SCIENTIFIC REPORT submitted to EFSA

Development of harmonised schemes for the monitoring and reporting of Q-fever in animals in the European Union[1]

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Abstract

Coxiella burnetii is the causative agent of Q fever, a well-known zoonosis. The clinical presentation of Q fever is non-specific in most animals, with the exception of ruminants where Q fever is responsible for late abortion and stillbirths. Q fever has only recently been included in the Community Summary Reports on Zoonoses. Reporting from the European Union Member States is not harmonised and the level of information available varies considerably. Therefore, a project on the development of harmonised schemes for the monitoring and reporting of Q fever in animals in the European Union was launched. More than 30 different animal species susceptible to Q fever have been recorded in Europe. However, domestic ruminants (cattle, sheep and goats) represent the source most often associated to human outbreaks. Thus, it is proposed to focus monitoring schemes on domestic ruminants. A standardised definition is suggested for a herd/flock considered as clinically affected with Q fever. This includes the occurrence of serial abortions, confirmation of the presence of *C. burnetii* by Polymerase Chain Reaction and positive serology by Enzyme-Linked Immunosorbent Assay. It is further proposed that the monitoring of Q fever should mainly rely on a passive system aiming at the identification of clinically affected herds and flocks and diagnostic methods should include a combination of Enzyme-Linked Immunosorbent Assay and Polymerase Chain Reactions. Guidelines for the interpretation of the test results are presented for cattle and small ruminants. Active monitoring schemes may be applied in countries that need to evaluate Q fever prevalence in their animal populations when the disease frequency in humans or animals is suspected to be high. Active monitoring can involve either bulk tank milk testing or sero-surveys. Harmonised reporting forms are suggested for submitting the information at Community level.

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Summary

Coxiella burnetii is the causative agent of Q fever, a well-known zoonosis. Q fever has spread worldwide and the infection is habitually asymptomatic both in humans and in animals. The clinical presentation of Q fever is non-specific in most animals, with the exception of ruminants where *C. burnetii* is responsible for late term abortion, stillbirths and low birth weight.

Q fever has only been included in the Community Summary Reports on zoonoses since 2005. Currently, data from Member States reporting Q fever cases in animals have improved. However, disease reporting from Member States is not harmonised and the level of information available varies considerably. Therefore, the European Food Safety Authority issued a grant for a project on the development of harmonised schemes for the monitoring and reporting of Q fever in animals in the European Union. The project objectives were to evaluate the current disease situation and the national level of monitoring and reporting; to identify animal species to monitor; to identify the most suitable diagnostic methods to be used; to define sample sizes, specimen types and sampling techniques; as well as to propose harmonised monitoring and reporting schemes.

In order to obtain relevant information a questionnaire survey was carried out among the Member States. The results indicated that in most Member States there are no regulations on Q fever in ruminants, and thus no official surveillance or centralisation of the data is organised. The disease in animals is notifiable in 14 Member States but a clear case definition is not available and the lack of apparent clinical symptoms is likely to lead to under-reporting. A literature survey indicated that more than 30 different animal species susceptible to Q fever were reported in Europe. However, domestic ruminants (cattle, sheep and goats) represent the source more often identified and associated to human outbreaks than other animal species. Consequently, it is proposed to focus monitoring schemes on domestic ruminants. The diagnosis of Q fever in these animal species involves the use of multiple techniques and can be interpreted validly only at herd or flock level. Polymerase Chain Reaction is regarded as a sensitive and rapid method for direct detection of *C. burnetii*, whereas Enzyme-Linked Immunosorbent Assay tests are recommended to be used for serological testing. In order to harmonise the reporting of Q fever outbreaks in domestic ruminants, it is proposed that a herd/flock be considered as clinically affected when serial abortions have occurred, the presence of *C. burnetii* is confirmed by Polymerase Chain Reaction from animals having aborted, and when serology by Enzyme-Linked Immunosorbent Assay is positive. Differential diagnosis with other abortive agents is essential. It is further proposed that monitoring of Q fever should mainly rely on a passive system aiming at identification of the clinically affected herds/flocks. This monitoring should include a combination of Enzyme-Linked Immunosorbent Assay and Polymerase Chain Reaction sampling techniques and diagnostics. Guidelines for the interpretation of test results are presented both for cattle and small ruminants. In addition, an active monitoring system is proposed for countries that may wish to evaluate the prevalence of Q fever in their animal population, especially when such information is needed whenever the disease frequency in humans or animals is suspected to be high. Such active monitoring may involve either bulk tank milk testing, both by Polymerase Chain Reaction and Enzyme-Linked Immunosorbent Assay, or a sero-survey using Enzyme-Linked Immunosorbent Assay on non-dairy herds and flocks. Finally, standardised reporting forms are proposed for both types of monitoring for use when submitting information at Community level.

Key words: Q fever, *Coxiella burnetii*, surveillance, monitoring, reporting, epidemiology, diagnostics, animals, ruminants.

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Background

The European Community (EC) system for the monitoring and collection of information on zoonoses is established by Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents[2]. This Directive requires Member States (MSs) to collect, evaluate and report data on zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks to the European Commission each year. The monitoring and reporting system used is based on that of MSs, and in a few cases it is harmonised by Community legislation to the extent that results from the monitoring are directly comparable between MSs.

According to the Directive, MSs have to send their zoonoses report to the European Commission annually by 31 May. The Commission is asked to submit this information to the European Food Safety Authority (EFSA), who is responsible for examining the data and for publishing the Community Summary Report from the results. The report is prepared by EFSA in close collaboration with the European Centre for Disease Prevention and Control (ECDC) and EFSA's Zoonoses Collaboration Centre. In practice, MSs report the information on zoonotic agents in animals and food through a web-based reporting application run by EFSA.

It should be noted that data on zoonoses cases in humans are provided through the Community networks for the epidemiological surveillance and control of communicable diseases established under Decision No 2119/98/EC and coordinated by ECDC.

According to Directive 2003/99/EC, the reporting of information on Q fever takes place on the basis of the epidemiological situation in the country, which means that MSs should report the information if those zoonotic agents are considered to be of importance in their country. For the reporting year 2006, 10 MSs reported information on Q fever (*Coxiella burnetii*) in animals.

In the Community Summary Report on zoonoses (EFSA, 2010), the information received from MSs is analysed and summarised specifically to identify trends in the occurrence of zoonotic agents and the sources of human infections. As there are currently no detailed harmonised rules or recommendations for reporting and monitoring Q fever, the data obtained is often difficult to analyse and interpret at the Community level.

EFSA's Scientific Panels on Biological Hazards and on Animal Health and Welfare have issued two opinions on the Review of the Community Summary Reports on Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004 and 2005 (EFSA, 2006; 2007). In these opinions the panels provide recommendations on the improvement of the monitoring and reporting of Q fever. The panels also stated that there is a need for a common strategy on data collection, monitoring and reporting as well as for the improvement of the harmonisation of definitions, in order to improve the usefulness of the data presented in the Community Summary Report.

[2] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC (OJ L 325, 12.12.2003 p. 31)

Terms of reference

The objective is to obtain proposals for the development of harmonised monitoring and reporting schemes for Q fever, respectively, in animals under Directive 2003/99/EC. The schemes shall be applicable in all European Union (EU) MSs and in compliance with relevant Community legislation.

The harmonised monitoring and reporting schemes shall, in particular, specify:

- the animal species, which should be monitored and the study populations (subgroups of the population) to be targeted. The animal species may cover farm animals, pet animals, zoo animals and wildlife;
- the stage when the sampling should take place (e.g. at farm, at slaughterhouse);
- the sampling strategy (the procedure on how to select samples) and the sample size (the number of samples to be collected);
- the type of specimen to be taken and the sampling techniques to be used;
- the diagnostic and analytical methods to be used;
- the information to be collected at national level and possibly at regional level; and
- the information to be reported.

The rationale for the specifications chosen in the monitoring and reporting schemes must be given. When developing the schemes, the following shall be taken into account: public health and animal health needs, the feasibility and cost-effectiveness of the schemes, different MS situations, existing Community legislation as well as the scientific advice of EFSA's scientific panels as well EFSA's guidance documents.

The schemes shall also include suggestions for the analyses of data at national and Community levels, and, in particular, indicate where the following of trends over the reporting years would be useful and where spatial analyses would be applicable.

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1. Introduction

The gram-negative, obligate-intracellular bacterium *Coxiella burnetii* is the causative agent of Q fever, a well-known but neglected zoonosis. Q fever has spread worldwide with the exception of New Zealand. The infection is habitually asymptomatic both in humans and in animals (Maurin and Raoult, 1999; Norlander, 2000).

In humans, two clinical patterns of Q fever can be observed. The acute disease, most often asymptomatic or resembling a flu-like syndrome, is usually a self-limiting febrile illness during which pneumonia or hepatitis can occur. The chronic disease, is a severe and possibly fatal illness, usually resulting in endocarditis and occasionally in a vascular infection, osteomyelitis and/or chronic hepatitis. Some patients may develop a chronic fatigue syndrome and *C. burnetii* infection can lead to abortions, stillbirth or premature deliveries in pregnant women (Maurin and Raoult, 1999).

The reservoir of *C. burnetii* is large and includes mammals, birds and arthropods, mainly ticks. Generally, the clinical presentation of Q fever is non-specific in most animals, with the exception of ruminants where *C. burnetii* is responsible for late term abortion, mortinatality, prematurity and low birth weight (Moore et al., 1991; Bildfell et al., 2000).

Many animal species are susceptible to infection by *C. burnetii*: ruminants, domestic carnivores, wildlife mammals, birds and arthropods, such as ticks. Different species may play a role in the dissemination or maintenance of the disease, either as pathogen-carriers or as vectors, although the role of some of them in the transmission of the disease has not been yet established with certainty. The source of human infection is often unknown, although sheep and goats are more frequently related to Q fever outbreaks in humans than are other animal species. For instance, most cases in man follow a direct or indirect exposure to livestock and could be attributed to livestock practices, such as spring lambing and shearing, leading to environmental contamination and spread of the microorganism. Therefore, human cases or outbreaks are considered a good indicator of disease activity, leading to the strengthening of the investigation into likely sources (Cutler et al., 2007, Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Berri et al., 2003; van der Hoek et al., 2010). Nevertheless, domestic ruminants are considered to be the main source of human infection as they may shed *C. burnetii* in urine, faeces, milk and birth products. High concentrations of *C. burnetii* are found in the placenta and vaginal secretions of infected animals (Arricau-Bouvery and Rodolakis, 2005; Arricau-Bouvery et al., 2003; Berri et al., 2000; Berri et al., 2007). The contamination of humans occurs after inhalation of aerosol or dust contaminated with parturient fluids of infected livestock. In addition, survival of bacteria in an unfavourable environment and long-term persistence (as a pseudo spore) are likely to contribute to the prevalence of enzoo-epizootic foci of Q fever.

In Europe, Q fever has been described in almost every country, but the epidemiological situation is not well-known because of a considerable variation in monitoring or the lack of specific Q fever surveillance systems across EU MSs. Moreover, the epidemiology of this disease is largely unstudied. Indeed, both human and animal *C. burnetii* infections are under-diagnosed and under-reported mostly because of the polymorphic nature of the disease, characterised by the absence of apparent clinical symptoms, and the lack of awareness of this disease in medical and veterinary communities. In addition, the diagnosis of Q fever is a laboratory-based diagnosis and requires expensive and elaborate methods necessitating well-

trained personnel to establish an unequivocal Q fever diagnosis.

Even though historically not perceived as an important public health threat in the medical or veterinary communities, *C burnetii* can cause debilitating disease and may result in potentially fatal chronic infections among humans. It is also considered a potential agent of bioterrorism because of its accessibility, low infectious dose, resistance to environmental degradation, and aerosol route of transmission (Centers for Disease Control and Prevention, USA; http://www.cdc.gov).

Moreover, human outbreaks in urban or residential areas have been recently reported in EU MSs (Bulgaria, Germany and the Netherlands) and in Croatia, involving large numbers of cases and being linked to small ruminant flocks (Panaiotov et al., 2009; Medic et al., 2005; Porten, et al., 2006; Gilsdorf et al., 2008; Schimmer et al., 2008). For instance, the situation in a country could rapidly become alarming, such as the Q fever outbreak in the Netherlands which has been ongoing since 2007 (accounting for 194 cases in 2007, 982 cases in 2008 and 2,305 cases in 2009) with deaths partly caused by Q fever (National Institute for Public Health and the Environment, the Netherlands; http://www.rivm.nl). This contrasts with the past situation where, between 1997 and 2006, relatively few cases of Q fever were reported in the country (5 to 16 cases per year). Indeed, until a few years ago, Q fever was not diagnosed as a clinical disease in ruminants in the Netherlands. The lack of efficient diagnostic methods could have hampered the diagnosis of Q fever at an earlier stage (Wouda and Dercksen, 2007). The situation in the Netherlands emphasises the role of ruminants, in particular goats, as an important reservoir of infection. Therefore, the health surveillance of ruminant herds or flocks prone to abortions should be encouraged.

Moreover, these recent and large outbreaks highlight how zoonoses such as Q fever may also represent a public health threat for urban populations and emphasise the need for strengthening surveillance regarding Q fever in MSs. Therefore, the implementation, development and standardisation of monitoring and detection methods are crucial for helping future preventive and control measures.

2. Current disease situation in the MSs, national level of monitoring and reporting information

2.1 Rationale

In the terms of reference it is specified that harmonised schemes should consider different situations in MSs and the schemes should be designed to be applicable in all EU MSs. Consideration should also be given to testing schemes currently carried out in MSs. A questionnaire was designed to gather data needed to assess public health needs, the current testing situation and to define epidemiological parameters.

2.2 Approach

A questionnaire related to Q fever was designed and distributed to EFSA's Zoonoses Task Force members in order to collect information from MS competent authorities by 10 April 2009. The questionnaire related to the epidemiological situation of the disease, the existence of a regulation on the disease, the current surveillance and data collection systems, as well as the sampling and diagnostic methods available in the country. In addition, the use of personal contacts or networks within the project team combined with literature searches were carried out in order to gather data about recent Q fever outbreaks in MSs.

2.3 Results

Twenty-four out of 27 MSs and two non-MSs responded to the questionnaire, which allowed the identification of official contacts from many MSs, represented by the Zoonoses Task Force members. The last response was received on 30 September 2009. The existence of national reference laboratories for Q fever were identified in several MSs (Appendix A). Information was received regarding the status of the disease in each country (Appendix B) as well as about the existence of specific national or local regulations on Q fever in ruminants (Appendix C).

According to the information collected, most of the MSs do not carry out official monitoring or control programmes for Q fever and usually diagnosis of Q fever is part of a differential diagnosis made for the confirmation of the agent responsible for abortions in ruminant herds/flocks.

3. Identification of animal study population species

3.1 Rationale

The variety of animal species susceptible to *C. burnetii* infection, constituting the host range is very large. Natural reservoirs playing a role in the transmission of Q fever, both for humans and animals, are numerous. Thus, the identification of the most relevant animal species for any monitoring to be implemented is important for gaining basic knowledge on Q fever prevalence and for identifying changes in the incidence and geographical distribution of the disease on an annual basis.

3.2 Approach

Literature (scientific publications, textbooks, official websites, OIE/WHO/ECDC) on *C. burnetii* and Q fever was reviewed. Susceptible animal species present in Europe were identified by literature searches and expert knowledge within the network. Outbreaks involving ruminants were identified in different countries starting from the year 2000 (reports in literature and databases). A common definition for livestock clinically infected by Q fever and also for non-clinical Q fever was also set up.

3.3 Source of contamination and rationale for the choice of the study population

The gram-negative, obligate-intracellular bacterium *C. burnetii* can infect a wide range of susceptible hosts including farm animals, pets, wild mammals and even non-mammalian species, such as domestic and wild birds, reptiles and ticks. Virtually all animals infected with *C. burnetii* can act as a reservoir, and should be regarded as possible sources of infection for humans (Babudieri 1959; Lang 1990; Rousset et al, 2009b). To highlight this, the project team compiled a list of susceptible animal species (excluding domestic ruminants) that have been investigated in different MSs (Appendix D). It is noticeable that many species of birds have been reported to be infected and may represent an under-estimated source of infection. They may also be involved in the long distance spreading of the disease between herds/flocks through their migration routes.

However, domestic ruminants represent the most often reported source of *C. burnetii* infection associated with human outbreaks compared to any other animal species. Moreover, the described outbreaks linked to domestic ruminants have involved large numbers of human cases (van der Hoek et al., 2010; Arricau-Bouvery and Rodolakis, 2005; Norlander 2000; Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Berri et al., 2003).

It is concluded that:

- ***domestic ruminant species (cattle, sheep, goats) are more frequently associated to human outbreaks than other animal species;***
- ***the role of pets is not well-documented and the role of wildlife is not yet understood in Q fever;***
- ***in special situations, for example when the source of infection remains unclear, it may be necessary to check for the* C. burnetii *status of wildlife in the vicinity of human cases;***
- ***consequently, the monitoring and reporting schemes proposed in this report will focus on domestic ruminant species.***

3.4 Establishment of a common case definition for livestock clinically infected by Q fever

The clinical pattern of Q fever in animals is pleiomorphic. In most cases, infection of cattle, sheep and goats with *C. burnetii* remains non-apparent, with or without serological response, and infected animals may shed the bacteria.

Due to some differences between these three domestic ruminant species, some details of the case definitions will be considered separately. The proposal is to distinguish cattle from small ruminants, mainly because the symptoms of clinical suspicion differ markedly between the two categories of ruminants, but only a little between the two species of small ruminants. The following two situations can be distinguished:

- when late-term abortions, stillbirths or birth of stunted animals are observed in sheep and goat flocks, Q fever is highly suspected as the cause, with up to 90% of the reproductive females within the flock being possibly affected (Arricau-Bouvery and Rodolakis, 2005);
- however, in cattle *C. burnetii* may be associated with metritis and infertility (Rodolakis 2009), and pneumonia has also been described. Given the lack of specificity of these latter symptoms, it is not recommended to retain them for clinical diagnosis of Q fever. The major warning sign to be taken into account is the number of abortions and calves with low birth weight in cattle herds (two or more abortions in a month or three abortions in the year for herds with less than 100 cows and more than 4% of cows aborting during the course of the year for herds of more than 100 cows).

In both situations, following an abortion storms, it is essential to include the diagnosis of Q fever in the clinical differential abortion diagnosis (see Section 3 on diagnostic and analytical methods).

Taking this into account and according to the expertise within the consortium, a more comprehensive case definition was established, as proposed hereafter.

A herd or flock should be considered clinically affected with Q fever when:

- abortions and/or stillbirths have occurred;
- positive PCR results confirming the presence of the agent of Q fever on specimens from affected animals in the herd or flock; and
- positive serology.

The types of samples for the clinical investigation of Q fever are vaginal mucus and placenta or foetal tissue (see Table 1 in Section 5 on diagnostic and sampling approach).

To prevent the transmission of the infection from a herd/flock to another, or from animals to humans, it is thus essential to identify the clinically affected animals and herds/flocks that shed *C. burnetii* on a massive scale in placentas, vaginal secretions and faeces.

Q fever case definition in animals	
The proposed definitions are as follow:	
Confirmed case	***Probable case***
• *Clinical pattern of coxiellosis: mainly abortion, stillbirth* • *Confirmation of agent presence (PCR-positive, isolation, staining, IFA[(a)])* • *Positive serology*	• *Clinical pattern of coxiellosis: mainly abortion, stillbirth* • *Positive serology*

(a) immunofluorescence assay tests

4. Identification of the most suitable diagnostic and analytical methods

4.1 Rationale

Regarding the diagnosis of Q fever in ruminants several tools are available for direct and indirect detection of *C. burnetii*. Testing for *C. burnetii* is highly advisable after an abortion storm in the flock/herd. In this case, it is recommended to carry out a group diagnosis (contrary to an individual diagnosis) and simultaneously seek other abortive agents (differential diagnosis for: *Brucella, Chlamydophila abortus, Toxoplasma, Salmonella*, etc) accordingly to major pathogens that may be involved among large and small ruminants in the targeted geographical area.

4.2 Approach

Existing analytical methods, as cited in publications or official methods (OIE diagnostic manual for terrestrial animals, 2008), and used within EU MSs were compiled and listed. Also the approximate specificity and sensitivity of these methods were considered based on expert knowledge and ring trial analyses in the framework of the EU funded project (MedVetNet; www.medvetnet.org). To date there is no prescribed test for Q fever and theoretically the gold standard method is the isolation of the bacterium. However, the isolation of these strictly intracellular bacteria is time-consuming, cannot be used routinely and may not be successful.

A cost estimate for the analysis was also included in the considerations as this is an important criterion when recommending analytical methods. An example of cost estimate is given in Appendix E.

4.3 Results

Feedback from the questionnaires to MSs revealed that, except for a few MSs from which information is lacking or incomplete, the principal methods necessary for the diagnosis of Q fever are already in use in the MSs listed in Appendix F.

It is important to note that there is no officially prescribed test for Q fever. However, the complement fixation test (CFT), was proposed as an alternative test for international trade by the Office International des Epizooties (OIE, 2008). According to the OIE, alternative tests may be used for the diagnosis of a disease within a local context, and can also be used when importing and exporting animals following bilateral agreements. Besides, indirect immunofluorescence assay tests (IFA) and Enzyme-Linked Immunosorbent Assay (ELISA) tests (commercial and in-house assays) are increasingly being used. At least three ELISA commercial kits for the diagnosis of Q fever in domestic ruminants are currently available.

Comparative analyses of these serological methods, through studies on the field as well as ring trial assessments in the framework of an EU funded project (MedVetNet: http://www.medvetnet.org), revealed that IFA and commercially available ELISAs were

comparable in sensitivity and exhibit a minimum discordance rate compared to the CFT assay. Indeed, the CFT's sensitivity was highly variable and weak compared to ELISA or IFA (Ruiz-Fons et al., 2010; Kittelberger et al., 2009; Rousset et al., 2007a; Rousset et al., 2009b; Roest et al., 2008). Most CFT results were negative or weakly positive in animals that have aborted from Q fever (Rousset et al., 2007a) and in *C. burnetii* shedding animals (Rousset et al., 2009a). The CFT failed to detect some cases when anti-complementary substances were present in the tested sera. Also, some antibodies were not revealed by CFT because of differences in the ability of the IgG sub-classes to activate the complement. In ruminants, only IgG1 antibodies are known to fix the complement in the CFT. Moreover, CFT titers may be reduced due to the presence of IgG2 and IgM antibodies which can suppress complement fixation by IgG1 antibodies (Rousset et al., 2009a).

IFA is not often used for the diagnosis of Q fever in animals since it is inconvenient for large scale screening, whereas ELISA requires a single dilution of sera and can be automated. No commercial kit using IFA for veterinary investigation is available. Despite these advantages of ELISA, it has recently been shown that ELISAs can display different sensitivities (Kittelberger et al., 2009). In addition, ELISA tests based on antigens prepared from a ruminant isolate are more sensitive than ELISAs based on antigens from the reference strain Nine Mile (isolated from ticks) (Touratier et al., 2007). Therefore, harmonisation of ELISA tests remains an important goal, especially for the determination of the positivity cut-off of the different tests used. ELISA tests are best suited for testing large numbers of animals and flocks.

It is concluded that:

- ***for the serological diagnosis of Q fever, it is highly advisable to use ELISA tests rather than CFT;***
- ***ELISA tests are more sensitive and more specific than CFT;***
- ***ELISA tests using antigens prepared from ruminant isolates are the most sensitive.***

Although the serological methods are useful they do not allow for the identification of *C. burnetii* shedding animals. Indeed, some infected animals can be seropositive without shedding *C. burnetii*, and others can shed the bacteria and remain seronegative, which is of great concern and could have an important impact on both animal and public health. In addition, there is no serological test which can distinguish between vaccinated and naturally infected animals.

Currently, the PCR is one of the most sensitive and rapid means for the direct detection of *C. burnetii* and the identification of shedding animals. PCR is adapted to a wide range of samples; it is sensitive and rapid, and is becoming increasingly common in diagnostic laboratories (Berri et al., 2000; Nicollet and Valognes, 2007). Moreover, the development of real-time PCR technology has recently allowed the quantification of *C. burnetii* in samples, and commercialised kits are now available.

It has been shown recently that PCR tests are commonly used to detect the bacteria in MSs

(Duquesne et al., 2008, EFSA questionnaire). Moreover, comparative ring trial analyses, involving seven MS laboratories, revealed that the specificity level was comparable among different laboratories detecting *C. burnetii* DNA from three different spiked matrices (Phosphate buffer saline (PBS), placenta and milk) as well as from Q fever-positive ruminant abortion samples (Duquesne et al., 2008; Jones et al., 2009). Regarding sensitivity, PCR tests directed to the multiple copy target IS*1111* (real time and conventional) were superior to tests detecting single copy genes. In addition, the real time PCR advantage over conventional PCR is the possibility of quantifying the number of bacteria in a biological sample. Indeed, the number of bacteria in the placenta could help the veterinarian to make or confirm an abortion diagnosis (Duquesne et al., 2008; Jones et al., 2009). Thus, detection of *C. burnetii* could be achieved by most laboratories with a good sensitivity rate. It is widely accepted that for routine diagnostics real-time PCR technology is preferable to conventional gel-based detection methods. It allows high sample throughput, has a reduced potential for carry-over contamination (as it is a closed system) and is best suited for quantification.

In conclusion, in veterinary practice, serology is often the only examination carried out in the diagnosis of abortive agents in ruminants. For the diagnosis of abortion in ruminants, ready-to-use serological kits (CFT and ELISA) are commercially available. These tests reveal only a suspicion of abortion being caused by Q fever. The result remains suggestive as long as it is not coupled with a search for the infectious agent (by PCR for example). The overall approach is differential and consists in looking for several abortive agents.

It is recommended that the strategy for screening animal infection to be followed is:

- ***a PCR test of vaginal swabs (generally, the bacterial burden is high in the placenta and vaginal secretions, lower in milk and scarcely known in faeces, urine and sperm);***
- ***associated with ELISA serology (technique more specific than CFT which allows the quick handling of large sera series), however serology cannot be used in vaccinated herds/flocks.***

The techniques to be used for the diagnosis of Q fever must be multiple and can be interpreted validly only at herd/flock level.

5. Specimen types and sampling techniques

5.1 Rationale

As stated above the diagnosis of Q fever involves the use of multiple techniques and can be interpreted validly only at herd or flock level. For instance, a serological positive result from an animal indicates only that the animal was in contact with the Q fever agent. Current serological methods do not provide information on the stage of the infection (recent or latent infection) or whether the animal is shedding or not.

For a herd/flock, it is still difficult to identify if it is shedding or will be shedding *C. burnetii*, without setting up a heavy protocol, involving the multiplication of samples from a sufficient number of animals.

A set of reasons led the members of the consortium to suggest making diagnoses at herd/flock level rather than at individual animal level, in particular:

- the non-specific clinical symptoms especially when cases are isolated;
- the high frequency of the non-apparent infection with regard to the symptomatic infection;
- the fact that the herds/flocks, where several animals are clinically affected with Q fever, are potentially the ones shedding most and thus more important in terms of risk to public health;
- the important circulation of *C. burnetii* within the herd/flock population.

5.2 Approach

Information gathered within the framework of Sections 1 to 3 helped to identify sampling stages and to establish a sampling strategy. Moreover, literature (scientific publications, textbooks, official websites, OIE/WHO/ECDC) on *C. burnetii* and Q fever was reviewed for this purpose.

5.3 Results

For direct identification of *C. burnetii*, sampling should be targeted at pregnant animals either giving birth normally or aborting. This is because infected female animals, even with normal parturition, are high shedders of *C. burnetii* into birth products (Arricau-Bouvery et al., 2003). *C. burnetii* shedding may persist over several months (Kim et al., 2005; Berri et al., 2005). However, the shedding level of the bacteria decreases after parturition or abortion. Thus, sampling should be carried out as soon as possible after this period and more precisely within the week following abortion or parturition. The identification of the presence of the bacteria in the vaginal mucus of animals having aborted, or in their foetuses, by molecular methods, will then be more reliable.

The most suitable samples will be:

- a vaginal swab;
- a placenta swab focusing on the zones of necrosis;
- from the placenta: cotyledons presenting lesions;
- the organs (spleen, lung, liver) or the stomach contents of the aborted foetus.

Note: it is strongly recommended that sampling be carried out as soon as possible after the abortion and at most within the following week.

In every case, it is recommended to carry out the analysis (at least the DNA extraction) within a maximum of 48-72 hours following sampling, with samples being sent and kept at 4°C.

Besides its presence in birth products, *C. burnetii* is also shed in faeces and milk. However, the quantity of the bacteria in these biological compartments may vary in function of the stage of infection. Concomitant shedding by several routes remains weak (Rodolakis, 2009). A high percentage of detected animals are positive only by a single route of shedding (especially by milk or vaginal mucus).

Studies carried out on bulk tank milk (BTM) samples indicate that:

- *C. burnetii* shedding seems to be very frequent in milk from asymptomatic dairy cows (Kim et al., 2005; Rodolakis, 2009);
- *C. burnetii* shedding in milk is less widespread in ewes than in cows. These differences in shedding patterns may depend on host species or *C. burnetii* strain (Rodolakis, 2009);
- In goats, shedding is observed in all three routes but shedding into milk seems to be a frequent route.

Therefore, BTM samples can be used to investigate the sanitary condition of dairy cattle and goat herds, by checking for the presence of *C. burnetii* by PCR and for antibodies by ELISA, but cannot be used in the context of abortion diagnosis.

The indirect diagnosis of Q fever should depend mainly on the ELISA test. Therefore, blood sampling would be carried out for this purpose. However, numerous seroconversions in animals are only indicative of the circulation of the bacterium in the herd/flock, without necessarily the presence of clinical symptoms. Serology should never be interpreted at individual level but at herd/flock level only. To this end, a minimum number of samples should be analysed (see below).

As a reminder, serological analyses:

- will be used as a complement to the PCR, if necessary (i.e. in the absence of abortion sample material);
- will be carried out, preferably, by means of a test using antigens from a ruminant *C. burnetii* isolate;
- will be carried out on sera from several animals having aborted or shown reproductive problems.

As for the sampling strategy and the sample size, these will be part of the proposed monitoring schemes and are set out in detail in Section 5.

6. Proposed harmonised schemes for monitoring and reporting

6.1 Proposition of monitoring schemes

Epidemiological monitoring and surveillance are essential for the protection of animal populations against exotic or new diseases, as well as for the implementation and for the evaluation of prophylaxis and control programmes. Monitoring and surveillance are also useful for public health safety and allow the collection of data on zoonoses that are enzootic, new or re-emerging.

Generally, surveillance consists of the systematic collection, analysis, interpretation and prompt dissemination of data on specific diseases or syndromes to those who need to know, for relevant action to be taken. The main purpose of a surveillance system is to determine the need for immediate or longer-term action in response to diseases. A surveillance programme can be described in terms of input, processing and analysis, and outputs. The input of a surveillance programme includes passively or actively collected data. The output of a surveillance system are, in general, technical reports on health conditions, resources available, their use and results obtained.

Hereafter, two monitoring schemes for Q fever, a passive and an active scheme, are set out.

6.1.1 Passive monitoring scheme

The following scheme aims at detecting herds of cattle or goats, or flocks of sheep that are clinically affected with Q fever in an endemic (infected) area, region or country.

In order to achieve the screening of the affected herds/flocks, the proposed scheme is based on recommendations from the French association for the working group on farm animal health certification "ACERSA" (Touratier et al., 2007) and has been developed as follows.

The plan is passive because it starts when several abortions in a herd/flock have been observed (see case definition of clinical Q fever). This is followed by an investigation within the herd/flock to confirm the diagnosis and to identify other cases. Thus, contrary to an active plan, it does not rely on a systematic search for Q fever in a animal population.

The differences in the clinical pattern between the three domestic ruminant species led to the proposition of adapted approaches distinguishing cattle and small ruminants (see Case definition of clinical Q fever).

Following the same logic, to widen the search of the presence of the bacterium in several animals or samples, while moderating the cost of the diagnosis, PCR analyses of pooled samples appears to be an alternative which should be considered.

Note: Including the systematic search for C. burnetii in *the process of differential abortion diagnoses (even if the case is not consistent with the case definition) is highly advisable and encouraged.*

6.1.1.1 Diagnostic and sampling approach in cattle

In cattle herds, the major warning sign to be taken into account is the occurrence of a series of abortions, as defined below:

- for herds < 100 animals: 2 abortions or more in the month or 3 abortions in the year;
- for herds > 100 animals: more than 4 % of cows with abortions in the year.

Thus, the observation of a series of abortions in cattle should lead to the mandatory follow-up of the herd by differential diagnoses including Q fever.

Note: In cattle C. burnetii *is capable of inducing pneumonia, possibly followed by a abortion storms and/or premature births and sick calves, and also a series of metritis and infertility. However, considering their lack of specificity, the symptoms of metritis and pneumonia should not solely be taken into account when making a clinical diagnosis of Q fever. Abortion is by far the most frequent and significant clinical symptom related to Q fever in cattle.*

Following a series of abortions in a herd, the following are recommended:

- the sampling of a vaginal swab, and/or a specimen of abortion materials (placenta and spleen, liver, lung or stomach contents of the foetus) from **one or two** animals having aborted **less than eight days previously** for PCR detection (preferably using quantitative PCR).
- serological blood sampling of at least **six animals (three multiparous and three primiparous)** including, preferably, animals having aborted more than **15 days** previously or female animals presenting reproductive problems (i.e. presenting symptoms such as metritis, late or changed returns to heat in the previous four months) and, if needed but without exceeding 50% of the totality, animals without reproduction problems from the same herd. Table 1 summarises the nature and number of samples and the type of analyses to be carried out.

Table 1. **Nature, number of samples and type of analysis to be carried out in the case of diagnosis of Q fever in cattle herds**

Target Animals	Nature of Sample	Type of Analysis	Number
Cows having aborted and foetuses within eight days following abortion	Vaginal swab or placental swab or specimen of abortion materials (placenta and stomach content, spleen, lung or liver of foetus)	PCR (preferably quantitative real-time PCR)	All concerned cows with a maximum of three cows
Cows having aborted from 15 days and less than four months ago	Blood	Serology by ELISA (preferably with kits using antigens prepared from *Coxiella* isolates from ruminant)	Minimum of six (if possible equilibrate between multi-and primiparous cows)
Cows presenting reproduction problems: late returns, metritis in the previous four months.			Only if necessary (to complete the sampling above up to six animals in total)
Control cows: those showing no reproductive problems			

Note: The choice of the number of animals to be sampled constitutes a compromise between, technical considerations, with the inclusion of a minimum number of animals allowing the interpretation of results at herd level, and economical considerations related to the cost of sampling and eventual serological analyses. The blood samples would possibly be analysed according to the results obtained from one or both PCRs.

6.1.1.2 Diagnostic and sampling approach in small ruminants

Regarding small ruminants, any occurence of abortion mainly at the end of gestation and/or of premature births, births of sick or stillborn animals should lead to a differential diagnosis in which it is advisable to include Q fever.

Note: It does not seem useful to define a number or a rate of affected animals to trigger the investigation since:

- the flock sizes of small ruminants might be very variable;
- the rate of abortions and/or premature births, sick new-born and stillborn linked to the circulation of Q fever can be very variable even if it seems to be higher in goats.

In the case of small ruminants it is more essential than in cattle to consider a diagnosis of clinical Q fever, within the framework of a differential diagnosis of abortions. In order to widen the search for the causative abortive agents from several animals, and in order to decrease the cost of the diagnosis, pooling samples for PCR analyses appears to be an optimal solution.

It is recommended that one of the following be carried out:

- either two PCRs analyses in the laboratory on individual samples, or
- two PCRs analyses of pooled samples comprising maximum of six individual samples.

Due to the current methods available and the lack of a multiplex PCR method for the detection of abortifacient agents, a PCR-based diagnosis requires, in most cases, separate PCRs to be carried out for each of the abortive agents considered from a single DNA extract.

Thus, based on these considerations, following a series of abortion in a flock the following is recommended (Table 2):

- the sampling of a vaginal swab, and/or a specimen of abortion materials (placenta and/or spleen, liver, lung or stomach contents of foetus) from **two to six animals** having aborted **less than eight days** previously with the aim of PCR detection (preferably quantitative PCR).

Note: depending on the size of the flock, analyses of pooled vaginal swab samples rather than individual samples can be envisaged. The samples of placenta, organs or stomach contents of foetus should be reserved for individual analyses.

- If a single sample for the purposes of PCR is available, serological sampling of **at least ten animals** of the affected flock should be carried out at once (i.e. animals having aborted or having stillbirths from at least 15 days to a maximum of three weeks previously, together with, if necessary, animals having given birth from at least 15 days to a maximum of three weeks previously from the same flock). These samples should be kept for ELISA testing, in case of a negative PCR result.

Table 2. **Nature, number of samples and type of analysis to be carried out in the case of diagnosis of Q fever in small ruminants flocks**

Target Animals	Nature of Sample	Type of Analysis	Number
Goats or ewes having aborted within eight days	Vaginal swab or placental swab or specimen of abortion materials (placenta and stomach content, spleen, lung or liver of foetus)	PCR (preferably quantitative real-time PCR) allowing differential diagnosis of abortions	Sample two to six animals to carry out two PCR analyses: two individual PCRs or two pooled PCRs (when more than two animals are sampled)
If only one sample is available for the PCR testing or if one of the two quantitative PCRs is inferior to the set threshold see below			
Goats or ewes having aborted from 15 days to three weeks ago	Blood	Serology by ELISA (preferably with kits using antigens prepared from *Coxiella* isolate from ruminant)	Minimal sampling of 10 animals privileging ones having aborted (if possible five or more)
Goats or ewes presenting stillbirth from 15 days to three weeks ago			
Goats or ewes from the same herd: with no reproductive problems within three weeks following lambing			

6.1.1.3 Guidelines for the interpretation of test results

As a general rule for the interpretation of the diagnostic tests results, three situations can be distinguished for the herds/flocks according to the various combinations of the obtained results (see Appendix G).

- Situation A: the herds/flocks are considered as clinically affected with Q fever.
- Situation B: the diagnosis of clinical Q fever cannot be extended to herd/flock level. Most likely an isolated case of abortion due to Q fever has occurred. However, it is recommended to follow up the evolution of the clinical pattern as follows:

  Additional investigations are to be carried out, for any new abortion during a period of one year from the first abortion in cattle or during the period of birth for small ruminants, in order to confirm or to exclude Q fever as being the cause of abortion.

  In the absence of new abortions during this period, Q fever is not considered as being the origin of the abortion storms at herd/flock level.
- Situation C: Q fever is not considered as being the origin of the abortion storms at herd/flock level.

Recommended threshold for quantitative PCR: Although a threshold is not officially approved at international level, a group of French experts has suggested that abortion in ruminants should be suggested to be caused by *C. burnetii* when at least 10^4 bacteria per gram

of placenta or vaginal swabs are detected (Touratier et al., 2007). In tissues or stomach contents from aborted foetuses, the same group considered that a positive result by quantitative PCR is sufficient to diagnose Q fever as the origin of abortion. For pooled samples, the proposed threshold is 10^3 bacteria per pool. These thresholds are indicative and may be revised especially if new scientific information becomes available.

Recommended threshold for ELISA: in the absence of reference sera, it is recommended to refer to the interpretation guidelines of the kit's supplier.

Interpretation in cattle herds:

For the diagnosis of Q fever in cattle herds two scenarios could be observed, as illustrated in Appendix G.

First scenario: samples of abortion products, for quantitative PCR, from two animals are available.

If both quantitative PCRs are superior or equal to the recommended threshold: the diagnosis of Q fever in the herd is confirmed (situation A).

If only a single quantitative PCR is superior or equal to the recommended threshold, the results from serology would guide the diagnosis:

- if more than 50% of tested animals are seropositive, the diagnosis of Q fever is confirmed (situation A).
- if less than 50% of tested animals are seropositive: the suspicion of abortions due to Q fever cannot be ruled out. It is then recommended to carry out new sampling within three weeks on the initially sampled animals (with the exception of the animals who have already been confirmed seropositive). If three weeks later (the positive animals in the first series of analyses are re-entered into the calculation):
    - 50% or more of tested animals are seropositive, the diagnosis of Q fever in the herd is confirmed (Situation A);
    - less than 50% of tested animals are seropositive, it is unlikely that the abortion storms is related to Q fever. However, it is recommended to monitor any development in clinical symptoms (situation B) during the period of surveillance (up to one year after the first abortion) and any new aborting animals or animal having a miscarriage must be immediately sampled with the aim of carrying out a PCR analysis.

If both quantitative PCRs are lower than the retained threshold, Q fever is not considered as being the origin of the abortion storms at herd level (Situation C).

Second scenario: samples of abortion products for quantitative PCRs from only one animal are available.

If the quantitative PCR is superior or equal to the retained threshold, the results from serology would guide the evaluation of the diagnosis:

- if 50% or more of the tested animals are seropositive, the diagnosis of Q fever is confirmed (Situation A);
- if less than 50% of the tested animals are seropositive: the suspicion of abortions due to Q fever cannot be excluded. It is then recommended to take new samples within three weeks on the initially sampled animals (with the exception of the animals who have already been

confirmed seropositive). If three weeks later (the positive animals in the first series of analyses are re-entered in the calculation):

- 50% or more of the tested animals are seropositive, the diagnosis of Q fever in the herd is confirmed (situation A);
- less than 50% of tested animals are seropositive, Q fever still cannot be excluded (situation B). It is then recommended to monitor any development of clinical symptoms during the period of surveillance (up to one year after the first abortion) and any new aborting animal or animal having a miscarriage must be sampled (vaginal swab and/or placenta and/or organs or stomach content of the runt) with the aim of carrying out a PCR analysis.

If the PCR is lower than the threshold and if no other abortive agent was identified, serological analyses are made on six animals and the results would allow guiding the evaluation of suspicion:

- if three to six animals are seropositive, Q fever cannot be excluded and a quantitative Q fever PCR is to be carried out on any new abortion (Situation B). If this one is superior or equal to the threshold, the diagnosis of Q fever is confirmed at herd level (situation A) and if it is lower than the retained threshold Q fever is not considered as being the origin of the abortion storms at herd level (Situation C);
- if less than three animals are seropositive: Q fever is not considered as being the origin of the abortion storms at herd level (Situation C).

Interpretation in small ruminant flocks:

As for cattle, two different Q fever diagnosis scenarios can be distinguished in small ruminant flocks (Appendix G).

First scenario: samples of abortion products from two to six animals are available for quantitative PCR (two individual PCRs or two pooled PCRs).

If both quantitative PCRs are superior or equal to the recommended threshold: the diagnosis of Q fever in the flock is confirmed even in the case of the simultaneous presence of another abortive agent (Situation A).

If only a single quantitative PCR is superior or equal to the recommended threshold, it is advisable to carry out, if possible, PCRs on samples from one (for individual PCRs) and preferably from several new cases of abortions (PCR of maximum three different animals) having taken place within eight days. If this new PCR is superior or equal to the recommended threshold, the diagnosis of Q fever in the flock is confirmed (Situation A), otherwise Q fever is not considered as being the origin of the abortion storms (Situation C).

In the absence of abortion product samples from recently aborted animals, serological tests on at least ten animals of the affected kidding/lambing animals will be carried out (i.e., animals having miscarried or aborted or with stillbirths from at least two to three weeks previously).

If 50% or more of the tested animals are seropositive, Q fever is confirmed at flock level. (Situation A).

If less than 50% of the tested animals are seropositive: the diagnosis of clinical Q fever cannot be extended to flock level (Situation B). The occurrence of a new abortion during the period

of birth then has to be the object of a new PCR testing. In case of results superior or equal to the retained threshold the flock is considered as clinically affected by Q fever (Situation A). In case of results lower than the threshold, Q fever is not considered as being at the origin of the abortion storms (Situation C).

If the two PCRs are lower than the threshold, Q fever is not considered as being the origin of the abortion storms at flock level (Situation C).

Second scenario: samples from a single animal having aborted or having had a miscarriage within eight previous days are available for an individual PCR analysis. Serological tests from blood samples are systematically carried out.

- The flock is considered as clinically affected with Q fever: if the individual PCR is superior or equal to the retained threshold and 50% or more of the sampled animals are seropositive (situation A).
- If the PCR is superior or equal to the retained threshold but less than 50% of the sampled animals are seropositive, this abortion could probably be considered as an isolated case. Thus, it is recommended to monitor for any development of clinical symptoms and to carry out PCR analyses on any new animal which would abort or miscarry during the birthing period (Situation B).
- In the case of a PCR lower than the threshold and 50% or more seropositive animals, it is recommended to carry out PCR analyses on any new animal which would abort or miscarry during the birthing period (Situation B).

Finally when the PCR is lower than the threshold and when less than 50% of tested animals are seropositive, it is considered that the abortion storm is not related to *C. burnetii* (Situation C).

6.1.2 Active monitoring scheme

Active monitoring aims at determining the prevalence of Q fever infection among the targeted animal population. MSs may wish to use such monitoring schemes for example when the disease frequency in animals and/or in humans is high.

Different sampling strategies for an active surveillance might be used (e.g. simple, systematic or stratified random sampling, multistage sampling). However they need to be elaborated in accordance with the needs and the epidemiological situation of a specific country. Harmonised approaches for determining appropriate sample sizes in the monitoring may be developed further by EFSA, if needed.

An active monitoring scheme for Q fever is cost-intensive because a large sample size is required and it involves complex practical and logistical organisation. However, some suggestions for making this monitoring feasible are set out below.

- Targeted sampling towards a sub-population at high risk could be envisaged, where the probability of finding cases is highest (e.g. to sample ruminants around the birthing period, to sample animals introduced/imported from a country or area of risk) rather than random sampling.
- Sampling can be targeted towards a sub-population considered as an epidemiological

emerging indicator group, where the probability of finding recent propagation is highest (e.g. on the basis of age group stratification: young, middle-aged, old).

- A reduction in costs can be achieved, when possible, by the use of screening tests of herds/flocks rather than individual tests (e.g. pools of individual samples such as vaginal swabs, milk or serum).
- In the case of dairy herds/flocks, bulk tank milk (BTM) samples could be used both for PCR and serological testing (BTM represents the herd/flock or a combination of several herds/flocks). Similarly in the case of investigation of non-dairy herds/flocks, the possibility of testing pools of individual milk samples should be considered.

When carrying out a Q fever survey, either transversal or longitudinal, it should be kept in mind that sampling should be carried out at the same time, especially relative to the kidding/lambing period, to obtain comparable data between herds/flocks.

Depending on laboratory tools and capacities, a serosurvey (ELISA test is recommended) is useful as a first screening, and prevalence could then be subsequently determined using PCR analyses assessing the shedding level (quantities, frequencies). If possible, two shedding routes (e.g. milk, faeces, birth products) should be tested. The variability of shedding among animals and over time is not well known.

Furthermore, other restrictions have to be considered, such as the case of vaccinated herds/flocks where serological testing at individual level or on BTM should be avoided because only antibodies induced by vaccination will be found. In addition, at present, no DIVA test (differentiating infected from vaccinated animals) is available for Q fever. In these herds/flocks the detection of the infectious agent is the only method to obtain information on Q fever status at individual or herd/flock level.

In the case of positive test results, if accurate prevalence is needed and funding is sufficient, it is recommended to carry out further analyses, to determine the infected herds/flocks, following the sampling procedure previously described for the passive monitoring scheme.

6.2 Harmonisation and improvement of Q fever reporting

Until recently, information on Q fever was not covered by the zoonotic diseases reporting system in the EU. Q fever has only been incorporated in EFSA's Community Summary Report on zoonoses since 2005. At the beginning only two out of 27 MSs reported data. Currently, the number of countries reporting Q fever cases has increased. However, disease reporting from the different MSs is not harmonised and the level of information available varies considerably. In this section, guidelines are provided in order to harmonise and collect additional data to improve the reporting of Q fever.

The primary objective of these guidelines is to provide and to establish comparable data on the occurrence of Q fever in the main animal reservoirs. If genuinely comparable data from different MSs are to be obtained, case definitions, diagnostic methods and sampling plans must be harmonised and standardised between the countries. The type of information to be collected by MSs is proposed in Tables 3, A and B, and in Appendix H.

Table 3: **Data reporting of "*Coxiella burnetii* (Q fever) in animals"**

A. Passive monitoring (clinical investigations)

Passive monitoring: please provide the results of testing for *Coxiella burnetii* (Q fever) in the context of clinical investigations. Specifically if data is available, report the number of herds/flocks tested and the number of herds/flocks testing positive in response to clinical investigations to allow an assessment of the number of outbreaks occurring in your country.	**Animal species**			
	Cattle	**Sheep**	**Goats**	**Other[(a)]**
Country:				
Sampling information				
Origin of herds/flocks (geographical location/region or national level)				
Sampling stage (where the samples have been collected (i.e. at farm/slaughterhouse etc.)				
Total number of herds/flocks tested for infectious abortive agents				
Total number of herds/flocks diagnosed for being infected with abortive agents including Q fever				
Sampling context (i.e. planned monitoring, investigation of outbreaks etc.)				
Q fever direct diagnosis (detection of *C. burnetii*)				
Sample type for direct diagnosis (vaginal swab, aborted placenta etc.)				
Size of pool for direct diagnosis if applicable				
Test type for direct diagnosis (i.e. PCR, staining, isolation etc.)				
Number of animals tested				
Number of herds/flocks tested				
Number of animals tested positive				
Number of herds/flocks tested positive				
Q fever indirect diagnosis (serological test)				
Sample type for indirect diagnosis (serum)				
Test type for indirect diagnosis (i.e. ELISA, IFA, CFT etc.)				
Number of animals tested				
Number of herds/flocks tested				
Number of animals tested positive				
Number of herds/flocks tested positive				
Results of testing for Q fever, herds/flocks clinically affected with Q fever				
Number of confirmed herds/flocks clinically affected with Q fever abortions (b)				
Comments				
Free text to be used for further information				

(a) Please specify the animal species
(b) According to the test interpretation guidelines

B. Active monitoring

Active Monitoring: please provide the results of testing for *Coxiella burnetii* (Q fever) in the context of monitoring. Specifically if data is available, report the number of animals tested and the number of animals testing positive to allow an assessment of the prevalence within your country.	**Animal species**			
	Cattle	**Sheep**	**Goats**	**Other[(a)]**
Country:				
Sampling information				
Origin of herds/flocks (geographical location/region or national level)				
Sampling stage (where the samples have been collected (i.e. at farm/slaughterhouse etc.)				
Total number of herds/flocks tested				
Sampling context (i.e. planned monitoring, voluntary survey etc.)				
Sampling frequency (constant effort throughout the year, restricted to kidding season, etc.)				
Sampling strategy (random selection, every animal, etc.)				
Q fever direct diagnosis (detection of *C. burnetii*)				
Sample type for direct diagnosis (vaginal swab, milk etc.)				
Size of pool, Bulk Tank Milk (BTM) for direct diagnosis if applicable				
Test Type for direct diagnosis (i.e. PCR, isolation etc.)				
Number of animals tested				
Number of herds/flocks tested				
Number of animals tested positive				
Number of herds/flocks tested positive				
Q fever indirect diagnosis (serological test)				
Sample type for indirect diagnosis (serum, BTM)				
Test Type for indirect diagnosis (i.e. ELISA, IFA etc.)				
Number of animals tested				
Number of herds/flocks tested				
Number of animals tested positive				
Number of Herds/flocks tested positive				
Comments				
Free text to be used for further information				

(a) Please specify the animal species

It is strongly recommended that samples be analysed using validated methods in laboratories applying quality assurance systems.

Conclusions and Recommendations

- Historically, Q fever has not been perceived as an important public health threat in medical or veterinary communities. However, the causative agent, *C burnetii* can cause debilitating disease and may result in potentially fatal chronic infections among humans. Moreover, many outbreaks in humans in urban or residential areas have been recently reported in certain MSs (Bulgaria, Germany and the Netherlands) and in Croatia, involving large numbers of human cases and linked to small ruminant flocks. This highlights the fact that Q fever may represent a real public health threat also for urban populations and emphasises the need for enhancing the reporting and monitoring of this emerging problem. As exemplified by the ongoing Q fever outbreak in the Netherlands since 2007, Q fever may affect a large number of humans.
- The implementation, development and standardisation of schemes for the monitoring and reporting of Q fever in animals in the EU are crucial for the prevention and control of this zoonosis. Since the first reporting of Q fever cases by MSs to EFSA in 2005, the quality of data has improved steadily. However, monitoring and reporting is not harmonised and information available varies considerably between MSs.
- In order to improve the reporting and to provide and establish comparable data on the occurrence of Q fever in the main animal reservoirs, recommendations to include additional data (information) to be collected are proposed. Moreover, schemes for the surveillance and monitoring of Q fever were designed taking into account the characteristics of Q fever, the traits of the bacterium, the situation of Q fever in most MSs, the availability of suitable diagnostic tools and a financial compromise. Consequently, it is proposed to focus monitoring and survey schemes on domestic ruminants. A passive monitoring system is recommended rather than active. This scheme is based upon identification of clinically affected herds/flocks (i.e. in which a series of abortions has occurred) by using laboratory-based diagnosis of Q fever. Alternatively, some principles of active surveillance are also proposed for countries that may wish to evaluate further the prevalence of Q fever in their domestic ruminant populations.
- Above all, due to the lack of clear clinical symptoms, in particular when they are isolated (observed on individuals) which most likely results in under-reporting, it was necessary to provide a clear case definition. Thus, it is proposed here that a herd/flock should be considered as clinically affected when serial abortions have occurred, the presence of *C. burnetii* is confirmed by PCR and serology by ELISA. Differential diagnoses with other abortive agents are essential.
- The efficiency of such a surveillance scheme and its sustainability could rely on several factors. Among these, the sensitisation and the training of the network (farmers, veterinarians) and their investment are essential (regular awareness campaigns and training could be implemented). The role of laboratories is also important for the detection of the disease. These laboratories should follow quality management systems, and are encouraged to participate regularly in inter-laboratory proficiency tests organised by National Reference Laboratories (NRLs). It is highly recommended that laboratories wishing to improve technical capacities regarding Q fever diagnostics establish a link with

NRLs in order to enable specific technical training support. This should promote more accurate and efficient diagnostics and therefore will result in more comparable and harmonised data on the occurrence of Q fever in MSs.

- Finally, it is understood, however, that the recommendations presented here cannot be regarded as definitive guidelines. They reflect current knowledge, and will certainly need to be revised and improved when new scientific knowledge will be made available.

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Appendices

APPENDIX A. NATIONAL REFERENCE LABORATORIES (NRLS) FOR Q FEVER OR EQUIVALENT IN MSS

Country	NRL for Animals	NRL for Humans	No NRL
Austria	No answer		
Belgium	X		
Bulgaria	X	X	
Cyprus	X	X	
Czech Republic			X
Denmark	X		
Estonia			X
Finland	X	X	
France	X	X	
Germany	X	X	
Greece	X		
Hungary		X	
Ireland	No answer		
Italy	X		
Latvia		X	
Lithuania	X		
Luxembourg	X		
Malta	No answer		
Netherlands			X
Poland	X	X	
Portugal	X		
Romania	X		
Slovakia			X
Slovenia			X
Spain	X	X	
Sweden	X	X	
United Kingdom	X	X	
Non-MSs			
Norway	X		
Switzerland	X		

APPENDIX B. OFFICIAL STATUS OF Q FEVER IN MSS AND TWO NON-MSS

Country	Notifiable disease in humans	Notifiable disease in animals	Occupational disease
Belgium			
Bulgaria	X	X	X
Cyprus	X		
Czech Republic	X	X	X
Denmark		X	X
Estonia			
Finland	X	X	
France			X
Germany	X	X	X
Greece	X	X	X (non confirmed)
Hungary	X		X
Italy	X	X	X
Latvia	X	X	X
Lithuania	X	X	
Luxembourg			
Netherlands	X	X (since 2008)	X
Poland	X	X	X
Portugal	X		
Romania			
Slovakia	X		X
Slovenia	X	X	X
Spain	X	X	
Sweden	X	X	
United Kingdom			X
Non-MSs			
Norway			
Switzerland	X (when outbreaks)	X	

APPENDIX C. EXISTING Q FEVER REGULATIONS IN MSS AND TWO NON-MSS

National or local regulations relative to Q-fever in ruminants for:					
Abortion	**Crude milk**	**Manure**	**Vaccination**	**Export or Import purposes**	**Governmental financial help (or project) for stockbreeders**
Bulgaria	Bulgaria	Bulgaria		Bulgaria	Bulgaria
Slovenia	Slovenia	Slovenia			Slovenia
Netherlands[(a)]	Netherlands[(a)]	Netherlands[(a)]	Netherlands[(b)]		
Italy	Italy	Italy			
Romania					
Germany	Germany				
	Finland				
	France				
					Denmark
Switzerland[(c)]					
				Norway[(c)]	

(a) since 2008
(b) since 2009
(c) Non-MSs

APPENDIX D. LIST OF ANIMAL SPECIES SUSCEPTIBLE TO Q FEVER IN EUROPE

Species	Country (examples)	Antibodies	Agent	Author
a) Domestic animals (excluding ruminants)				
Pig	Germany	X		Henning and Sting (unpublished)
Horse	Germany	X		Krauss et al. (1977)
	Germany	X		Jaspers et al. (1994)
	France	X		Pitre (1960)
Chicken	Czechoslovakia[(a)]	X		Raska und Syrucek (1956)
Duck	Czechoslovakia[(a)]	X		Raska and Syrucek (1956)
Goose	Czechoslovakia[(a)]	X		Raska and Syrucek (1956)
Pigeon	Czechoslovakia[(a)]	X		Raska and Syrucek (1956)
	France		X	Stein and Raoult (1999)
Turkey	Czechoslovakia[(a)]	X		Raska and Syrucek (1956)
Dog	Belgium	X		EFSA (2007)
	Germany	X		Krauss et al. (1977)
	Greece	X		Dragonas et al. (1967)
	Italy	X		Baldelli et al. (1992)
Cat	Germany	X		Werth et al. (1987)
	Switzerland	X		Kaaserer et al. (1976)
b) Wildlife				
Roe deer *(Capreolus capreolus)*	Czech Republic	X		Hubálek et al. (1993)
	Spain	X		Ruiz-Fons et al. 2008
	Germany	X		Weber et al. (1978)
Red deer (*Cervus elaphus*)	Czech Republic	X		Hubálek et al. (1993)
	Spain			Ruiz-Fons et al. (2008)
Fallow deer (*Dama dama*)	Czech Republic	X		Hubálek et al. (1993)
Mouflon *(Ovis musimon)*	Czech Republic	X		Hubálek et al. (1993)
	Germany	X		Krauss et al. (1977)
Wild boar *(Sus scrofa)*	Czech Republic	X		Hubálek et al. (1993)
Fox *(Vulpes vulpes)*	Germany		X	Schaaf (1961)
	Czechoslovakia[(a)]	X		Raska and Syrucek (1956)
Hedgehog *(Erinaceus europaeus)*	Romania		X	Zarnea et al. (1959)

APPENDIX D (CONTD.). LIST OF ANIMAL SPECIES SUSCEPTIBLE TO Q FEVER IN EUROPE

Species		Country (examples)	Antibodies	Agent	Author
b) Wildlife (contd.)					
Rodents	Wild brown rat *(Rattus norvegicus)*	United Kingdom	X	X	Webster et al. (1996) Raska und Syrucek (1956)
	Rat *(Rattus rattus)*	Czechoslovakia[(a)]	X		Raska und Syrucek (1956)
	Bank vole *(Clethrionomys glareolus)*	Czechoslovakia[(a)]		X	Raska und Syrucek (1956)
	Common vole *(Microtus arvalis)*	Czechoslovakia[(a)]	X		Rehacek et al. (1976)
	Mouse *(Mus musculus)*	Czechoslovakia[(a)]	X	X	Raska und Syrucek (1956)
	Rabbit (*Oryctolagus cuniculus*)	Czechoslovakia[(a)]	X		Vosta et al. (1990)
c) Birds					
Common Redstart *(Phoenicurus phoenicurus)*		Czechoslovakia[(a)]	X	X	Raska und Syrucek (1956)
Wagtail *(Motacilla alba)*			X	X	
Swallow *(Hirundo rustica)*			X		
House Martin *(Delichon urbica)*			X		
Sparrow *(Passer domesticus)*			X		
Yellowhammer *(Emberiza citrinella)*			X		
Greenfinch *(Carduelis chloris syn. Chloris chloris)*			X		
Woodpecker *(Dryobates maior)*			X		
d) Zoo animals					
Lion *(Panthera leo)*		Italy		X	Torina et al. (2007)
Waterbuck (*Kobus ellipsiprymnus*)		Portugal		X	Clemente et al. (2008)
Sable antelope (*Hippotragus niger niger*)		Portugal		X	Clemente et al. (2008)
Musk oxen (*Ovibos moschatus*)		Germany	X		Schroder (1998)
e) Ticks					
Dermacentor marginatus		Germany		X	Hellenbrand et al. (2005)
Haemaphysalis punctata		Spain		X	Barandika et al. (2008)
Rhipicephalus sanguineus species complex		Switzerland		X	Bernasconi et al. (2002)

(a) References made to 'Czechoslovakia' refer to the period previous to 1 January 1993.

APPENDIX E. COST ESTIMATE FOR Q FEVER MONITORING

In France, the global cost of the proposed protocols for passive monitoring of Q fever in herds was estimated on the basis of a survey from 34 departmental laboratories (Nicollet and Valognes, 2007).

- In cattle, in most cases the cost of a conclusive diagnosis at herd level of the implication of *C. burnetii* in spates of abortions, depending on the speed of the diagnosis, is estimated to be within a range of 85 € to 200 €.
- In small ruminants, calculations were made by including the differential diagnosis of the following three diseases: chlamydiosis, Q fever and toxoplasmosis with a hypothesis of sampling of six animals and the carrying-out of two pooled analyses with quantitative PCR (from six swabs). The cost of the diagnosis at flock level, depending on the speed of the diagnosis, is estimated to be between 140 € and 240 €.

APPENDIX F1. SEROLOGICAL METHODS AVAILABLE IN MSS AND TWO NON-MSS

Country	IFA	CFT	ELISA
Belgium		X (Virion-Serion)	X (LSI Kit)
Bulgaria	X ("home-made")	X (commercial)	
Cyprus	X		X
Czech Republic		X (Virion)	X (IDVET)
Denmark		X (dogs, cats, pigs)	X (ruminants)
Estonia			
Finland			X
France	X	X	X (LSI, IDEXX, Id-Vet)
Germany		X	X (IDEXX)
Greece		X	X (IDEXX)
Hungary	X focus (humans)	X (Vet)	
Italy	X (humans)		X (CHEKIT)
Latvia [(a)]			
Lithuania			
Luxembourg			
Netherlands		X	X (IDEXX, LSI)
Poland	X	X	X
Portugal			X (IDEXX)
Romania			X (IDEXX)
Slovakia		X	
Slovenia		X (Virion/Serion)	X (IDEXX)
Spain	X	X	X
Sweden	X Q-Focus (humans)	X (Behring Antigen)	X (IDEXX),Virion (humans)
United Kingdom		X	X (under-evaluation)

Non-MSs

Norway			X (IDEXX)
Switzerland			X (IDEXX)

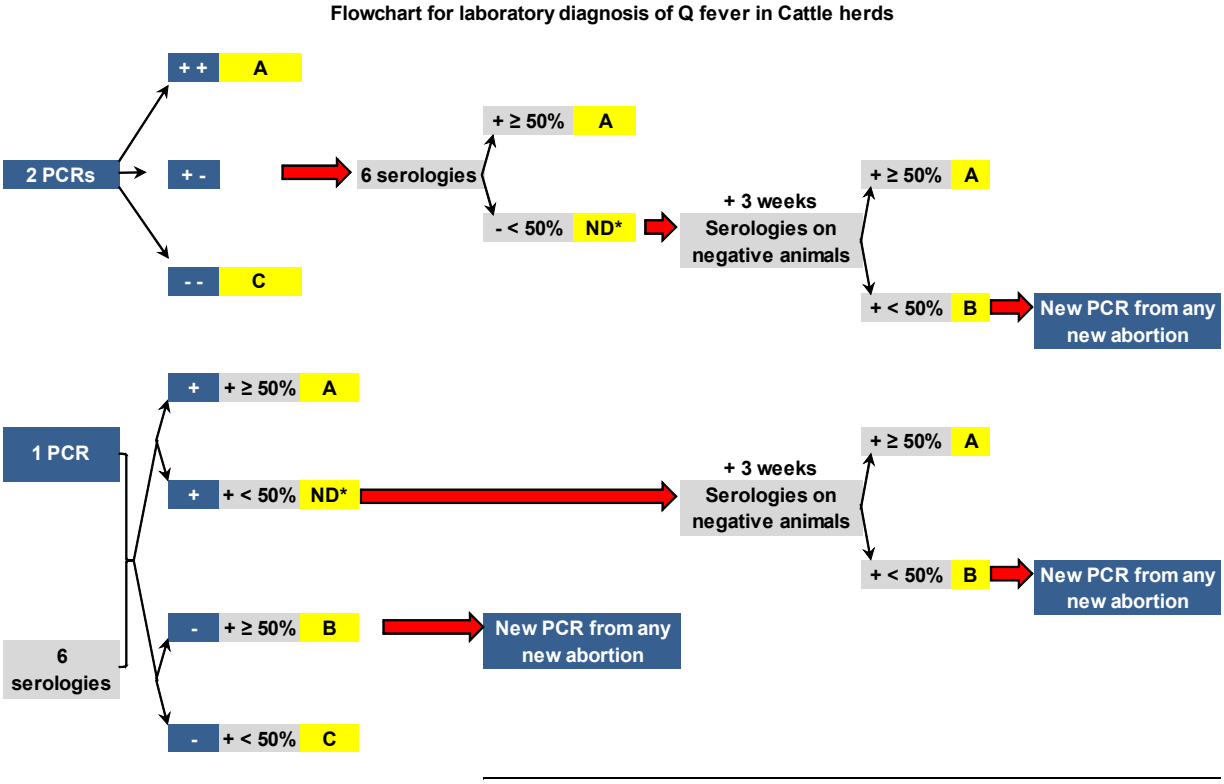
(a) Answer provided refers to animal health sector

APPENDIX F2. DIRECT IDENTIFICATION AND ISOLATION METHODS OF *C. BURNETII* CURRENTLY USED IN MSS AND TWO NON-MSS

Country	Conventional	RT-PCR	Cells	Eggs	Animals	Staining
Belgium		X (Taqvet Kit LSI)		X		X
Bulgaria			X	X	X	X
Cyprus						X
Czech Republic						
Denmark	X	X				X[(a)]
Estonia						
Finland	X (Adiagene)					X
France	X	X	X	X	X	X
Germany	X	X	X			X
Greece	X	X				X
Hungary	X (Hum HM)					
Italy	X					
Latvia						
Lithuania						
Luxembourg						
Netherlands		X	X			X[(b)]
Poland	X		X	X		X
Portugal	X					
Romania						
Slovakia						
Slovenia	X (Adiagene)	X				
Spain	X	X	X	X	X	X
Sweden		X (Adiagene)	X			
United Kingdom		X			X	X
Non-MSs						
Norway						
Switzerland	X		X	X		X

(a) Fluorescent in situ hybridization (FISH) with oligonucleotide probe targeting 16S rRNA; formalin-fixed placenta
(b) Immuno Histo Chemistry (IHC)

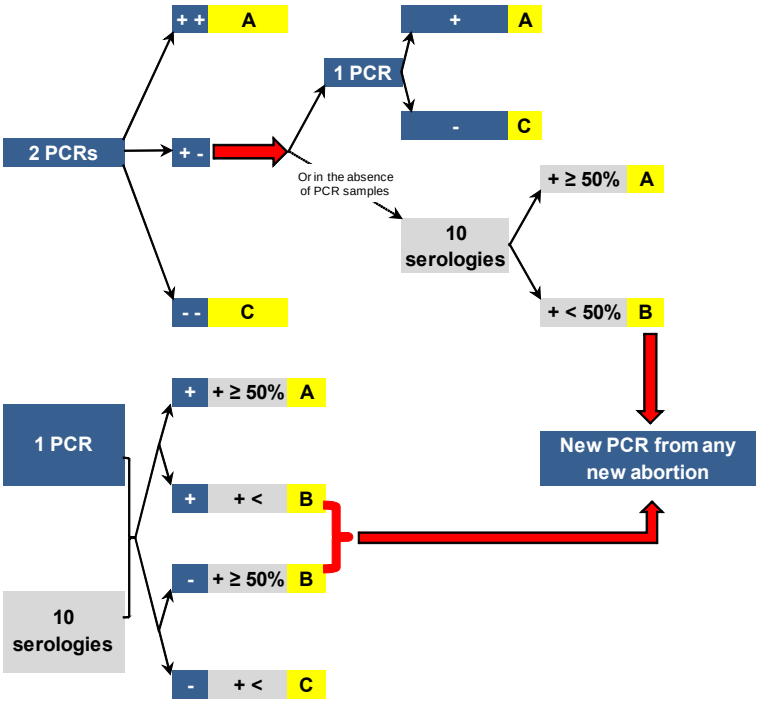
APPENDIX G. GUIDELINES FOR RESULTS INTERPRETATION



A: Situation A, the herd/flock is considered as clinically affected.
B: Situation B, Q fever cannot be excluded at the herd/flock level.
C: Situation C, abortion is not related to *C. burnetii* at the herd/flock level
ND: Not determined.

APPENDIX G (CONTD). GUIDELINES FOR RESULTS INTERPRETATION

Flowchart for laboratory diagnosis of Q fever in small ruminants herds



A: situation A, the herd/flock is considered as clinically affected.
B: situation B, Q fever cannot be excluded at the herd/flock level.
C: situation C, abortion is not related to *C. burnetii* at the herd/flock level
ND: Not determined.

APPENDIX H. GUIDELINES FOR REPORTING DATA ON Q FEVER IN ANIMALS

- **Relevant animal species for reporting:** cattle, sheep and goats, other ruminants.
- **Case definition:** a positive case is a herd/flock with clinical symptoms (abortion and/or stillbirth) for which the presence of the agent has been confirmed (PCR, etc.) in association with a positive serological test (ELISA, etc).
- **Definition of a positive sample:** a sample which tested postive for *Coxiella burnetii* in the test carried out.
- Diagnostic/analytical methods available:
  - Direct diagnosis: PCR, staining, isolation of the agent by cell culture;
  - Indirect diagnosis: ELISA, IFA, CFT.
- Sampling information:
  - **Sample type:** the sample type should be reported (i.e. vaginal mucus, aborted placenta, serum, milk);
  - **Sampling stage:** where the samples have been collected (i.e. at farm/at slaughterhouse) and the sample type (i.e. animal sample/faeces) should be reported;
  - **Sampling context:** information on the context of the sampling (i.e. planned monitoring, passive or active monitoring, voluntary investigation of abortion incidents);
  - **Sampling strategy:** (i.e. random selection, every animal, etc. should be inserted;
  - **Sampling frequency:** (i.e. constant effort throughout the year, restricted to kidding season, etc.).
- **Results of testing for Q fever:** the number of herds/flocks tested positive according to the case definition.
- **Comments:** free text to be used for further information that could be of interest.

Glossary

Case definition: definition stating when the sample is considered to be positive for the zoonotic agent or when the person, animal, herd or flock is considered to be infected with the zoonotic agent. In this document case definition should be interpreted as "Q fever in animals with clinical symptoms".

Epidemiology: the study of the cause, distribution and control of a disease in a population.

Herd/flock: an animal or group of animals kept on a holding as an epidemiological unit (Regulation (EC) No 2160/2003); if more than one herd is kept on a holding, each of these herds shall form a distinct unit and shall have the same health status (Directive 64/432/EEC). In the present document the term "herd" was used for cattle and "flock" for small ruminants.

Med-Vet-Net: is the European Network of Excellence for zoonoses research. Med-Vet-Net aims to develop a network of excellence for the integration of veterinary, medical and food scientists, in the field of food safety, at European Level, in order to improve research on the prevention and control of zoonoses, including food-borne diseases. The network also takes into account public health concerns of consumers and other stakeholders throughout the food chain. (http://www.medvetnet.org).

Monitoring: system of collecting, analysing and disseminating data on the occurrence of zoonoses, zoonotic agents and antimicrobial resistance related thereto (Directive 2003/99/EC). As opposed to surveillance, no active control measures are taken when positive cases are detected.

Multiparous: A female that has given birth more than once.

Primiparous: 1. A young female that is pregnant for the first time.

2. A female that has given birth once.

Surveillance: In general, a close and continuous observation for the purpose of control. As opposed to monitoring, active control measures are taken after detection of a positive case. This type of programme does not necessarily have a defined target for diseases/contamination occurrence reduction.

Zoonosis: any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans (Directive 2003/99/EC).

Zoonotic agent: any virus, bacteria, fungus, parasite or other biological entity which is likely to cause a zoonosis (Directive 2003/99/EC).

Abbreviations

AFSSA	Agence Française de Sécurité Sanitaire des Aliments
BTM	Bulk Tank Milk
CFT	Complement Fixation Test
CVI	Central Veterinary Institute, the Netherlands
DIVA	Differentiating infected from vaccinated animals
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FLI	Friedrich Loeffler Institute, Germany
IFA	Immuno3-fluorescence Assay
MS	Member State of the European Union
NRL	National Reference Laboratory
NVRI	National Veterinary Research Institute, Poland
OIE	Office International des Epizooties, World Organization for Animal Health
PCR	Polymerase Chain Reaction
PBS	Phosphate buffer saline
WHO	World Health Organisation