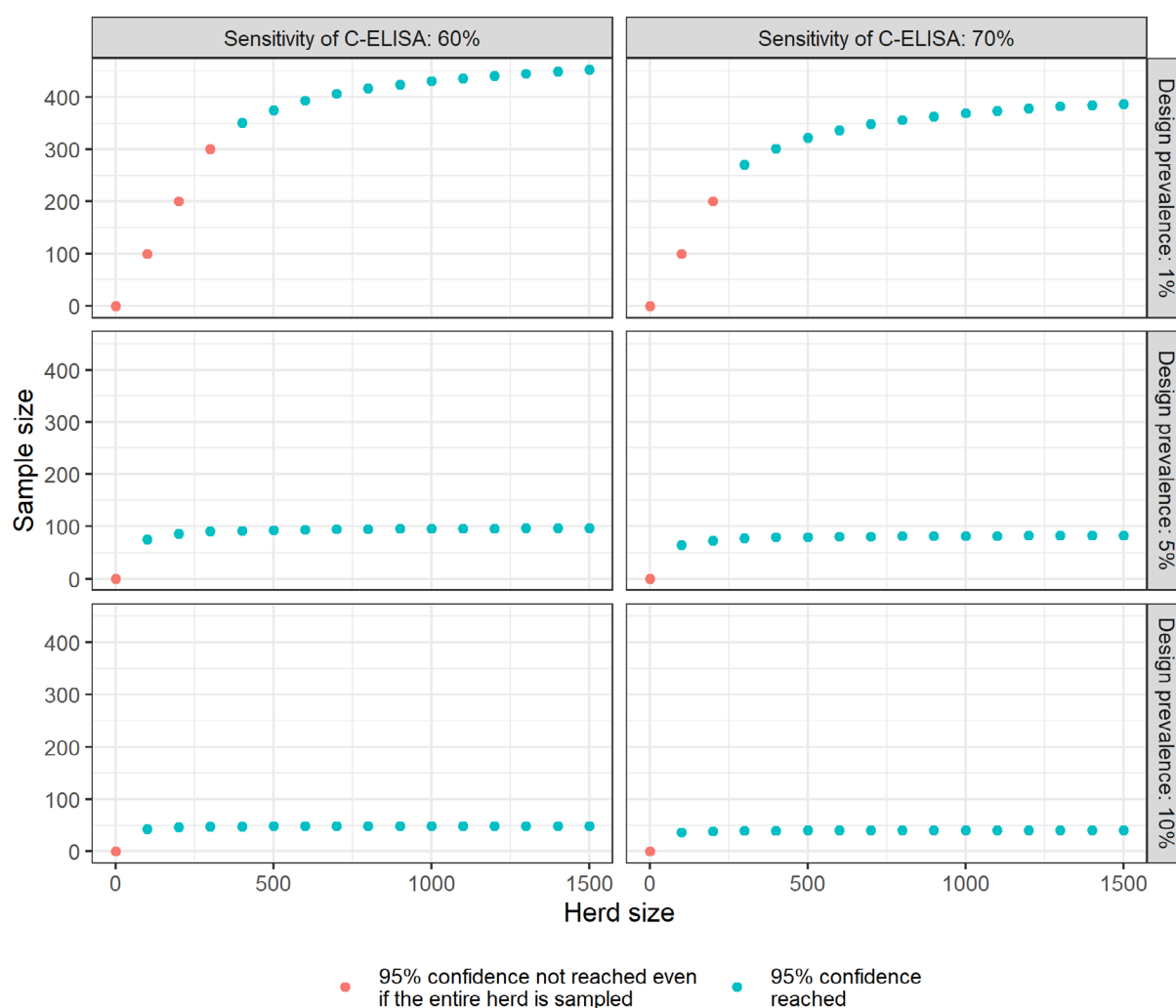


Figure 7: Minimum sample size, to detect animals with CBPP with confidence level of 95%, assuming design prevalence of 1%, 5% and 10% using c-ELISA with different sensitivity levels (Se.: 60%, 70%)

In case of negative or doubtful results and based on the epidemiological situation and the risk assessment contacted at national level, the sampling procedures could be repeated at least 90 days later. If the establishment is infected, the prevalence will increase during this period and the likelihood to detect CBPP as well (Figure 7 and Table 4).

Overall, and considering the uncertainties regarding the sensitivity of the serological tests depending on the stage of infection of tested animals and the possible stage in which affected herds may be when sampling takes place (e.g. in an early stage of infection with many animals in the incubation period, or in a more advanced stage with more animals in a chronic stage of infection), **it was concluded with a 90–100% certainty that the proposed sampling strategy** (c-ELISA of all animals for herds with < 255 animals or 255/369 animals depending on herd size up to two times separated by three months if no reactors are detected in the first herd test) **would be able to detect the infection in 95% or more of all CBPP affected establishments in which the suspicion is raised in an establishment in the absence of clinical signs or CBPP-related mortality.** Some uncertainty exists due to the possible existence of animals in early stages of infection (whose proportion should nevertheless be low in the second sampling), the presence of very low antibody titres in infected animals due to the circulation of low virulence strains, and the involvement of small herds in which available sample sizes will be reduced and therefore limit the potential of the proposed sampling strategy to detect infected herds at low prevalence levels. Again, this judgement is based on an adequate performance of the diagnostic techniques, which may not be always expected if testing procedures are not well standardised in the laboratories conducting the testing.

Given the limitations of knowledge on the quantitative characteristics of the laboratory diagnostic methods, the confidence for the CBPP diagnosis can be increased by: (i) targeted sampling from animals with clinical signs or from lesions in carcasses or animals epidemiologically linked to the affected ones (closest animals or with common origins) that will increase the sensitivity, (ii) implementing a combination of laboratory methods in different samples, (iii) using experienced and trained veterinarians able to recognise the characteristic lesions of CBPP, and (iv) training on sampling collection and transport.

In addition, the nomination of a European Reference Laboratory (EURL) for CBPP will increase the preparedness and the capacity of the National Reference Laboratories (NRL) to early detect the CBPP in case it enters the EU territory. A EURL may drive studies and test validations adjusted to the needs of EU Countries that are mainly focused on highly sensitive and specific tests able to detect the disease at early stages.

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 CBPP

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

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

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

- 1) It concerns an affected establishment officially confirmed
- 2) Kept animals of listed species found dead or before/when they are killed are sampled
- 3) The competent authority shall collect samples for laboratory examination
- 4) The purposes of the sampling are:
 - a) to support the epidemiological enquiry:
 - i) to identify the likely origin of the disease;
 - ii) to calculate the likely length of time that the disease has been present;
 - iii) to identify establishments where the animals could have contracted the disease and movements from the affected establishment that could have led to the spread of the disease; and
 - iv) to obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors.
 - b) to confirm/rule out disease in the event of preventive killing.

Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 2nd scenario.

Assessment

When CBPP has been officially confirmed in an establishment, further sampling procedures will support the needs of the epidemiological enquiry to obtain information on the origin of the disease, the length of time that the disease is present. In addition, in case preventive killing is applied in establishments where the disease has not been yet confirmed, sampling procedures will confirm or rule out the disease.

Development of new procedures

Estimate the prevalence of infected animals within the affected establishment

It is difficult to estimate the prevalence of CBPP infected animals. Information on clinically affected animals may be collected during the epidemiological enquiry. Serological tests may help, but they may underestimate the situation due to limited sensitivity.

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

This will be obtained by the epidemiological investigation and interview of the owner on the health history of the establishment and animal movements. Laboratory tests cannot be used for this purpose.

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

Since the disease has been confirmed in the establishment and assuming this has not been already done during the investigation of the suspicion, some additional samples may be taken for *Mmm* isolation according to the instructions provided by the laboratory (see also Laboratory Examination in Section 4.1.1.1).

Isolation of *Mmm* strains is of paramount importance as it may allow a retrospective study through whole genome sequencing results (Loire et al., 2020).

Confirm the disease in case a preventive killing is decided

In the Delegated Regulation, preventive killing may be implemented for the animals of listed species for CBPP (*Bison ssp.*, *Bos ssp.*, *Bubalus ssp.*, *Syncerus caffer*) in three cases: (i) in an establishment suspected of CBPP, (ii) in the establishments in temporary restricted zones (Article 9 of Delegated Regulation), and (iii) in the establishments of the restricted zone (that is the protection and surveillance zones and further restricted zones).

In case preventive killing is applied, all animals in the establishment should be subjected to clinical examination to identify those with clinical signs in order to proceed with further laboratory examinations and the whole procedure as described in the first scenario in Section 4.1.1.1 in the event of the suspicion of the disease, should be implemented.

In case of preventive killing in the absence of clinical signs, and given that the animals are going to be culled, the confirmation of CBPP in the establishment will be based on one or a combination of the following:

- i) detection of lung lesions in culled animals (acute lesions may be pathognomonic);
- ii) cultivation of mycoplasmas from lung lesions, pleural fluid, regional lymph nodes. Extra caution should be taken to distinguish *Mycoplasma bovis* from *Mmm* and avoid any confusion. *Mycoplasma bovis* is common in the EU and might be isolated from several establishments, especially in areas where the prevalence is high, or the infection is endemic;
- iii) PCR from lung lesions, pleural fluid, and regional lymph nodes;
- iv) blood sampling for c-ELISA as described in Table 4 can be collected. In some cases, serological tests might be positive although cultivation and PCR yield negative results.

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

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

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

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 3rd scenario.

Assessment

The following considerations should be taken into account when designing derogations from killing animals in CBPP affected establishments:

- i) the lack of specificity of clinical examination;
- ii) animals without clinical signs may be incubating CBPP which cannot be detected by laboratory tests; (long incubation period of the disease; more often 3 to 8 weeks but it can be extended up to 6 months);
- iii) some animals may become '*carriers*' following their exposure and may remain a source of *Mmm*;
- iv) the length of infectious period is not known;
- v) data on sensitivity and specificity of diagnostic tests are sparse, complicating interpretation of test results and estimates of predictive values;
- vi) the identification of infectious animals is often not possible;
- vii) airborne transmission up to 200 m may occur.

Consequently, sampling procedures (clinical and laboratory) cannot provide a high level of confidence that these animals do not pose a risk for transmission if they are kept alive.

Development of new procedures

All the animals intended for derogation from killing should be subjected to thorough individual clinical examination and samples for laboratory examination with serological tests (c-ELISA, LAT) should be collected from all the animals irrespective of the presence of clinical signs.

Regular clinical examination should be carried out, preferably every week for the first 45 days, to detect early the onset of clinical signs, and then every 45 days. Sampling for laboratory examination can be repeated at least every 45 days combined with the clinical examination for all the animals in the establishment.

This procedure should be carried out for at least 1-year calculated forwards from the day of confirmation of the latest case within the establishment.

The animals with clinical signs and/or those found positive to serological tests should be culled, and thorough post-mortem inspection should be implemented. Samples from carcasses (lung lesions, pleural fluid, lymph nodes) could additionally be examined by PCR and isolation to detect or rule out the presence of *Mmm*.

Sampling procedures for laboratory examinations in order to detect or rule out the presence of *Mmm* should follow the procedures described in the Section 4.1.1.1.

However, even with these new procedures the EFSA AHAW Panel considers that, given the currently available laboratory tests, it is very difficult to provide a high level of confidence that the animals from an affected establishment without clinical signs and with negative results in serological tests do not pose a risk of transmission and therefore this practice should be discouraged.

4.1.1.4. For the animals of non-listed species kept in an CBPP 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 detect the agent if the agent is present in these species. For further details, see Annexes B and C.

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

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 4th scenario.

Assessment

In the scientific literature, there are few references of other non-listed species becoming infected with *Mmm* and able to transmit CBPP. Brandao (1995) reported that in Portugal, post-mortem examination of sheep and goats with clinical signs of mastitis or pneumonia detected three strains of *Mmm* from the milk of sheep and two strains from goats with pneumonia. Mycoplasma from milk and lungs were isolated after four to five days culture. Gonçalves et al. (2002) used the European strains of *Mmm* from the outbreaks in Portugal and inoculated sheep. No clinical signs were observed in the first trial, but a second infectious dose did induce an immune response, although there was some variability in the response to specific antigens and there was low transmissibility from infected sheep. Xin et al. (2012) reported that, during experimental CBPP vaccine development in sheep, animals developed respiratory signs, but the main purpose of the study was to produce a greater yield of vaccine suitable for bovines in the eradication programme. The fact that disease has been successfully eradicated in countries by only controlling infection in cattle or other bovines without any measures for sheep and goats, indicates that cattle are the natural hosts and sheep and goats only become infected as the result of a spill-over event. Therefore, a sheep or goat reservoir is almost impossible (0–1%), but transmission from cattle to small ruminants cannot be ruled out, where these two groups are in close contact.

There is no evidence to demonstrate the epidemiological involvement of other non-listed species in the spread or maintenance of CBPP in the field.

Development of new procedures

In the scenario where sheep and goats are kept in a CBPP affected establishment, they should be monitored for clinical signs. On the occurrence of clinical signs or deaths, samples should be collected for laboratory analysis following the procedures of the 1st scenario in Section 4.1.1.1.

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

4.1.1.5. For wild animals of the listed species within the CBPP 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 agent, if the agent is present in these wild species. For further details, see Annexes B and C.

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

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

- 1) It concerns an affected establishment officially confirmed;
- 2) They may exist wild animals of listed species within the establishment and in the surroundings of the establishment;
- 3) As listed in Commission Implementing Regulation (EU) 2018/1882 for CBPP; the wild animals of listed species animals are those of *Bison* spp., *Bos* spp., *Bubalus* spp., *Syncerus caffer*;
- 4) The competent authority may establish these sampling procedures in addition to other measures;
- 5) The purpose of the sampling procedures in wild animals of listed species is to ensure the detection of the agent, if the agent is present in these wild species.

Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 5th scenario.

Assessment

The literature search for CBPP in the listed species of *Bison* spp. and *Bubalus* spp. has not yielded any evidence for infection, seroconversion or clinical manifestation of the disease. There was only one study with CBPP challenge in African buffalo (*Syncerus caffer*), which was experimentally infected with subcutaneous inoculation of *Mmm*, leading to isolation of *Mmm* 53 days after infection, however without any gross lesions typical of CBPP (Shifrine et al., 1970).

Wild *Bos* species (including stray or feral animals) could be infected as a result of close contact with infected cattle (e.g. absence of fences, free ranging herds) and therefore may play a role in the spread or maintenance of CBPP. For the CBPP control around an affected establishment, the presence of wild or feral cattle must therefore be considered.

Development of new procedures

The detection of CBPP in wild animals is more complicated than in kept animals because of the practical difficulties and limitations of surveillance and monitoring activities of wildlife in the natural environment.

The surveillance of wild animals (including stray or feral animals) of listed species around an affected establishment may include: (i) visual inspection of these animals from a distance, (ii) clinical examination of trapped animals and (iii) thorough examination of animals found dead or hunted to identify lesions compatible with CBPP and sampling for laboratory analysis by PCR and or isolation according to the procedures described in Section 4.1.1.1 (1st scenario).

In the scenario where wild animals of *Bos* spp. are living in the surrounding area of the affected establishment, and the risk assessment carried out by the Competent Authority may conclude that sampling live animals is necessary, then blood samples may be collected for laboratory analysis with c-ELISA. Wildlife population health experts would be able to provide additional advice in these circumstances.

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

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

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

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

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the 6th scenario.

Assessment

According to the Delegated Regulation, the protection zone for CBPP is limited to the affected establishment while the minimum radius for the surveillance zone is 3 km (Annex V of the Delegated Regulation). This means there is no protection zone as defined for other diseases and only the affected establishment is considered as a protection zone with no other establishments included, not even those neighbouring the affected one.

The absence of a protection zone is not considered effective as explained in Section 4.3.1. The reason is that animals in adjacent establishments can have contact over the fences and the causative agent can be transmitted by air over 200 meters (OIE, 2020).

Development of new procedures

It is advised to implement a protection zone including the establishments adjacent to the infected establishment (e.g. a 1 km zone depending on the local situation). Clinical inspection of animals of the listed species in establishments neighbouring the infected ones is recommended, in addition to animals in herds kept on pastures or yards adjacent to the infected establishment or pastures thereof. Animals should be clinically inspected for signs pointing at CBPP. Moreover, the health history in the establishment should be investigated mainly focusing on the occurrence of clinical signs compatible with CBPP the past half year and the use of antimicrobials effective against CBPP.

Where animals show signs suggestive of acute CBPP, sampling to detect the pathogen should be undertaken as described in 1st scenario in Section 4.1.1.1.

In the absence of animals with clinical manifestation of typical signs, blood should be collected from the holding according to the Table 4 (to detect a 1% seroprevalence with 95% confidence), including all animals with a clinically suspect history.

In the event of negative or inconclusive results, based on the epidemiological situation in the protection zone, the sampling procedures should be repeated after 90 days (duration of the protection zone recommended by the EFSA AHAW Panel see Section 4.3.2), aiming to detect a seroprevalence higher than 1%, e.g. 5%, 10%.

The protection zone can be lifted if all samples prove negative. Increased awareness should be raised in the protection zone in order to enhance passive surveillance and immediate reporting of signs suggestive for CBPP. Animals brought to slaughter (Scenario 9) should be thoroughly examined for CBPP like lesions followed by sampling according to Scenario 9.

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

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

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

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the 8th scenario.

Assessment

According to the Delegated Regulation, the minimum radius for the surveillance zone is 3 km (Annex V of the Delegated Regulation).

Based on EFSA AHAW Panel assessment, a development of the protection zone is recommended as explained in Section 4.3.1 and specific sampling procedures in this zone have been proposed in Section 4.1.1.6.

Development of new procedures

Because *Mmm* is mainly transmitted by direct contact between animals and airborne transmission is not expected to go beyond 200 meters (OIE, 2020), for the surveillance zone, it is recommended that the efforts will be allocated to enhance immediate notification and passive surveillance by increasing awareness in all establishments, industry and public.

In addition, the awareness of the veterinarians at the slaughterhouses should be high when undertaking the ante-mortem animal inspection and post-mortem inspection of the pleural cavity. Animals from establishments located in the surveillance zone should be thoroughly examined at slaughterhouse for CBPP-like lesions followed by sampling in case of suspicion according to the procedures described in Section 4.1.1.1 (1st scenario).

Any establishment, where more generic signs of the disease such as fever, lethargy, lost appetite, feed intake and productivity are reported, should be visited; the animals should be clinically examined and samples should be collected following the procedures described in 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 Sections 4.1.1.1 and 4.1.1.2.

Given the limited transmission, a 3-km zoAne is considered effective and the zone should be implemented for 90 days according to the incubation period of the disease (see Section 4.3.2) and not be lifted before the sampling of all the establishments in the protection zone has been completed with negative results.

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

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

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

- **9th scenario of sampling procedures;**

- ToR 1.4 in accordance with Article 28(5) of the Delegated Regulation (EU) 2020/687;
- Article 29 of the Delegated Regulation;

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 9th scenario in EU legislation.

Assessment

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

During CBPP outbreaks, the following considerations should be included when designing animal movement derogations:

- i) the lack of specificity of clinical examination;
- ii) animals without clinical signs may be incubating CBPP which cannot be detected by laboratory tests; (long incubation period of the disease; more often 3 to 8 weeks but it can be as long as 6 months)
- iii) some animals may become 'carriers' following their exposure and may remain a source of *Mmm*;
- iv) the length of the infectious period is not known;
- v) data on sensitivity and specificity of diagnostic tests are sparse, complicating interpretation of test results and estimates of predictive values;
- vi) identification of infectious animals is often not possible and;
- vii) airborne transmission up to 200 m can occur.

Consequently, sampling procedures (clinical and laboratory) cannot provide a high level of confidence that these animals do not pose a risk for transmission if moved to slaughterhouses.

The highest risk of spread due to movement of undiagnosed animals is associated with sub-scenario c, then b and finally a. Nevertheless, because the destination of these animals is the slaughterhouse, provided all biosecurity measures are implemented and given that the animals should be slaughtered within 24 h, the risk is reduced. In addition, slaughtering animals from the establishments in the protection zone could have a further beneficial effect reducing the number of potential hosts for the further spread of CBPP agent.

Since the lesions in pleural cavity are pathognomonic for CBPP diagnosis, post-mortem inspection at the slaughterhouse is crucial for the detection of the disease.

Development of new procedures

Even though the clinical examination is not specific for CBPP diagnosis, it can identify the animals with respiratory clinical signs.

All the animals in the establishment of origin should be clinically examined before their movement, following the procedures described in the Section 4.1.1.1 and a thorough investigation of the health history of the establishment for at least 90 days (monitoring period recommended by the EFSA AHAW Panel) backwards should be performed to identify any sign compatible to CBPP. In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in that case, the individual clinical examination can be restricted to those animals that are intended to be moved and the whole establishment should be visually inspected for clinical signs from respiratory system.

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

At the slaughterhouse, a thorough post-mortem inspection should be routinely performed on animals coming from the protection zone, to identify the lesions of CBPP. Any suspect lesion attributable to CBPP should be further investigated with laboratory examinations to rule out the presence of *Mmm* following the procedures described in the 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 (Art. 37). For further details, see Annexes B and C.

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

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

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

Summary of sampling procedures

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

Assessment

This scenario is very similar to the 9th scenario of the Section 4.1.2.1; therefore, the assessment is the same.

Development of new procedures

This scenario is very similar to the 9th scenario of the 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 article 43(5) and article 44 of the Delegated Regulation (EU) 2020/687;

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

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

Summary of sampling procedures

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

Assessment

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

The same considerations as described in the 9th scenario should be included in the assessment when designing animal movement derogations.

Development of new procedures

This scenario is very similar to the 9th scenario of the Section 4.1.2.1, and therefore, the procedure is the same.

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

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

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

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

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

Summary of sampling procedures

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

Assessment

The same considerations as described in the 9th scenario should be included in the assessment when designing animal movement derogations.

Consequently, sampling procedures (clinical and laboratory) are not able to ensure with a high level of confidence that animal movements to pastures do not pose a risk for transmission.

Development of new procedures

The animal movements from the establishments located in the surveillance zone to pastures within the surveillance zone should be allowed once the first clinical inspection of the establishments in the protection zone have been completed and the results of the initial laboratory tests in these establishments are negative.

All the animals in the establishment of origin should be clinically examined before their movement, following the procedures described in the Section 4.1.1.1 and a thorough investigation of the health history of the establishment for at least 90 days (monitoring period recommended by the EFSA AHAW Panel) backwards should be performed to identify any sign compatible to CBPP. In an establishment, where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in that case, the individual clinical examination can be restricted to those animals that are intended to be moved and the whole establishment should be visually inspected for clinical signs from respiratory system.

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

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

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

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

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

- 1) It concerns the surveillance zone;
- 2) Grant derogation for movement of kept animals of listed species from the surveillance zone;
- 3) To be moved to an establishment belonging to the same supply chain, located in or outside the surveillance zone, to complete the production cycle before slaughter;
- 4) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved.

Summary of sampling procedures

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

Assessment

The same considerations as described in the 9th scenario should be included in the assessment when designing animal movement derogations.

Consequently, it is very difficult to develop sampling procedures that will ensure with a high level of confidence that the disease will not spread if live animals are allowed to be moved.

Moreover, it is noteworthy to emphasise that allowing movements from establishment in a surveillance zone to an establishment belonging to the same supply chain, located outside the surveillance zone may increase the risk of CBPP expansion outside the surveillance zone.

Development of new procedures

The animal movements from the establishments located in the surveillance zone to an establishment belonging to the same supply chain should be allowed once the first clinical inspection of the establishments in the protection zone have been completed and the results of the initial laboratory tests in these establishments are negative.

All the animals in the establishment of origin should be clinically examined before their movement to an establishment belonging to the same supply chain, following the procedures described in the Section 4.1.1.1. In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible. In that case then clinical inspection of the whole establishment and thorough investigation of the health history of the establishment for at least 90 days backwards should be performed to identify any clinical sign compatible with CBPP.

In case clinical signs compatible with CBPP have been identified in the last 90 days, the establishment is considered suspect and the procedures for the laboratory confirmation as described in the Section 4.1.1.1 should be followed and any movements should be prohibited.

In addition to clinical examination, a minimum sample of animals (including all animals to be moved) should be tested with c-ELISA as described in the Section 4.1.1.1 based on the total number of animals in the establishment.

Additional measures are recommended also for the establishment of destination, where the animals should be tested again with c-ELISA 90 days after their introduction. Moreover, during that period, animal movements from the establishment of destination, slaughterhouses excluded, should not be allowed.

Nevertheless, the EFSA AHAW Panel considers that, given the current available laboratory tests, it is very difficult to state with high confidence, that live animals without clinical signs and with negative results in serological tests do not pose a risk of transmission, and therefore live animals movements from the surveillance zone outside the restricted zone should be discouraged.

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

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

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

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

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

Summary of sampling procedures

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

Assessment

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

Development of new procedures

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

4.1.3. Assessment of sampling procedures for repopulation purposes

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

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

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

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

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

Summary of sampling procedures

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

Assessment

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

When designing the sampling procedures for repopulation the following elements should be taken into consideration:

- i) the lack of specificity of clinical examination;
- ii) animals without clinical signs may be incubating CBPP which cannot be detected by laboratory tests; (long incubation period of the disease; more often 3 to 8 weeks but it can be extended up to 6 months);
- iii) some animals may become 'carriers' following their exposure and may remain a source of *Mmm*;
- iv) the length of the infectious period is not known;
- v) data on sensitivity and specificity of diagnostic tests are sparse, complicating interpretation of test results and estimates of predictive values;
- vi) identification of infectious animals is often not possible and
- vii) airborne transmission up to 200 m can occur.

Development of new procedures

All the animals in the establishment of origin (if belonging to the restricted zones) should be clinically examined before movement, following the procedures described in the Section 4.1.1.1. In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible. In that case clinical inspection of the whole herd and thorough investigation of the health history of the establishment for at least 90 days backwards should be performed to identify any symptom compatible to CBPP.

In case clinical signs compatible with CBPP are identified, the establishment is considered suspect and the procedures for the laboratory confirmation as described in the Section 4.1.1.1 should be

followed. The animals intended for the repopulation, even if clinically healthy, should not be dispatched.

If animals intended to repopulate previously affected establishments originate from areas outside the restricted zones of CBPP, there is no need for laboratory examination if there are no other reasons based on the authority risk assessment to recommend it (e.g. epidemiological link with an affected establishment or with an affected or high-risk area). Clinical examination as described above would be enough.

When animals originate from restricted areas established around different index cases, in addition to clinical examination a minimum sample of animals (including all animals to be moved) should be tested with c-ELISA as described in the Section 4.1.1.1 based on the total number of animals in the establishment before the movement.

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

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

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

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

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

Summary of sampling procedures

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

Assessment

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

Development of new procedures

In the event of animals with clinical signs, or deaths, or lesions compatible with CBPP, as they have been described in Section 4.1.1.1, being identified in an establishment during the repopulation, the establishment is considered suspect. The repopulation should be stopped and the procedures for the laboratory confirmation as described in the Section 4.1.1.1 should be followed.

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

4.1.3.3. For animals that have been repopulated

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

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

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

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

Summary of sampling procedures

No specific guidelines on sampling procedures for laboratory examination were found for the 21st scenario.

Assessment

During the repopulation of an establishment previously affected by CBPP, there is still a risk of re-introduction of the disease with the new animals being infected either at the establishment of origin or during their transport, and a risk of re-emergence of the disease if the new animals are infected after their arrival at the establishment of destination.

The animals that have been used for the repopulation should be submitted to thorough clinical and laboratory examination in order to rule out the presence of the disease.

Development of new procedures

Animals must be subjected to clinical inspection weekly starting 3 weeks after introduction to the establishment up to 90 days (monitoring period as recommended by EFSA AHAW Panel) after introduction. The last day of the monitoring period following the latest day of the animal introduction, all the animals should be subjected to thorough clinical examination.

If clinical signs are identified, then the procedures for the laboratory confirmation that are described in the Section 4.1.1.1 should be followed. In addition, the establishments from where the suspected or confirmed animals coming from, should be considered as suspects. The procedures that are described in Section 4.1.1.1 should be followed as well in the establishments of origin.

Laboratory examination is not recommended if there are no clinical signs and there are no other reasons based on the authority risk assessment to recommend it.

4.2. Assessment of the length of the monitoring period

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

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

Annex D in this Opinion describes the seven scenarios, for which an assessment of the length of the monitoring period for CBPP had been requested.

For the assessment of this ToR, the methodology described in Section 2.3 of the Technical Report published by EFSA (2020) was followed. In essence, in order to assess the length of the monitoring period, the purpose of this monitoring period for each of the scenarios was ascertained.

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

To answer Scenario 5, a literature search was conducted by EFSA on the seroconversion period, as well as the earliest time of antibody detection in blood, with the outputs being discussed with relevant experts.

4.2.1. Results

Extensive Literature Search

The search was carried out on 10/06/2021 identifying 98 unique references. As no references were available for outbreak data from the EU/EEA, the search was extended to data from the rest of the world and to simulation data. Among the 98 references, six were selected to be included in the qualitative review. The full selection process is displayed in Figure 8.

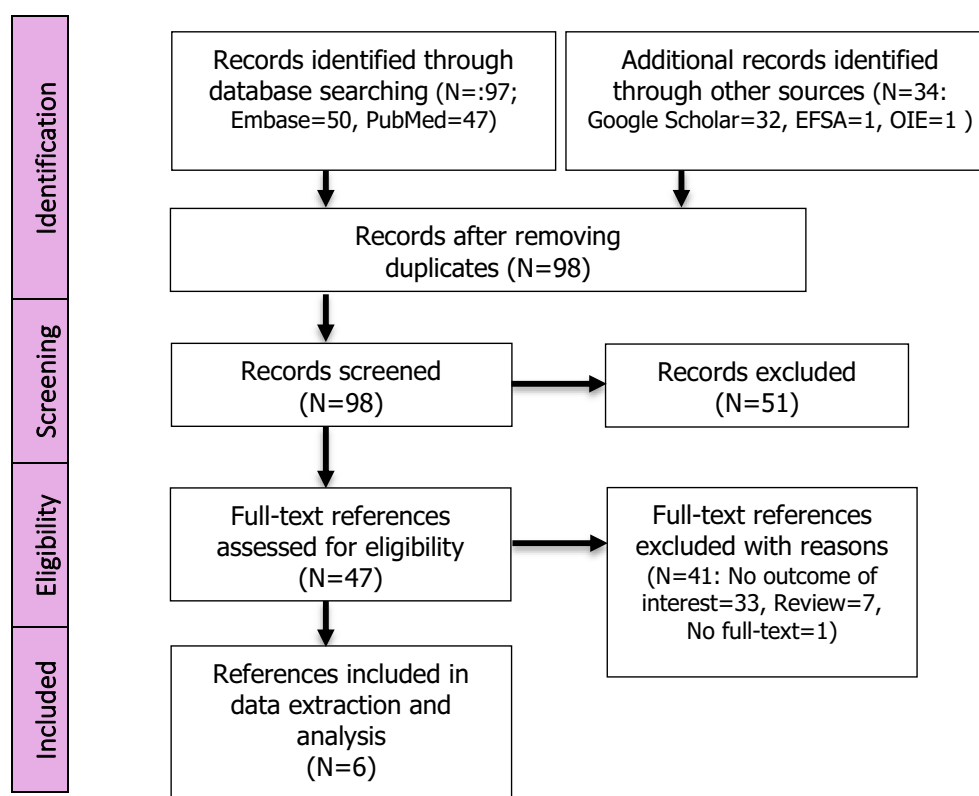


Figure 8: PRISMA diagram CBPP Monitoring period ELS

Four of the six references reported dates instead of periods. Therefore, the dates were used to calculate the different periods of interest (as described in Section 2.1 – PICOS table).

Tables 5 and **6** 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 three references were retrieved:

Table 5: Summary of the CBPP extraction for the period between earliest point of infection and suspicion report: Outbreak data

Reference	Country	Year	Host/Breed	Period (days)
ProMED (2003)	Eritrea	2002	Cattle/Raya-Azebo	61 ⁽¹⁾
ProMED (2004)	Democratic Republic of Congo	2004	Cattle/Ankole longhorn	108 ⁽¹⁾

(1): Primary outbreak.

Table 6: Summary of the CBPP extraction for the period between earliest point of infection and suspicion report: Simulation data

Reference	Country	Year	Host/breed	Period (days)
EFSA AHAW Panel (2017)	EU	NA	NA	90 ⁽¹⁾

NA: not applicable.

(1): Assumption used in the context of the time interval between cycles of monitoring and culling; defined as the time needed to allow animals in the incubation period to show symptoms and to be detected either by clinical surveillance or by serology.

As described in **Table 5** (outbreak data), the shortest period between the earliest point of infection and the suspicion report was 61 days. It was found in the context of a primary outbreak that occurred in 2002 in Eritrea, where CBPP was well known for a long time (endemic areas or already affected). The outbreak source consisted of a group of adult Raya-Azebo trade cattle imported illegally into Eritrea (ProMED, 2003). The longest period, 108 days, was retrieved in a reference reporting a primary outbreak, which occurred in 2004 in the Ituri region of the Democratic Republic of Congo (DRC) among Ankole longhorn cattle. This DRC region is bordering CBPP-infected countries. The source of the outbreak is described in the reference as the return of war of displaced people and refugees that led to uncontrolled cattle movements (ProMED, 2004).

The extracted values for ($n = 3$) (Tables 5 and 6) can be summarised as follows:

- 1) Average (mean) period = 86 days (median = 90 days).
- 2) Shortest period = 61 days.
- 3) Longest period = 108 days.

Although it is important to bear in mind that the results from Eritrea and RDC are not directly transferable to the EU context, based on the available information, the shortest reported period between infection and transmission is 61 days (longer than the monitoring period of 45 days defined in the legislation) and in an area where the disease was well known (endemic areas or already affected). Moreover, the OIE reports an incubation period of 3 weeks to 6 months, with most cases becoming apparent in 3–8 weeks (OIE, 2020).

Seroconversion in animals

Several publications describing experimental infection with *Mmm* were consulted (Table 7) and the time of seroconversion after infection/inoculation and contact was retrieved from the serological results described. Nevertheless, these studies were not designed to estimate the time between infection and seroconversion (first time when antibodies can be detected) and they can only provide an estimation. In addition, animals that were infected by endobronchial intubation (EBI) with the highly virulent Gladysdale strain usually died within the first 1 to 2 weeks post-infection, prior to antibody production, thus limiting the amount of scientific information which may be obtained from these studies (Garba and Terry, 1986; Thiaucourt et al., 2000; Wesonga and Thiaucourt, 2000).

In experimental studies (Table 7), where non-vaccinated naïve cattle were infected by EBI the latest day of seroconversion identified was: (i) 3 weeks post-infection by CFT (Hübschle et al., 2003) and (ii) within the first 2–3 months post-infection by c-ELISA (Nkando et al., 2016).

In experimental studies (Table 7), where non-vaccinated naïve cattle were infected through direct contact with animals infected with *Mmm* through EBI, the latest day of seroconversion identified was 30 weeks post-contact both by CFT and c-ELISA (Niang et al., 2006). It should be mentioned that the time of seroconversion has been calculated forwards from the day when the animals joined the infected animals and not from the day of infection, which is not known. This fact may explain the delay in detection of seroconversion in these experiments.

Table 7: Range of days/weeks for seroconversion and latest day of antibody detection in cattle after experimental infection with *Mycoplasma mycoides* subsp. *mycoides*

Laboratory method	Infection	Blood collection	Range of days/weeks for seroconversion (dpi ⁽¹⁾)		Latest day/week of antibodies detection/end of experiment	Reference
			Earliest day/week of seroconversion	Latest day/week of seroconversion		
CFT	EBI	Weekly	3 weeks post-challenge	NS	8 weeks post-challenge	Hübschle et al. (2003)
		Weekly	7 days post-challenge	13 days post-challenge	60 days post-challenge	Scacchia et al. (2007)

Laboratory method	Infection	Blood collection	Range of days/weeks for seroconversion (dpi ⁽¹⁾)		Latest day/week of antibodies detection/end of experiment	Reference
			Earliest day/week of seroconversion	Latest day/week of seroconversion		
		Monthly	Not clear. Probably within the first 2-3 months after challenge, since 53% of challenged animals were positive at 3 months post-challenge. Not all challenged animals seroconverted.		16 months post-challenge	Nkando et al. (2012)
		1 blood sample	2 weeks post-challenge	NS	NS	Nkando et al. (2016)
	In-contact with EBI-infected animals	Weekly	5 weeks post-contact	7 weeks post-contact	22-25 weeks post-contact	Gray et al. (1986)
		Weekly	7 weeks post-contact	15 weeks post-contact	15 weeks post-contact	Hübschle et al. (2003)
		Weekly	11 weeks post-contact	30 weeks post-contact	46 weeks post-contact	Niang et al. (2006)
		Weekly	14 days post-contact	42 days post-contact. 1 animal was a late reactor at 77 dpi	77 days post-contact	Scacchia et al. (2007)
c-ELISA	EBI	Weekly	Not clear. Probably within the first 2-3 months post-challenge, since 53% of challenged animals were positive at 3 months post-challenge. Not all challenged animals seroconverted.		16 months post-challenge	Nkando et al. (2012)
	In-contact with EBI-infected animals	Weekly	11 weeks post-contact	30 weeks post-contact	16 months post-contact	Niang et al. (2006)
SAST	In-contact with EBI-infected animals	Weekly	5 weeks post-contact	10 weeks post-contact	20-25 weeks post-contact	Gray et al. (1986)
PMPT	In-contact with EBI-infected animals	Weekly	3.5 weeks post-contact	NS	20-25 weeks post-contact	Gray et al. (1986)

CFT: complement fixation test; c-ELISA: competitive ELISA; SAST: slide agglutination serum test; PMPT: passive mouse-protection test; NS: not specified; EBI: endobronchial intubation.

(1): dpi: days post-infection/inoculation.

In the experimental studies mentioned above, the antibodies remained detectable until the end of the observation period of the trial or until the animals were euthanised or died. The longest period of antibody detection found in experimental studies was 46 weeks (≈ 322 days) by CFT (Gray et al., 1986; Hübschle et al., 2003; Niang et al., 2006; Scacchia et al., 2007) and 16 months (≈ 480 days) by c-ELISA (Niang et al., 2006) and it was the end of the follow up period of the trial.

However, not all challenged animals (EBI or in-contact animals) seroconvert, and some remain seronegative for the entire period of study. Therefore, serological tests for CBPP are reliable at the herd level only, since tests on single animals may be misleading (OIE, 2018). Negative serological results have to be carefully interpreted, especially in acute or peracute cases, where death occurs and in subclinical or chronic forms with nonspecific clinical signs. Additional laboratory tests are necessary to be implemented (PCR, post-mortem and bacteriological examinations and other serological tests, performed in the same or other animals) (FAO, 2007; OIE, 2018; Di Teodoro et al., 2020). When CBPP

prevalence is very low or zero, CFT-positive samples can usually be confirmed using the western-blotting (EFSA AHAW Panel, 2017).

4.2.2. Assessment

Considering the results presented above, an assessment of the effectiveness of the monitoring period for CBPP, depending on the purpose of that period in the different scenarios shown in Annex D, was carried out. For CBPP, the length of the monitoring period as defined in Annex II of the Delegated Regulation is 45 days.

Scenarios 1, 2 and 3

1st scenario of monitoring period

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

2nd scenario of monitoring period

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

3rd scenario of monitoring period

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

For the first three scenarios, the main purpose of the use of the monitoring period is to be able to carry out a full epidemiological investigation (i.e. in scenarios 1 and 2, at the time of the suspicion and confirmation, respectively), or part of the epidemiological investigation (i.e. scenario 3, where the aim is to identify any possible epidemiological links between the affected establishment and any separated non-affected epidemiological units).

The length of the monitoring period should then dictate how far, backward or forward, tracing activities (and other activities needed during an epidemiological investigation) should go (checks for production records, animal movement records, etc.). This monitoring period is the time, where the infection could have been present and remains undetected in an establishment, and due to the regular activities carried out in this establishment, could have spread to other epidemiological units.

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

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

According to the ELS presented above, there are no available data from the Europe and the available information is very limited and restricted to Eritrea and DRC that cannot represent the European conditions. Nevertheless, in Eritrea where the disease was well known (e.g. endemic or affected) and in areas of DRC bordering CBPP affected countries, the suspicion of the disease was

reported 61 and 108 days accordingly after the infection. However, the incubation period defined by OIE is from 3 weeks to 6 months with most cases becoming apparent in 3–8 weeks (OIE, 2020). Therefore, the length of the monitoring period of 45 days as defined in the Delegated Regulation is short and is not considered effective to capture the period between the earliest point of infection and the suspicion. Based on the available evidence and expert opinion, **it was concluded with a 70–100% certainty that out of all CBPP-infected herds in an already affected region, infection would have occurred within the 90 days before the suspicion report, and therefore a monitoring period of at least 90 days is recommended in areas where the disease is present** (QoI 2b). There is some uncertainty mostly related with the difficulties in assessing the sensitivity of the surveillance activities for detection of newly infected herds. This uncertainty is much larger in the case of the index case in a region where the disease was not known to circulate, since in this context a low awareness is expected; because of this, **in the case of previously CBPP-unaaffected regions, this period should be extended to at least 180 days (6 months) for index cases** (QoI 2a).

In the case of independent epidemiological units within CBPP affected cattle establishments that eventually become infected (QoI 2c) the certainty of the efficacy of the 90 days proposed monitoring period is limited due to potential complications to maintain truly independent epidemiological units within an affected cattle establishment for such a long period of time. Nevertheless, considering the very low risk of CBPP-transmission due to the effect of fomites, and provided that units are truly independent, **it was concluded with a 66-90% certainty that all independent epidemiological units within the CBPP affected cattle establishments that eventually became infected, would have been infected within the 90 days prior to the date of suspicion and that this monitoring period could be considered effective.**

Scenario 4

4th scenario of monitoring period

- ToR 2 in accordance with article 27(3)c and Annex II of the Delegated Regulation (EU) 2020/687;
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of the CBPP outbreak in the protection zone. Products or other materials likely to spread the disease, must have been obtained or produced, before this time period in order to be exempted from prohibitions of movements.

The main purpose of the monitoring period in scenario 4 is to ensure that certain products or materials, likely to spread the disease, that have been produced in a non-affected establishment located in the protection zone of an affected establishment, can be moved safely and without posing a risk of disease spread. In this scenario, and in contrast with the previous three scenarios, the establishment of concern is neither a suspect nor an affected establishment, but restrictions are still in place, for establishments in the protection zone.

For the assessment of this scenario, we assume that the earliest plausible point of infection of these products or materials in the establishment of concern would be the earliest plausible point of infection of the establishment that originated the protection zone. If these products have been obtained or produced before the earliest point of infection of the affected establishment, then they could be exempted from prohibitions to be moved, as long as other conditions specified in the legislation are met (e.g. the products must have been clearly separated during the production process, storage and transport, from products not eligible for dispatch outside the restricted zone).

Even if the disease has already been detected in the area, and high awareness is expected, the length of the monitoring period of 45 days is not considered effective in this scenario. Therefore, a longer monitoring period of at least 90 days (3 months) is recommended while for the early phases of the outbreak in the area where the awareness is low (for example, if the index case was in a slaughterhouse and the epidemiological enquiry is taking time) the monitoring period should be at least 180 days (6 months).

Because the assessment of the effectiveness of the proposed alternative monitoring period is subjected to the same uncertainties described for scenarios 1, 2 and 3, the same certainty regarding its effectiveness was reached for scenario 4.

Scenario 5

5th scenario of monitoring period

- ToR 2 in accordance with article 32 (c), article 48(c) and Annex II of the Delegated Regulation (EU) 2020/687;
- The purpose of this Section is to assess the effectiveness of the length of the Monitoring Period, as the time period calculated forwards from the date of semen collection from animals of listed species kept in approved germinal product establishments in the protection or in the surveillance zone, to prove that the donor animal has tested favourable on a sample taken not earlier than 7 days after the monitoring period.

The aim of the monitoring period is to ensure that semen from animals in the non-affected establishments (located in a protection or surveillance zone) that has been collected and frozen after the earliest time of infection of the affected establishment that originated the protection zone, is safe to be moved without posing a risk of disease spread. In this scenario, EFSA is requested to assess the length of time, after the semen was taken, when the animal should be tested in order to allow that semen to be moved. Here, it is assumed that the earliest point of infection of the animal would be on, or after the earliest point of infection of the affected establishment that originated the protection zone, and the latest date the semen could have become contaminated would be the date the semen was collected.

Mmm has been isolated and identified in semen of bulls. Stradaoli et al. (1999) have isolated and identified by PCR methods *Mmm* in semen from serological negative bulls without clinical signs derived from an outbreak in 1994 in Italy. In addition, *Mmm* strains have been isolated in semen from bulls collated in Portugal in 1991 (Cheng et al., 1995).

Nonetheless, the limited number of the available experimental studies and the small number of animals included, suggest further investigation on the role of semen in the transmission of the disease. In addition, the lack of field studies cannot conclude on the significance of the semen in natural transmission of CBPP.

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

The latest date of seroconversion for non-vaccinated, naïve animals infected through contact with already infected animals was identified by CFT and c-ELISA as 30 weeks (7.5 months) post-contact with infected animals and 45 days after EBI infection as reported by Niang et al. (2006) and Nkando et al. (2012) accordingly.

Considering that results from serological tests for CBPP should be interpreted at a herd level and not at individual animals; use of c-ELISA at the establishment from where the donor is coming from (45 days+7) days after semen collection as foreseen in the Delegated Regulation is not considered effective to detect antibodies, given that the infection may have occurred at the latest on the day of semen collection. Therefore, a longer monitoring period of at least 90 days (3 months) is recommended while for the index case in the area where the awareness is low the monitoring period should be at least 180 days (6 months).

Scenarios 6 and 7

6th scenario of monitoring period

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

7th scenario of monitoring period

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

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

In Scenario 6, the monitoring period is used to ensure that the repopulation process is not put at risk due to the disease still being present unknowingly in establishments within the surrounding area of the establishment to be repopulated (if an establishment tested positive to CBPP within a distance equal or lower to the radius of the surveillance zone, the repopulation process could not take place).

Repopulation can only take place after a period equal to the monitoring period has elapsed, since the final cleaning, and disinfection of the affected establishment.

In this regard, the number of days of the monitoring period for CBPP, counted from the day of the final cleaning and disinfection, must ensure enough time for any potentially affected surrounding establishment to be reported as a suspicion. Considering the results presented in Section 4.1.2, and taking into account that a high level of awareness is expected due to the disease having been present in the area, the EFSA AHAW Panel considers the existing length of the monitoring period (45 days) is not considered effective, as it would not allow the identification of any potentially affected establishment in the surrounding area prior to the repopulation taking place.

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

The aim of the monitoring period in this scenario is to ensure the early detection of any potentially recently infected animal intended for repopulation once all animals have been moved into the repopulated establishment. Although the preferred option is that all animals are introduced into the establishment to be repopulated at the same time, this is not always feasible. The first clinical and laboratory sampling of the repopulated animals takes place once all the animals are in situ. By restricting the period of time during which animals may be introduced into the establishment, the period of time during which the disease could be unknowingly spreading within the establishment is reduced. Assuming that the latest point of infection of an animal introduced into the repopulated establishment is the day when it is moved, and considering that the shortest length of time to detection found in the literature is 61 days, it would be likely that clinical signs might not be present in animals if this visit is carried out 45 days after the last introduction. The EFSA AHAW Panel considers the existing length of the monitoring period (45 days) not effective, as it would not allow the early detection of potentially infected animals at the first visit following re-stocking.

Based on the available evidence, it was concluded with a 90–100% certainty that 95% or more of all repopulated CBPP-affected cattle establishments that become reinfected would be infected within the 90 days following the introduction of the animals. Therefore, a longer monitoring period of at least 90 days is recommended.

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

4.3.1. Assessment of the minimum radius

The purpose of this Section is to assess the effectiveness to control the spread of CBPP of the minimum radius of the protection and surveillance zones as set out in Annex V of the Delegated Regulation for CBPP. According to this regulation, protection zone is at the level of establishment and the minimum radius for the surveillance zone is 3 km for CBPP (Annex V of the Delegated Regulation).

Results

No transmission kernels either specific for CBPP or for diseases that have similar transmission routes to CBPP were found in the literature, nor were data suitable to estimate kernels identified. Accordingly, the zone sizes for CBPP were assessed using expert knowledge.

Assessment

Since transmission kernels are not available to allow an estimation of CBPP transmission beyond an affected establishment, given that the transmission occurs, the assessment of the effectiveness of the length of the radius of the surveillance zone and the fact that only the affected establishment constitutes the protection zone (in fact there is no protection zone), cannot be quantified. Based on the WG expert opinion, the absence of a protection zone is not considered effective. The reason is that

animals in adjacent establishments can have contact over the fences and the causative agent can be transmitted by air up to 200 meters (OIE, 2020).

It is advised to develop a protection zone including the establishments adjacent to the infected establishment or establishment with pastures or yards adjacent to the infected one (e.g. 1 km zone depending on the local situation and the establishments distribution). All the establishments in the protection zone should be visited and clinical inspection of cattle is recommended. **It was concluded with a 90–100% certainty that in 95% or more of all the protection zones built as proposed, transmission would not occur beyond the zone, and therefore it would be considered effective.**

Taken into consideration that *Mmm* is mainly transmitted by direct contact between animals and airborne transmission is not expected to go beyond 200 meters (OIE, 2020), **it was concluded with a 95–100% certainty that in 95% or more of all the surveillance zones transmission would not occur beyond a 3 km radius, and therefore the length of the radius of the surveillance zone is considered effective.**

Nevertheless, transmission across longer distances cannot be excluded if infected animals are moved outside the zones.

4.3.2. Assessment of the minimum period

The purpose of this Section is to assess the effectiveness to control the spread of CBPP of the minimum periods during which the competent authority should apply the restriction measures in the protection and surveillance zones as set out in Annexes X and XI for the CBPP. The minimum period for the protection zone and the surveillance zone is 45 days.

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

Based on the results of the ELS as presented in the Section 4.2.1, it follows that the average time between infection and notification of the suspicion is 86 days (median = 90 days) and the shortest period reported is 61 days (longer than the one defined in the legislation monitoring period of 45 days). Moreover, the OIE reports an incubation period of 3 weeks to 6 months, with most cases becoming apparent in 3–8 weeks (OIE, 2020).

Consequently, the minimum period of 45 days indicated in the Delegated Regulation for the restriction measures in the protection and surveillance zone is not considered effective to detect effected establishments and to prevent the movement of infected animals from the protection zone. Therefore, a longer duration of at least 90 days (3 months) is recommended, since it is concluded with a 90–100% certainty that this minimum period would allow the detection of 95% or more of the new outbreaks due to infections starting before control measures were implemented.

4.3.3. Uncertainty analysis

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

5. Conclusions and Recommendations

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
ToR 1: In the event of suspicion or confirmation			
1st scenario Section 4.1.1.1 In the event of a suspicion of CBPP in an establishment where animals of the listed species are kept	See Annex C	<p><u>Clinical examination and Inspection of Lesions</u></p> <p>The clinical signs are not pathognomonic to CBPP but are similar to very common respiratory diseases in bovine establishments. In the chronic phase, the clinical signs are mild and cannot be easily detected.</p> <p>The lesions identified in lungs and in the thoracic cavity in carcasses are considered pathognomonic and can play a crucial role to the diagnosis since they contain a large amount of <i>Mmm</i>. In case of antibiotic treatment, typical lesions may not be observed, and the results of the laboratory examinations may be affected.</p> <p>In non-endemic areas or in areas away from the affected ones, clinical signs most likely will not trigger the suspicion of CBPP; other more common respiratory diseases will be suspected and probably treated with antimicrobials. The suspicion is usually triggered at the slaughterhouses during post-mortem inspection of the lungs and the thoracic cavity or during necropsy of dead animals submitted to post-mortem examination.</p> <p>In endemic, affected, or close to the affected areas, when the awareness is higher, clinical signs may raise a suspicion of CBPP. No data on the sensitivity and specificity of clinical examination exist in the literature: i) the specificity cannot be considered high and ii) the sensitivity decreases when the animals enter the chronic phase.</p> <p>For the purposes of this assessment the sensitivity of clinical examination is considered 90% and the specificity 80%.</p> <p><u>Laboratory examination</u></p> <p>c-ELISA is highly specific ($Sp > 99.5\%$) and the sensitivity can be considered 70%.</p>	<p>Clinical examination is recommended to identify animals most suitable for sampling for further laboratory examinations.</p> <p>Samples from dead animals with a history of respiratory disease and preferably without receiving antimicrobial treatment (lungs with lesions, regional lymph nodes and pleural fluid when available) should be collected to be cultured and/or tested with PCR.</p> <p>In addition to dead animals, or if dead animals are not available for sampling, animals with clinical signs associated with CBPP are recommended to be killed for further examinations: i) up to 20 animals to be tested in LAT and c-ELISA, and ii) at least five animals positive in LAT should be killed and submitted for post-mortem inspection, to identify the pathognomonic lesions and to collect samples to be tested by PCR to detect an outbreak.</p> <p>In the event that all 20 samples are negative by LAT it can be considered that the animals are in the later stages of infection and no more IgM is present. In that case there will be some c-ELISA positives.</p> <p>If both the LAT and c-ELISA are negative, it is very, if not extremely unlikely that the clinical signs are caused by <i>Mmm</i>. Restrictions on the establishment are maintained until negative testing with c-ELISA and further clinical examination of the animals can resolve the suspicion. To further increase the certainty of absence of CBPP a random sample of animals (according to Table 4) could be tested by c-ELISA (which will detect 'older infections').</p> <p>The visit to the establishment and the sampling procedures should be repeated after a period of 90 days to have a very high certainty of the absence of the infection.</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
		<p>For the laboratory methods for CBPP there are no proper validation studies to estimate the performance (sensitivity, specificity) of these methods in conditions similar to EU Countries (free from the disease) where the prevalence of CBPP in the establishments is expected zero or very low (in case of occurrence).</p> <p>The quality of the samples and the conditions of their transport to the laboratory, may affect the final diagnosis.</p>	<p>It was concluded with a 90-100% certainty that the proposed sampling strategy would be able to detect the infection in 95% or more of all CBPP affected establishments in which suspicion was triggered due to the occurrence of clinical signs or dead animals resembling CBPP.</p> <p>In the <u>absence of clinical signs</u> collecting serum samples to be tested by c-ELISA allowing the detection of a 1% design prevalence with 95% confidence, is recommended. In case of negative or doubtful results, based on the epidemiological situation and the risk assessment contacted at national level, the sampling procedures should be repeated at least 90 days later.</p> <p>In case of the absence of clinical signs, it was concluded with a 90-100% certainty that the proposed sampling strategy would be able to detect the infection in 95% or more of all CBPP affected establishments in which the suspicion is raised in an establishment in the absence of clinical signs or CBPP-related mortality.</p> <p>EFSA AHAW Panel recommends also some additional actions that will increase the level of confidence to CBPP diagnosis: i) the validation of the performance of the existing laboratory methods, ii) the development of alternative to c-ELISA and LAT serological methods, and iii) the nomination of EURL for CBPP, in order to support the preparatory activities of the NRL of the EU Countries..</p>
2nd scenario Section 4.1.1.2. For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU)	No specific guidelines on sampling procedures for clinical or laboratory examination	<p><u>Epidemiological enquiry</u></p> <p>Due to the difficulties with determining the age of lesions or the true prevalence on an affected establishment initial enquiry should investigate movement records and an interview with the owner.</p>	<p><u>Epidemiological enquiry</u></p> <p>Additional samples for c-ELISA can be collected in a confirmed affected establishment to investigate the distribution of infected animals in the establishment.</p> <p>Isolation and sequencing are recommended to determine the origin of the Mmm and to perform a retrospective study.</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
2016/429 in an CBPP officially confirmed establishment	were found for the 2nd scenario.	<p>Molecular methods such as whole genome sequencing may be used to determine the geographical origin of the pathogenic agent.</p> <p><u>Preventive Killing</u></p> <p>Confirm and rule out the disease in case of preventing killing will be based on clinical and laboratory examination of the animals.</p>	<p><u>Preventive Killing</u></p> <p>In case of preventive killing, all animals should be subjected to clinical examination and in case of clinical signs the procedures described in the 1st Scenario should be followed.</p> <p>In case of no clinical signs:</p> <ul style="list-style-type: none"> i) lungs should be inspected to detect lesions in culled animals (acute lesions may be pathognomonic) ii) cultivation of mycoplasmas from lung lesions and regional lymph nodes. iii) PCR from lung lesions, pleural fluid, and regional lymph nodes iv) collecting blood samples for c-ELISA according to Table 4.
3rd scenario Section 4.1.1.3 For granting a specific derogation from killing animals of the categories of article 13.2 of the Delegated Regulation in an CBPP affected establishment	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 3rd scenario.	<p>In an CBPP affected establishment, the following considerations should be taken into account when designing derogations from killing animals in CBPP affected establishments:</p> <ul style="list-style-type: none"> i) the lack of specificity of clinical examination; ii) animals without clinical signs may be incubating CBPP which cannot be detected by laboratory tests; (long incubation period of the disease; more often 3 to 8 weeks but it can be extended up to 6 months) iii) some animals may become 'carriers' following their exposure and may remain a source of <i>Mmm</i>; iv) the length of infectious period is not known; v) data on sensitivity and specificity of diagnostic tests are sparse, complicating interpretation of test results and estimates of predictive values; vi) the identification of infectious animals is often not possible; vii) airborne transmission up to 200 m may occur. <p>Consequently, sampling procedures (clinical and laboratory) cannot provide a high level of confidence that individual animals do not pose a risk for transmission if they are kept alive.</p>	<p>All animals intended for derogation from killing should be subjected to thorough individual clinical examination and samples for laboratory examination with serological tests (c-ELISA, LAT) should be collected from all the animals irrespective of the presence of clinical signs. Any animal testing positive should be killed.</p> <p>Regular clinical examination should be carried out, preferably every week for the first 45 days to detect early the onset of clinical signs and then every 45 days. Sampling for laboratory examination can be repeated at least every 45 days combined with the clinical examination for all the animals in the establishment. This procedure should be carried out for at least one-year calculated forwards from the day of confirmation of the latest case within the establishment.</p> <p>The animals with clinical signs and/or those found positive to serological tests should be culled, and thorough post-mortem inspection should be implemented.</p> <p>Samples from carcasses (lung lesions, pleural fluid, lymph nodes) could additionally be examined by PCR and isolation to detect or rule out the presence of <i>Mmm</i>.</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
			<p>Sampling procedures for laboratory examinations in order to detect or rule out the presence of <i>Mmm</i> should follow the procedures described in the Section 4.1.1.2.</p> <p>The EFSA AHAW Panel considers that, given the currently available laboratory tests, it is very difficult to provide a high level of confidence that the animals from an affected establishment without clinical signs and with negative results in serological tests do not pose a risk of transmission and therefore this practice should be discouraged.</p>
4th scenario Section 4.1.1.4 For the animals of non-listed species kept in a CBPP affected establishment.	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 4th scenario.	<p>There is no evidence to demonstrate the epidemiological involvement of other non-listed species in the spread or maintenance of CBPP in the field.</p> <p>A few publications report on sheep and goats infected with <i>Mmm</i>, A sheep or goat reservoir is almost impossible (0-1%), but transmission from cattle to small ruminants cannot be ruled out, where these two groups are in close contact.</p> <p>The lack of information on the performance of laboratory tests (sensitivity, specificity) for animal species other than cattle along with the lack of validation of the diagnostic methods in them will increase the uncertainty on the reliability of the sampling strategy.</p>	<p>If clinical signs or deaths occur in animals of non-listed species, such as sheep and goats, kept in an affected establishment, samples from these animals should be collected for further laboratory examinations following the procedures of the 1st Scenario in Section 4.1.1.1.</p>
5th scenario Section 4.1.1.5 For wild animals of the listed species within the CBPP affected establishment and its surroundings.	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 5th scenario.	<p>The literature search for CBPP in the other listed species of <i>Bison</i> spp. and <i>Bubalus</i> spp. have not yielded any evidence for infection, seroconversion or clinical manifestation of the disease. Only one study with CBPP experimental infection in African buffalo (<i>Syncerus caffer</i>) was found without however any lesions typical of CBPP.</p> <p>Wild <i>Bos</i> spp. (including stray or feral animals) could be infected as a result of close contact with infected cattle and therefore may play a role to the transmission as a reservoir host.</p>	<p>The surveillance of wild animals (including stray or feral animals) of listed species around an affected establishment may include: i) visual inspection of these animals from a distance, ii) clinical examination of trapped animals and iii) thorough examination of animals found dead or hunted to identify lesions compatible with CBPP and sampling for laboratory analysis by PCR and or isolation following the procedures of the 1st Scenario in Section 4.1.1.1.</p> <p>In the event when wild animals of <i>Bos</i> spp., are living in the surrounding area of the affected establishment, and the risk</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
		The lack of information on the performance of laboratory tests (sensitivity, specificity) for animal species other than cattle along with the lack of validation of the diagnostic methods in them, will increase the uncertainty on the reliability of the sampling strategy.	<p>assessment carried out by the Competent Authority may conclude that sampling live animals is necessary, then blood samples may be collected for laboratory analysis with c-ELISA. Wildlife population health experts would be able to provide additional advice in these circumstances.</p> <p>Investigation of development and validation of non-invasive diagnostic procedures by using alternative sample matrices (e.g. faeces, chewing baits) to detect antibodies.</p>
6th scenario Section 4.1.1.6 For animals of listed species in the non-affected establishments located in a protection zone	No specific guidelines on sampling procedures for a clinical or laboratory examination were found for the 6th Scenario.	<p>The causative agent may be transmitted by air over 200 meters and therefore CBPP may be transmitted to adjacent establishments.</p> <p>The absence of a protection zone and consequently visits and sampling procedures to establishments adjacent to the affected one, is not considered effective.</p>	<p>A protection zone including establishments adjacent to the infected establishment is recommended.</p> <p>Upon implementation clinical inspection and laboratory testing should take place in all establishments in the protection zone according to the 1st scenario (based on the presence or not of clinical signs or deaths).</p> <p>In case results are negative, serological sampling (aiming to detect seroprevalence higher than 1%) should be performed after 90 days (duration of the protection zone as proposed by EFSA AHAW Panel see Section 4.3.2) and the protection zone can be lifted if all samples prove negative.</p> <p>Increased awareness should be raised in the protection zone to enhance passive surveillance and immediate reporting of signs suggestive for CBPP. Animals brought to slaughter (Scenario 9) should be thoroughly examined for CBPP like lesions followed by sampling according to Scenario 9.</p>
8th scenario Section 4.1.1.7 For non-affected establishments located in a surveillance zone	No specific guidelines on sampling procedures for a clinical or laboratory examination were found for	<p>The causative agent can be transmitted by air over 200 meters and therefore CBPP can be transmitted to adjacent establishments.</p> <p>Given the limited transmission, a 3 km zone is considered effective and the zone should be implemented for 90 days according to the incubation period of the disease (see Section 4.3.2) and not be lifted before the sampling of all the</p>	<p>For the surveillance zone, it is recommended that the efforts will be allocated to enhance immediate notification and passive surveillance by increasing awareness in all establishments, industry and public.</p> <p>High awareness at the slaughterhouses during the ante-mortem animal inspection and post-mortem inspection of the pleural cavity. Animals from establishments located in the surveillance zone should be thoroughly examined CBPP like lesions followed</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
	the 8th Scenario.	establishment in the protection zone has been completed with negative results.	<p>by sampling in case of suspicion according to the procedures described in Section 4.1.1.1 (1st Scenario).</p> <p>Any establishment where more generic signs of the disease such as fever, lethargy, lost appetite, in the feed intake and productivity are reported should be visited, the animals should be clinically examined and samples should be collected following the procedures described in Section 4.1.1.1.</p> <p>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 following the procedures described in Section 4.1.1.1.</p> <p>The zone should not be lifted before the second negative test (90 days after the initiation of the zone and the first sampling) of the establishments in the protection zone.</p>

ToR 1: To grant derogations for animal movements

9th scenario Section 4.1.2.1. From non-affected establishments located in the protection zone to slaughterhouses located within the protection zone or in the surveillance zone or outside the restricted zone	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 9th Scenario in EU legislation.	<p>During CBPP outbreaks, the following considerations should be included when designing animal movement derogations:</p> <ul style="list-style-type: none"> i) the lack of specificity of clinical examination; ii) animals without clinical signs may be incubating CBPP which cannot be detected by laboratory tests; (long incubation period of the disease; more often 3 to 8 weeks but it can be extended up to 6 months) iii) some animals may become 'carriers' following their exposure and may remain a source of <i>Mmm</i>; iv) the length of the infectious period is not known; v) data on sensitivity and specificity of diagnostic tests are sparse, complicating interpretation of test results and estimates of predictive values; vi) identification of infectious animals is often not possible and vii) airborne transmission up to 200 m can be occurred. <p>The fact that the destination of these animals is the slaughterhouse, all biosecurity measures are implemented and</p>	<p>All the animals in the establishment of origin should be clinically examined before their movement, following the procedures described in the Section 4.1.1.1 and a thorough investigation of the health history of the establishment for at least 90 days backwards should be performed to identify any sign compatible to CBPP.</p> <p>In an establishment where the number of animals is large, the individual clinical examination of all the animals may not be feasible; in that case, the individual clinical examination can be restricted to those animals that are intended to be moved and the whole establishment should be visually inspected for clinical signs from respiratory system.</p> <p>In case clinical signs compatible with CBPP are identified, the establishment is considered suspect and the procedures for the laboratory confirmation that are described in the Section 4.1.1.1 should be followed and any movements should be prohibited.</p>
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Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
		<p>given that the animals should be slaughtered within 24 hours reduces the risk.</p> <p>Animal slaughtering from the establishments in the protection zone could have beneficial effect encompassing the reduction of the number of potential hosts for the further spread of CBPP agent.</p> <p>Since the lesions in pleural cavity are pathognomonic for CBPP diagnoses the post-mortem inspection at slaughterhouse is crucial for the detection of the disease.</p>	<p>At slaughterhouse a thorough post-mortem inspection should be performed for each animal, to identify lesions of CBPP. Any suspected lesion attributable to CBPP should be further investigated with laboratory examinations to rule out the presence of <i>Mmm</i> following the procedures described in the Section 4.1.1.1.</p>
<p>12th scenario Section 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</p>	<p>No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 12th Scenario in EU legislation.</p>	<p>This scenario is very similar to the scenario 9th of the Section 4.1.2.1.</p>	<p>This scenario is very similar to the 9th scenario of the Section 4.1.2.1; therefore, the same procedures will be followed for this scenario as well.</p>
<p>13th scenario Section 4.1.2.3. From an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone and from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone</p>	<p>No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 13th scenario in EU legislation.</p>	<p>This scenario is very similar to the scenario 9th of the Section 4.1.2.1.</p>	<p>This scenario is very similar to the 9th scenario of the Section 4.1.2.1; therefore, the same procedures will be followed for this scenario as well.</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
14th scenario Section 4.1.2.4 From an establishment in a surveillance zone to pastures situated within the surveillance zone	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 14th Scenario in EU legislation.	<p>The same considerations as described in the 9th scenario should be included in the assessment when designing animal movement derogations.</p> <p>Consequently, sampling procedures (clinical and laboratory) are not able to ensure with high level of confidence that animal movements to pastures do not pose a risk for transmission.</p>	<p>The animal movements from the establishments located in the surveillance zone to pastures within the surveillance zone should be allowed once the first clinical inspection of the establishments in the protection zone have been completed and the results of the initial laboratory tests in these establishments are negative. All the animals in the establishment of origin should be clinically examined before movement.</p> <p>In case clinical signs compatible with CBPP are identified, the establishment is considered suspect and the procedures for the laboratory confirmation that are described in the Section 4.1.1.1 should be followed and any movements should be prohibited.</p>
15th scenario Section 4.1.2.5 From an establishment in a surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 15th Scenario in EU legislation.	<p>The same considerations as described in the 9th scenario should be included in the assessment when designing animal movement derogations.</p> <p>Taken into consideration the above-mentioned limitations (scenario 9) it is very difficult to develop sampling procedures that will ensure with high level of confidence that the disease will not spread if live animals are allowed to be moved. Consequently, it is noteworthy to emphasise that allowing movements from establishment in a surveillance zone to an establishment belonging to the same supply chain, located outside the surveillance zone increases the risk of CBPP expansion.</p>	<p>The animal movements from the establishments located in the surveillance zone to an establishment belonging to the same supply chain should be allowed once the first clinical inspection of the establishments in the protection zone have been completed and the results of the initial laboratory tests in these establishments are negative.</p> <p>All animals in the establishment of origin should be clinically examined before any movement to an establishment belonging to the same supply chain.</p> <p>In case clinical signs compatible with CBPP are identified or evidence of clinical signs the last 3 months, the establishment is considered suspected and the procedures for the laboratory confirmation as described in the Section 4.1.1.1 should be followed and any movements should be prohibited.</p> <p>In addition to clinical examination a minimum sample of animals (including all animals to be moved) should be tested with c-ELISA as described in the Section 4.1.1.1 based on the total number of animals in the establishment (Table 4) and should be negative before moving the animals.</p> <p>Additional measures are recommended also for the establishment of destination where the animals should be tested again with c-ELISA 90 days after their introduction in the establishment of destination. Moreover, during that period, animal movements</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
			<p>from the establishments of destination, slaughterhouses excluded, should not be allowed.</p> <p>Nevertheless, the EFSA AHAW Panel considers that given the current available laboratory tests, it is very difficult to guarantee with high confidence, that live animals without clinical signs and with negative results in serological tests do not pose a risk of transmission and therefore live animals movements from the surveillance zone outside the restricted zone should be discouraged.</p>
18th scenario Section 4.1.2.6 From an establishment located in the restricted zone to move within the restricted zone when restriction measures are maintained beyond the period set out in Annex XI of the Delegated Regulation	No specific guidelines on sampling procedures for clinical or laboratory examination were found for the 18th scenario.	Same conclusions as described in Sections 4.1.2.1, 4.1.2.3, 4.1.2.4 and 4.1.2.5.	The same sampling procedures, according to different scenarios, should be implemented as those described in Sections 4.1.2.1, 4.1.2.3, 4.1.2.4 and 4.1.2.5.
ToR 1: For repopulation purposes			
19th scenario Section 4.1.3.1 For the animals that are kept for the repopulation prior to their introduction	No specific guidelines on sampling procedures for laboratory examination were found for the 19th scenario.	The following elements should be taken into consideration in case animals intended to be used for repopulation: i) the lack of specificity of clinical examination; ii) animals without clinical signs may be incubating CBPP which cannot be detected by laboratory tests; (long incubation period of the disease; more often 3 to 8 weeks but it can be extended up to 6 months) iii) some animals may become 'carriers' following their exposure and may remain a source of <i>Mmm</i> ; iv) the length of the infectious period is not known;	<p>Animals intended for repopulation should be subjected to clinical examinations because for animals originate from establishments located in free areas, there is no need for laboratory examination if there are no other reasons based on the authorities' risk assessment to recommend it.</p> <p>When animals originate from restricted areas established around different index cases, in addition to clinical examination a minimum sample of animals (including all animals to be moved) should be tested with c-ELISA as described in the Section 4.1.1.1 based on the total number of animals in the establishment before the movement.</p>

Sampling procedure	Existing Sampling Procedures	Conclusions	Recommendations
		v) data on sensitivity and specificity of diagnostic tests are sparse, complicating interpretation of test results and estimates of predictive values; vi) identification of infectious animals is often not possible vii) airborne transmission up to 200 m can occur.	
20th scenario Section 4.1.3.2 In the event of unusual mortalities or clinical signs being notified during the repopulation	No specific guidelines on sampling procedures for laboratory examination were found for the 20th scenario.	In case of unusual mortalities or clinical signs compatible to CBPP, notified during the repopulation is important to rule out the presence of the disease.	In the event of animals with clinical signs or lesions compatible with CBPP, as they have been described in Section 4.1.1.1, being identified in an establishment during the repopulation, the establishment is considered suspected. The repopulation should be stopped and the procedures for the laboratory confirmation as described in the Section 4.1.1.1 should be followed. In addition, the establishments from where the suspected animals originated, should be considered as suspected; the procedures described in the Section 4.1.1.1 should be followed as well.
21st scenario Section 4.1.3.3 For animals that have been already repopulated	No specific guidelines on sampling procedures for laboratory examination were found for the 21st scenario.	Following restocking, animals should be thoroughly examined clinically and by laboratory examinations in order to rule out the presence of the disease.	Animals must be subjected to weekly clinical inspection up to 90 days (monitoring period as proposed by EFSA AHAW Panel) after re-introduction. The last day of the monitoring period following the latest day of animals' introduction, all the animals should be subjected to thorough clinical examination as described in Section 4.1.1.1. Laboratory examination is not recommended if there are no other reasons based on the authorities' risk assessment to recommend.

ToR 2

Description	Conclusions	Recommendations
Section 4.2. Assessment of the length of the monitoring period of CBPP	Scenarios 1, 2, 3, 4, 6 and 7}} Based on the results of the ELS as presented in the Tables 5 and 6 in Section 4.2.1: – the longest length of the period between infection and suspicion of CBPP was 108 days – the average (mean) length was 86 days (median=90 days) – the shortest length was 61 days	<u>Scenarios 1,2,3,5,6,7</u> A monitoring period of at least 90 days (3 months) is recommended case of high awareness since it was concluded with a 70-100% certainty it would be effective (66-90% certainty in the case of independent epidemiological units in scenario 3 and 90-100% for

ToR 2

	<p>Scenario 5 Based on the results of the scientific publications as presented in Table 7 in Section 4.2.1, the latest date of seroconversion identified by CFT and c-ELISA was 3 months after EBI infection and 30 weeks (7.5 months) post-contact with infected animals. The results of the serological tests for CBPP should be interpreted at herd basis and not individual animals.</p> <p>The length of the monitoring period of 45 days, as defined in the Delegate Regulation, is not considered effective.</p>	<p>scenario 6, while for the index case in the area where the awareness is low the monitoring period should be at least 180 days (6 months).</p> <p>Scenario 4</p> <p>Even if the disease has already been detected in the area, and high awareness is expected, the length of the monitoring period of 45 days is not considered effective in this scenario. Therefore, a longer monitoring period of at least 90 days (3 months) is recommended while for the early phases of the outbreak in the area where the awareness is low (for example, if the index case was in a slaughterhouse and the epidemiological enquiry is taking time) the monitoring period should be at least 180 days (6 months).</p>
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ToR 3

Description	Conclusions	Recommendations
<p>Section 4.3.1 Assessment of the minimum radius</p>	<p>No transmission kernels either specific for CBPP or for diseases that have similar transmission routes to CBPP were found in the literature, nor were data suitable to estimate kernels identified. Accordingly, the zone sizes for CBPP were assessed using expert knowledge.</p> <p>The causative agent can be transmitted by air up to 200 metres and therefore animals in adjacent establishments can be infected.</p> <p>Based on the WG expert opinion, the absence of a protection zone is not considered effective while the defined minimum radiuses of 3 km of the surveillance zone, is considered effective (95-100% certainty) to restrain the spread of CBPP.</p> <p>Transmission across longer distances cannot be excluded if infected animals are moved outside the zones.</p>	<p>A protection zone including the establishments adjacent to the infected establishment or establishment with pastures or yards adjacent to the infected one (e.g. 1 km zone depending on the local situation and the establishments distribution) since this would prevent disease spread (90-100% certainty).</p> <p>All the establishments in the protection zone should be visited and clinical inspection of bovines is recommended (see Section 4.3.1).</p> <p>Taken into consideration the local epidemiological situation, the density of the establishments and the commercial activities different combinations of radiuses in the protection and the surveillance zones may be selected to further decrease the spread of the disease.</p>
<p>Section 4.3.2 Assessment of the minimum period</p>	<p>The results of the ELS, showed that the average time between infection and notification of the suspicion is 86 days (median=90 days) and the shortest period reported is 61 days.</p> <p>The OIE reports an incubation period of 3 weeks to 6 months, with most cases becoming apparent in 3–8 weeks.</p> <p>The minimum period of 45 days indicated in the Delegated Regulation for the restriction measures in the protection and surveillance zone is not considered effective to detect affected establishments and to prevent the movement of infected animals from the zones.</p>	<p>A minimum period of at least 90 days is recommended for both protection and surveillance zone, since it is concluded with a 90-100% certainty it would be effective to detect affected establishments and prevent movement of infected animals from the zones.</p>

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Abbreviations

ASF	African swine fever
AHS	African horse sickness
c-ELISA	competitive enzyme linked immunosorbent assay
CFT	complement fixation test
CSF	classical swine fever
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
CO	cut-off (of a diagnostic test)
dpi	days post-inoculation/infection
dpc	days post-contact
ELISA	enzyme-linked immunosorbent assay
ELS	extensive literature search
FMD	foot and mouth disease
FMDV	foot and mouth disease virus
HPAI	highly pathogenic avian influenza
LSDV	lumpy skin disease virus
NCDV	Newcastle disease virus
OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
PZ	protection zone
RP	rinderpest virus
RT-PCR	reverse transcription polymerase chain reaction
RVFV	Rift Valley fever virus
SPGP	sheep pox and goat pox
SZ	surveillance zone
ToR	Terms of Reference

Annex A – Definitions in EU legislation

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

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

Annex B – Scenarios of ToR 1

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.1 ToR 1.2	6(2) of the Delegated Regulation	1st scenario	To assess the effectiveness of disease-specific sampling procedures of animals of listed species in a suspected establishment, based on clinical examination (ToR 1.1) and laboratory examination (ToR 1.2), in their ability to detect a category A disease in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2)).	<ul style="list-style-type: none"> • event of suspicion of a category A disease • in an establishment • kept animals of listed species • the competent authority shall immediately conduct an investigation to confirm or rule out the presence of the suspected listed disease • official veterinarians perform clinical examinations and collect samples for laboratory examinations
ToR 1.2	Art. 12(3), Art. 7 (4) (Preventive killing) of the Delegated Regulation, and Art. 57 Reg. 2016/429	2nd scenario	To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, agent identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU)2016/429.	<ul style="list-style-type: none"> • affected establishment officially confirmed • kept animals of listed species found dead or before/when they are killed • competent authority collects samples for laboratory examination for the purposes of: <ul style="list-style-type: none"> a) supporting the epidemiological enquiry: <ul style="list-style-type: none"> – to identify the likely origin of the disease – to calculate the likely length of time that the disease is present – to identify establishments where the animals could have contracted the disease and movements from the affected establishment that could have led to the spread of the disease – to obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors b) confirming/ruling out disease in the event of preventive killing
ToR 1.1 ToR 1.2	Article 13(3)c of the Delegated Regulation	3rd scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment, in order to grant a specific	<ul style="list-style-type: none"> • affected establishment officially confirmed • kept animals of listed species of specific categories • animal categories based on article 13(2): <ul style="list-style-type: none"> a) animals kept in a confined establishment

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease.	<ul style="list-style-type: none"> 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 <ul style="list-style-type: none"> 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 agent if the agent is present in these species.	<ul style="list-style-type: none"> kept animals of non-listed species of epidemiological relevance for the control of the disease animals of non-listed species are those animals that are not listed in Commission Implementing Regulation (EU) 2018/1882 for each of the category A diseases animal species acting purely as mechanical carriers of the agent will not be covered The competent authority is not obliged to carry out the sampling of non-listed species, but they may establish it in addition to other measures sampling procedures to ensure detection of the agent 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 agent, if the agent is present in these wild species	<ul style="list-style-type: none"> affected establishment officially confirmed wild animals of listed species within the establishment and in the surroundings of the establishment the competent authority may establish these sampling procedures in addition to other measures sampling procedures in wild animals of listed species to ensure the detection of the agent, if the agent is present in these wild species

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.1 ToR 1.2	Article 26(2) of the Delegated Regulation	6th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the agent, if the agent is present in these animals.	<ul style="list-style-type: none"> • protection zone with radius up to 3 km • non-affected establishments with kept animals of listed species • all the non-affected establishments within the protection zone • 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 agent if the agent is present in establishments within the protection zone	<ul style="list-style-type: none"> • protection zone with radius larger than 3 km • non-affected establishments of kept animals of listed species • sample of the non-affected establishments in the protection zone • in a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km • In case of a radius larger than 3 km, official veterinarians may not visit all establishments, but a sample of those. EFSA is requested to assess how many of these establishments should be inspected, in order to ensure the detection of the agent, if the agent 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	<ul style="list-style-type: none"> • surveillance zone • establishments of kept animals of listed species • sample of the establishments in the surveillance zone • official veterinarians carry out visits to a sample of the establishments

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			is to ensure disease detection if the agent is present in establishments within the surveillance zone	<ul style="list-style-type: none"> 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 within the protection zone or in the surveillance zone or outside the restricted zone (Art. 29)	<ul style="list-style-type: none"> protection zone kept animals of listed species grant derogation for movement from a non-affected establishment in the protection zone to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 30(1) of the Delegated Regulation	10th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of day-old-chicks located in the protection zone and hatched from eggs originating in the restricted zone or outside the restricted zone. The sampling procedures should ensure that the movement of these day-old-chicks to an establishment located in the same Member State but if possible, outside the restricted zone	<ul style="list-style-type: none"> protection zone grant derogation for movement from a non-affected establishment in the protection zone day-old-chicks from non-affected establishment located in the protection zone, hatched from eggs originating in or outside the restricted zone to be moved to an establishment located in the same Member State but if possible, outside the restricted zone clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 30(2) of the Delegated Regulation	11th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone to establishments located in the same MS and if possible within the restricted zone.	<ul style="list-style-type: none"> protection zone ready-to-lay poultry grant derogation for movement from a non-affected establishment in the protection zone to be moved to an establishment located in the same Member State and if possible, within the restricted zone clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.4	Article 28(5) and Article 37 of the Delegated Regulation	12th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art. 37)	<ul style="list-style-type: none"> • protection zone • kept animals of listed species • grant derogation for movement from a non-affected establishment in the protection zone • to be moved to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed • clinical examinations and laboratory examinations of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 44 of the Delegated Regulation	13th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of listed species in order to grant derogation from prohibitions and allow for these animals to be moved: a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, b) from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone	<ul style="list-style-type: none"> • surveillance zone • kept animals of listed species • grant derogation for movement from an establishment in the surveillance zone to be moved to a slaughterhouse within the restricted zone or outside the restricted zone • grant derogation for movement from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 45(1) of the Delegated Regulation	14th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone	<ul style="list-style-type: none"> • surveillance zone • kept ungulates of listed species • grant derogation for movement from an establishment in the surveillance zone • to be moved to pastures situated within the surveillance zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 45(2) of the Delegated Regulation	15th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant derogation and allow to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside	<ul style="list-style-type: none"> • surveillance zone • kept animals of listed species • grant derogation for movement from the surveillance zone • to be moved to an establishment belonging to the same supply chain, located in or outside the surveillance zone, to complete the production cycle before slaughter

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			the surveillance zone, in order to complete the production cycle before slaughter	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> surveillance zone ready-to-lay poultry to be moved to an establishment located in the same Member State clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 56(1)c of the Delegated Regulation	18th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI	<ul style="list-style-type: none"> restricted zone when restriction measures are maintained beyond the period set out in Annex XI kept animals of listed species grant derogation for movement from an establishment within the restricted zone clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
Repopulation				
ToR 1.5	Article 59(2),(3) of the Delegated Regulation	19th scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease.	<ul style="list-style-type: none"> repopulation of a previous affected establishment kept animals of listed species animals intended to repopulation shall be sampled prior to their introduction into the establishment of destination samples shall be collected from a representative number of animals to be introduced of each consignment from each establishment or from a representative number of animals

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
				<p>of each consignment (if animals are all to be introduced at different times or from different establishments of origin)</p> <ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • repopulated establishment • unusual mortalities or clinical signs during the repopulation • the official veterinarians shall without delay collect samples for laboratory examination • sampling procedures to rule out the presence of the disease
ToR 1.5	Article 59(5) of the Delegated Regulation	21st scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment.	<ul style="list-style-type: none"> • repopulated establishment • kept animals of listed species • animals that have been used for repopulation • laboratory examinations • sampling procedures to rule out the presence of the disease

Annex C – Existing sampling procedures for Contagious bovine pleuropneumonia (CBPP)

Laboratory and clinical guidelines as described in the relevant documents

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
1st	To assess the effectiveness of disease-specific sampling procedures of animals of listed species in a suspected establishment, based on clinical examination (ToR 1.1) and laboratory examination (ToR 1.2), in their ability to detect a category A disease in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2)).	<p><i>OIE Terrestrial Code (OIE, 2019):</i></p> <p><u>Article 11.5.1.</u> <u>General provisions</u> For the purposes of the Terrestrial Code, the incubation period for contagious bovine pleuropneumonia (CBPP) shall be six months. ...</p> <p><u>Article 11.5.8.</u> <u>Recommendations for importation from CBPP infected countries or zones</u> For domestic bovids and water buffaloes for slaughter Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</p> <ol style="list-style-type: none"> 1) showed no clinical sign of CBPP on the day of shipment; 2) originate from an establishment where no case of CBPP was officially reported for the past six months; and 3) are transported directly to the slaughterhouse/abattoir in sealed vehicles. <p><i>Notes:</i></p> <p><i>OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2021):</i></p> <p>B. Diagnostic techniques <u>Clinical diagnosis of CBPP is unreliable as initial signs may be slight or non-existent and may be indistinguishable from any severe pneumonia.</u> Therefore, CBPP should be investigated by pathological, microbiological, molecular or serological diagnostic methods. As the <u>pathological lesions of CBPP are distinctive, and pathognomonic</u>, abattoir surveillance for CBPP involving lung examination is a practical method for disease monitoring. ...</p>	<p><i>OIE Terrestrial Code (OIE, 2019):</i></p> <p><u>Article 11.5.1.</u> <u>General provisions</u> The following defines the occurrence of MmmSC infection:</p> <ol style="list-style-type: none"> 1) MmmSC has been isolated and identified as such from an animal, semen, oocytes or embryos; or 2) antibodies to MmmSC antigens which are not the consequence of vaccination, or MmmSC deoxyribonucleic acid have been detected in one or more animals showing pathological lesions consistent with infection with MmmSC with or without clinical signs, and epidemiological links to a confirmed outbreak of CBPP in susceptible animals. <p>...</p> <p><u>Article 11.5.10.</u> <u>Recommendations for importation from CBPP infected countries For bovine semen</u> Veterinary Authorities should require the presentation of an international veterinary certificate attesting that:</p> <ol style="list-style-type: none"> 1) the donor animals: <ol style="list-style-type: none"> a) showed no clinical sign of CBPP on the day of collection of the semen; b) were subjected to the complement fixation test for CBPP with negative results, on two occasions, with an interval of not less than 21 days and not more than 30 days between each test, the second test being performed within 14 days prior to collection; c) were isolated from other domestic bovids and water buffaloes from the day of the first complement fixation test until collection; d) were kept since birth, or for the past six months, in an establishment where no case of CBPP was reported during that period, and that the establishment was not situated in a CBPP infected zone;

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		<p>CFSPH factsheet on CBPP (CFSPH, 2015): <u>Clinical Signs</u> A few cattle with CBPP may die peracutely <u>with no clinical signs</u> other than fever. <u>Acute cases</u> in cattle are characterized by <u>nonspecific signs</u> of fever, loss of appetite, depression and a drop in milk production, followed by respiratory signs, which may include coughing, purulent or mucoid nasal discharges, and rapid respiration. Clinical signs can differ in severity between outbreaks . . . <u>In calves up to six months</u> of age, the primary sign may be polyarthritis, especially of the carpal and tarsal joints, often without respiratory signs. The affected joints may be so painful that the animal is very reluctant to bend them. <u>Chronic CBPP</u> is characterized by recurrent low-grade fever, loss of condition, and respiratory signs that may be apparent only when the animal is exercised.</p> <p>Foreign animal diseases (USAHA, 2008): CHAPTER 14: Contagious bovine pleuropneumonia <u>6. clinical signs</u> . . . Contagious bovine pleuropneumonia often evolves into a chronic disease. This form, characterized by ill thrift and recurrent low-grade fever, may be difficult to recognize as pneumonia. Forced exercise may precipitate coughing. In calves, the mycoplasmaemia results in a polyarthritis which may be the primary presenting complaint, often without an accompanying pneumonia. . .</p>	<p>e) AND EITHER: i) have not been vaccinated against CBPP; OR ii) were vaccinated using a vaccine complying with the standards described in the Terrestrial Manual not more than four months prior to collection; <u>in this case, the condition laid down in point b) above is not required;</u> . . . <u>Article 11.5.12.</u> Recommendations for importation from CBPP infected countries For in vivo derived or in vitro produced oocytes or embryos of domestic bovids and water buffaloes Veterinary Authorities should require the presentation of an international veterinary certificate attesting that: 1) the donor animals: → <u>same rules for the certificate as for semen (see Article 11.5.10)</u></p> <p>Notes:</p> <p>OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2021): <u>B. Diagnostic techniques</u> . . . <u>Table 1:</u> Laboratory methods currently used for diagnosis of CBPP and their purpose: purpose of "<u>confirmation of clinical cases</u>": - Confirmation of the agent: In-vitro culture isolation (followed by species identification tests) (recommended method), direct molecular test (PCR) - Detection of immune response: CFT, Immunoblotting, C-ELISA (recommended method)</p> <p><u>1. Detection of the agent</u> <u>1.1. Samples</u> A key to isolation success lies in collecting good quality samples. MmmSC can be isolated from samples taken either from live animals or at necropsy. <u>Samples taken from live</u></p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>animals are: nasal swabs or nasal discharges, broncho-alveolar lavage or transtracheal washing and pleural fluid collected aseptically by puncture made in the lower part of the thoracic cavity between the seventh and eighth ribs. Samples taken at necropsy are: lungs with lesions, pleural fluid ('lymph'), lymph nodes of the broncho-pulmonary tract, and synovial fluid from those animals with arthritis. The lung samples should be collected from lesions at the interface between diseased and normal tissue.</p> <p>When collecting nasal swab samples, a <u>transport medium should be used</u> to protect the mycoplasmas and prevent proliferation of cell-walled bacteria ...</p> <p>After collection <u>all samples must be kept refrigerated at 4°C</u> and sent to the laboratory within 24 hours. For longer periods they should be frozen at or below -20°C.</p> <p><u>1.2. In-vitro cultures</u></p> <p>The presence of the pathogen varies greatly with the stage of development of the lesions, and <u>a negative result is not conclusive</u>, particularly if the animal was treated with an antibiotic.</p> <p>...</p> <p><u>1.4. Molecular identification and typing methods (PCR-based tests).</u></p> <p>... <u>PCR has become the method of choice for the rapid and specific identification of MmmSC</u> when the organism is isolated from a clinical sample. ...</p> <p><u>2. Serological tests</u></p> <p>Serological test results for CBPP should not be analysed and interpreted individually but in groups of animals from the same herd or region because false positive or false negative results may occur in individual animals. Tests on single animals can be misleading, either because the animal is in the early stage of disease, which may last for several months, before specific antibodies are produced, or it may be in the chronic stage of the disease when very few animals are seropositive. False-positive results can occur (2%), of which an important cause is <u>serological cross-reactions with other mycoplasmas</u>, particularly other members of the <i>M. mycoides</i> cluster. The validity of the</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>results has to be confirmed by post-mortem and bacteriological examination, and serological tests on blood taken at the time of slaughter.</p> <p>The complement fixation test (CFT) and enzyme-linked immunosorbent assays (ELISAs) are recommended for screening and eradication programmes. The highly specific immunoblotting test is useful as a confirmatory test but is not fit for mass screening.</p> <p><u>2.1. Complement fixation</u> ... With a sensitivity of 63.8% and a specificity of 98% (Bellini et al., 1998), the CFT can detect nearly all sick animals with <u>acute lesions</u>, but a rather smaller proportion of animals in the early stages of the disease or of animals with chronic lesions.</p> <p><u>2.2. Competitive ELISA</u> ... Validation tests (Amanfu et al., 1998; Le Goff & Thiaucourt, 1998) that have been carried out in several African and European countries would indicate: i) that the <u>true specificity of the C-ELISA</u> has been reported to be at least 99.9%; ii) that the <u>sensitivity of the C-ELISA</u> and the CFT are similar; and iii) antibodies are detected by the C-ELISA in an infected herd very soon after they can be detected by the CFT, and <u>C-ELISA antibody persists for a longer period of time</u> (Niang et al., 2006).</p> <p><u>2.3. Immunoblotting test</u> An immunoblotting test (IBT) is an immunoenzymatic test that has been developed to <u>confirm doubtful CFT or C-ELISA results</u>. A field evaluation indicated a higher specificity than the CFT enabling the <u>detection of CFT false positives</u> (Gonçalves et al. 1998).</p> <p><i>EFSA Scientific opinion on CBPP (EFSA, 2017):</i> <u>3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools</u> <u>Diagnostic tools</u> <u>Parameter 1 – Existence of diagnostic tools</u> <u>Direct detection of <i>Mmm</i>:</u> <u>Isolation of <i>Mmm</i> is quite easy</u> if the laboratory has some experience in Mycoplasma isolation and that the quality of the</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>sample is adequate (no bacterial contamination, no antibiotic residue). ...</p> <p><u>Specific PCR and quantitative PCR (Q-PCR)</u> have been developed ... There is a PCR that can detect specifically the T1 vaccine strains</p> <p><u>Indirect detection:</u></p> <p>There are two prescribed serological tests: the CFT and the cELISA. ...</p> <p>- <u>The CFT is not strictly specific (97–99%) and false-positive results are to be expected</u> when large enquiries are performed, ... When CBPP prevalence is very low or zero, CFT-positive samples can usually be <u>confirmed using the western-blotting technique</u>.</p> <p>A specific <u>competitive ELISA</u> has been developed (Le Goff and Thiaucourt, 1998). Its <u>specificity is higher than 99.5%</u> when the test is performed under quality procedures. It is difficult to determine its <u>sensitivity</u>. It is lower than the CFT at an <u>early stage after infection</u> (as CFT detects mostly immunoglobulins M (IgMs)) but it is higher at a later stage, notably to detect chronic cases ...</p> <p>This test is produced by IDEXX ref: P05410-10 and all batches are controlled by CIRAD before release. It is important to note that <u>CFT and cELISA are both considered herd tests</u>. They <u>will not be able to detect latent Mmm shedders</u>, which do not display circulating antibodies and they will have a <u>low sensitivity for the detection of chronic carriers</u> (Mycoplasma-induced antibodies are short-lived). A practical consequence is that serological results should be expressed in 'herd prevalence' with sampling frames including the random selection of herds in the various compartments but a <u>targeted selection of animals in the chosen herds to increase the sensitivity of the results</u>.</p> <p>...</p> <p>3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures</p> <p>3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities</p> <p>Availability</p> <p><u>Parameter 1 – Officially/internationally recognised diagnostic tool, OIE certified</u></p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>... The cELISA may be more reliable because of its specificity and facility for quality monitoring.</p> <p><u>Parameter 2 – Se and Sp of diagnostic test</u></p> <p>The accuracy of the <u>cELISA at individual level</u>: specificity is near 100% when the test is performed under quality management, while the sensitivity varies by disease stage and time of sampling after the onset of outbreak. It can be 60–70% for up to 6 months and much lower after 1 year. This is the reason why the epidemiological unit to be considered should be the herd.</p> <p>The accuracy of the <u>cELISA at herd level</u>: specificity remains near 100% when the test is performed under quality management ... Sensitivity is much higher when targeting animals in the herd, which have suffered from suspicious signs during the past 12 months, hence improving the sensitivity of detection, although a specific estimate of the herd sensitivity is available.</p> <p><i>Diagnostics tests for CBPP (European Commission. 2001),</i></p> <p><u>5. Diagnosis of CBPP</u></p> <p><u>5.1. Histology</u></p> <p>The detection of <u>specific lesions</u> is an important factor in identifying cattle infected subclinically. The “organising centres” observed in the interlobular septa of lungs with lesions are considered <u>pathognomonic for CBPP</u></p> <p><u>5.2. Detection and identification of MmmSC</u></p> <p><u>5.2.1. Culture</u></p> <p><u>5.2.1.1. Sample collection for culture</u></p> <p>From the <u>living animal</u>: nasal swabs and secretions, tracheal and bronchoalveolar washes and pleural fluid and occasionally blood, urine and synovial fluid should be obtained.</p> <p>From the <u>dead animal</u>: pleural fluid, portions of affected lungs and lung sequestra (scrapings from inside the capsule) and lung-associated lymph nodes, and kidneys should be taken.</p> <p><u>5.2.2. Immunobinding</u></p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>At present, immunobinding assays are the most reliable tests for routine identification of <i>Mycoplasma</i> species isolated from clinical material.</p> <p>...</p> <p>5.2.4. PCR PCR is a rapid and sensitive diagnostic method. It allows detection of MmmSC directly in samples of lungs, bronchial lymph nodes, nasal swabs, pleural fluid and blood.</p> <p>...</p> <p>5.3. Detection and quantification of antibodies 5.3.1. Conventional tests Antigenic cross-reactivity among <i>Mycoplasmas</i> species, especially those of the "M. mycoides cluster", has been widely observed, raising practical problems for CBPP serodiagnosis. The first conventional test described is the complement fixation test, followed by the agglutination test and the passive haemagglutination test. The latter ones may be used as screening tests as they detect the IgM response in <u>2 weeks after infection</u>, but they generally lack sensitivity and specificity. ...</p> <p>Foreign animal diseases (USAHA, 2008): 9. DIAGNOSIS b. Laboratory diagnosis i. Samples From a live animal, nasal swabs, transtracheal washes, or pleural fluid obtained by thoracic puncture all provide good samples for isolation attempts. From a dead animal that has had severe clinical disease, the best specimens to submit are affected lung, swabs of major bronchi, tracheo-bronchial or mediastinal lymph nodes, and joint fluid from those animals with arthritis. All samples should be collected aseptically and, if possible, placed in transport medium ... Samples should be kept cool and shipped on wet ice as soon as possible. If transport to the laboratory is delayed (more than a few days), samples may be frozen. Blood should be collected for serum.</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
2nd	To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, agent 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.	<p>No specific guidelines described in legislation</p> <p>Notes:</p> <p>Foreign animal diseases (USAHA, 2008): <u>CHAPTER 14: Contagious bovine pleuropneumonia</u> <u>7. POST- MORTEM LESIONS</u> <u>a. Gross</u> In the lung, gross pathologic features of CBPP are characteristic. If the animal dies, there is usually extensive and marked inflammation of the lung and associated pleurae. In severe cases there can be abundant fluid in the thoracic cavity. The inflammation is not uncommonly unilateral. ... <u>9. DIAGNOSIS</u> <u>a. Field diagnosis</u> Clinical diagnosis of CBPP is difficult. At post-mortem the gross lesions of CBPP are somewhat distinct. Unlike most other pneumonias, CBPP is usually unilateral. Often there is an extensive deposition of fibrin and a large quantity of straw-colored fluid in the thoracic cavity with a prominent marbling of pulmonary parenchyma. In some chronic cases the nodules of inflammation may not be readily apparent from the pleural surface but can be palpated within the parenchyma.</p>	No specific guidelines described in legislation
3rd	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment,	No specific guidelines described in legislation	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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.		
4th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the agent if the agent is present in these species.	<p>No specific guidelines described in legislation</p> <p>Notes:</p> <p>CFSPH factsheet on CBPP (CFSPH, 2015): The effects of <i>M. mycoides</i> SC on small ruminants are still unclear. There have been reports of its isolation from sheep with mastitis and goats with respiratory disease. Other agents, including other mycoplasmas, were also detected in some outbreaks, but not others.</p>	No specific guidelines described in legislation
5th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the agent, if the agent is	<p>No specific guidelines described in legislation</p> <p>Notes:</p> <p>COMMISSION IMPLEMENTING REGULATION (EU) 2018/1882 of 3 December 2018 According to the Table in Annex of this EU regulation, the listed species for CBPP are: Bison ssp., Bos ssp., Bubalus ssp., Syncerus caffer</p> <p>CFSPH factsheet on CBPP (CFSPH, 2015): <u>Species affected</u> ... White-tailed deer (<i>Odocoileus virginianus</i>) have been infected experimentally. There is little published surveillance for <i>M. mycoides</i> SC in wildlife, with the exception of two studies</p>	<p>No specific guidelines described in legislation</p> <p>Notes:</p> <p>EFSA Scientific opinion on CBPP (EFSA, 2017): 3.1.1.1. Article 7(a)(i) Animal species concerned by the disease <u>Susceptible animal species</u> <u>Parameter 1</u> – Naturally susceptible wildlife species (or family/orders) No naturally susceptible wild species are identified. Although slightly positive complement fixation tests (CFT) have been recorded from wildebeest and hippopotami (Shifrine and Domermuth, 1967), subcutaneous inoculation of live <i>Mycoplasma</i> did not induce any typical lesion as compared to</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	present in these wild species.	<p>conducted before 1970, which reported that African wildlife were unlikely to be infected.</p> <p>...</p> <p><u>Clinical signs</u></p> <p>... One of two experimentally infected white-tailed deer developed a fever and died with severe respiratory lesions; the second deer remained healthy.</p> <p><i>US SOP on CBPP (USDA, 2017)</i></p> <p><u>Susceptible species:</u></p> <p>Sheep and goats in Africa, Portugal, and India have been infected with CBPP. African buffalos (<i>Syncerus caffer</i>) seem unaffected by CBPP. Other wildlife have not been shown to play a role in CBPP disease or transmission.</p>	<p>control bovines inoculated in parallel, so these species are not likely natural hosts despite these findings.</p> <p>Positive complement fixation tests have been detected only in camels (Paling et al., 1988) but no mycoplasmas were isolated from nasal secretions from camels or buffaloes, which is similar to findings in American camelids (Hung et al., 1991). While CFT was performed with a crude antigen, it is highly probable that the positives were in fact cross-reactors with other mycoplasmas or bacteria.</p> <p>...</p> <p><u>Parameter 3</u> – Experimentally susceptible wildlife species (or family/orders)</p> <p>African buffalo (<i>Syncerus caffer</i>) (Shifrine et al., 1970) has been experimentally infected with subcutaneous inoculation of <i>Mmm</i> sometimes leading to re-isolation of <i>Mmm</i>, 53 days after inoculation, although no gross lesions were observed.</p>
6th	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the agent, if the agent is present in these animals.	No specific guidelines described in legislation	No specific guidelines described in legislation
7th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and	No specific guidelines described in legislation	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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 agent if the agent is present in establishments within the protection zone.		
8th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the agent is present in establishments within the surveillance zone.	<p>No specific guidelines described in legislation</p> <p>Note: OIE Terrestrial Code (OIE, 2019): Article 11.5.14. <u>General conditions and methods for surveillance</u> 1) A surveillance system in accordance with Chapter 1.4. should be under the responsibility of the Veterinary Authority. A procedure should be in place for the <u>rapid collection and transport of samples from suspect cases of CBPP to a laboratory for CBPP diagnoses.</u> 2) The CBPP surveillance programme should: a) include an early warning system throughout the production, marketing and processing chain for reporting suspicious cases. Farmers and workers (such as community animal health workers) who have day-to-day contact with livestock, meat inspectors as well as laboratory diagnosticians, should report promptly any suspicion of CBPP. They should be integrated directly or indirectly (e.g. through private veterinarians or veterinary paraprofessionals) into the surveillance system. <u>All suspect cases of CBPP should be investigated immediately. Where suspicion cannot be resolved by epidemiological and clinical investigation, samples should be taken and submitted to a laboratory.</u> This requires that sampling kits and other</p>	<p>No specific guidelines described in legislation</p> <p>Note: OIE Terrestrial Code (OIE, 2019): Article 11.5.15. <u>Surveillance strategies</u> ... Irrespective of the survey design selected, the <u>sensitivity and specificity of the diagnostic tests</u> employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated. Irrespective of the surveillance system employed, the design should anticipate the <u>occurrence of false positive reactions</u>. If the characteristics of the testing system are known, the rate at which these false positives are likely to occur can be calculated in advance. There should be an effective <u>procedure for following-up positives to ultimately determine with a high level of confidence, whether they are indicative of infection or not.</u> This should involve follow-up with supplementary tests, clinical investigation and post-mortem examination in the original sampling unit as well as herds which may be epidemiologically linked to it. ...</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		<p>equipment are available for those responsible for surveillance. Personnel responsible for surveillance should be able to call for assistance from a team with expertise in CBPP diagnosis and control;</p> <p>b) implement, when relevant, <u>regular and frequent clinical inspection and testing of high-risk groups of animals, such as those adjacent to a CBPP infected country or zone</u> (for example, areas of transhumant production systems);</p> <p>c) take into consideration additional factors such as animal movement, different production systems, geographical and socio-economic factors that may influence the risk of disease occurrence.</p> <p>An effective surveillance system will periodically identify suspicious cases that require follow-up and investigation to confirm or exclude that the cause of the condition is CBPP. <u>The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably.</u></p> <p>...</p> <p><u>Article 11.5.15.</u> <u>Surveillance strategies</u> <u>1. Introduction</u></p> <p>The target population for surveillance aimed at identifying disease and infection should cover <u>all the susceptible species (Bos taurus, B. indicus, B. grunniens and Bubalus bubalis)</u> within the country or zone.</p> <p>Given the limitations of the diagnostic tools available, the interpretation of surveillance results should be at the <u>herd level</u> rather than at the individual animal level.</p> <p><u>Randomised surveillance may not be the preferred approach given the epidemiology of the disease (usually uneven distribution and potential for occult foci of infection in small populations) and the limited sensitivity and specificity of currently available tests.</u> <u>Targeted surveillance</u> (e.g. based on the increased likelihood of infection in particular localities or species, <u>focusing on slaughter findings, and active clinical surveillance</u>) may be the most appropriate strategy. The applicant Member Country should justify the surveillance</p>	<p><u>4. Serological testing</u> <u>Serological surveillance is not the preferred strategy for CBPP.</u> However, in the framework of epidemiological investigations, serological testing may be used.</p> <p>The limitations of available serological tests for CBPP make the interpretation of results difficult and <u>useful only at the herd level.</u> <u>Positive findings should be followed up by clinical and pathological investigations and agent identification.</u></p> <p><u>Clustering of seropositive reactions should be expected in CBPP infections and is usually accompanied by clinical signs.</u> As clustering may signal field strain infection, the investigation of all instances should be incorporated in the surveillance strategy. Following the identification of a CBPP infected herd, <u>contact herds should be tested serologically. Repeated testing may be necessary</u> to reach an acceptable level of confidence in herd classification.</p> <p><u>5. Agent surveillance</u> Agent surveillance should be conducted <u>to follow up and confirm or exclude suspect cases. Isolates should be typed to confirm MmmSC.</u></p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		<p>strategy chosen as adequate to detect the presence of CBPP infection in accordance with Chapter 1.4. and the epidemiological situation.</p> <p><u>Targeted surveillance</u> may involve testing of the entire target subpopulation or a sample from it. In the latter case the sampling strategy should incorporate an epidemiologically appropriate design prevalence. The sample size selected for testing should be large enough to detect infection if it were to occur at a predetermined minimum rate. The sample size and expected disease prevalence determine the level of confidence in the results of the survey.</p> <p>The applicant Member Country should justify the choice of design prevalence and confidence level based on the objectives of surveillance and the epidemiological situation, in accordance with Chapter 1.4. Selection of the design prevalence in particular should be clearly based on the prevailing or historical epidemiological situation.</p> <p><u>Irrespective of the survey design selected, the sensitivity and specificity of the diagnostic tests employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated.</u></p> <p>...</p> <p><u>2. Clinical surveillance</u></p> <p><u>Clinical surveillance aims at detecting clinical signs of CBPP in a herd by close physical examination of susceptible animals.</u></p> <p><u>Clinical inspection</u> is an important component of CBPP surveillance contributing to reach the desired level of confidence of detection of disease if <u>a sufficiently large number of clinically susceptible animals is examined.</u></p> <p><u>Clinical surveillance and laboratory testing should always be applied in series to clarify the status of CBPP suspects detected by either of these complementary diagnostic approaches.</u></p> <p><u>Laboratory testing and post-mortem examination may contribute to confirm clinical suspicion, while clinical surveillance may contribute to confirmation of positive serology.</u></p> <p>Any sampling unit within which suspicious animals are detected</p>	

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		<p>should be classified as infected until contrary evidence is produced.</p> <p><u>3. Pathological surveillance</u></p> <p>Systematic pathological surveillance for CBPP is the most effective approach and should be conducted at slaughterhouses/abattoirs and other slaughter facilities. Suspect pathological findings should be confirmed by agent identification. Training courses for slaughter personnel and meat inspectors are recommended. ...</p>	
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 (Art. 29).	No specific guidelines described in legislation	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	NA	NA

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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.		
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	No specific guidelines described in legislation	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art. 37).		
13th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of listed species in order to grant derogation from prohibitions and allow for these animals to be moved: a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, b) from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone.	No specific guidelines described in legislation	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.	No specific guidelines described in legislation	No specific guidelines described in legislation
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.	No specific guidelines described in legislation	No specific guidelines described in legislation
16th	To assess the effectiveness of disease-specific sampling	NA	NA

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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.		
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	No specific guidelines described in legislation	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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.		
Repopulation			
19th	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease.	No specific guidelines described in legislation	No specific guidelines described in legislation
20th	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease.	No specific guidelines described in legislation	No specific guidelines described in legislation
21st	To assess the effectiveness of disease-specific sampling procedures based on	No specific guidelines described in legislation	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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.		

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Annex D – Scenarios of ToR 2

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	<p>Article 8 of the Delegated Regulation</p> <p>Article 57 of 2016/429 Regulation</p> <p>Annex II of the Delegated Regulation</p>	1st scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of the notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of a suspicion.	<ul style="list-style-type: none"> • event of suspicion of a category A disease • in an establishment with kept animals of listed species • time period calculated backwards from the date of the notification of the suspicion • time period before the suspicion, during which the pathogenic agent may have been introduced in the establishment and may have spread outside the establishment • the aim of the epidemiological enquiry is: • identify the likely origin of the listed disease in question and the means of its spread • calculate the likely length of time that the listed disease has been present • 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 • 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 • obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors
ToR 2	<p>Article 17(2) and Article 57 of 2016/429 Regulation</p> <p>Annex II of the Delegated Regulation</p>	2nd scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of confirmation of the disease.	<ul style="list-style-type: none"> • event of confirmation of a category A disease • in an establishment with kept animals of listed species • time period calculated backwards from the date of the notification of the suspicion • time period before the suspicion, during which the pathogenic agent was introduced in the establishment and during which it could have spread outside the establishment. • The aim of the epidemiological enquiry is the same as above.

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	Article 13(b) of the Delegated Regulation Annex II of the Delegated Regulation	3rd scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a category A disease in an establishment with kept animals of listed species, during which the epidemiological units in which the disease has not been confirmed were kept completely separated and handled by different personnel, in order to provide derogations from killing.	<ul style="list-style-type: none"> • event of confirmation of a category A disease • in an affected establishment with kept animals of listed species • non-affected epidemiological units kept separated • to provide derogation from killing for animals in non-affected separated epidemiological units • to exclude any possible contact between the affected establishment and the separated epidemiological units as per the epidemiological enquiry • time period calculated backwards from the date of the confirmation • time period before the confirmation, during which the pathogenic agent may have been introduced in the separated non-affected epidemiological units of the affected establishment.
ToR 2	Article 27(3)c of the Delegated Regulation Annex II of the Delegated Regulation	4th scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of the latest outbreak of a category A disease in the protection zone. Products or other materials likely to spread the disease, must have been obtained or produced, before this time period in order to be exempted from prohibitions of movements.	<ul style="list-style-type: none"> • protection zone • non-affected establishments • Products or other materials likely to spread the disease, obtained or produced, before the start of the monitoring period of the affected establishment that originated the protection zone • time period calculated backwards from the date of suspicion of the latest outbreak in the protection zone • time period before the notification of the suspicion, during which the products and materials produced in the non-affected establishments of a protection zone may have been contaminated by the pathogenic agent of the disease.
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.	<ul style="list-style-type: none"> • protection or surveillance zone • non-affected approved germinal establishments • semen from kept animals (donor) of listed species • semen collected after the estimated date of the earliest infection of the earliest affected establishment that originated the protection zone/surveillance zone (if belonging to more than one protection or surveillance zones) • to take samples from the donor for laboratory analysis at least 7 days after the end of the monitoring period • to authorise movements of semen from approved germinal product establishments located in the protection or surveillance zones in case of favourable laboratory results

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
				<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> repopulation of a previous affected establishment kept animals of listed species to allow the repopulation of an affected establishment time period calculated forwards from the date of the final cleaning and disinfection of the establishment time period to ensure that the repopulation exercise is not put at risk due to the disease being unknowingly present in an establishment in the surrounding area.
ToR 2	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.	<ul style="list-style-type: none"> repopulation of a previous affected establishment kept animals of listed species to be repopulated the animals may not be introduced at the same time 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 (CBPP)	3 km	10 km	15 days	15 days	30 days
Infection with rinderpest virus (RP)	3 km	10 km	21 days	9 days	30 days
Infection with Rift Valley fever virus (RVFV)	20 km	50 km	30 days	15 days	45 days
Infection with lumpy skin disease virus (LSD)	20 km	50 km	28 days	17 days	45 days
Infection with <i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC (Contagious bovine pleuropneumonia) (CBPP)	Establishment	3 km	45 days	Not applicable	45 days
Sheep pox and goat pox (SPGP)	3 km	10 km	21 days	9 days	30 days
Infection with peste des petits ruminant virus (PPR)	3 km	10 km	21 days	9 days	30 days
Contagious caprine pleuropneumonia (CCPP)	Establishment	3 km	45 days	Not applicable	45 days
African horse sickness (AHS)	100 km	150 km	12 months	Not applicable	12 months
Infection with <i>Burkholderia mallei</i> (Glanders)	Establishment	Establishment	6 months	Not applicable	Not applicable
Classical swine fever (CSF)	3 km	10 km	15 days	15 days	30 days
African swine fever (ASF)	3 km	10 km	15 days	15 days	30 days
Highly pathogenic avian influenza (HPAI)	3 km	10 km	21 day	9 days	30 days
Infection with Newcastle disease virus (NCD)	3 km	10 km	21 days	9 days	30 days

Annex F – Uncertainty

Source or location of the uncertainty	#	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the assessment
ToR 1	1	There is limited data on the performance of the diagnostic tests considered in the assessment, particularly regarding the sensitivity and specificity of clinical examination, in the different species.	The effectiveness of the sampling strategies could be over or underestimated.
ToR 2 and ToR 3	2	Information on the period elapsed between the earliest point of infection and the suspicion report could only be retrieved from references obtained in countries in Africa where the disease was already present and therefore a higher awareness was expected	The effectiveness of the proposed monitoring period based on the limited available evidence could be overestimated.
	3	The two references originated from countries in Africa where surveillance systems may perform very differently, and therefore data may not be representative for other regions/periods due to differences in production systems affecting the effectiveness of surveillance systems.	The effectiveness of the proposed monitoring period could be over or underestimated.