SCIENTIFIC OPINION

Scientific Opinion on Q fever[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2, 3]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3] (Chapter 4 on Food Safety)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, a scientific opinion was prepared by EFSA's Animal Health and Welfare Panel to determine the magnitude, distribution, impact and significance of infection and disease in domestic ruminants and humans, risk factors for the maintenance (in domestic ruminant populations) and spillover (from these populations to humans) of *Coxiella burnetii* (the causative agent of Q fever), and control options in domestic ruminant populations. A range of approaches were used, including an assessment of monitoring/surveillance data, the development of a simple conceptual model, a critical review of available literature, and several country case studies. Control options for *C. burnetii* infection in small ruminants were qualitatively assessed. Infection is endemic in domestic ruminants in most, if not all, EU member states, however, disease is rare and impact is limited. In the EU, Q fever is a zoonotic disease with limited public health impact, except under certain epidemiological circumstances and for particular risk groups. Human cases are often associated with proximity to small ruminants (particularly at parturition or during abortions) and dry, windy weather. Currently, there is no clear evidence of an association between bacterial genotypes/isolates and virulence. A number of longer-term options to control *C. burnetii* infection in domestic ruminants were identified; these should be considered in those situations where the public health risk is considered unacceptable. Some additional options were not considered sustainable for long-term control, but may have a role in the face of an outbreak. Persistent environmental contamination may confound animal-based control efforts. Vaccination should be considered a long-term control option, noting that effectiveness may not be observed in the short-term. Antibiotic treatment of animals is not recommended. There is no conclusive evidence that the consumption of milk and milk products containing *C. burnetii* has resulted in clinical Q fever in humans.

KEY WORDS: Q fever, EU, Coxiella burnetii, domestic ruminants, public health, risk factors, control options

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SUMMARY

The recent developments in the EU, especially the increase in confirmed human cases of Q fever in the Netherlands, call for special consideration as regards the risks posed by Q fever for humans and animals. The European Commission requested further scientific advice and risk assessment, as regards Q fever in animals. The mandate posed three questions:

- to assess the significance of the occurrence of Q fever in the EU Member States for a better understanding of the scale and distribution of the disease and infection (with the focus on farm animals and humans)
- to assess the risk factors for Q fever occurrence and persistence in animal husbandry and the related risks for humans, and
- to assess the effectiveness and efficiency of disease control options.

An opinion, in response to the mandate, was prepared by the EFSA Panel on Animal Health and Welfare, with input from EFSA's Panel on Biological Hazards (BIOHAZ) and the Zoonoses and Assessment Methodologies Unit, and in close collaboration with the European Centre for Disease Prevention and Control (ECDC).

Several approaches were adopted during the preparation of this scientific opinion. An assessment of the magnitude and distribution of infection and disease in domestic ruminants and in humans was conducted after considering diagnostic methods for *Coxiella burnetii* infection (the causal agent of Q fever) and monitoring/surveillance for *C. burnetii* infection in different member states of the European Union (MS). Impact and significance was assessed, based on expert opinion. Several methods were used to clarify risk factors for maintenance of *C. burnetii* infection in domestic ruminants and spillover of infection from domestic ruminants to humans, including the development of a simple conceptual model, a critical review of available literature, and several country case studies. Control options for *C. burnetii* infection in domestic ruminant populations were evaluated, following the development of a generic framework for the control of infectious diseases, and based on available data about individual control interventions.

Infection with *Coxiella burnetii* (the causal agent of Q fever) is endemic in domestic ruminants (cattle, sheep, goats) in most, if not all, EU MS. Although infection in domestic ruminants is common, disease is rare. The overall impact of *C. burnetii* infection on the health of domestic ruminants in EU MS is limited. In humans, *C. burnetii* infection is present in most, if not all, EU MS. Q fever is a zoonotic disease with limited public health impact in the EU, however, in certain epidemiological circumstances and for particular risk groups the public health impact can be significant. As yet, the Q fever burden of disease in humans has not been determined.

It seems likely that *C. burnetii* infection can be maintained in domestic ruminants in a wide range of husbandry systems. There is considerable uncertainty about the relative importance of risk factors for maintenance of *C. burnetii* infection in domestic ruminant populations, and for spillover from domestic ruminants to humans. Nonetheless, maintenance of *C. burnetii* infection within farms might be favoured by persistently infected animals, other animal reservoirs of infection, ticks, husbandry practices that favour within-herd transmission and/or environmental contamination. Currently, there is no clear evidence of an association between bacterial genotypes/isolates and virulence. The common risk factors associated with spillover of infection from domestic ruminants to humans in different EU MS include an association between human infection and small ruminants (sheep and goats), an indication of proximity between animals and human populations, particularly in association with parturition in animals (and to abortions, in the case of goats), and specific climatic conditions, in particular dry, windy weather. In humans, the risk of exposure to *C. burnetii* is increased, either following close contact to animals infected with *C. burnetii*, or following community-based exposure (caused by an elevation of *C. burnetii* in the wider environment following release and dissemination from infected animal hosts). There is no conclusive evidence in support of a link between an increased density of animals and/or farms and spillover of *C. burnetii* from infected farms to humans. The factors leading to outbreaks of Q fever in the human population are not fully understood.

Regarding disease control, the opinion focused on control options applicable to domestic ruminants, and on the effectiveness of these options to reduce within-herd transmission, between-herd spread (each in domestic ruminant populations), and/or the spillover of infection from domestic ruminants to humans. For almost all of the control options, there is a medium to high level of uncertainty associated with estimates of control effectiveness, as little relevant published information is available. It is likely that control methods would need to be used in combination. There is variation in the sustainability of the assessed control options. A number of long-term control options were identified, including preventive vaccination, manure management, changes to farm characteristics, wool shearing management, a segregated lambing/kidding area, removal of risk material, visitor ban, control of other animal reservoirs and tick control. All but the latter two relate specifically to small ruminants. These options are ranked according to effectiveness, as assessed by expert opinion, in reducing spillover from domestic ruminants to humans. Several options were not considered sustainable for long-term control, but may have a role in the face of an outbreak, including the culling of pregnant animals, a temporary breeding ban, stamping out, identification and culling of shedders, control of animal movements and stand still. These options all relate specifically to small ruminants. *C. burnetii* is highly resistant in the environment; consequently, persistent environmental contamination is a matter of concern. Vaccination can be used both to reduce the risk of future outbreaks (preventive vaccination) and in the face of an outbreak (outbreak vaccination), noting that preventive vaccination is more effective than outbreak vaccination, phase I is more effective than phase II vaccination, vaccination is more effective in non-infected than infected animals, vaccination does not appear to be effective if used in pregnant females, and effectiveness may not be observed in the short-term.

The Animal Health and Welfare Panel recommended harmonized field and laboratory data collection about *C. burnetii* infection in animals in EU MS, to allow comparison of prevalence/incidence estimates over time and between countries. Further, there is a need to strengthen systems to promote rapid identification and reporting of Q fever outbreaks in animals (abortion episodes). Consideration should be given for support of early information exchange between veterinarians and public health counterparts regarding potential events with zoonotic potential, including Q fever. Further studies on the estimation of Q fever burden of disease in humans are needed. Prevalence and incidence studies in domestic ruminants should place emphasis on small ruminants, rather than cattle, to provide a clearer picture of the risk of exposure for humans. Further investigations and research are needed to clarify the relationship between genotype and virulence, the molecular basis for virulence, and the relationship between mice models and virulence studies in domestic ruminants, determine the host specificity of different bacterial isolates, identify factors influencing the maintenance of infection, including an improved understanding of transmission pathways, quantify the number of bacteria excreted under different conditions, quantify survival of *C. burnetii* in the environment, clarify the role of environmental contamination and climatic factors in the spillover of infection from animals to humans, and clarify the trade off between farm density and farm size in the maintenance and spillover of infection. Future investigations are needed to objectively assess the effectiveness of control options for *C. burnetii* infection in domestic ruminant populations. Assessment should focus on relevant epidemiological parameters, including rates of within-herd transmission, between-herd spread and spillover from animal populations to humans. Control options should generally be used in combination, given that within-herd transmission, between-herd spread and spillover to humans are each likely to involve more than one transmission pathway, and that no control option is likely to be completely effective in limiting within-herd transmission, between-herd spread and spillover to humans. Longer-term options to control *C. burnetii* infection in domestic ruminants should be considered in those situations where the public health risk is considered unacceptable. Control options where effectiveness at reducing spillover was assessed as either high or medium include preventive vaccination, appropriate manure management, changes to farm characteristics, wool shearing management, segregated lambing/kidding area, removal of risk material and visitor ban. Control options to address proximity between humans and small ruminants, particularly around the time of lambing/kidding, would be expected to meaningfully contribute to a reduction in spillover from animal populations to humans. There is a need to build awareness among farmers and veterinarians of *C. burnetii* infection in farmed ruminants, including risk factors for spillover from domestic ruminant populations to humans. Short term options such as the culling of pregnant animals, a temporary

breeding ban, stamping out, identification and culling of shedders, control of animal movements and stand still could be used to reduce shedding from infected animals. However, other options (including preventive vaccination, manure management, wool shearing management, segregated lambing/kidding area, removal of risk material) should be considered to reduce human exposure through environmental contamination. Vaccination should be considered as a long-term control option. In order to better assess vaccine efficacy, it is recommended that field and experimental data are gathered, to improve our understanding of the epidemiology of *C. burnetii* infection in, and between, infected flocks, both prior to and following vaccination. Antibiotic treatment is not effective in substantially reducing either the level or duration of bacterial shedding in domestic ruminant populations; therefore the use of antibiotics cannot be recommended.

The widespread distribution of *C. burnetii* in food producing animals and its occurrence in the milk supply necessitates questioning the role of food as a vehicle for the transmission of this zoonotic bacterium to humans. *C. burnetii* infection in occupationally or otherwise exposed people is mainly due to inhalation of infected aerosols rather than consumption of contaminated food (e.g. dairy, meat) products. However, *C. burnetii* is excreted in milk of infected animals (cattle, sheep and goats) for variable periods during lactation irrespective whether these animals are showing clinical signs or not and in addition, milk can be contaminated with *C. burnetii* by faecal materials or from sites of infection in the periparturient and/or lactating animal. Consumption of raw milk and raw milk products represent a relatively greater risk of human exposure to *C. burnetii* than the consumption of both milk and dairy products made with milk that has undergone appropriate heat treatment. There are epidemiological indications that consumption of milk and/or milk products *c*ontaining *C. burnetii* has been associated with sero-conversion in humans. However, there is no conclusive evidence that the consumption of milk and milk products containing *C. burnetii* has resulted in clinical Q fever in humans.

The scientific opinion was adopted by the Scientific Panel on Animal Health and Welfare (AHAW) on 27 April 2010. Chapter 4 (focusing on food safety issues) and related conclusions were adopted by the Scientific Panel on Biological Hazards (BIOHAZ) on 22 April 2010.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Q fever is a highly contagious zoonotic disease caused by the pathogen *Coxiella burnetii* which is commonly present in all countries worldwide, except in New Zealand. It is listed within the category of multiple species diseases in the OIE (World Organisation for Animal Health) list (Article 1.2.3. of the OIE terrestrial Animal Health Code) and many domesticated and wild animals including mammals, birds, reptiles and arthropods can be carriers of the pathogen but cattle, goats and sheep are the main reservoirs. In these animals, infection is mostly asymptomatic except for the increase of abortions or stillbirths.

Both symptomatic and asymptomatic animals shed *C. burnetii* in large quantities when giving birth. Shedding can also occur in faeces, milk and urine. *C. burnetii* is highly resistant to environmental conditions and can be resistant to heat, drying, and many common disinfectants. These features enable the bacteria to survive for long periods in the environment and to be spread by the wind. Ticks may be important in transmission among wildlife, and can also spread infections to domesticated ruminants.

Little is known about the therapeutical treatment of ruminants or other domestic animals. Prophylactic antibiotic treatment is sometimes recommended to reduce the risk of abortion. Antibiotics may suppress rather than eliminate infections. Vaccines are available and may prevent infections in calves, decrease shedding of organisms and improve fertility in infected animals. They do not eliminate shedding of the organism. As a general rule, in an infected flock, isolating infected pregnant animals and burning or burying the reproductive membranes and placenta can decrease transmission.

In humans, Q fever occurs in either an acute form (pneumonia, hepatitis) or a severe chronic form (endocarditis) following an early infection that may have passed unnoticed. In rare cases, Q fever can be fatal, although the disease is usually treatable with antibiotics. Most human cases are associated with exposure to ruminants, particularly when the animals have given birth. Human outbreaks generally involve farmers or other occupationally exposed people (veterinarians, slaughterhouse personnel). Humans are mainly infected via aerosols, but transmission may also occur by the ingestion of unpasteurised milk or other contaminated material.

In the EU, there are no harmonized rules or recommendations for the monitoring and reporting of Q fever in animals. Q fever is not explicitly listed in Annex I to Directive 2003/99/EC of the European Parliament and of the Council on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC.

Q fever (in humans) is listed in Annex I of Commission Decision 2000/96/EC of 22 December 1999 on the communicable diseases to be progressively covered by the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. For the communicable diseases and special health issues listed in this Annex, epidemiological surveillance within the Community network is to be performed by the standardised collection and analysis of data in a way that is to be determined for each communicable disease and special health issue when specific surveillance networks are put in place.

Disease control measures are normally taken on national, regional or even farm level. Currently, EFSA is working on a guidance document for the harmonized reporting of Q fever and rabies. The outcome of this work should be available by the end of this year.

As regards the infection in animals, the information provided by the EU Member States in the context of Directive 2003/99/EC on Q fever in animals for the years 2008 (18 EU MS's) and 2007 (17 EU MS's) show that the proportion of reported positive cases in cattle, sheep and goats increased from 7.4% to 10.0% in 2008 at the EU level. The highest proportion of positive cases was reported for goats: 9.7% and 15.7% in 2007 and 2008, respectively. In particular, the Netherlands reported a three-fold increase in 2008 from 9.5% to 31.9%. The proportion of positive cases in sheep was 6.3% in 2008 and 7.9% in 2007.

In the EU Member States, Q fever appears to be present in cattle, sheep and goat holdings. Nevertheless, human cases of Q fever were rarely known until 2007. In 2008, a total of 1554 confirmed cases of Q fever were reported in the EU. This figure represents a 165.5% increase compared with the number of confirmed cases reported in 2007. The Netherlands and Germany accounted for the majority of this increase. In the Netherlands, for the year 2009, the number of human cases appears to have already doubled to almost 2300.

It is not clear what has caused this major increase of reported human outbreaks in the Netherlands. It appears that the majority of the human cases are not linked to farm-visits or occupational exposure. The geographical locations of the human cases indicate that there might be a connection with the presence of dairy goat holdings in the area, where this particular type of milk production has developed rapidly over the past decade.

In response to this unusually large number of human cases, special preventive and control measures have been taken to control Q fever in the Netherlands, in particular in dairy goats and dairy sheep:

- The culling of all pregnant animals and infected male animals on infected farms;
- Q fever in 2010, vaccination will be compulsory nationwide;
- all dairy goat and dairy sheep farms are obliged to participate in a regular milk test for Q fever;
- Special measures are imposed on farms where the bacterium is found in milk, such as restrictions on movements of animals and compulsory composting of manure.

The recent developments in the EU, especially in the Netherlands call for special consideration as regards the risks posed by Q fever for humans and animals. The Commission is in need of further scientific advice and risk assessment, as regards Q fever in animals.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In view of the above, and in accordance with Article 29 of Regulation (EC) 178/2002, the Commission asks EFSA for a scientific opinion and specifically:

1. to assess the significance of the occurrence of Q fever in the EU Member States for a better understanding of the scale and distribution of the disease and infection (with the focus on farm animals and humans);
2. to assess the risk factors for Q fever occurrence and persistence in animal husbandry, and the related risks for humans, taking into account at least the presence and density of susceptible livestock and the type of husbandry in which they are kept;
3. to assess the effectiveness and efficiency of disease control options such as vaccination, pharmaceutical treatments, establishing animal movement restrictions, the culling of animals, etcetera.

ASSESSMENT

1. Introduction

1.1. Context

Q fever has emerged as an important public health problem in the Netherlands. In May 2007, the first community Q fever outbreak was reported around a single village in the province of Noord-Brabant (Schimmer et al., 2010). Subsequently, human outbreaks of unprecedented size have occurred in the Netherlands during 2007 (Karagiannis et al., 2007), 2008 (Schimmer et al., 2008) and 2009 (Schimmer et al., 2009). Further, there is early evidence of ongoing transmission of *Coxiella burnetii* (the causative agent of Q fever) to humans in 2010 (van Duynhoven et al., 2010).

In response to these public health concerns, the European Commission has sought scientific advice from the European Food Safety Authority. The current EFSA report focuses on three broad areas, to address the above-mentioned terms of reference:

- *Chapter 2:* The magnitude and distribution of both infection and disease, and associated impact and significance, in human and animal populations in Europe; the focus regarding animal population was on domestic ruminants (cattle, sheep and goats).

- *Chapters 3:* Risk factors for *C. burnetii* maintenance of infection in domestic ruminants, and spillover of infection to humans. Risk factors relating to food safety are addressed in *Chapter 4;* and

- *Chapter 5:* Potential animal-focused control options, to limit adverse impact on both animal health (reducing within-herd transmission, reducing between-herd spread) and public health (reducing spillover to humans), assessed on the basis of effectiveness, certainty with respect to this estimate, sustainability and limitations. Control options relating to food safety are addressed in *Chapter 4.*

This *Chapter 4* and conclusions and recommendations related with food safety have been adopted by EFSA-BIOHAZ Panel.

1.2. Approaches

Several approaches were adopted during the preparation of this scientific opinion, after considering important constraints, as discussed below. The drafting of this opinion was substantially constrained by the time available which limited the choices of methodologies that could be applied. In particular it was not possible to complete a formal systematic review of the literature and meta analysis of risk factors and control options.

1.2.1. Magnitude and distribution of infection and disease

A number of important constraints were identified during project design, as follows:

- In the EU, there are no harmonized rules or recommendations for either monitoring or reporting of *C. burnetii* infection and Q fever in animals. Further, comparability of data between EU MS will be affected by variations in regulatory aspects (including case definitions), laboratory capacity and monitoring/surveillance intensity. These concerns are more problematic for *C. burnetii* infection and Q fever in animals compared to humans; and

- In its current form, EU-level data (as compiled in the EFSA/ECDC zoonoses database) needs to be interpreted with considerable care, for a range of reasons including incomplete and uneven reporting, the use of inconsistent case definitions, and difficulties in distinguishing active and passive data collection.

After considering each of the above-mentioned concerns, this opinion has utilised the following methodology to determine the magnitude and distribution of infection and disease in both domestic ruminants and humans:

- *A brief review of the diagnosis of* C. burnetii, based on a review of the published literature, and a recent report commissioned by EFSA under Article 36 (Sidi-Boumedine et al., 2010);

- *A descriptive assessment of monitoring and surveillance for* C. burnetii *infection in different EU MS.* For animals, this was based on an evaluation of notification, laboratory capacity and monitoring/surveillance intensity. A range of data sources were used, including the recent Article 36 report (Sidi-Boumedine et al., 2010), monitoring/surveillance data collected from the annual EFSA/ECDC Zoonoses Reports for 2006 to 2008 (EFSA/ECDC, 2007, 2009, 2010), and feedback from an *ad hoc* EFSA consultation in 2010 of *C. burnetii* testing that was conducted in EU MS during 2009. Additional information from WG members was also taken into account. For humans, a review was conducted of notification (including case definition) and monitoring/surveillance programmes in EU MS.

- *Insights into the magnitude and distribution of infection and disease.* For animals, this was based on an understanding of testing intensity (irrespective of whether the tests were conducted in association with active or passive data collection), of clinical investigation (a disease event in animals that was initially triggered by passive data collection), and of animal- and herd-level seroprevalence (active data collection). For the first of these two points [testing intensity, clinical investigation], data were obtained from the annual EFSA/ECDC Zoonoses Reports for 2006 to 2008 (EFSA/ECDC, 2007, 2009, 2010) and from feedback from an *ad hoc* EFSA consultation in 2010 of *C. burnetii* testing that was conducted in EU MS during 2009 (the questionnaire is included in appendix D, responses were received from 24 of 29 countries [27 EU MS, plus Norway and Switzerland]). For the final point [seroprevalence], an earlier review (Arricau-Bouvery and Rodolakis, 2005) was updated after considering all relevant literature published after 2004 (the literature search protocol is included in appendix A). For humans, an analysis was undertaken of data collected under the European Surveillance System. Note that Q fever incidence in human populations for 2009 was not available entirely at the time of drafting of this opinion.

- *A critical evaluation of impact and significance.* The evaluation of impact and significance of *C. burnetii* infection on human and on animal health in EU MS was prepared, drawing on expert opinion, and after considering each of the issues listed previously, including diagnosis, monitoring and surveillance, and estimates of magnitude and distribution. The impact of *C. burnetii* infection on other issues, such as the economic impact following the imposition of control options by authorities was not considered. Relevant literature was also considered. A summary of human outbreaks was prepared, updating an earlier review (Arricau-Bouvery and Rodolakis, 2005) with all relevant literature published after 2004.

The first term of reference *(significance of the occurrence of infection and disease, for both domestic ruminants and humans)* was addressed, after considering:

- A brief review of the diagnosis of *C. burnetii* infection,
- A descriptive assessment of measures of monitoring/surveillance of *C. burnetii* infection in different MS,
- Insights into the magnitude and distribution of infection and disease, and
- A critical qualitative evaluation of impact and significance.

1.2.2. Risk factors for Q fever maintenance and spillover

A number of important constraints were identified during project design, as follows:

- There is considerable variation in the degree (depth, intensity) to which reported outbreak investigations have been conducted, both in terms of epidemiological methods and laboratory support;
- Risk factor identification/assessment has generally been conducted using qualitative methods, including implication on the basis of association and consistency with previous reports. The level of scientific certainty associated with study conclusions is often relatively low; and
- The published literature on Q fever outbreaks (and associated risk factors) is fragmented, with a particular focus on the large and the unusual outbreaks.

After considering each of the above-mentioned concerns, this opinion has utilised the following methodology:

- *The development of a simple conceptual model,* separately highlighting the maintenance of infection in animal populations (focusing on domestic ruminants), and the spillover of infection from animal populations to humans through a process of amplification, transmission and exposure;
- *A critical review of available literature,* identifying risk factors for *C. burnetii* maintenance and for spillover. Limits to the literature search are presented in Appendix A; and
- *Several country case studies* (overview, magnitude and distribution, risk factors for maintenance and spillover, control options, implications/lessons learned), based on a detailed review of relevant published literature, and based on expert knowledge and opinion.

The second term of reference *(risk factors for Q fever maintenance and spillover)* was addressed, after considering:
- The development of a simple conceptual model;
- A critical review of available literature; and
- Several country case studies.

1.2.3. Control options in domestic ruminant populations

The third TOR requested the assessment of possible options to control the maintenance of infection in domestic ruminants and spillover from animal to human populations. A number of important constraints were identified during project design, as follows:

- There is limited published information about animal-based control options adopted in response to outbreaks of disease in humans;
- With most outbreaks, there has been little to no robust evaluation of control effectiveness. Further, available information is rarely quantitative; and
- The published literature on Q fever outbreaks (and associated control options) is fragmented, with a particular focus on the large and the unusual.

After considering each of the above-mentioned concerns, this opinion has utilised the following methodology:

- *The development of a generic framework for the control of infectious diseases*, based on a brief review of concepts and applications in infectious disease epidemiology. This framework was then used as the basis for a critical evaluation of available control options, as outlined below. Further, control options focused specifically on small ruminants (goats, sheep), given that cattle appear to have a very minor role in the spillover of *C. burnetii* infection from domestic ruminants to humans;

- *A critical evaluation of available control options*, based on expert opinion within the WG, after considering all available information in both the published and grey literature. For each option, each of the following were undertaken:

  - A description;

  - A qualitative assessment of effectiveness (high, very effective; medium, moderately effective; *low*, very limited effect; *none*, no effect):

    - To influence animal health through reduced within-herd transmission and between-herd spread;

    - To influence spillover from domestic ruminant populations to humans;

  - The qualitative assessment of the level of uncertainty associated with each of these estimates of effectiveness:

    - *High:* Scarce or no data available; evidence provided in unpublished reports, or few observations and personal communications, and/or authors' or experts' conclusions vary considerably

    - *Medium:* Some or only incomplete data available; evidence provided in small number of references; authors' or experts' conclusions vary, or limited evidence from field observations, or solid and complete data available from other species which can be extrapolated to the species being considered

    - *Low:* Solid and complete data available; strong evidence in multiple references with most authors coming to the same conclusions, or considerable and consistent experience from field observations.

  - A qualitative assessment of sustainability (high, sustainable in the long-term; medium, sustainable only in the short-to-medium term; low, sustainable only in the short term.); and

  - Associated limitations.

The third term of reference *(effectiveness and efficiency of disease control options in farmed animal populations)* was addressed, after considering:
- The development of a generic framework for the control of infectious diseases; and
- A critical evaluation of available control options.

2. Magnitude and distribution of Q fever in the EU Member States

2.1. Magnitude and distribution of *C. burnetii* infection and Q fever in domestic ruminant

2.1.1. Diagnosis of *C. burnetii* infection

Several assays have been described for the diagnosis of *C. burnetii* in animals, including both direct identification of the agent and serological testing (OIE, 2009; Table 1).

Direct identification of the agent

Samples should be collected from aborted fetuses, placenta and vaginal discharges soon after abortion or parturition. Milk (bulk tank milk; milk or colostrum from individual cows), and faeces samples can also be taken. Conventional staining techniques (Stamp, Gimenez, Macchiavello, Giemsa and modified Koster) are available within the context of the diagnostic of abortion and are used on tissues from fetus or placenta and on vaginal discharge. However these tests have low diagnostic sensitivities and specificities. Attention must be taken in the interpretation of the results as, microscopically, *C. burnetii* can be confused with *Chlamydophila abortus* or *Brucella* spp.

Isolation of *C. burnetii* can be done by cell or embryonated chicken egg culture. Such isolation is possible when microscopic examination indicates a large number of *C. burnetii* and a low level of contamination. However, such methods are labour intensive and are not usually used in routine diagnostics laboratories. In addition, level 3 containment facilities are required. With heavily contaminated samples, such as placentas, vaginal discharges, faeces or milk, the inoculation of laboratory animals may be necessary. Mice and guinea pigs are the most appropriate, but infected animals also have to be manipulated in level 3 biocontainment facilities.

Detection of *C. burnetii* can also be achieved by immunohistochemistry (IHC). Immunohistology may be used with paraffin-embedded tissues or on acetone fixed smears (Raoult et al., 1994a). The method uses either indirect immunofluorescence or an immunoperoxidase assay, using polyclonal *C. burnetii* antibodies (either a well characterized antiserum of human origin or a specific antiserum produced in either rabbits or guinea pigs). An anti-species (human, rabbit or guinea pig) anti-IgG conjugate labeled with Fluorescein isothiocyanate (FITC) or peroxidase is then used to visualize the bacteria. No specific antibodies for immunohistochemistry are commercially available (OIE, 2009).

Currently, the polymerase chain reaction (PCR) is one of the most analytically sensitive and rapid means for both the direct detection of *C. burnetii* and the identification of shedders. PCR can be used on a wide range of samples (vaginal discharge, abortion material, faeces and milk [bulk or individual]). It has become increasingly common in diagnostic laboratories (Berri et al., 2000; Nicollet and Valognes, 2007). The level of detection of conventional PCR is related to the sample under investigation (1–500 bacteria/ml of milk; 1 bacteria/mg of faeces). Several target genes have been used, such as the multicopy insertion sequence (IS1111) or single copy genes encoding various proteins (e.g dismutase [sodB]; com1 encoding a 27 kDa outer membrane protein; heat shock proteins [htpA and htpB]; isocitrate dehydrogenase [icd]; macrophage infectivity potentiator protein [cbmip]). Real-time PCR techniques have also been described (Kim et al., 2005; Klee et al., 2006; Stemmler and Meyer, 2002). For routine diagnostics, it is widely accepted that 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 and is best suited for quantification of *C. burnetii* in biological samples. As with conventional PCR, various target genes are used, including IS1111, com1 and icd. Quantitative PCR kits are now commercially available.

Several typing methods have been used for the characterisation of *C. burnetii* strains, including restriction endonucleae of genomic DNA (Hendrix et al., 1991), PFGE (pulsed-field gel electrophoresis) (Heinzen et al., 1990; Jager et al., 1998), and sequence and/or PCR-RFLP (restriction fragment length polymorphism) analysis of icd, com1 and mucZ genes. More recently, two PCR-based typing methods have been described, MLVA (multi-locus variable number of tandem repeats analysis) (Arricau-Bouvery et al., 2006; Svraka et al., 2006) and multispacer sequence typing (MST) (Glazunova et al., 2005) that each permit the typing of *C. burnetii* without the need for isolation of the organism. To date, MLVA and MST are considered to be the most discriminating methods for *C. burnetii*, allowing the identification of up to 36 distinct genotypes. Moreover, databases have been established (http://minisatellites.u-psud.fr/MLVAnet/ and http://ifr48.timone.univ-mrs.fr for MLVA and MST, respectively). The availability of such databases allows for easy interlaboratory comparisons, leading to a better understanding of different *C. burnetii* isolates. Furthermore, their use in the characterisation of field samples or isolates is increasing (Chmielewski et al., 2009; Klaassen

et al., 2009), and efforts to produce a standardised scheme for MLVA (based on common decisions for allele calling and marker panels) are in progress and should be available in the near future. These tools will be very useful for epidemiological investigation, particularly toclarify linkages regarding source of infection.

Serological testing

Although there is no officially (OIE) designated serological test for Q fever, the complement fixation (CFT) was considered the reference test for historical reasons. However, there is increasing use of indirect immunofluorescence assay (IFA) and ELISAs (commercial and in-house assays). Serological assays are suitable for screening herds, but interpretation at the individual animal level can be difficult. Indeed, animals may remain seropositive for several years following an acute infection, some animals may shed *C. burnetii* and pose a risk for infection prior to the development of antibodies, and some infected animals seem not to seroconvert. Three ELISA commercial kits for the diagnosis of Q fever in ruminants are currently available. Comparative analyses of available serological methods has been conducted during ring trials assessments as part of a EU-funded, Framework 6 project (MedVetNet; a European network of excellence working for the prevention and control of zoonoses and food borne diseases; www.medvetnet.org). The IFA and commercially available ELISAs were each reproducible, with comparable diagnostic sensitivity. In contrast, the diagnostic sensitivity of the CFT was highly variable (Roest et al., 2009). The analytical sensitivity of the ELISA was found to be 8-16 times higher than that of the best CFTs (Roest et al., 2009). Based on recent work, it was found that two commercial ELISAs can display different diagnostic sensitivities (81 and 95%, respectively) using a panel of sera from cattle, goat and sheep (Kittelberger et al., 2009). ELISA tests showed higher diagnostic sensitivities that the CFT. An ELISA test based on antigens of *C. burnetii* isolated from ruminants was found to have a higher diagnostic sensitivity than ELISAs based on antigens from the Nine Mile reference strain isolated from ticks, as assessed on goat sera, and may allow premature detection of infection (Rousset et al., unpublished).

Serological testing is useful in clarifying the infection status in herds but provides less certainty about the infection status of individuals (Kennerman et al., 2010). A seronegative result does not provide assurance that the animal is not infected. Indeed, this state may be associated with both early and long-term *C. burnetii* infection. In humans, tests are available (based on detection of antibodies to phase I and phase II antigens) to distinguish acute and chronic infection (see section 2.2.1). Such tests are not yet available for animals. Serological methods do not allow the identification of *C. burnetii*-shedding animals (Rodolakis, 2006; Rousset 2009). Currently, there is no serological test which can distinguish between vaccinated and naturally infected animals, although skin tests are recently being developed to select negative animals for vaccination (Guatteo et al., unpublished).

Table 1: Overview of laboratory tests used for diagnosis of *C. burnetii* infection in domestic ruminants

	Detects	Analytical Se	Comparative Se	Comparative Sp	Used for	Remarks
Direct						
Culture	viable bacteria		Culture <staining Staining < IHC IHC << PCR	Culture < staining Staining < IHC IHC << PCR	Research/ Clinical investigation	
Staining	bacteria				Clinical investigation	
IHC	bacteria in situ				Clinical investigation	Pathology diagnosis, skills are important.
PCR	DNA	1-10 bacteria [99,99%]			Clinical investigation/ Bulk milk tank	Multicopy target more sensitive than single copy target.
Serology						
CFT	Ab		CFT < ELISA ELISA = IFA	CFT < ELISA ELISA = IFA	Herd/ Individual testing	
ELISA	Ab				Herd/ Individual testing	
IFA	Ab				Herd/ Individual testing	The differentiation between antibodies to phase I and phase II antigens is possible, however, such tests for animals are not commercially available.

Se: Sensitivity, Sp: Specificity, IHC: immunohistochemical staining, PCR: Polymerase Chain Reaction (conventional as well as real time), CFT: Complement Fixation Test, IFA: immunofluorescent assay.

- Several methods are available for diagnosis of *C. burnetii* infection in animals, including both direct identification of the agent and serological testing.
- *Direct identification of the agent:*
  - There is no officially designated test for the direct identification of *C. burnetii* in domestic ruminants.
  - Immunohistochemistry can be very useful when considering potential causes of abortion in domestic ruminants.
  - In animals, multicopy polymerase chain reaction (PCR) is currently the most sensitive and rapid mean for the direct detection of *C. burnetii*, and the identification of shedders.
  - Currently it is not possible to reliably detect infected animals which are not shedding.
  - PCR can be used on a wide range of samples, including vaginal discharges, abortion material, faeces and milk (either bulk or individual).
  - Two PCR-based typing methods have been described (multi-locus variable number of tandem repeats analysis, MLVA; multispacer sequence typing, MST). These methods may become very useful for epidemiological investigations.
- *Serological testing:*
  - There is no officially designated serologic test for *C. burnetii* infection in domestic ruminants.
  - Serological assays are suitable for screening herds, but interpretation at the individual animal level can be difficult.
- Current serological methods provide limited information about stage of disease, whether infection is recent or latent, or whether the animal is currently shedding/ infectious. The present serological methods do not allow for differentiation between vaccinated or naturally infected animals.

2.1.2. Q fever monitoring and surveillance

Notification

There are no EU rules about the notification (i.e. the disease is not listed in Directive 82/849/EEC) or monitoring/surveillance of *C. burnetii* infection and/or Q fever in domestic ruminants. There are also no EU rules, relevant to Q fever, concerning control options, intra-community trade or import. In many European countries, there is also no national requirement for notification of Q fever in ruminants. The notification status of Q fever in selected European countries, based on a questionnaire by the recent Article 36 review (Sidi-Boumedine et al., 2010), is presented in Table 2. No data were specifically sought about the criteria for notification in different EU MS. Nonetheless, it is likely that there is considerable variation between EU MS, both in the notification criteria and the animal species for which disease is notifiable.

Table 2: The notification status of Q fever in European countries, in domestic ruminants

Country	Notifiable in domestic ruminants
Austria	NR
Belgium	NN
Bulgaria	✓
Cyprus	NN
Czech Republic	✓
Denmark	✓
Estonia	NN
Finland	✓
France	NN
Germany	✓
Greece	✓
Hungary	NN
Ireland	NR
Italy	✓
Latvia	✓
Lithuania	✓
Luxembourg	NN
Malta	NR
Netherlands	✓*
Poland	✓
Portugal	NN
Romania	NN
Slovakia	NN
Slovenia	✓
Spain	✓
Sweden	✓
UK	NN
Norway	NN
Switzerland	✓

*Notifiable in dairy sheep and goats from 2008
NN Not notifiable
NR No response to the questionaire

National reference laboratories

The answers to a questionnaire (Sidi-Boumedine et al., 2010) concerning the presence of a national reference laboratory for Q fever diagnosis in animals are presented in Table 3. A national reference laboratory (NRL) for Q fever detection is reported in all but 4 of the 24 EU MS that participated in the survey (Czech Republic, Estonia, Slovakia and Slovenia). Note that the lack of a NRL does not necessarily reflect a lack of laboratory capacity for monitoring/surveillance.

Table 3: National reference laboratories for C burnetii diagnosis in animals in EU MS

Country	NRL
Austria	NR
Belgium	✓
Bulgaria	✓
Cyprus	✓
Czech Republic	No NRL
Denmark	✓
Estonia	No NRL
Finland	✓
France	✓
Germany	✓
Greece	✓
Hungary	No NRL
Ireland	NR
Italy	✓
Latvia	No NRL
Lithuania	✓
Luxembourg	✓
Malta	NR
Netherlands	✓*
Poland	✓
Portugal	✓
Romania	✓
Slovakia	No NRL
Slovenia	No NRL
Spain	✓
Sweden	✓
UK	✓
Norway	✓
Switzerland	✓

*Central Veterinary Institute (CVI) is the NRL for all notifiable animal diseases in the Netherlands, including Q fever
NR No response to the questionaire

Monitoring/surveillance intensity

Q fever is included as a list B disease *('Other zoonoses and zoonotic agents that shall be monitored according to the epidemiological situation in a MS')* in Annex I of Directive 2003/99/EC. The disease is not specifically listed in Regulation 2160/2003/EC for zoonosis control options. In the EFSA/ECDC Zoonoses Report, data on *C. burnetii* infection and Q fever have been available since 2005. In 2005, data were available from only 2 countries, however, this has progressively increased to include data in 2008 from 17 EU MS and 2 non-EU MS. However, disease reporting from EU MS is not harmonized, and data completeness varies considerably.

Limited monitoring/surveillance programmes are conducted in European countries. Table 4 summarises all monitoring activities for Q fever in domestic ruminants in European countries, based on data from the EFSA/ECDC Zoonoses Reports (2006, 2007, 2008), an *ad hoc* consultation with EU MS in association with the current opinion (see Table 17), the published scientific literature (see Table 5) and additional information provided by the Q fever WG. It does not include data about clinical investigation, which is presented in Table 16. Testing for Q fever is generally conducted as part of the differential diagnosis for abortion.

Table 4: Monitoring/surveillance for *C. burnetii* infection/Q fever in domestic ruminants in European countries

European countries	Information source(s)[a]	Monitoring/surveillance in domestic ruminants[b]				
		2005	2006	2007	2008	2009
Austria	*N/A*[c]					
Belgium	R					c
Bulgaria	R, L	c, s (R, L)	c, s, (R, L)			c, s, g, (R)
Cyprus	L		c, s, g			
Czech Republic	R					No[d]
Denmark	R, L			c (R)	c (R, L)	
Estonia	R					No[d]
Finland	R					c
France	R, L	g (L)	g (L)		g (L)	g (R)
Germany	R				c, s, g	
Greece	R, L	s, g (R)	s, g (R)	s, g (L)		
Hungary	R					No[d]
Ireland	R, L					c, s,
Italy	R		c, s, g	c, s, g	c, s, g	
Latvia	*N/A*					
Lithuania	*N/A*					
Luxembourg	*R*					g
Malta	N/A					
Netherlands	R, L				c, s, g	s, g
Poland	R				c	
Portugal	R				s	
Romania	*N/A*					
Slovakia	*N/A*					
Slovenia	R				c, s, g	c
Spain	L	s	s	c, s, g	c, s, g	c, s, g
Sweden	R				c	c
United Kingdom	R				c	s, g
Norway	R				c	c, s, g
Switzerland	R	c, s, g	c, s, g			

a. R: as reported to EFSA (EFSA/ECDC Zoonoses Reports or in response to an ad hoc consultation with member states in 2010 in association with the current opinion), L: as reported in the scientific literature (source Table 5)
b. c: cattle, s: sheep, g: goats
c. N/A: no information available, either as reported to EFSA or in the scientific literature
d. No: no monitoring/ surveillance for Q fever

- There are currently no EU rules concerning notification and monitoring/surveillance of *C. burnetii* infection and/or Q fever in domestic ruminants. In many European countries, there are also no national notification requirements.
- There is also variable laboratory capacity in European countries for diagnosis of *C. burnetii* infection.
- There is limited monitoring/surveillance for *C. burnetii* infection in domestic ruminants in European countries, based on available data.
- Disease reporting from EU MS is not harmonized, and the level of reporting is highly variable. It is not currently possible, with any confidence, to provide accurate, comparative information about the occurrence of *C. burnetii* infection and Q fever in domestic ruminant populations among EU MS.

2.1.3. Infection and disease information in domestic ruminants

Testing intensity

Based on feedback from an *ad hoc* EFSA consultation with EU MS in 2010, in association with the current opinion (as detailed in section 1.2.1), testing for *C. burnetii* infection was conducted during 2009 in 16 (in cattle) 11 (in sheep) and 13 (in goats) of 23 European countries that replied to the consultation. Detailed data about testing for *C. burnetii* infection within Europe, by animal species, is presented in Table 15 (Appendix C).

Clinical investigations

Based on feedback from an *ad hoc* EFSA consultation with EU MS in 2010, in association with the current opinion (as detailed in section 1.2.1), Q fever testing in association with clinical investigations was conducted during 2009 in 15 (in cattle), 9 (in sheep) and 9 (in goats) of 23 European countries that replied to the consultation, as highlighted in Figures 1. Details of tests conducted in European countries during 2009 as part of a clinical investigation for Q fever in domestic ruminants, by species and countries, is presented in Table 16 (Appendix C).

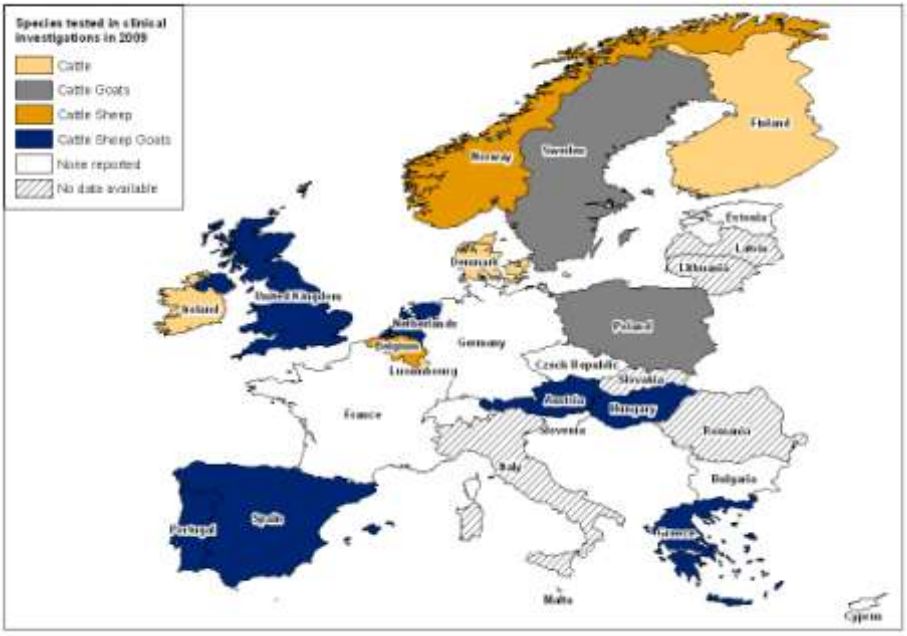

Figure 1: European countries where clinical investigation activities for Q fever in domestic ruminants were conducted in 2009.

Monitoring/surveillance activities

Based on feedback from an *ad hoc* EFSA consultation in 2010, in association with the current opinion (as detailed in section 1.2.1), testing for *C. burnetii* infection as part of a monitoring and/or surveillance activities was conducted during 2009 in 8 (in cattle), 4 (in sheep) and 6 (in goats) of 23 European countries that replied to the consultation, as highlighted in Figures 2. A detailed presentation of available data, by species and country, are presented in Table 17 (Appendix C). Animal- and herd-level seroprevalence for *C. burnetii* infection in domestic ruminants in European and neighbouring countries, by species and country, is also presented in Table 5. These data were initially presented in a 2005 publication by Arricau Bouvery and Rodolakis (2005), but have been updated following a review of literature published since 2004. Seroprevalence estimates are based on antibody detection.

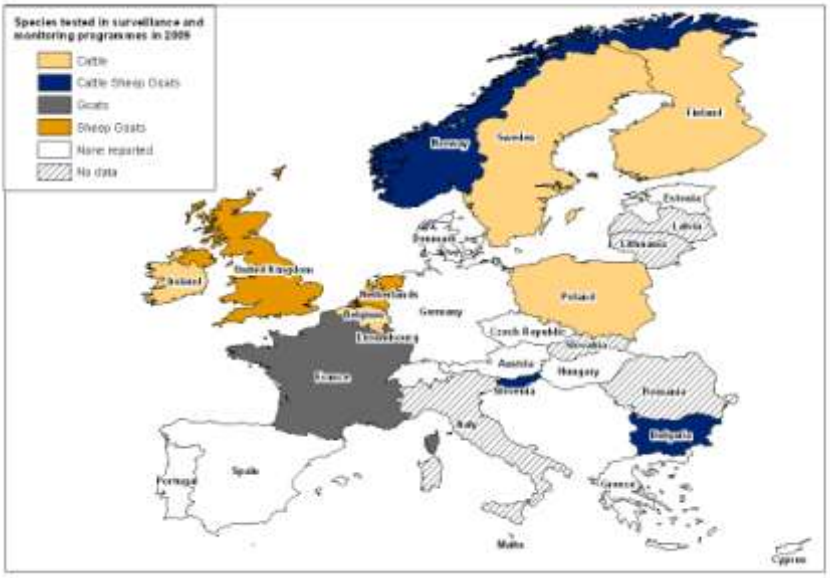

Figure 2: European countries where monitoring and/or surveillance investigation activities for *Coxiella burnetii* infection in domestic ruminants were conducted in 2009

Table 5: Prevalence of *C. burnetii* (animal and herd level) in domestic ruminants in Europe and neighbouring countries.

CATTLE							
Country	**Year of study**	**No. tested**		**% positive**		**Test[a]**	**Reference**
		Cattle	**Herds**	**Cattle**	**Herds**		
Albania	1999	552		8.5		ELISA	Cekani et al., 2008
	1995-1997	311		10.9		ELISA	Cekani et al., 2008
Bulgaria	2002	3,006		8.2		CFT	Martinov 2007a
	2003	3,714		6.5		CFT	Martinov 2007a
	2004	120		20.8*		IFA	Panaiotov et al., 2009
	2004	3,188		9.7		CFT	Martinov 2007a
	2005	3,026		8.1		CFT	Martinov 2007a
	2006	2,932		10.6		CFT	Martinov 2007a
	1989-2006	95,737		5.4		CFT	Martinov 2007a
	1977-1988	20,086		11.8		CFT	Martinov 2007a
	1950-1976	4,749		19.8		CFT	Martinov 2007a
Cyprus	NA	75		24.0		IFA	Psaroulaki et al., 2006a
Denmark	2008		100		59.0	ELISA-milk	Agger et al., 2010
	2007		742		57	ELISA-milk	Bodker and Christoffersen, 2008
Italy	1998	544***	21	13	nd	IFA	In Arricau-Bouvery and Rodolakis 2005
		155***	6	2	nd	IFA	In Arricau-Bouvery and Rodolakis 2005
		486	26	20	nd	IFA	In Arricau-Bouvery and Rodolakis 2005
	NA	650		44.9**		ELISA	Cabassi et al., 2006
	NA	600		22.0		ELISA	Cabassi et al., 2006
Germany	1998-2000	1,167	*105*	1.4 to 2**	nd	ELISA	Sting et al., 2002
	1998	21,191	544	8	nd	ELISA	In Arricau-Bouvery 2005

	1996-1997	826	*38*	14.3** 0.6	nd	ELISA	Sting et al., 2000
	1992-1993	500 665 383** 612 Bulls	*NA* *39* *33* *1*	7.6 9.6 19.3 5.6	nd 76.9 78.8 100	CFT	Wittenbrink et al., 1994
	1991	1,095	*21*	11.8	81	ELISA	Rehacek et al., 1993
	1989-1990	3,500	*155*	13.3	57.4	ELISA	Klemt and Krauss, 1991
Netherlands	1987	1,160**	*234*	21	37	ELISA	Muskens et al., 2007
Spain	2006-2007	79		35.4		IFA	Ruiz-Fons et al., 2008
	2008-2009?	626		6.7		ELISA	Ruiz-Fons et al., 2010
	2008-2009?		42		42.9	ELISA	Ruiz-Fons et al., 2010
Turkey	2006-2008	92		16.3		ELISA	Ceylan et al., 2009
	2005	230		9.6		ELISA	Seyitoglu et al., 2005
	1998	416	48	6	nd		In Arricau-Bouvery and Rodolakis 2005
UK (NI)	2009	5,182		6.2		ELISA	McCaughey et al., 2010
	2009		273		48.4	ELISA	McCaughey et al., 2010

SHEEP							
Country	**Year**	**No. tested**		**%**		**Test**	**Reference**
		Sheep	**Herds**	**positive**			
Albania	1999	292		12.3		ELISA	Cekani et al., 2008
	1995-1997	350		8.9		ELISA	Cekani et al., 2008
Bulgaria	2002	1,819		12.7		CFT	Martinov 2007a
	2003	1,811		8.3		CFT	Martinov 2007a
	2004	100		21.0*		IFA	Panaiotov et al., 2009
	2004	1,258		14.1		CFT	Martinov 2007a
	2005	1,911		15.2		CFT	Martinov 2007a
	2006	1,925		8.4		CFT	Martinov 2007a
	1950-1976	17,088		16.7		CFT	Martinov 2007a
	1977-1988	16,593		18.8		CFT	Martinov 2007a
	1989-2006	99,189		4.8		CFT	Martinov 2007a
	NA 2006??	153		56.9**		CFT	Martinov. 2007b
Croatia	2004	182		11.0*		CFT	Medic et al., 2005
Cyprus	NA	481		18.9		IFA	Psaroulaki et al., 2006
Germany	NA		*95*		2.7		Runge and Ganter, 2008
	1998	1,346		1.3		ELISA	In Arricau-Bouvery and Rodolakis 2005
	1999	100	1	57		ELISA	In Arricau-Bouvery and Rodolakis 2005
		3,460		8.7		ELISA	Sting et al., 2002
Greece	NA	554		10.5		IFA	Pape et al., 2009a
Italy	1999-2002	7,194	675	9/38		ELISA	In Arricau-Bouvery and Rodolakis 2005
Netherlands	1987	3,603		3.5		ELISA	In Muskens et al., 2007
	2008	12,363		2.4		ELISA	Van den Brom and P. Vellema, 2009
Spain	1999-2003		148	8.8**		PCR	Oporto et al., 2006
	1999-2003		148	2.7**		CFT	Oporto et al., 2006
	1999-2003	38		42.1**		CFT	Oporto et al., 2006
	2005	34		67.6**		ELISA	Garcia-Perez et al., 2009
	2005	1,011		8.9		ELISA	Garcia-Perez et al., 2009
	2005		154	22.1		ELISA	Garcia-Perez et al., 2009
	2007-2008	1,379		11.7		ELISA	Ruiz-Fons et al., 2010
			46	34		ELISA	Ruiz-Fons et al., 2010
Turkey	NA	465		21.1**		ELISA	Karaca et al., 2009

	2001-2004	743	42	20	83	ELISA	Kennerman et al., 2010
	1998	411		10.5		IFA	In Arricau-Bouvery and Rodolakis 2005
UK (NI)	NA 2009?	1,022		12.3		ELISA	McCaughey et al., 2010
	NA 2009?		58	62.1		ELISA	McCaughey et al., 2010

GOATS							
Country	Year	**No. tested**		**% positive**		**Test** [(a)]	**Reference**
		Goats	**Herds**				
Albania	1999	260		4.2		ELISA	Cekani et al., 2008
	1995-1997	443		8.8		ELISA	Cekani et al., 2008
Bulgaria	2002	677		11.8		CFT	Martinov, 2007a
	2003	1,044		7.4		CFT	Martinov, 2007a
	2004	50		40.0*		IFA	Panaiotov et al., 2009
	2004	1,016		21.7		CFT	Martinov, 2007a
	2005	832		11.1		CFT	Martinov, 2007a
	2006	359		19.2		CFT	Martinov, 2007a
	1950-1976	1,417		20.5		CFT	Martinov, 2007a
	1977-1988	1,791		10.8		CFT	Martinov, 2007a
	1989-2006	54,175		7.6		CFT	Martinov, 2007a
Cyprus	NA	417		48.2		IFA	Psaroulaki et al.2006a
France	2006	359		36.0		ELISA	Dubuc-Forfait et al., 2009
	2006		42	88.1		ELISA	Dubuc-Forfait et al., 2009
	2006	75		65.3**		ELISA	Chaillon et al., 2008
	2008	1,057		32.0		ELISA	Dubuc-Forfait et al., 2009
	2008	42		88.1		ELISA	Dubuc-Forfait et al., 2009
Germany	1998	278		2.5		ELISA	In Arricau-Bouvery and Rodolakis 2005
Greece	NA	61		6.6		IFA	Pape et al., 2009a
Italy	1999-2002	2,155	104	13	47	ELISA	In Arricau-Bouvery and Rodolakis 2005
Netherlands	1987	498		1 goat		ELISA	Muskens et al., 2007
	2008	3,409		7.8		ELISA	Van den Brom and P. Vellema, 2009
Poland	NA, ML after 1997	98		79.6**		MAT	Platt-Samoraj et al., 2005
Spain	2007-2008	115		8.7		ELISA	Ruiz-Fons et al., 2010
	2007-2008		11		45.5	ELISA	Ruiz-Fons et al., 2010
Turkey	2006-2008	92		5.4		ELISA	Ceylan et al., 2009
UK (NI)	NA, ML 2008-2009?	54		9.3		ELISA	McCaughey et al., 2010
	NA, ML 2008-2009?		7		42.9	ELISA	McCaughey et al., 2010

(a) Indirect Immunofluorescence assay (IFA), Enzyme Linked Immunosorbent assay (ELISA), Complement fixation test (CFT), Microagglutination test (MAT)

* Investigation in relation to a human outbreak

** Investigation in relation to clinical symptoms in the population (animals)

*** The study was conducted to compare animals kept indoors (544) and outdoors (155)

NA - not available; ML- most likely

- There were differences between European countries in testing intensity, and in the reported level of clinical investigations and monitoring/surveillance activities, for *C. burnetii* infection and Q fever
- *C. burnetii* infection is prevalent in domestic ruminants (cattle, sheep, and goats) in a wide range of European and neighbouring countries, based on the results of serological testing over the last several decades. These results are indicative of infection, but not necessarily disease.

2.1.4. Interpretation/evaluation of the surveillance data

Information of the incidence and prevalence of *C. burnetii* infection and Q fever in the animal population in EU is based either on passively collected data (the animal owner or veterinarian reports a case or outbreak to the authorities) or on a combination of data collected passively and actively (that is, samples are collected according to a planned framework). The latter is, without doubt, the best way to get a sound picture of the disease situation in animals, provided:

- There is a clear case definition,
- There is a commitment among farmers and veterinarians to report suspect (and confirmed) cases, and
- a well designed active monitoring protocol is implemented, including formal random sampling protocols (sample size based on predefined accuracy and precision, taking into account clustering of infection in herds and possibly regions) and information on the diagnostic performance (sensitivity and specificity) of the test(s) being used.

Interpretation of the annual EFSA/ECDC Zoonoses Reports (2006, 2007 and 2008) is currently problematic. It is difficult to make comparisons between species, countries and years as there are considerable differences in testing protocol and data availability. Factors such as the reason for testing (e.g. an abortion/outbreak investigation, milk controls programme, prevalence survey), number of animals tested, type of tissue sampled (in particular bulk milk or individual animal samples) and laboratory technique (some animals testing positive for ELISA then test negative for PCR) can each have a significant effect on the number of animals testing positive. Herd prevalence is higher than animal prevalence, highlighting concerns about data collection, including the completeness of herd-level data. In many EU MS, notification is not mandatory. In those EU MS where notification is mandatory, the differences in the relative number of Q fever tests that have been performed (number of tests relative to the ruminant population) suggest that compliance to notification is quite variable. This is to be expected, noting that the clinical signs of Q fever in animals are not very specific, the causative agent seems to be present in most EU MS, and diagnosis does not result in specific intervention measures in most EU MS. In addition, there is quite a broad variation in tests being used for the diagnosis, while at the same time there is a lack of sound information on the diagnostic sensitivity and specificity of these tests. For the active monitoring and surveillance programmes, comparison between EU MS is hampered by more or less the same problems. It is not performed in most EU MS, the sampling design is not consistent among EU MS where it has been implemented and sound information on diagnostic sensitivity and specificity is lacking (section 2.1.1.). In conclusion, it is not possible to make a sound comparison of outbreak data or of measures of Q fever occurrence such as incidence or prevalence.

From the available information and the above, we can conclude that *C. burnetii* is present in most, if not all, EU MS. However, because of the lack of harmonization of the monitoring programme and tests being used in the various EU MS, a comparison of the occurrence of *C. burnetii* infection in animal populations between different EU MS is subject to considerable bias and therefore associated with considerable uncertainty. Within EU MS, however, the monitoring is likely more comparable over the years (although the variation in tested samples also varies within EU MS, this variation is less than the variation between EU MS), with a possible exception of 2009 when the Dutch problem may have boosted Q fever interest in other EU MS as well. Based on the information in the Annex, there are no indications for an obvious upward trend in Q fever incidence/prevalence within EU MS although some increase in seroprevalence has been observed in some EU MS.

Questions could be raised about the potential value of a shift towards mandatory Q fever notification in animal populations in EU MS. However, such a change could be counterproductive in the absence of clear advantages, such as compensation for affected farmers. If genuinely comparable data from different EU MS were needed, a range of issues (including case definitions, diagnostic methods and sampling plans) need to be harmonised and standardised. In addition, laboratories need to operate under agreed quality management systems.

- Based on available data, *C. burnetii* is present in most, if not all, MS.
- Interpretation of the annual EFSA/ECDC Zoonoses Reports is currently problematic, specifically because data collection between MS is not harmonised. Consequently, it is not possible to conduct an unbiased comparison of Q fever prevalence/incidence in animals between MS.
- Based on available data, there does not appear to be an upward trend in Q fever prevalence/incidence in EU MS. However, comparison over time and between countries (or regions) is associated with considerable uncertainty.
- If an unbiased comparison of Q fever occurrence in animals between EU MS and over time is needed, then monitoring/surveillance would need to be conducted in a harmonised manner.

2.2. Magnitude and distribution of Q fever in humans

2.2.1. Q fever diagnosis in humans

Q fever clinical symptoms in humans are usually non-specific and often relatively mild; hence, classical differential diagnosis must be supported by laboratory tests for accurate diagnosis of clinical disease.

Serological testing

The classical method for Q fever diagnosis is serological analysis to detect the presence of antibodies to *C. burnetii* antigens. Typically, this relies on ELISA, Complement Fixation or Immunofluroescense Assay platforms using commercially available or in-house testing kits (Scola, 2002; Fournier et al., 1998; Schimmer et al., 2009). Serology has an advantage because it can theoretically differentiate between acute and chronic infection of *C. burnetii*. This is because there are two antigenic phases called phase I and phase II that vary depending on the clinical progression of infection. Each phase has a different antigen profile; in acute Q fever, the immune response is primarily driven by IgM and IgG antibodies directed against the avirulent form of *C. burnetii* (Phase II). In the chronic form, IgG and IgA antibodies predominate and are directed against both the virulent and avirulent forms of bacteria (Phase I). Acute infection is therefore characterised by elevated phase II antibody levels, and generally is first detectable after the second week of illness. In chronic Q fever, typically the opposite is true: i.e. phase I antigens significantly predominate over Phase II (Maurin and Raoult, 1999). This is because antibodies to phase I antigens of *C. burnetii* generally require longer to appear and indicate continued exposure to the bacteria. Thus, high levels of antibody to phase I in later specimens, in combination with constant or falling levels of phase II antibodies and other signs of inflammatory disease, suggest chronic Q fever. Antibodies to phase I and II antigens have been known to persist for months or years after initial infection (CDC, Fact sheet- Q fever[4]).

Broadly the same diagnostic methods are also used for seroprevalence studies to estimate the extent to which a given population has been infected by *C. burnetii* following possible exposure. However this type of study is not designed to determine the incidence of clinical disease; it is only through the

[4] CDC fact sheet; Q fever: www.cdc.gov.ncidod/dvrd/qfever/index.htm (accessed 15/3/10)

combination of clinical symptoms and confirmatory laboratory diagnostics that clinical disease can be determined.

Direct identification of the agent

In addition to serology, DNA detection methods are becoming increasingly common and widely used. They have the obviously advantage that they are able to detect the presence of the etiological agent before the occurrence of seroconversion events that is prerequisite for diagnosis using serological methods. For example, Real-Time PCR tests have recently been developed in the Netherlands. Typically they are able to detect the presence of *C. burnetii* within 10 days of symptom onset. (Schneeberger et al., 2009).

2.2.2. Q fever monitoring and surveillance in humans

Within the EU legal framework on communicable disease surveillance and notification, Q fever is one of the 47 communicable diseases for which surveillance is mandatory in the EU and three EEA/EFTA countries (Commission Decision 2000/96/EC[5]). This legal framework requires EU MS to report surveillance data to ECDC. This underlying legislative requirement is supported by harmonised case definition of human Q fever under EU legislation (CD 2008/426/EC[6]) (Table 6):

Table 6: EU harmonised Q fever case definition (adapted from CD 2008/426/EC)

Q Fever *(Coxiella burnetii)*

Clinical criteria

Any person with at least one of the following three symptoms:
— Fever
— Pneumonia
— Hepatitis

Laboratory criteria

At least one of the following three:
— Isolation of *Coxiella burnetii* from a clinical specimen
— Detection of *Coxiella burnetii* nucleic acid in a clinical specimen
— *Coxiella burnetii* specific antibody response (IgG or IgM phase II)

Epidemiological criteria

At least one of the following two epidemiological links:
— Exposure to a common source
— Animal to human transmission

Case classification

A. **Possible case**

- NA

B. **Probable case**

- Any person meeting the clinical criteria and with an epidemiological link

C. **Confirmed case**

- Any person meeting the clinical and the laboratory criteria

[5] Commission Decision 2000/96/EC of 22 December 1999 on the communicable diseases to be progressively covered by the Community network under Decision No 2119/98/EC of the European Parliament and of the Council

[6] Commission Decision 2008/426/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council

However, the range and type of surveillance systems for Q fever throughout the EU varies significantly and although each system may be adapted to local circumstances in order to maximise detection of cases (Table 7), there is likely to be significant variability in the case ascertainment between and within EU MS; it is certainly recognised that case notification rates are dependent on the level of local medical and scientific interest in Q fever (Raoult, 1996).

Table 7: Q fever - EU surveillance systems overview in humans (from ECDC Annual Epidemiological Report 2009)

						Data reported by				
Country	**Data Source Description**	**Compulsory (Cp)/Voluntary (V)**	**Comprehensive (Co)/Sentinel (Se)**	**Active (A) /Passive (P)**	**Case-Based (C)/Aggregated (A)**	**Laboratories**	**Physicians**	**Hospitals**	**Others**	**National coverage**
Belgium	Reference Laboratories	V	Co	P	C	Y	N	N	N	Y
Bulgaria	National Surveillance System	Cp	Co	P	A	Y	Y	Y	Y	Y
Cyprus	System for Mandatory Notified Diseases	Cp	Co	P	C	N	Y	N	N	Y
Czech Republic	EPIDAT	Cp	Co	A	C	-	Y	Y	N	Y
Estonia	Obligatory, countrywide, based on a double system of reporting Hemorrhagic fevers	Cp	Co	P	C	Y	Y	Y	Y	Y
Finland	National Infectious Disease Register (NIDR)	Cp	Co	P	C	Y	N	N	N	Y
France	National reference Centres	V	Co	P	C	Y	N	N	N	Y
Germany	SurvNet@RKI IfSG 7.1	Cp	Co	P	C	Y	Y	Y	Y	Y
Greece	Notifiable Diseases System	Cp	Co	P	C	Y	Y	Y	N	Y
Hungary	Notification System for Infectious Diseases	Cp	Co	P	C	N	Y	Y	N	Y
Ireland	CIDR	Cp	Co	P	C	Y	Y	Y	N	Y
Italy	National Reporting System	Cp	Se	P	-	N	Y	Y	-	Y
Latvia	Visums	Cp	Co	P	C	N	Y	Y	N	Y
Lithuania	National Communicable diseases surveillance System	Cp	Co	P	C	Y	Y	N	N	Y
Luxembourg	System 1 mandatory notification system	Cp	Co	P	C	N	Y	N	N	Y
Malta	Infectious Disease Prevention and Control Unit	Cp	Co	P	C	Y	Y	Y	Y	Y
Netherlands	Osiris	Cp	Co	P	C	Y	Y	N	Y	Y
Norway	MSIS (group A diseases)	Cp	Co	P	C	Y	Y	Y	N	Y
Poland	National Surveillance System of Infectious Diseases	Cp	Co	P	C	Y	Y	N	N	Y
Portugal	Q- fever Surveillance System	Cp	Co	P	C	N	Y	N	N	Y
Romania	Romanian National	Cp	Co	P	A	N	N	Y	N	Y

	Surveillance System									
Slovakia	EPIS - Epidemiological Information System	Cp	Co	A	C	Y	Y	Y	Y	Y
Slovenia	SURVIVAL	Cp	Co	P	C	Y	Y	N	N	N
Spain	Microbiological Information System	V	Se	P	C	Y	N	N	N	N
Sweden	SmiNet	Cp	Co	P	C	Y	Y	Y	N	Y
United Kingdom	Q fever Surveillance System	V	Co	P	C	Y	N	Y	Y	Y

2.2.3. Infection and disease information in humans

Clinical disease

Harmonised data on Q fever notifications throughout the EU are collected under the European Surveillance System. This is a single repository under which EU MS upload data collected at national level on diseases for which surveillance is mandatory in the EU. The most recent information is presented in two separate outputs: 2007 data is presented in the 2009 Annual Epidemiology report from ECDC (Annual epidemiologic report on Commuinicable diseases in Europe 2009), and data form 2008 is presented in the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne outbreaks in the European Union (EFSA, 2008).

In 2007, the broad epidemiological situation from reported data was that 22 EU and EEA/EFTA countries reported a total of 669 cases of Q fever in 2007 (8 countries reported zero cases), 637 of which were confirmed. Outbreaks of human infection were reported in two countries (Netherlands (168 cases) and Slovenia (93 cases) and both these EU MS obtained notification rates above one per 100,000. The majority of other cases were reporting in Bulgaria (36), Germany (83), Spain (159) and the UK (62). In 2008, 24 EU/EFTA countries reported 1,599 Q fever cases (1,594 confirmed), representing an increase of over 170% from the previous year. The increase was mainly attributed to the increase in the Netherlands and in Germany, while 13 EU/EFTA countries reported zero cases (Table 8). In 2008, the increasingly widespread epidemic in the Netherlands resulted in over 1,000 human cases being notified.

Table 8: Reported confirmed Q fever cases in humans reported to the ECDC, 2007-2008

		2008 [4]			**2007** [3]		
Country	**Report Type**[1]	**Total cases**	**Confirmed Cases**	**Cases/ 100,000**	**Total cases**	**Confirmed Cases**	**Cases/ 100,000**
Austria	–[2]	–	–	–	-	–	–
Belgium	A	0	0	0	14	0	0
Bulgaria	A	17	17	0.2	36	33	0.43
Cyprus	C	0	0	0	8	8	0
Czech Republic	–	–	–	–	-	–	–
Denmark	–[2]	–	–	–	-	–	–
Estonia	C	0	0	0	0	0	0
Finland	C	2	2	<0.1	2	2	<0.1
France	–	–	–	–	-	–	–
Germany	C	370	370	0.5	83	83	0.1
Greece	C	3	3	<0.1	0	0	0
Hungary	C	0	0	0	7	7	<0.1
Ireland	C	13	10	0.2	4	4	<0.1
Italy	–	–	–	–	-	–	–
Latvia	C	0	0	0	0	0	0
Lithuania	A	0	0	0	0	0	0

Luxembourg	–[2]	–	–	–	-	–	–
Malta	C	0	0	–	0	0	0
Netherlands	C	1,013	1,011	6.2	168	168	1.03
Poland	C	0	0	0	0	0	0
Portugal	C	12	12	0.1	10	8	<0.1
Romania	A	3	3	<0.1	6	6	<0.1
Slovakia	C	0	0	0	1	1	<0.1
Slovenia	C	0	0	0	93	93	4.6
Spain	C	119	119	0.3	159	159	-
Sweden	C	7	7	0.1	3	3	<0.1
United Kingdom	A	40	40	<0.1	62	62	0.1
EU Total		**1,599**	**1,594**	**0.5**	**669**	**637**	**0.16**[(a)]
Iceland	C	0	0	0	-	-	-
Liechtenstein	C	0	0	0	-	-	-
Norway	C	0	0	0	0	0	0
Total		**1,599**	**1,594**	–	**669**	**637**	**0.16**[(a)]

1. A: aggregated data report; C: case-based report; –: no report;
2. No surveillance system exists
3. Data from ECDC 2009 Annual epidemiological report
4. Data from Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and food-borne outbreaks in the European Union in 2008

(a) overall rate excludes data from Spain

Descriptive epidemiology on the age, gender and seasonal distribution on Q fever notifications in 2007 is presented in the ECDC Annual epidemiological Report for 2009, and is reproduced below:

- Age and gender distribution: The highest rates were seen in the age groups of 15–24 year-olds and 45–64 year-olds, with notification rates of 0.24 and 0.23 per 100 000 population, respectively. Only seven of the 501 cases for which such information was available were reported among children under the age of 15. The overall rate was higher in men than in women (0.23 and 0.13 per 100 000, respectively), with a male-to female ratio of 1.78:1.

- *Seasonality:* The information on seasonality was available for the 585 confirmed cases during 2007. The months with the highest number of reported cases were July and August (84 and 69 cases, respectively). In 2006 the majority of cases occurred during June and July which was linked to spring lambing season in many European countries. The distribution by date of notification of cases is not very useful as day of onset of illness can be earlier than day of notification for such an insidious disease.

Seroprevalence

The date collected under EU surveillance capture notified clinical cases of Q fever but there is no EU harmonised surveillance system to assess the levels of exposure or infection to *C. burnetii*. However, many serological studies have been performed to determine the extent to which defined population groups may have been infected with *C. burnetii* (see Table 9). Generally this work has been done to assess levels of seroconversion in the general population and risk groups associated with known outbreaks, or comparative studies to assess seroconversion in occupational groups. The design, focus and scale of these studies vary significantly including different diagnostic tests cut off values and, population groups. This makes direct comparison impossible. However, it can broadly summarised that, like the clinical case data, there is significant variation in the levels of seroconversion throughout the EU, but that at the general population level, there are typically a small percentage of individuals (often between 2-10%) that have evidence of having been infected with *C. burnetii*. This figure inevitably rises in areas with ongoing community-based outbreaks of human Q fever, or where outbreaks are commonly reported and there is some endemicity of *C. burnetii*. Comparatively high seroprevalence are also commonly seen in occupational and other groups that have extended exposure

to animal hosts for *C. burnetii* (farmers, veterinarians, slaughterhouse workers, laboratory workers etc) (Table 10).

Table 9: Prevalence for *C burnetii* in human populations in Europe and neighbouring countries

Country	Year of study	Number of tested	% positive	Test	Reference
Cyprus	NA	141**	57.4	IFA	Psaroulaki et al., 2006a
Cyprus	NA	131**	44.3	IFA	Psaroulaki et al., 2006a
Cyprus	NA	121**	43.0	IFA	Psaroulaki et al., 2006a
Cyprus	NA	63**	52.4	IFA	Psaroulaki et al., 2006a
Cyprus	NA	127**	64.6	IFA	Psaroulaki et al., 2006a
France	1982-1990	22,496**	23		In Maurin et Raoult, 1999
Netherlands	1982	222**	83.8		In Van den Brom and Vellema, 2009
Netherlands	1983	359*	24		In Van den Brom and Vellema, 2009
UK (NI)	1986-1987	2 394	12.8	ELISA	McCaughey et al 2008
Greece	1987	231**	13.8 to 38.1	IFA	In Psaroulakis et al., 2006a
Italy	NA (1987?)	NA	6.1	IFA	In Psaroulakis et al., 2006a
Greece	1987	238**	42.0	IFA	In Psaroulakis et al., 2006a
France	1988	924**	4.03	IFA	In Psaroulakis et al., 2006a
Spain	1989	NA	12.7	IFA	In Psaroulakis et al., 2006a
Spain	1990	NA	50.2	IFA	In Psaroulakis et al., 2006a
Spain	1993	400	38.4	IFA	In Psaroulakis et al., 2006a
Bulgaria	1993-2000	1,4353**	15	CFT+ MIFT	Martinov 2007a
Spain	1994	NA	40.6	IFA	In Psaroulakis et al., 2006a
France	1995	790*	1	IFA	In Arricau-Bouvery and Rodolakis 2005
France	1995-1996	785	5	IFA	In Arricau-Bouvery and Rodolakis 2005
Spain	1996-1997	1,654	5	IFA	In Arricau-Bouvery and Rodolakis 2005
France	1996	620*	3	IFA	In Arricau-Bouvery and Rodolakis 2005
France	1996	12,716	0.2	IFA	In Arricau-Bouvery and Rodolakis 2005
France	1996	208**	71	IFA	In Psaroulakis et al., 2006a
Turkey	1998	102	8	IFA	In Arricau-Bouvery and Rodolakis 2005
Spain	1998	595	48.1	IFA	In Psaroulakis et al., 2006a
Spain	1999	1 654	5.1	IFA	In Psaroulakis et al., 2006a
Bulgaria	2001-2004	5 207**	18	CFT+ MIFT	Martinov, 2007a
France	2002	376***	2.9	IFA	Tissot-Dupont et al., 2007
France	2002	91***	5.5	IFA	Tissot-Dupont et al., 2007
France	2002	578***	14.7	IFA	Tissot-Dupont et al., 2007
Bosnia Herzegovina	2002	76***	34.2	IFA	Sukrija et al., 2006
Poland	2003	90	18	IFA	In Arricau-Bouvery and Rodolakis 2005
Canary Island	2003	662	21.5	IFA	In Psaroulakis et al., 2006a
Bulgaria	2004	104***	7.7	IFA	Panaiotov et al., 2009
Croatia	2004	56***	14	CF	Medic et al., 2005
Turkey	2004?	92	19.6	ELISA	Seyitoglu et al., 2005
Turkey	2004	339	1.8 to 13.2	IFA	In Psaroulakis et al., 2006a
Greece	2007	1007	7.5	IFA + ELISA	Pape et al., 2009b
Greece	2007	850**	6.7	IFA +	Pape et al., 2009c

				ELISA	
Netherlands	2006-2007	5,654	1.5	ELISA	Duynhoven et al. 2010

*Blood donors
** Risk group
***humans in the outbreak area

2.2.4. Interpretation/evaluation of the surveillance data

Overall, it can broadly be concluded from the EU surveillance data that human Q fever persists as a relatively infrequent clinical disease, but one that occurs throughout the EU/EFTA. Typically human cases are reported either as single sporadic cases or discrete cluster of cases from a specific source with no obvious association to an increased general disease risk (Wilson et al., 2009, Orr et al., 2006), or as part of clusters of infections associated with a known community-based outbreak. The underlying basic EU/EFTA case numbers have some variance, but the annual notification rates have the same order of magnitude over time; typically between 500-2000 cases per annum, of which a significant proportion are associated with outbreaks related with animals. The number of outbreaks in the EU/EFTA also varies each year, but generally is relatively infrequent: typically between 1-3 outbreak clusters of human infections are reported annually in the EU/EFTA. These are commonly isolated to a discreet geographical area within a EU MS, although there may be separate clusters and multiple sources within each outbreak. Typically a relatively small number of infected individuals are identified in each outbreak (between 10-500), and at least in the last 10 years, they do not usually persist over consecutive years (Table 11).

Although the underlying epidemiology remains broadly consistent in the EU, it is likely that most human cases of Q fever are not diagnosed and therefore not reported. This is because prior infection in animal hosts is often undetected or not notified, which means that public health authorities may not receive early warning of the presence of the known zoonotic agent in animal populations. In addition, most infected humans are either asymptomatic, or present with non-specific respiratory symptoms and mild self-limited infection. Hence the number of cases both at national and EU level are likely to be significantly underestimated, and therefore it is difficult to assess the real number of *C. burnetii* infections in the EU (ECDC, 2009).

Although it is clear that surveillance systems are unlikely to pick up all the clinical cases of Q fever, increasing levels of general respiratory illness, particularly atypical pneumonia have alerted medical practitioners to emergent outbreaks in several EU MS (see Table 11). However given the variance in routine and active surveillance in the EU, it remains uncertain if all such outbreaks within the EU are identified. It certainly can not be discounted that clusters of human cases that may represent a potential outbreak event may be missed given the relatively mild unspecific symptomology of most acute human cases of Q fever, together with the mild and non-discriminatory clinical manifestation of infection in animals and the fact that the infection in animals is non-notifiable in many EU countries. Indeed, it is likely that humans act as sentinels for infection; human disease may be the first indication that *C. burnetii* is present in a locality. Even in this case, many factors must align before infection is recognised; alert medical practitioners testing on the basis of clinical symptoms; accurate sample collection and submission; diagnostic laboratory capacity and capability and result feedback. A recent study in the Netherlands retrospectively examining hospitalisations for lower respiratory tract infections has suggested that some local Q fever clusters may have passed unnoticed; six clusters of excess hospitalisations in adults in areas with Q fever in small ruminants were detected between 2005 and 2007, next to the first recognised human outbreak in 2007 (Schimmer et al., 2007) However, there is no suggestion that proactive syndromic surveillance would have identified these cases at the time.

The Q fever epidemic in the Netherlands that was first reported in 2007 has shown some divergence from the general epidemiology of Q fever seen in the EU as a whole. In this case, the infection appears to be persisting over consecutive years, and disease is becoming both more prevalent and more widespread in humans following initial notification of an outbreak cluster. Although the outbreaks were initially confined to a small region within the province of Noord-Brabant in the Netherlands in

2007, the foci of animal infection and associated human disease has expanded to include other neighbouring provinces over subsequent years.

- Human Q fever cases must be notified under EU law. Typically, between 600-1500 cases are notified annually throughout the EU, of which the majority is associated with specific Q fever outbreaks in animals. Most MS commonly report cases indicating the widespread distribution of the *C. burnetii* throughout the EU.
- Human cases are likely to be underreported; Q fever symptoms are commonly mild and non-specific and hence the infection is not commonly considered in differential diagnosis unless in the locality of known outbreaks.
- Humans may act as sentinels for *C. burnetii* infection; human disease may be the first indication that *C. burnetii* is present in a locality.
- The challenges of case ascertainment, and the associated underreporting of clinical infection in the EU, means that it is difficult to draw conclusions on the underlying trend in *C.burnetii* infections in the EU. However, the epidemiological pattern commonly seen in EU outbreaks are localised events of limited duration with numbers of clincial cases in the order to magtude of 10-500.
- The Q fever epidemic in the Netherlands that was first reported in 2007 has shown some divergence from the outbreak epidemiology of Q fever seen in the EU; the infection appears to be persisting over consecutive years, and has become more prevalent and more widespread in humans following initial notification of a outbreak cluster.

2.3. Significance and impact of Q fever

2.3.1. Significance and impact on public health

Clinical manifestation and treatment

C. burnetii in humans demonstrates a wide variability in clinical presentation, but broadly can be classified into acute and chronic infection. Acute and chronic infection can be distinguished on the basis of clinical expression, temporal course and serological profiles.

Acute Q fever

- It is commonly reported that approximately 60% of infections are asymptomatic seroconversions (Arricau-Bouvery and Rodolakis, 2005). Patients with clinical disease present a range of non-specific symptoms, the most common of which are flu-like symptoms that is typically characterised by high fever, headache and cough, atypical pneumonia or hepatitis. Other rare clinical manifestations have also been described inducing skin rash, nausea myocarditis, pericarditis, meningoencephalitis, pancreatitis and abortion.
- Most symptomatic cases suffer a relatively mild self-limiting infection, but some may need medical attention (20%), and approximately 1-5% requires hospital treatment that in some cases requires ventilation support and intensive care.
- Patients presenting with acute illness can be effectively treated (Delsing et al., 2009).

Chronic Q fever

- The prevalence of chronic Q fever is uncertain, and there are relatively wide ranging estimates in the literature; Tissot-Dupont et al. report that 0.5 % of patients having had the acute disease become chronic. Other authors report that approximately 1 % of acutely ill patients become chronic, while others have proposed up to 5% (Tissot-Dupont et al., 2007; Arricau-Bouvery and Rodolakis, 2005).
- The clinical manifestation of chronic Q fever can be broad, but the most common symptoms relate to heart and circulatory impairment, of which endocarditits is the most common and

reported in about 60-70% of all chronic cases. Other conditions associated with chronic Q fever include osteoarticular infections, and chronic pulmonary infections.

- In addition to circulatory effects, chronic hepatitis is another common feature as is chronic fatigue syndrome (Wildman 2002) and fever of unknown origin. Rare manifestations are osteomyelitis, pericarditis, meningitis, Guillain-Barré syndrome, vertebral infections (Landais et al., 2007) skin rash and chronic itch (Rustscheff, 2005).
- Available antibiotic treatment is effective and well tolerated (Tissot Dupont et al., 2007).

Pregnant women and other risk groups

- There are some population groups that have some enhanced risk of developing more severe clinical outcomes in comparison to the general population.
    - There is some evidence that Q fever has increased significance in pregnancy. Acute infection during pregnancy, whether symptomatic or asymptomatic, may result in abortion and adverse pregnancy outcomes such as premature birth and low birth rate, the risk being greater in the first trimester. Furthermore, the risks of developing chronic Q fever may be increased in pregnant women and if chronic infection is established, *C. burnetii* may undergo reactivation during pregnancy (even years after primary infection) which in turn has been associated with higher rates of abortion, prematurity, and low birth weight (Stein and Raoult, 1998).
    - Because the clinical manifestation of chronic Q fever commonly impacts on heart and circulation, individuals with pre-existing heart conditions, and particularly those with heart valve defects, heart valve prosthesis or an arterial grafts have some enhanced risk of developing chronic disease.
    - Disease is more likely to develop in immunocompromised individuals and in patients with renal failure.

Overview of human outbreaks and associated public health impact

Although annual notification rates of clinical disease remains relatively low in the EU, serological data indicate that infection is more common than disease notification data indicates. Seroprevalence data varies significantly in EU (Table 9). Crude extrapolations from basic serological data, could quickly generate figures that suggest that several million EU citizens may have been infected with *C burnetii* during their lifetime. However, there are many differences in sampling and diagnostic methodology that prevent generalised assessment of this data, and the clinical significance of positive serology remains uncertain in general populations. Hence, the underlying public health impact from such infection is also impossible to assess. Overall, it seems likely that even taking well acknowledged underreporting of Q fever into account, the assumption of Q fever as a disease with relative minimal general impact in the EU is justifiable.

Notwithstanding the generally low public health impact of Q fever, there are circumstances in which the risk to public health and the public health impact is significantly elevated. Broadly, this is either because of:

- direct exposure to *C.burnetii* infection from close contact to susceptible animals that may be infected; typically occupational or similar exposure (direct contact); or
- general community-wide exposure where levels of *C. burnetii* is elevated in the wider environment because of significant bacterial shedding from infected animals (indirect contact).

Direct contact

In most parts of the world, Q fever is primarily seen as an occupational disease. Certainly seroprevalence studies in occupational groups consistently show higher levels of seroconversion, indicating that such groups are more likely to have been infected with *C. burnetii* (Table 9 and 10). Case control studies have also indicated an association between Q fever and occupational exposure to

animals or animal products (Orr et al., 2006). However there is generally limited evidence in the literature to indicate that such groups have higher incidence of clinical disease in either acute or chronic forms than general populations. Hence the clinical significance of the serology studies is difficult to interpret, but it has been presumed that many cases in occupational groups are not identified at the time of illness (Orr et al., 2006). This may be a case ascertainment artefact because sporadic Q fever caused by direct exposure is less likely to be diagnosed than in an outbreak setting, or occupationally at risk groups may be more resistant to clinical disease following infection. Additional consideration of routes of exposure via direct contact can be found in Chapter 3.2.2.

Table 10: Seroprevalence of C burnetii in occupational groups and the general populations

	Farmers	**Veterinarians**	**General population**	**References**
Netherlands	68% (94)	84% (221)	2,4% (5654)	Richardus et al., 1987 RIVM 2009
Poland	17,8% (90)			Cisak et al., 2003
France	37% (168	25%(12)	7,8% (22496)	Thibon et al., 1996, Tissot Dupont et al., 1992
Italy	73,4% (128)	100%(12)	13,6%(280)	Monno et al., 2009
Spain		11%(472)	48,6%(595) 23,1%(863)	Bartolome et al., 2007 Pascual et al., 1998
Sweden	28% (147)	13%		Macellaro et al., 1993
Denmark	3%(163)	36% (87)		Bosnjak et al., 2009

Indirect contact

The dispersal of *C. burnetii* and the associated indirect exposure to the pathogen in community settings has greater public health significance because it can lead to multiple infections and clusters of cases. An overview of human outbreaks of Q fever in EU MS and neighbouring counties, based on literature published since 1999, is presented in Table 10. In the largest outbreaks, in Bulgaria (in Panagyurische 1993) and the Netherlands (2008), at least 1,000 human cases were recorded. Another outbreak in Bulgaria (2004) resulted in 220 hospitalisations alone (Kamenov and Tiholova, 2004). Although no information is published on the level of Q fever associated symptoms in the general population, one can quickly extrapolate for the hospitalisation data that several hundreds of people are likely to have suffered some immediate public health impact due to acute infection. Of those, some will have developed chronic infection with associated negative long term health impact. In the Netherlands, at least 20% of approximately 3,500 cases reported between2007-2009 were hospitalized, and 7 fatalities have been associated with acute infection. Furthermore, clinical follow-up demonstrates that even acute Q fever is not always a mild disease with short duration. Patients diagnosed with acute infection may suffer from persisting fatigue for several months after disease onset (Schimmer et al., 2007). In addition, some patients, particularly those with known risk factors, will develop chronic infection and associated clinical symtomology such as endocarditis. Previous studies suggest that between 1-5% of those that contract acute infection will go on to develop chronic Q fever. Crude extrapolation from the Q fever notification rates in the Netherlands would suggest that of the 3,500 acute clinical cases reported since 2007, between 35- 175 could develop chronic infection. The impact on such individuals is particularly significant, and will lead to premature death and lower quality of life in many cases, even if treatment is administered.

An update of the literature review for human outbreaks in European countries made by Arricau-Bouvery and Rodolakis (2005) was done for this opinion; the results are presented in Table 11. Comparisons between outbreaks are difficult since outbreak investigations vary on case definition laboratory tests used.

Table 11: Human outbreaks of Q fever in European countries

Year		Country	Most-likely Source	No. of cases	Laboratory diagnosis	Reference(s)
Start	End					
1982	1983	**Germany**	Ruminants	156	CFT	Stelzner et al., 1986 Kramer 1990
1984		**Bulgaria**	Ruminants	725	CFT	In Martinov 2007a
1985		**Bulgaria**	Ruminants	544	CFT	In Martinov 2007a
1987	1988	**Italy**	Sheep	235	NA	In Arricau-Bouvery and Rodolakis 2005
1990	1995	**France**	Sheep	289	IFA	In Arricau-Bouvery and Rodolakis 2005
1992		**Germany**	Sheep	80	CFT	Schneider et al., 1993, Molle et al., 1995
1993		**Bulgaria**	livestock	>1000	CFT	In Martinov 2007a
1994		**Germany**	Sheep	>18	CFT	Schulze et al., 1996
1996	2000	**Bulgaria**	livestock	NA	CFT	In Martinov, S. 2007
1996		**France**	Sheep	29	IFA	In Arricau-Bouvery and Rodolakis 2005
1996		**Germany**	Sheep	56	ELISA	Lyytikainen et al., 1997, 1998
1997		**Bosnia**	Sheep	26	serology	In Arricau-Bouvery and Rodolakis 2005
1999		**Germany**	Sheep manure	82	NA	In Arricau-Bouvery and Rodolakis 2005
2000		**France**	Goat manure	10	NA	In Arricau-Bouvery and Rodolakis 2005
2000		**France**	Sheep manure	5	IFA	In Arricau-Bouvery and Rodolakis 2005
2000	2001	**Germany**	Sheep	75	NA	RKI 2001
2001		**Germany**	Sheep	3	NA	RKI 2001
2002		**Bulgaria**	livestock	121	CFT	Martinov, S. (2007)
2002		**France**	Sheep	88	IFA	In Arricau-Bouvery and Rodolakis 2005
2002	Jul-Sep	**United Kingdom**	Straw board	95	CFT	VanWoerden et al., 2004
2003		**Germany**	Sheep	299	ELISA	Porten et al., 2006
2003		**Germany**	Cattle	8		RKI 2004
2003		**Italy**	Sheep	133	IFA	In Arricau-Bouvery and Rodolakis 2005
2003	Jan-Feb	**Italy**	Sheep and goats	133	IFA	Starnini et al., 2005
2003	Jan-Feb	**Spain**	NA	60	IFA	Garcia-Clemente et al., 2007
2004		**Bulgaria**	Sheep and goats	220	IFA, CFT	Panaiotov et al. 2009 Martinov, 2007
2004		**Croatia**	Sheep	14	CFT	Medic et al., 2005
2004		**Spain**	Sheep and goats	22	IFA	De los Rios Martin et al., 2006
2005		**Germany**	Sheep	331	ELISA	Gilsdorf et al., 2008
2006		**United Kingdom**	Sheep	110	IFA	Wilson et al., 2009
2007		**Netherlands**	Goats	182	IFA	Van den Brom and Vellema, 2009
2007	Apr	**Slovenia**	Sheep	35	IFA	Grilc et al. 2007
2007	Apr-	**France**	Sheep	18		INVS 2009[7]

[7] http://www.invs.sante.fr/publications/2009/fievreq_ florac2007/RAPP_FIEVRE_Q_Florac_2007.pdf

	May					
2008		**Netherlands**	Goats	1000	IFA	Schimmer et al. 2009, Van den Brom and Vellema, 2009
2008	Jan-Mar	**Germany**	sheep	>46		RKI, 2008, Hamann et al., 2009
2008		**Germany**	sheep	>56		RKI, 2008
2009		**Germany**	sheep	5		Henning et al., 2009
2009	Jan-May	**Netherlands**		345	IFA	Schimmer et al., 2009
2009		**Netherlands**	Goats	2357	IFA	Van der Hoek et al 2010
2010		**Germany**		235		Brockmann et al., in press

Indirect Immunofluorescence Assay (IFA); Enzyme Linked Immunosorbent assay (ELISA); Polymerase Chain Reaction (PCR); Complement fixation test (CFT)

In addition to the general elevation of risk to populations exposed to *C. burnetii* through either direct or indirect contact with known infection sources, there is also significant variation within populations in both susceptibility to clinical infection and resultant clinical manifestations. Risk groups such as pregnant women, the immunocompromised and those with pre-existing heart conditions have an enhanced risk of serious clinical manifestation to acute infection, and also to developing chronic disease with associated long term health impairment.

Broader implications of the current Q fever outbreak in the Netherlands

It is difficult to determine whether the expanded epidemic in the Netherlands equates to a more general elevation of risk for human populations in the EU as a whole. Certain epidemiological factors associated with the Netherlands outbreak are relatively unique, such as the high density of intensively farmed goat herds, and their proximity to large urban areas and human populations. It may be that as-yet poorly defined factors may also play an important role in creating the Dutch situtation. Many such contributory factors could be proposed including agent factors (such as increased virulence or transmissibility of circulating strains; this remains poorly defined), unique environmental factors (associated with climate, wind and temperature), 'public health' factors (including improved diagnostics and local awareness contributing to high case asecertainment), and host factors (such as waning immunity in the local Dutch population due to specific population demographics and urbanisation). However, while the convergent of many risk factors are clearly specific to the Dutch situtation, there are much in the epidemiological background that is not unique to the Netherlands: the general mechanisms of *C. burnetii* maintenance in animals populations, its environmental persistence, its widespread prevalence, and the presence of domestic ruminants and farming systems that could lead to amplification of the pathogen in animals are all relatively common in the EU. Hence it is likely that pathogen spillover from animal hosts will continue to occur, including particularly following abortion episodes when the bacterial load is greatly elevated. This means that humans will continue to be exposed to the *C. burnetii* in the EU both through occupational exposure and direct contact with infected animals, and through the aerosolisation and dissemination of the pathogen into the wider environment. It is noteworthy that a study of the evolving epidemiology of Q fever in Germany also revealed an increase in human disease in recent years (Hellenbrand et al 2001). Increased urbanisation in rural areas is hypothesised as a contributing factor. If this pattern is mirrored in other EU MS, it is likely that more people may be exposed to the pathogen in the EU, which in turn is likely to result in an increase in clincial cases. This is particularly the case if exposed urban populations are more immunologically naïve to *C. burnetii* that established rural communities.

Taken together, it is clear that while the impact of Q fever on public health is negligible in most circumstances, there are clearly specific circumstances and certain population groups where this underlying assessment of negligible risk is no longer valid. Direct exposure to domestic ruminant livestock or associated animal products that are the primary reservoir of *C. burnetii* clearly poses an enhanced risk of *C.burnetii* infection in comparison to the general population. However, the greatest public health risk, and the most significant public health burden arise from Q fever is associated with

community-acquired infection due to spillover from animal reservoirs to humans. As discussed above, and considered in more detail in Chapter 3, the cause of spillover is multifactoral and complex, but the resultant impact on public health can be very significant.

In the final analysis, although many uncertainties remain concerning the emergence of Q fever in humans, strengthening general routine surveillance and reporting in both animal and human populations will increase the probability that these episodes will be identified early. More specifically, a risk-based surviellance approach could be helpful in supporting public health action; at base level some active monitoring to determine the early local presence of *C. burnetii* in domestic ruminants would support early awareness of the presence of the pathogen. Where *C. burnetii* is identified in a locality, enhanced surveillance, including particularly following abortions in domestic ruminants, would give early indication of possible amplification and an elavation of public health risk. In addition, rapid notification of abortion and associated diagnostic testing, irrespective of the presumed status of *C burnetii*, would be valuable to ascertain cause and thereby support both animal health and, if necessary public health action such systems must be complemented by strong and well-defined communication channels between veterinarians and public health counterparts, to ensure mutual information exchange on potential Q fever events in both animals and humans. The 'one health' approach to reporting and communication of potential Q fever outbreaks is vital to ensure that early action is taken to reduce public health risks.

- Overall, it is justifiable to consider Q fever as a disease with minimal general public health impact in the EU; even taking underreporting into account, notified case numbers are low and human to human transmission is so rare as to be epidemiologically insignificant.
- There are circumstances in which the risk to public health and the impact on public health is significantly elevated; primarily from either direct (occupational) exposure to Q fever infected animals, or community based exposure caused by an elevation of *C. burnetii* in the wider environment following release and dissemination from infected animal hosts. The latter has greater public health concern.
- There is some consistency in the incidence of acute and chronic disease in EU outbreaks; typically 50% of those with acute infection report symptoms of which self limiting flu-like febrile illness is the most commonly described. Of those, approximately 1% will develop chronic infection with more severe long term sequelae.
- There is huge variability in clinical manifestation within exposed populations and the basis for this is poorly understood. However, there are some specific at-risk groups where likelihood and clinical manifestation may be more severe in comparison to the general population: those with underlying heart valve disorders, the immunocompromised and pregnant women. In some circumstances, specific targeted measures to minimise exposure and monitor infection status may be justified for such groups.
- Treatment with appropriate antibiotics is effective and indicated for the management of both acute and chronic Q fever.

2.3.2. Significance and impact on animal health

In ruminants, *C. burnetii* may induce reproductive disorders including abortion, stillbirth, and the delivery of weak and non-viable neonates. Signs such as placentitis, endometritis, abortion, stillbirth, infertility, and weak offspring (Davis et al., 2007) should trigger an investigation, considering Q fever among the differential diagnoses. *C. burnetii* localises in the uterus and mammary glands of infected animals (Babudieri, 1959; Martinov 2007b). Cattle may present with metritis and infertility, and mastitis has been described (Moffa et al., 1970). Data from Germany indicate that a majority of the reported cases involved cattle (see section 5.2.1). Experimentally induced Q fever with respiratory

manifestations in lambs and a sheep has been described by Martinov (2007a). Note, however, that infection in ruminants is frequently subclinical, and Q fever incidence in domestic ruminants remains unclear because abortion rates – other than in some herds of goats – are frequently low (Rodolakis, 2009) and probably not investigated. Ewes with naturally acquired *C. burnetii* infection can show no clinical signs and lamb normally (Berri et al., 2005a). Studies on the impact of Q fever in milk production are, to our knowledge, not available. In goats, where the lactation period can be extended, milk production is unlikely to be significantly affected. The impact of *C. burnetii* infection on the incidence of mastitis and metritis is uncertain

Based on the data available the impact of Q fever on animal health in Europe is unlikely to be very high. Although infection with *C. burnetii* in domestic ruminants is common, clinical disease is rare. In small ruminants, clinical cases are generally sporadic, and abortion episodes are relatively rare. In the current Dutch outbreak, however, abortion episodes affecting up to 60% of at-risk goats have been observed, particularly during the first Q fever outbreak on affected farms. Nonetheless, Q fever has a much lesser impact than conditions such as ovine brucellosis and enzootic abortion (caused by infection with *Chlamydophila* spp.) as a cause of late abortion and associated reproductive disorders in small ruminants.

- Based on the available information, there is limited impact of Q fever on the health of domestic ruminants (cattle, sheep, goats) in Europe
  - In small ruminants, clinical cases are generally sporadic, and abortion episodes relatively rare. In general, Q fever has a much lesser impact on small ruminants than conditions such as ovine brucellosis and enzootic abortion (caused by infection with *Chlamydophila* spp.). However, the impact on individual goat farms during an abortion episode can be significant.

3. Risk factors for *C. burnetii* maintenance and spillover

3.1. Transmission routes

A broad range of species has been identified as reservoirs for *C. burnetii*, including mammals, birds, and arthropods (ticks). Sheep and goats are the most frequently implicated as the source of human infection (Rodolakis, 2006). *C. burnetii* infection occurs mainly after inhalation of aerosols (aerial transmission) generated from excreta from infected animals (abortion and birth material, faeces, urine, milk) (Angelakis and Raoult, 2010). Direct transmission (close contact) or indirect (long distance) through aerosols may occur. (Schimmer et al., 2010). A simplified diagram of the transmission routes is presented in Figure 3.

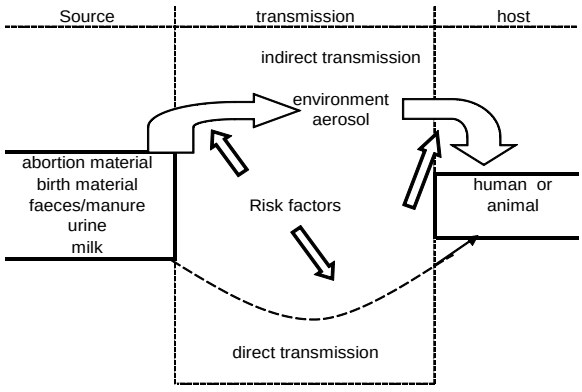

Figure 3: Transmission routes for *Coxiella burnetii* infection in humans and animals (adapted from Roest 2010)

The WG developed a simple conceptual model, separately highlighting:

- the maintenance of infection in animal populations (focusing on domestic ruminants), and
- the spillover of infection from animal populations to humans through a process of amplification, transmission and exposure (Figure 4).

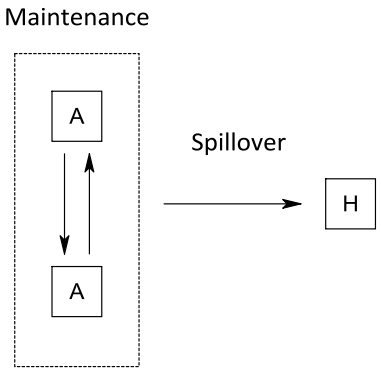

Figure 4: A conceptual model for maintenance of *C. burnetii* infection in domestic ruminant populations, and spillover from animals to humans (A, animal, H, human)

- Farmed ruminants, and particularly sheep and goats, are implicated as the main source of human infection
- *C. burnetii* infection occurs mainly after inhalation of aerosols (aerial trtansmission) generated from infected animal excreta (abortion and birth material, faeces, urine, milk).

3.2. A review of published literature

3.2.1. Factors affecting the maintenance of *C. burnetii* infection in domestic ruminants

Factors affecting the maintenance of *C. burnetii* infection in animal populations can be grouped according to:

a) *Agent factors*, relating to the characteristics of *C. burnetii*, and in particular infectivity, virulence and resistance to environmental conditions;
b) *Host factors*, including animal species, susceptibility, infectiousness, age and sex; and
c) *Environment factors* , related to animal management, as well as manure management and farm characteristics.

Agent factors

Q fever was described as a febrile illness, which had started to occur in 1933 in abattoir workers in Brisbane, Queensland, Australia (Derrick, 1937). It was originally identified as a species of Rickettsia and also isolated from ticks (Davis and Cox, 1938). Based on philogenetic investigations, however, C *burnetii* is no longer regarded as closely related to Rickettsiae (Labrenz and Hirsch, 2003) and had been placed in the *Coxiella*ceae family in the order Legionalles of the gamma subdivision of Proteobacteria. It is a gram negative obligate intracellular bacterium, a small pleomorphic rod (0.2–0.4 mm wide, 0.4–1.0mm long) adapted to resist and thrive within the phagolysosome of the phagocytic cells. The organism may occur as a large cell variant (LCV), small-cell variant (SCV) or small dense cell (SDC). The LCV of *C. burnetii* is intracellular and metabolically active, undergoing sporogenic differentiation to produce resistant, spore-like forms. The SDC and SCV forms are able to survive extracellularly as infectious particles (OIE, 2009). *C. burnetii* can survive for up to 42 months at 4-6°C in milk, 12 to 16 months in wool, 120 days in dust, 49 days in dried urine, 30 days in dried sputum (NABC, 2010). *C. burnetii* has a number of important physical characteristics including stability

against acids (up to pH 4.5), temperature (62 C for 30 minutes), UV light and pressure (up to 300.000 kPa). Further, the organism can survive for more than 6 months in 10% saline. *C. burnetii* is killed following exposure to 5% chloroform or formaldehyde gas (in an 80% humidified environment) *(with less than 30 minutes exposure),* to 5% H_2O_2, 0.5% hypochlorite and 70% ethanol *(all with 30 minute exposure),* and following pasteurization (at least 72°C for 40 seconds) (Frangoulidis, 2010).

C. burnetii is extremely infectious (Byrne, 1997; Kim et al., 2005; Nochimson, 2004). Under experimental conditions, the inhalation of a single *Coxiella* cell can produce infection and clinical disease in humans (Tigertt, 1961); similar studies have not been done in animals. It is likely that the dose of inoculum is relevant to the course of the infection. Mice inoculated with 10^5 *C. burnetii* CbC1 developed an efficient immune response with a weak level of IL10 and a high level of IFN-γ that reached a peak after 2 weeks. In contrast, mice inoculated with 10^7 *C. burnetii* CbC1 produced a low level of IFN-γ and a very high level of IL-10, with possible evolution to chronic disease. The level and kinetics of IFN-γ production varied according to the strain isolated (Rodolakis A. unpublished data).
Pulsed field gel electrophoresis (PFGE) and RFLP patterns of 80 *C. burnetii* isolates derived from animals and humans in Europe, USA, Africa and Asia allow the distinction of twenty different groups corresponding to geographical origin of the isolate. However, no correlation between restriction group and virulence of isolates was detected (Jager et al., 1998). The importance of strain as a risk factor for both maintenance of infection and disease progression is unknown. Knowledge of the virulence determinants of *C. burnetii* has been hampered by a lack of methods for genetic manipulation. Further, accurate methods are not available to assess strain virulence. Strains isolated from cattle and goats have been observed as more virulent that those isolated from aborted ewes in mouse models as well as presenting lower growth in embryonated eggs (A. Rodolakis, unpublished) but this work is still ongoing.

Using MLVA and MST genotyping methods, it has been possible to identify up to 36 distinct genotypes of *C. burnetii.* As yet, there is no evidence of species specificity.

- *C. burnetii* is highly resistant to environmental conditions, surviving for many months under a range of conditions.
- The organism is extremely infectious (in humans, infection could occur following inhalation of a few organisms). The initial dose is likely to affect the subsequent course of infection.
- The relationship between genotype/isolates and virulence is at the moment unclear. There is no clear evidence of species specificity.
- The importance of bacterial genotype/isolates on maintenance of infection and disease progression is uncertain.

Host factors

Host factors play a key role in the natural history of *C. burnetii* infection in human; similar information for ruminants is lacking. Nonetheless, seroprevalence varies between species of domestic ruminants in the same geographical area. Further, the evolution of disease, including clinical signs, is not the same in cattle, sheep or goats.

During chronic infection, *C. burnetii* is mainly found in the uterus and mammary glands (Babudieri, 1959). Shedding of *C. burnetii* into the environment mainly occurs during parturition; over 10^9 bacteria are released at the time of delivery (Babudieri, 1959). Goats and cows mostly shed *C. burnetii* in milk and vaginal mucus (Rodolakis et al., 2007; Guatteo et al., 2006a) whereas ovines shed mostly in faeces (Rodolakis et al., 2007; unpublished GDS data). Goats and cows shed *C. burnetii* in milk for several months or years. The udder (Behymer et al., 1977; Bell et al., 1949; Moretti, 1984) and retromammary lymph nodes (Plommet et al., 1973) can remain infected for more than 20 months.

Goats can be chronically infected and may shed *C. burnetii* for up to two pregnancies after being infected (Hachette et al., 2003). In addition, goats can abort twice following infection (Berri et al., 2007; unpublished ANICAP data) and shed *C. burnetii* in placenta and vaginal mucus during two subsequent kiddings (unpublished ANICAP data). In contrast, ewes aborted only once (Berri et al., 2002; unpublished GDS data) and did not shed in vaginal mucus at subsequent lambings. The duration of excretion (Table 12) of the agent, husbandry conditions and other factors may each play an important factor in the differences observed in animal seroprevalence and the persistence of infection.

Table 12: Longest observed duration of excretion during the follow up of naturally or experimentally infected animals (from Arricau-Bouvery, 2005)

	Duration of shedding		
	Vaginal mucus	**Feces**	**Milk**
Cow	Not determined	14 days	13 months
Goat	14 days	20 days	52 days
Ewe	71 days	8 days after lambing	8 days

There is a significant relationship between age and seroprevalence in sheep (Kennerman et al., 2010), most likely as a consequence of opportunity for exposure. In infected flocks, seroprevalence is very high among adults, noting that replacement ewes (<10 months) are not exposed to the bacteria until the lambing season (Garcia-Peres et al., 2008). At their first kidding, young goats shed more C. burnetii than adults (6.2 versus 2.8 log for previously infected adults; unpublished ANICAP data). A similar pattern is observed in cattle herds (Guatteo et al. 2008). The role of males in the persistence of infection is not well studied, although C. burnetii has been isolated from bull semen (Kruszewska et al., 1997).

Other animals may also play a role in the maintenance of the infection in domestic ruminants. The reservoirs for *C. burnetii* are extensive, but only partially known, including mammals, birds and arthropods (mainly ticks). Cats and dogs, but perhaps also foxes, may play a role in the persistence of the disease on a farm. Over 40 ticks species can be naturally infected (Maurin and Raoult, 1999). They are likely to play a significant role in transmission among wild vertebrates, but are not considered to be essential in the cycle of *C. burnetii* infection in livestock (Babudieri, 1959). Nonetheless, a strong correlation has been reported between seropositivity and ticks infestation in animals (Psaroulakis et al., 2006). Wild rats may constitute a reservoir of *C. burnetii* and play a role in the maintenance of infection (Webster et al., 1995)

- The evolution of infection (including duration and routes of excretion, clinical presentation) varies between different farmed animal species, which may influence animal seroprevalence and persistence of infection.
- Shedding of C. *burnetii* mainly occurs around parturition. Abortion products are where largest bacterial concentrations are found but birth products, vaginal mucus, milk urineand faeces are also sources.
- In general, seroprevalence increases with age, most likely as a consequence of opportunity for exposure.
- The role of males and of transplacental transmission in the persistence of infection is uncertain.
- A range of animal species may play a role in the maintenance of infection in domestic ruminants, including cats, dogs and possibly foxes and rats.
- Ticks can be naturally infected with *C. burnetii*, but seem not to be important in the maintenance of infection in domestic ruminants.

Environment factors

As in humans, it is likely that infection of livestock occurs mainly by inhalation of contaminated aerosols. Consequently, pathogen pressure is likely to be increased with factors that increase the concentration of C *burnetii* in the environment (including herd size, synchronization of lambing/kidding, regrouping of animals from different flocks). Animal density and husbandry practices have been investigated as potential risk factors for increased seroprevalence of Q fever in animals (Martinov et al., 2007). In sheep, a significant association was found between seroprevalence, but not PCR status, and size of flock (Kennerman et al., 2010; Garcia-Perez et al., 2008). In Danish dairy herds, there was no significant association between the regional density of dairy farms and the prevalence of antibody-positive farms (Agger et al., 2010). Abortions have also been identified as an important risk factor for herd status. Abortions in goat herds were linked to exposure, at a state fair, to another herd where goats had kidded prematurely (Sanford et al., 1994). Similarly, a link has been identified between cattle abortion and the serological status of the herd to Q fever (Cabassi et al., 2006). Consequently, husbandry practices that control the exposure of animals to infectious doses of *C. burnetii*, such as segregation of areas for lambing and kidding as well as removal of placenta and abortion materials, will be of use in reducing bacterial exposure.

The role of manure in transmission of Q fever between ruminants and human is well documented (Berri et al., 2003; Reintjes et al., 2002). Using PCR methods, transplacental infection has been demonstrated in the aborted foetus of experimentally infected goats, however, neither lesions nor *C. burnetii* antigens were detected in foetuses from goats killed during gestation (Sanchez et al., 2006). A recent study implicated macrophages in the pathogenesis of *C. burnetii* infection, suggesting that they transport the organism to the foetus (Bildfell et al., 2000).

- A range of environment factors have been sugested to influence the maintenance of infection in farmed animal populations, including increased herd/flock size, animal density, and herd/flock density. Pathogen pressure is likely to increase in association with farm factors that increase the concentration of *C. burnetii* in the environment.
- *C. burnetii* contaminated manure has been identified as a source for Q fever in human outbreaks. It is likely that it also plays a role on the maintenance of infection in animal populations.

3.2.2. Factors affecting the spillover of *C. burnetii* from domestic ruminants to humans

The mechanisms for spillover of infection from farmed livestock to human populations may be best understood if considered as three related stages stages:

i. Amplification, in the domestic ruminants population;
ii. Transmission, either directly or indirectly; and
iii. Exposure of humans.

Amplification

A number of factors are associated with the maintenance of infection, as discussed in section 3.1.2. In several human outbreaks, human disease as linked with parturition, and particularly abortion, in domestic ruminants. Several authors have described a seasonal variation in the incidence of human disease in the spring and summer, which has been attributed to spring lambing (Maurin and Raoult 1999; Hellenbrandt et al., 2001) or kidding (Schimmer et al., 2010). An infected dairy goat farm with abortion problems has been identified as the most likely source of a cluster of human cases in the Netherlands in the 2008 outbreak (Schimmer et al., 2010). In a study in France, human cases have

been associated with reported abortions in goats, but not cattle (Chaillon et al., 2008). Parturition, and particularly abortion, is associated with increased concentrations of bacteria in the environment.

- Parturition in animals, and particularly abortion, is associated with increased concentrations of bacteria in the environment.

Transmission and exposure

A range of factors are associated with transmission and exposure, as follows:

- From experimental and epidemiological evidence, there is no doubt that contaminated aerosols are the major mechanism whereby *C. burnetii* is transmitted to humans (Benenson and Tigertt, 1956; Tigertt et al., 1961; Gonder et al., 1979; Marrie et al., 1989).
- Persons in contact with domestic ruminants can be infected by inhalation of contaminated aerosols from amniotic fluid or placenta. Some studies have identified a higher human seroprevalence in healthy populations living in rural instead of urban areas and also in humans with occupations involving contact with livestock (Cyprus, Psaroulaki, 2006a; Greece, Pape, 2009b), whereas others have not (Spain, Cardenosa, 2006; Turkey, Kilic et al., 2008). Several studies have identified a higher seroprevalence to Q fever among farmers, abattoir workers and veterinarians, compared to the general population (Moffa et al., 1970; Bosnjak et al., 2009; Whitney et al., 2009). In 2009, in its assessment of meat-associated microbiological hazards, the Australian Food Standards[8] suggested that *'Cattle [sheep and goats] may carry pathogens normally associated with handling, which could potentially be transmitted via meat consumption. Notes: Examples include Anthrax (*Bacillus anthracis*), Melioidosis (*Burkolderia pseudomallei*), Q fever (Coxiella burnetii)'.* In California, 29% of workers at a local meatpacking plant were seropositive to Q fever, with the majority having recently experienced a clinical illness compatible with Q fever (Anonymous, 1986). A Q fever outbreak in a meat processing plant in Scotland has been reported (HPS, 2006; Donaghy et al., 2006).
- Many cases of Q fever in humans are unrelated to occupation. In several Q fever outbreaks, including the current outbreak in the Netherlands, cases have occurred in both rural and urban areas (Hawker et al., 1998; Schimmer et al., 2008; Schimmer et al., 2009).
- Several recent outbreaks have been associated with visits to school/hobby farms (de los Rios Martin et al., 2006, Porten et al., 2006, Tissot-Dupont et al., 2005). Laboratory personnel who work with infected animals are also at risk (Johnson and Kadull, 1966; Marrie 1990a, 1990b, 1990c).
- Humans may also be infected by handling contaminated wool (Abinanti et al., 1955).
- In intensive farming premises, the handling of manure (which can be heavily contaminated with *C. burnetii* from faeces, urine, reproductive fluids and tissues such as placenta etc) can generate infective aerosols (Hatchette et al., 2001). Use of manure in gardens has been identified as a risk factor for human infection (Psaroulaki, 2006a). In the Dutch outbreak, a seasonal variation was observed during 2007 and 2008, with peak incidence from December to May correlating with the spread of manure from goat stables (Delsing and Kulberg, 2008). In the Netherlands, substantial amounts of manure are transported from goat farms (generally in the south of the country) to other locations. There has been no evidence of a rise in human cases at locations where this manure has been spread (Vellema et al., 2010, Berri et al., 2003).
- There is significant variation in Q fever notificaiton rates between women and men: men are almost twice as likely to develop clinical disease. These differences have been proposed to be due to protective effects of female hormones (Leone et al., 2004). There is also a significant

[8] Australian Food Standards (2009). Assessment of Microbiological Hazards associated with the Four Main Meat Species.

age-related bias in clincial notification, with adults 5 times more likely to present clinical disease in comparison to those under 15 years of age.(Rault et al 2005)

- There are also instances in the global literature of human Q fever cases being associated with direct exposure to other animal hosts of *C. burnetii* , including wild animals (rabbits and rats) (Marrie et al., 1996, Webster 1995) and pet animals (cats and dogs) (Werth 1989). In North America, outbreaks of Q fever have resulted from direct and indirect contact with parturient cats (Marrie et al., 1988a; Marrie and Raoult, 2002). However, such cases appear rare and where they have occurred they have usually been associated with exposure to animals that have either recently aborted or given birth.
- Environmental factors such as wind and precipitation have been linked with human outbreaks (Karagiannis et al., 2009; Porten et al., 2006; Gilsdorf et al., 2007). A recent Dutch study investigated the relationship between environmental factors (vegetation index, land cover, soil characteristics, soil humidity, past weather conditions) and transmission to humans from infected farms. Key risk factors of importance included farms with a low vegetation density, and areas with drier soils (Hunink et al., 2010), highlighting the importance of wind in Q fever spread. A relationship between farm topography and human cases was found in several outbreaks in Bulgaria and France.
- It is likely that windborne transmission plays an important role in the dissemination of *C. burnetii* (Tissot Dupont et al., 2004). In the current Dutch outbreak, humans living within 2 km of an large infected dairy goat farm had a much higher infection risk than those living more than 5 km away (Schimmer et al., 2010). In an earlier study, humans within 50m of a presumed infection source (a sheep meadow) at 8.7 times greater risk that those 400m away (Gilsdorf et al., 2007). The influence of distance on risk is likely to vary depending on wind and other weather conditions. Other human cases have been linked to transmission in association with helicopters (Carrieri et al., 2002), and dust storms (Panaiotov et al., 2009).
- Ticks are known hosts of *C. burnetii*. Over 40 tick species (including *Dermacentor marginatus, Ixodes ricinus*, and *Rhipicephalus sanguineus*) can be naturally infected with *C. burnetii*, but they do not appear to be important in the maintenance of infections in livestock or humans (Maurin and Raoult, 1999). The organism does, however, multiply in the gut cells of ticks, and large numbers of *C. burnetii* are shed in tick faeces. Ticks may be more relevant for the transmission of *C. burnetii* to domestic mammals than to humans (Kazar, 1996).and is thought to play a role in maintenance of the pathogen in wildlife reservoirs. Ticks have been implicated in human infections on rare occasions, including via inhalation of contaminated tick faeces in wool (Hellenbrand et al., 2001).

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- Major outbreaks in humans may occur both in rural and urban areas and many cases of Q fever in humans are unrelated to occupation.
- Persons can be infected by inhalation of contaminated aerosols. This probability increases with proximity with farm animals.
- The handling of manure (which can be heavily contaminated with C. *burnetii* from faeces, urine, reproductive fluids and tissues such as placenta etc) can generate infective aerosols.
- A range of environmental factors (wind, farms with a low vegetation index, drier soils, farm topography) have been associated with human outbreaks. Wind dispersion is likely to play an important role in the dissemination of C. *burnetii*.

3.3. Country case studies

3.3.1. Bulgaria

a. Overview

Bulgaria has a long history of Q fever, and Q fever awareness. Human cases were first reported in the Balkan region (including Bulgaria) in 1940, among visiting soldiers (Imhauser, 1949; Bieling, 1950; Robbins et al., 1946; Serbezov et al., 1999, Teoharova et al., 2002, Martinov 2006). The first case in a Bulgarian resident was diagnosed in 1949 (Mitov, 1949). Pioner of the studies in animals in Bulgaria is S. Angelov (BAN 1951). Subsequently, there have been both sporadic cases and outbreaks. In Bulgaria, the disease is notifiable both in humans and animals, and diagnostic facilities are available (see section 3.1.).

b. Magnitude and distribution

In the last 25 years, there have been 12 outbreaks in humans, leading to between 121 and more than 1,000 serological confirmed cases per outbreak (Martinov, 2007a). Outbreaks have occurred in several areas and years (e.g. Kneyzha, 1984; Pavvlikeni, 1985; Etropole, 2002; Botevgrad, 2004), sometimes in the same place on more than one occasion (e.g. in Panagyurische, 1992-1993, 1995) (Serbezov et al., 1999).

Prevalence estimates are available for humans, animals and ticks, based on a large number of samples (19,560 patients with atypical pneumonia; 319,993 sera from domestic and wild animals collected over the last 50 years; 1,769 collected ticks from animals):

- Seroprevalence among patients with symptoms of Q fever was 15.0% (of 14,353 patients) during 1993-2000, and 18.0% (of 5,207 patients) during 2001-2004 (Martinov, 2007a; using MIFT and CFT). During a general community survey in the 90s (most probably 1995-97) from 9 Bulgarian regions (a random sample of 224 sera from the human serum bank), a seroprevalence of 38% was measured and 167 out of 252 patients (66%) with bronchopneumonia and acute flulike symptoms had antibodies to phase II *C. burnetii* detected by CFT or microagglutination test (MA) in 6 regions of the country (Serbezov et al., 1999).

- In animal populations, prevalence estimates vary by species, area and time. Four-fold differences were observed among domestic ruminants in different areas of Bulgaria (Serbezov, 1973). Seroprevalence estimates were obtained between 1950-2006, from goats, sheep and cattle presented in Martinov (2007a), respectively:
    - 1950-1976: Goats (20.5 %), sheep (15.3%), cattle (19.8 %)
    - 1977-1988: Goats (10.8 %), sheep (18.2%), cattle (11.8%)
    - 1989-2006: Goats (7.6 %), sheep (4.8 %), cattle (5.4 %)
- In ixodic ticks, 16.8% (of 1,769 tested) were infected with *C. burnetii* (Martinov, 2007a; using the immunofluorescent haemocytic test). In a detailed investigation, 22-26% ticks were infected (Georgieva, 1984).

c. Risk factors

In Bulgaria, a range of risk factors have been described for the maintenance of *C. burnetii* in animal populations and spillover to humans:

- *Strain.* Different pathogenic effects have been observed in guinea pigs and mice when infected with different isolates from domestic ruminants (Martinov, 2007a, 2007b). These results may partly explain the observed differences in the clinical presentation of Q fever in humans in different areas in Bulgaria. However, further work is needed, including the genetic characterisation of isolated strains, and the investigation of their pathogenic effect in well recognised model(s).

- *Host maintenance species. C. burnetii* infection (based on antibody response and/or strain isolation) has been observed in a wide variety of animals, including several species of domestic livestock, domestic pets, a wide range of wild mammals, birds and ticks (Martinov, 2007a). Two cycles for persistence were proposed, including:

- *[a] natural foci* (ticks, wild mammals, birds, with spillover to domestic animals), resulting in ongoing maintenance of *C. burnetii* in the environment, and cyclic recurrence of the disease in both wild and domestic animals, and

- *[an] agricultural foci* (cattle, sheep and goats, with spillover to humans), which presents the main source for infection to humans.

- *Host spillover species.* Goats were considered the most-likely infection source for humans during the Panagyurische outbreaks in 1992, 1993 and 1995, as these were the only domestic livestock species in the proximity during the outbreak (Serbezov et al., 1999). In other cases, a sharp increase in seroprevalence in goats, sheep and cattle was observed in association with human outbreaks (Martinov, 2007a; Panaiotov et al., 2009). During the Etropole (2002) ourbreak, 60.5 % of small ruminants (sheep and goat) were seropositive. In the Etropole (2002) and Botevgrade (2004) outbreaks, there were no reports of abortion episodes in domestic ruminants, (Ivan Yordanov, Animal Health Department, RVS Sofia district, 30 March, 2010, pers. communication).

- *Animal husbandry systems.* In Bulgaria, there have been substantial changes in animal husbandry systems over time: extensive animal housing systems were in place during 1950-1976, centralised, industrial-type housing was introduced during 1977-1988, and numerous small private farms have been operating since 1989 (Martinov, 2006). These changes may have contributed to the observed drop in *C. burnetii* seroprevalence in domestic livestock during this period. Outbreaks in humans have continued despite this observed fall in seroprevalence (Kneyzha, 1984; with 725 human cases; Pavlikeni, 1985; with 544 human cases Panagyurishe, 1992, 1993, 1995; Sopot, Troyan, Blagoevgrad, Pleven, 1996-2000; Etropole, 2002; Botevgrad, 2004).

- *Herd size and structure.* There has been a fundamental change in herd size since the late 1980s, following the collapse of large state premises and cooperative farms. This has resulted in a substantial reduction in sheep numbers (8 million in 1990, 3 million in 1997) and an increase in the number of goats (430 000 in 1990, 1 million in 1997) (Serbezov et al., 1999). Cattle herds and sheep flocks tend to be large, but managed separately from the human population, whereas goats are kept in a large number of small herds, including some that are held in towns and close to human settlements.

- *Seroprevalence in animals.* Human outbreaks have often occurred in areas where seroprevalence in domestic ruminants was high. In the Etropole (2002) and Botevgrad (2004) outbreaks, the following serological results were recorded in livestock close to the outbreak:

  - *In cattle:* 11.6-33%, compared with 7.1-13.1% in the population more broadly,

  - *In sheep:* 46.7-59.5%, compared with 8.3-14.1% in the population more broadly, and

  - *In goats:* 63.3 to almost 100%, compared with 7.4-21.7% in the population more broadly (Martinov et al., 2007a).

- *Animal proximity.* The gathering and movement of domestic ruminants through towns is believed to be a risk factor of a number of epidemics, including the outbreaks in Botevgrad in 2004 and in Panagyurische in 1992, 1993 and 1995. In Botevgrad, sheep and goats were gathered around the town by shepherds then subsequently moved to grazing areas (Panaiotov et al., 2009). Similar practices were observed in association with large human outbreaks (more than 500 cases) in Kneyzha in 1984 and Pavlikeni in 1985 (Serbezov et al., 1999). Most of the human cases were not employed in agriculture or the processing of animal products (Serbezov et al., 1999); further, in Botevgrad outbreak, most human cases were not

owners of goats, sheep or cattle (Ivan Yordanov, Animal Health Department, RVS Sofia district, 30 March, 2010, pers. communication).

- *Timing/concentration of lambing/kidding.* There has been a shift in the seasonal presentation of human cases in Bulgaria since the 1990s (from Jan-Apr to Apr-Jul; in Serbezov et al., 1999), coincident with a change in the timing of lambing and kidding. Following the collapse of collective farming, the practice of artificial insemination of ewes was stopped. Until 1990, artificial insemination of ewes was conducted over a defined insemination period in early autumn; in the 1990s, this practice was abandoned and the delivery period was extended. This change in practice, in association with an increase of goat numbers, has lead to an increase of goat deliveries over a more-extended kidding period. Sheep deliver in January-February, whereas goats deliver in March-April.

- *Geography.* Most of the most-recent 25 outbreaks have occurred in small towns located in valleys close to mountains or semi-mountains areas (Panagyurische, Sopot, Troyan, Etropole), or in regions with specific climatic conditions with strong winds (Kneyzha). In the most recent outbreak in Botevgrad (2004), cases were observed in a town of approximately 20,000 individuals in a valley close to the St. Planina mountain (Panaiotov et al., 2009).

- *Weather conditions.* During the Botevgrad outbreak in May-June 2004, the weather conditions including a dust storm (at the beginning of May, as reported by residents of the town), a thunderstorm without rain precipitation (on 2 May), heavy rain (3 May), and strong wind (greater than 5m/s), without further rain (during 4-6 May) (Panaiotov et al., 2009).

d. Control options

The following control options were undertaken during the Botevgrad outbreak in 2004:

- *Humans:* All patients were hospitalised. Schools were closed for a month during the outbreak, coincident with the summer holiday. Relevant authorities undertook a campaign of public awareness.

- *Animals:* All animals were kept in controlled yards outside the town. Those passing through the town had to follow certain roads, which were disinfected (aerosol disinfection with chlorenol) every day. Sheep and goats were treated by washing, whereas cows were disinfected by spraying acaricides (ectomine 3%). Acaricides were also sprayed on public parks and roads. Protective facial masks were used by people when applying these measures. Farmers were required to remove animal excrement and disinfect farm places. Potentially contaminated material were transported outside the town, and disinfected or burned. Milk collection was stopped. Also, meetings were organised between the local authorities, veterinary and public health government bodies (Ivan Yordanov, Animal Health Department, RVS Sofia district, 30 March, 2010, pers. communication).

e. Implications/lessons learned

- Two different maintenance cycles for *C. burnetii* are suggested ([a] natural foci, [an] agricultural foci). Domestic ruminants (cattle, sheep, goats) are considered the common link between these two foci.

- Risk factors have mainly been identified based on epidemiological observation and association.

- Key risk factors are believed to include:

    - An increase in goat numbers, and a change in goat husbandry,
    - Proximity with goats and sheep of naïve population, and

  - Dry, windy weather conditions.
  - Massive reservoir of the infection into the natural foci

- Change of the seasonal pattern of human cases appears to be associated with changes in livestock husbandry systems, insemination practices, population and proximity.

- Human outbreaks have continued despite an observed decrease in seroprevalence among domestic ruminants, particularly goats.

- Reporting of Q fever in a given territory depends on the attention of public health authorities, and the availability of diagnostic methods.

The efficacy of control options has not been formally assessed. Nonetheless, the rapid fall in human cases during the outbreak in Botevgrad (2004) could be attributed to measures leading to a rapid segregation of the animal and human population, the removal of farms, and the disinfection of the environment on farms and paths. There was also an associated increase in public health awareness following the outbreak.

- There is a long history of Q fever, and of Q fever awareness, in *Bulgaria.*
- In the last 25 years, there have been a number of large human outbreaks.
- Infection with *C. burnetii* is prevalent in humans, domestic ruminants and ticks, varying by area and time.
- A number of *risk factors for maintenance* of *C. burnetii (in domestic ruminants)* have been identified including bacterial strain, the presence of wildlife, the type of domestic ruminant and husbandry system. Additional *risk factors for spillover* of *C. burnetii (to humans)* include herd size and structure, seroprevalence in animals, animal proximity, the time and concentration of lambing/kidding, geography and weather conditions. Outbreaks appear to be linked to an increase in goat numbers, a change in goat husbandry, proximity to goats and sheep, and dry, windy weather conditions. Evidence in support of these risk factors is mainly based on epidemiological associations.
- Human outbreaks have continued, despite an observed decrease in seroprevalence among farmed ruminants, particularly goats.
- No formal assessment of control options has been conducted. Nonetheless, control effectiveness has been attributed to :
  - rapid segregation of the animal and human population;
  - Increased attention of public health authorities (earlier diagnosis and proper antibiotic treatment of humans), and
  - Measures applied to reduce environmental contamination

3.3.2. France

a. Overview

Q fever is a well-recognised disease in France. However, the disease is not notifiable, either in human or animals and informationabout prevalence and incidence are not centralized at a national level. In recent years, Q fever has been a public health concern on several occasions. A comprehensive report was published in 2004 by the French Food Safety Agency (AFSSA, 2004). In addition, some recommendations for a better control of Q fever in clinically affected herds or flocks were produced in 2007 by a group of experts under the auspice of the ACERSA (the French organisation in charge of animal health certification) (De Crémoux et al., 2007).

b. Magnitude and distribution

In humans

Several studies have been published, mainly by the NRL (Prof D. Raoult, University of Marseilles) The seroprevalence of Q fever in blood donors in the Marseilles area was estimated to be about 4 % (Tissot Dupont, 1992), consistent with other data from the south of France (5 %) and Burgundy (4.4%) (AFSSA, 2004). In the southern part of the country (Provence Alpes Cote d'Azur region), a seroprevalence study conducted in pregnant woman indicated that between 0.8 and 1.3 women per 1,000 had antibodies levels which could be attributed to recent infections (less than one year) (Rey et al., 2000). When these data were extrapolated to the whole country, it was suggested that the annual incidence in France would be in the range of 0.1-1 per 1,000 (Maurin and Raoult, 1999; Tissot-Dupont et al., 1992). The Chamonix Valley outbreak in 2002 is the most recent episode of major public health concern, with 89 confirmed human cases including 71 with clinical signs. Nine people, in high risk groups, received long term therapy. In May 2007, 5 patients with Q fever-like symptoms were reported in the rural southern French town of Florac with 18 cases confirmed after an epidemiological survey

In animals

There is no official epidemiological database for Q fever in animals in France. A national reference laboratory was recently nominated by the French Ministry of Agriculture (Laboratory for Ruminant and Bee Pathology Studies, AFSSA Sophia Antipolis, France). However, data are available mainly from local studies conducted with the aim to estimate the *C burnetii* infection situation in a geographical area or during a limited period of time.
Q fever diagnostics is not mandatory in France when abortion occurs in herds or flocks, and therefore Q fever incidence is probably underestimated. During a 5 year period in the district of Deux-Sevres, 5 out of 21 flocks of goats experienced abortion due to Q fever (Chartier et al., 1997). Surveys conducted between 1993 and 1996 in bovine herds from several administrative districts indicated that Q fever was the cause of abortion in between 0.5 and 3.8 % of the cases (Berger, 1999). In the past, the prevalence of the infection has mainly been estimated using seroepidemiological studies, even though data are not directly comparable due to the lack of harmonization of the techniques. There is a huge variability of the rate of seropositive domestic ruminants in France (AFSSA, 2004). Despite these limitations, animal-level prevalence based on various local studies done in France was estimated by AFSSA (2004) as :

- 1 to 15% for cattle,
- 0 to 20% for sheep, and
- 2 to 12 % for goats.

At the herd or flock level, the estimated prevalence is:

- 39 to 73% (cattle),
- 0 to 89% (sheep), and
- 10 to 40% (goats).

No national survey has been conducted to estimate the prevalence of Q fever in domestic ruminants. Local and regional data are available, but comparison is problematic. Nevertheless it appears that *C burnetii* infection is common in ovine and goat flocks, and is probably more widespread in the south compared to the north of France. Data regarding cattle are lacking since abortion is less frequently reported .

c. Risk factors

The wind is considered an important risk factor in France. In the La Crau area where sheep farming is important and where there is a tradition of outside lambing, the incidence of acute Q fever is 5.4 times higher than in the city of Marseilles. There is a seasonal pattern of human cases, being highest in spring in association with lambing, and a dry and windy environment. Further, human cases mainly located downwind from lambing (Tissot-Dupont et al., 1999, 2004). In 1996, 120 cases were reported

in the town of Briançon, and were attributed to aerosols dispersed by intense helicopter activity in the vicinity of an old slaughtering house (Carrieri et al., 2002).

A case-control study was performed following the Chamonix valley outbreak in summer 2002. In this study, cases were more likely to have been in close contact with sheep, or to have attended or otherwise watched the migration of sheep to their summer location. It is likely that aerosol contamination could explain most cases (INVS, 2005[9]). Aerosol contamination consecutive to the cleaning of contaminated pigeon faeces was also reported to be the cause of acute Q fever case in five members from the same family (Stein and Raoult, 1999). Professional exposure was investigated in a cohort of 323 human cases (Tissot-Dupont et al., 1992), but was found not to be significant. Of 477 patients affected by Q fever, 8% were agriculture workers or veterinarians, 38% were people living in rural areas, and 35% had had contact with new born or pregnant animals (Raoult et al., 2000). Of 80 French patients with pneumopathy due to *C. burnetii*, 32 had contact with animals (59% with sheep, 15.6% with cattle, 28.1% with goat and14.6% with cats) (Caron et al., 1998). A case-control study was carried-out in a hyperendemic area (Etang de Berre, south of France) from 1996 to 1999. Besides the role of wind, this study identified exposure to a teaching farm as a key risk factor (Tissot-Dupont et al., 2005).

The risk factor analysis conducted during the human outbreak in Florac (2007) revealed a significant association between acute infection and living or working near an area where manure had been spread, outdoor sport activities, attending the training center's canteen for eating, working or social activities. No link between any particular dairy food item and the occurrence of acute infection was observed (INVS, 2009[10], King et al., submitted). Of all (26 tested) goat, sheep and cattle herds/flocks located within a five kilometre radius of Florac, 11 were ELISA-positive to *C. burnetii* infection. Nine herds/flocks were positive by quantitative PCR, including 3 flocks of sheep and 1 flock of goats with low shedding levels, 2 flocks of sheep and 1 flock of goats with medium shedding levels and 2 sheep flocks with high shedding levels. It was concluded that the observed excess of cases of Q fever in Florac, an area endemic for this infection, in spring 2007 could be explained by airborne transmission from infectious ovine flocks situated close to the town.

d. Control options

During the last two outbreaks in France (Chamonix, 2002; Florac, 2007), control options were taken in order to limit the outcome of the disease in affected humans and to prevent further infections in humans. Measures may depend on the local situations. Some examples are given:

- Local surgeons were informed about the situation and received recommendations to screen humans at risk using serology. Pregnant woman were treated and received medical care until giving birth. Humans with symptoms had a cardiac echography and received a preventive treatment, on evidence of a valvulopathy, to avoid the risk of chronic Q fever. A national press bulletin was released recommending medical care (serology and preventive treatment, if positive) for people who had stayed temporarily in the affected areas during the epidemic.

- Blood donation was forbidden from people who stayed in the affected areas during the outbreaks, and blood products collected during this time were discarded.

- Regarding domestic ruminants, several sanitary measures regarding animal movements were taken (control of animal gathering, no participation in exhibitions, allowing transportation only by trucks). Recommendations for good-practice, in particular for manure spreading and dealing with animal birth and abortion products, were implemented. The vaccination of non-infected animals using a phase I vaccine on seropositive farms was recommended. Declaration of abortions among herds and the burying of biological materials, such as

[9] http://www.invs.sante.fr/publications/2005/fievre_q_chamonix/rapport_fievre_q.pdf
[10] http://www.invs.sante.fr/publications/2009/fievreq_florac2007/index.html

placentas and birth products, was recommended. Farmers were advised to carry out the removal of carcasses by quartering, to store manure a minimum distance from human dwellings, to spread it onto fields with the least exposure to wind, and to plough it in immediately after spreading.

- During the Florac outbreak, dairy production from herds around Florac was pasteurized before distribution in accordance with recommendations of the French Agency for Food Safety (AFSSA, 2004). However, since no clinical human case has been associated with consumption of crude milk products, either in this or other recent Q fever outbreaks in France, systematic pasteurization of milk is no longer required.

e. Implications/lessons learned

Q fever is endemic in France in domestic ruminants but regional and species differences are noticeable. The impact of Q fever on domestic ruminant health does not appear high, but improved surveillance is needed, especially for abortion. Q fever is periodically a public health issue and outbreaks have been reported in recent years. However, there is no apparent trend towards an increase of human case throughout the country. All recent human outbreaks were linked to an animal source and contaminated dust or aerosols. Therefore, control options in affected farms should focus on sanitary measures to avoid spreading of contaminated materials and to limit the extent of excretion of *C. burnetii* by infected flocks or herds. Vaccination of ruminants with a phase I vaccine may be the most-effective method to decrease *C. burnetii* excretion into the environment, but is more effective if given to seronegative animals. Because *C. burnetii* is highly resistant in the environment, vaccination is likely to have an impact only in the longer term. Finally, further work is needed to evaluate the efficacy of the various control options that can be implemented.

- Q fever is well-recognised in France. The disease is not notifiable in either humans or animals, and there is currently no centralized reporting system.
- There have been several recent outbreaks of Q fever in humans: in Chamonix in 2002 (89 confirmed cases) and Florac in 2007 (19 confirmed cases).
- No national survey has been conducted to estimate the prevalence of *C burnetii* infection in domestic ruminants. Local and regional data are available, but comparison is problematic. Nevertheless it appears that *C burnetii* infection is common in ovine and goat flocks, and is probably more widespread in the south compared to the north of France.
- Human cases have been linked to environmental conditions (dry, windy weather) and proximity to domestic ruminants (contact with animals particularly during parturition, observing sheep movement, in the locality of manure spreading, a seasonal pattern in humans that is coincident with time of lambing).
- A broad range of control options were applied during the recent human outbreaks in Chamonix and Florac. On infected farms, control options should focus on sanitary measures to avoid spreading of contaminated materials and to limit the extent of excretion of *C. burnetii.*
- Vaccination of ruminants with a phase I vaccine may be the most-effective method to decrease *C. burnetii* excretion into the environment, but is more effective if given to seronegative animals. Because *C. burnetii* is highly resistant in the environment, vaccination is likely to have an impact only in the longer term.

3.3.3. Germany

a. Overview

It is now commonly accepted that Q fever was introduced into Germany shortly after WWII. Infection was first observed in the southern parts of Western Germany (Württemberg, Baden and Bavaria). Prior to 1950, a total of 3,000 patients and 20 deaths were registered, with attack rates of 20% to 46% in small villages. Contact with domestic ruminants was identified as the main source of infection, although outbreaks in slaughterhouses were also reported. A large outbreak with more than 500 cases and 3 deaths has been reported at a dairy fair in Krefeld, Nordrhine-Westphalia in 1958 (Schaaf, 1969). Subsequently, Q fever has spread throughout the two former German states, and is now considered endemic in Germany.

b. Magnitude and distribution

Q fever has been notifiable in Western Germany since 1962, and in Eastern Germany since 1979 (Hellenbrand et al., 2001). Hellenbrand et al. (2001) present a synopsis of Q fever between 1947 and 1999. They calculated the average annual incidence of Q fever of 1.1 per million population (1979-1999), with Baden-Württemberg (4.1 per million), Hesse (2.8 per million), Rhineland-Palatinate (0.9 per million) and Bavaria (0.8 per million) being most affected. In a recent publication, Conraths et al. (in press) stated that roughly 200 to 400 human cases were registered each year from 2007 to 2009, again most frequently in Baden-Württemberg, Hesse and Bavaria. No significant increase in cases was seen in Northrhine-Westphalia or Lower Saxony. During a SurvStat@RKI survey conducted during the years 2004 to 2009, it was clear that many regions notify sporadic cases or outbreaks regularly, or even every year, e.g. Jena (Thuringia), Göppingen (Baden Württemberg), Lahn-Dill Kreis (Hesse), Aschaffenburg (Bavaria) etc. These findings demonstrate that Q fever, once it has become endemic, will remain within local reservoirs for years or even decades. Consequently, greater outbreaks in humans are registered periodically in these regions. In an update of their work, Hellenbrand et al. (2005) published some further larger outbreaks during the time period 1999 to 2003; i.e. outbreaks in Miltenberg, Bavaria (1999; n>100, due to sheep shearing), Ebersberg, Bavaria (1999, n=19, due to occupational contact to sheep), Rottweil-Göllersdorf, Baden-Württemberg (1999; n=118, due to exposure to sheep and shearing), Stetten, Baden-Württemberg (1999; n=39, due to a sheep fair with a shearing show) and Tübingen, Baden-Württemberg (1999; n=13, due to sheep farm). In Table 18 Appendix C, selected outbreaks from 1990 to 2009 are listed. Each has been published in local or international journal, with exception of an outbreak due to deer in 1997. All outbreaks involving more than two cases have to be investigated by local authorities; a final report has been prepared, but often not published. Reports in the local daily press are not included.

Seroprevalence studies in humans have been conducted only sporadically, mostly triggered by human outbreaks. In a Germany-wide survey amongst blood donors (n=1,611 during 1983-1986), 22% of samples were positive (Schmeer et al., 1987). Further, 22% of blood donors from the German army (n=1,651) had anti-*Coxiella* antibodies (Werth et al., 1991). These sera were collected during 1985 to 1987. Both studies used phase I/II antigen ELISAs. 15.5% of sera from Hesse (n=207, healthy blood donors) were positive. In 1993, Frost and Hengst found 2.4% to 19.8%, and 16.2% to 56.6%, of sera (n=1.208) positive when using CFT and ELISA, respectively. The survey was done on the population of Wiesbaden, Darmstadt, Frankfurt and Giessen (south and middle Hesse). However, in groups of diseased patients, 30.9% to 60.0% of sera were positive. In 2002, Sting et al. found 56 of 255 farmers whose dairy cows had abortion problems seropositive for anti-*Coxiella* antibodies. Abortion and seroprevalence of cows >20% was correlated with a higher seroprevalence rate in their owners. Homuth et al. (2006) investigated the sera of 145 farmers, 26 vets and 40 visitors at the agricultural fair 'Eurotier', Hannover during 2004, and found 1.8%, 0.0%, 0.0% to be truly seropositive, respectively. Brockmann et al. (in press) report a seroprevalence among inhabitants (n=1,036) from rural and urban municipalities in Baden-Württemberg of 0 to 18%. In the former German Democratic Republic, the first cases of Q fever were reported in the beginning of the 1980s. Klug and Maeincke report on a survey in personnel of a dairy farm: of 556 serum samples tested, 38 (6.8%) were positive (Klug and Maenicke, 1985). From 1979 to 1990, a total of 209 human cases, but no deaths, were

reported (Kramer, 1991). A large outbreak, involving 156 patients, in Meinungen, Thuringia was observed in 1982/83. Kramer (1991) also reports on single cases in the northern districts. Lange and Hunstock (1993) report on surveys conducted in a veterinary diagnostic institute, and on farms, during 1983 to 1990. Of 767 serum samples, 7.8% were positive. Seroprevalence was higher among milkers (19.1%), plant farmers (16.4%) and veterinarians (14.3%), in comparison with shepherds (7.4%). In a veterinary institute, seroprevalence among stockmen was 14.3%, in comparison to drivers (6.3%), vets (4.3%) and technicians (9.1%), which suggests that time of exposure, direct contact and the type of occupation were associated with a higher risk for serological conversion. The high seroprevalence among tractor drivers was attributed to the high risk of inhaling contaminated aerosols whilst spreading manure and slurry onto the fields. The high seroprevalence connected to 'calf production' is probably related to milk preparation. The positive titers in the institute personnel were caused either by using laboratory animals as 'diagnostic tools', working in the autopsy area or transporting samples (=drivers). However, clinical presentations in all groups were rarely seen.

Q fever in animals in Germany is *'meldepflichtig'*; i.e. owners of infected animals, vets and diagnostic facilities have to inform the local authorities on the occurrence of Q fever (including serological positive results). State veterinarians then report cases to the federal authorities who are in charge for gathering these notifications. Hellenbrand et al. (2009) note reports of approximately 300 herds affected annually with Q fever during the period between 1980 and 1998. In the last decade, Conraths et al. (in press) found that approximately 130 herds have been notified annually. Germany-wide testing is not mandatory. Data are generated mainly during veterinary practice (increased abortion rate), from dairy animals, whose milk is sold without pasteurisation and from human outbreak surveys (Hellenbrand et al., 2009). In herds/flocks with clinical symptoms, 75% of animals may test positive. These authors also stated that in 1998, 7.8% of cattle (n=21,191), 1.3% of sheep (n=1,346) and 2.5% of goats (n=278) were Q fever positive using various tests. Thirteen of 16 German states contributed to this data survey. In the 1980s, approximately 5 to 10% of cattle herds without, and up to 69% with, fertility problems were positive for *C. burnetii* infection, respectively (Hellenbrand et al., 2005). The seroprevalence in cattle was high in Bavaria, Hesse, Sotuthern Westphalia, Baden-Würtemberg (Woernle and Müller, 1986; Krauss et al., 1987; Hellenbrand et al., 2001). In a recent study from Baden-Württemberg, Wagner-Wiening and Brockmann (2009) found that 85% of the reported cases involved cattle. During 2001 to 2008, a total of 55,373 serological (ELISA and CFT) and 3,491 direct (PCR and STAMP) tests were conducted: 10.5% (cattle: 8.7%; sheep: 26.4%; goat: 20.1%) and 8% of these samples were positive, respectively. Most reports concern Q fever in cattle (see Table 19 Appendix C). Interestingly, high seroprevalence was also found in dogs and cats from Germany: 13% (n=1.127) and cats 26% (n=108) using ELISA, respectively (Werth et al., 1987). Very rarely, culture was attempted: Heil-Franke et al. (1993) found that 2.0% of aborted calves were positive for *C. burnetii.*

In the former German Democratic Republic (GDR), *C. burnetii* infection was found by means of serological investigation: during the years 1980 to 1989, 8.3% of cattle, 0.7% of sheep, and 2.8% of dogs were seropositive, whereas goats, pigs and pheasants tested negative for Q fever (Kramer, 1991). Regional differences were noted: e.g. in the district of Neubrandenburg, 20% of sheep flocks were positive and 12.6% of dogs were positive in the district of Rostock. In 11 of 13 districts, positive cows were present (1.7-23.3%). Kramer (1991) subsequently stated that Q fever was endemic in cattle herds in the GDR. In some natural foci, Q fever was found in roe deer *(Capreolus capreolus)* and red deer *(Cervus elaphus).* In middle Thuringia, 100% of tested dairy farms and 47% of tested sheep farms (n=17) were positive for Q fever (Lange and Klaus, 1992). A total of 4.337 ovine serum samples were investigated during 1983-1986 using CFT: 0.6 to 4.3% of animals in each herd were positive. A correlation between the number of animals in a herd and the level of management (specifically, poor management) was noted. A good overview of Q fever in the GDR is available (Kramer, 1990).

Infection with *C. burnetii* is ubiquitous in sheep and cattle, and probably also in goats, throughout Germany. Regional differences in distribution are found. Natural reservoirs of infection are present, which make eradication unlikely. Currently, there is no vaccine licensed for use in cattle, sheep or

goats. The role of dogs and cats in the infective cycle is unknown. The prevalence in ruminant herds is high, and appears to have remained unchanged during the last three decades.

c. Risk factors

Of 40 outbreaks investigated during the period, 1979 to 1999, 24 were associated with sheep.Sources of infection included products of conception, contaminated manure and dust from shearing. In most cases, either migrating sheep or neighbouring sheep farms could be identified as possible sources. Dry weather and/or wind were also identified as risk factors. In 6 cases, cattle were identified as the source of infection (as a result of contact with aborted material, contaminated milk or with cows at abattoirs). These authors noted a shift from winter to summer outbreaks, possibly due to changes in the sheep production system; nowadays lambing occurs throughout the year with the introduction of at least two mating periods per year. Recent outbreaks are also been more frequently associated with urban areas.

In a recent study from Baden-Württemberg, Wagner-Wiening and Brockmann (2009) correlated available data from human cases and sheep density per km^2. They found that human Q fever risk was higher in areas of higher sheep density (a relative risk of 2.8 if 1 sheep/km^2; 19.16 if 10 sheep/km^2; 182.55 if 100 sheep/km^2, compared to areas without sheep). In municipalities without any notified case, 7.4% of human residents were seropositive for Q fever compared with 20% in endemic municipalities. Interestingly, the presence of cattle in municipalities was considered protective. They found no correlation for 'dry climate'. In Baden-Württemberg 191 of 1,109 municipalities reported Q fever cases.

No significant and recurrent risk factors for Q fever have been identified, apart from direct or indirect contacts (e.g. dust, aerosols, manure) with infected animals and their contaminated products (birth products, faeces) (see also Table 18 Appendix C). Diseased animals may pose a higher risk to those who are taking care for them. Obstetric procedure conducted on animals without clinical signs may lead to human disease. The role of cats and dogs has not been clarified. The role of natural reservoirs e.g. wild animals, arthropods or the environment to maintain the infectious cycle within farm animals i.e. ruminants or cervids is unclear. The quality of farm management is important for clinical disease in farm animals.

d. Control options

The following options have been applied in herds, to reduce losses for farmers:

- antibiotics
- antibiotics with vaccination (phase 1 and phase 2)
- antibiotics (shortly before parturition to reduce the number of shed bacteria) and vaccination and culling of seropositive animals
- exclusion of seropositive males from breeding
- single boxes for birth
- regular disinfection with effective disinfectant
- use of acaricides (before grazing period, sheep)
- disinfection of the fleece (bathing, sheep)
- introducing only of *Coxiella*-negative new animals; quarantine
- removing aborted offspring and placenta material (sheep!) immediately
- composting of manure for 6 months under plastic foil / earth with non-hydrated lime; spreading only of manure only without wind and when the weather is dry; manure should be ploughed under immediately
- separation of gravid ewes
- reimbursement for all countermeasures for the animals owners
- good management practise: feed, water, etc
- sentinel animals
- no 'sharing' of male animals

The following options have been used to reduce the potential for transmission of *C. burnetii* to humans:

keeping distance to human settlements
no exhibition of gravid domestic ruminants in the last trimester or tick infested animals, no birth in the public
serological surveys in domestic ruminants and reservoir animals
reduction of stock drive; using tracks far away from human settlements
control of pet sheep in zoos, schools, nursing homes and culling of positive animals
using only *Coxiella* negative animals in animal experiments e.g. sheep
control of fences (sheep)
restriction of entry for farm visitors etc.
changing of clothes before leaving the farm
special movable equipment
pasteurisation of milk; no selling of raw milk

The following options have been applied during outbreak management:

interchange of information between veterinary public health – public health
serological control of all contact persons, identification of persons with special risk e.g. immunosuppressed persons

e. Implications/lessons learned

Q fever is endemic in Germany. A constant but underreported number of sporadic cases and outbreaks in man and domestic ruminants are notified every year. The disease is most often seen in humans but has no significant impact on animal health. Therefore, new strategies combining the interests of public health and veterinary public health have to be developed to control Q fever in the future.

Infection with *C. burnetii* is ubiquitous in sheep and cattle, and probably also in goats, throughout Germany. Regional differences in distribution are found. There exist natural reservoirs which make eradication unlikely. Currently, there is no vaccine licensed for use in cattle, sheep or goats. The role of dogs and cats in the infective cycle is unknown. The prevalence in ruminant herds was always high and appears to have remained unchanged during the last three decades.

- There is a long history of Q fever and infection in Germany. In humans, both sporadic cases and outbreaks are reported each year. Sheep are the most-frequently implicated source for human outbreaks. Human seroprevalence is variable, and highest among those with close contact with livestock.
- *C. burnetii* is endemic among domestic ruminants (sheep, cattle, and probably goats) in Germany. Seroprevalence is high, with no evidence of change over the last 3 decades.
- Q fever is notifiable in Germany, and Q fever cases (clinical signs and/or serological evidence) are relatively common.
- Currently, there is no vaccine licensed for use in domestic ruminants.
- Human Q fever risk is strongly associated with direct and/or indirect contact with infected animals, including those without clinical signs. In one study human cases have been associated with sheep density per km^2.

3.3.4. The Netherlands

a. Overview

Q fever was first diagnosed in the Netherlands in 1956 (Westra et al., 1958). There was no evidence of infection in the several years prior to this (Wolff and Kouwenaar, 1954). No antibodies against Q fever were found in 6,000 samples collected between 1954 and 1956 from humans with an atypical

pneumonia (Dekking and Zanen, 1958). In a seroepidemiological study performed between 1968 and 1983, an average seroprevalence of 76% was found in high-risk groups, including veterinarians, taxidermists and female wool spinners. In 1982, 186 of 222 (84%) farm animal veterinary practitioners were seropositive to Q fever, compared to 86 of 359 (24%) blood donors sampled in 1983. The seropositive results of the veterinarians were equally distributed over all age groups, suggesting most infections had occurred in early childhood. Males were more often infected than females (Richardus et al., 1984; Houwers and Richardus, 1987; Richardus et al., 1987).

In 1978, Q fever became a notifiable disease in humans. The number of notifications between 1978 and 2006, ranged between 1 and 32 cases annually, with an average of 17 cases per year. These cases predominantly involved patients with occupational risk. The total number of hospitalized persons in the period 1994–2001 was 49 (Delsing and Kullberg, 2008; Schimmer et al., 2009).

Since May 2007, the Netherlands is facing an increasing human Q fever problem.

b. Magnitude and distribution

In humans:

- In May 2007, several cases of atypical pneumonia were reported in the province of Noord-Brabant (Steenbergen et al., 2007). Retrospective investigation identified *C. burnetii* was the causal agent. In 2007, a total of 168 confirmed human cases were reported. The majority of cases presented between weeks 18 and 24 (May- June). The cases ranged from 7 to 87 years, the female to male ratio was 1:1.7, and the hospitalization rate was 50.0% (Schimmer et al., 2008; van der Hoek et al., 2010). Many patients suffered from persisting fatigue for several months after the onset of the disease (Nabuurs-Franssen et al., 2009).

- In 2008, Q fever returned and at the end of the year 1,000 human cases had been registered, making it the largest community outbreak of Q fever ever recorded in the world. The main symptoms were fever, fatigue, night sweating, severe headache and general malaise. In 65% of the cases, pneumonia was reported (Delsing and Kullberg, 2008; Schimmer et al., 2008; Schimmer et al., 2009), with a hospitalization rate of 20.9% (van der Hoek et al., 2010).

- In 2009, a total of 2,357 new Q fever patients were registered on the national infectious disease notification database. As clinical signs and symptoms of Q fever are aspecific, this high number is probably influenced by increasing awareness among patients and doctors. Nonetheless, the hospitalization rate was 19.7%, comparable to the situation in 2008 (van der Hoek et al., 2010).

- In the first ten weeks of 2010, 237 cases were notified, which was not expected, given experiences from the preceding 2 years. Further, the epidemic is expanding geographically, although most notifications are still from the province of Noord-Brabant.

- The seroprevalence of *C. burnetii* was investigated in 5,654 nationally representative samples taken prior to the human outbreaks (February 2006-June 2007) (van Duynhoven et al., 2010). Overall, the seroprevalence (past infections) was 2.4%. Ongoing studies in the high incidence areas show higher seroprevalence and among specific risk groups more than 80% have antibodies, suggesting old infection.

In animals:

- In 2005, Q fever was diagnosed for the first time in the Netherlands, as a cause of abortion on a dairy goat farm. Diagnosis was confirmed by using immunohistochemistry on sections of placenta (Wouda and Dercksen, 2007). A second case was diagnosed later in 2005. In 2006, 2007, 2008, and 2009, six, seven, seven, and six new cases, respectively, were confirmed on dairy goat farms, mainly in the southern part of the country. In the same period, two cases of

abortion caused by *C. burnetii* were found on dairy sheep farms, one in the southern and one in the northern part of the country. The average number of goats per infected farm was 900, of which 20% of the pregnant goats on average (10% to 60%) had aborted. The average number of sheep for the two infected sheep farms was 400 and the abortion rate was 5% (van de Brom and Vellema, 2009).

- In 2008, all 15,772 blood samples from small ruminants to be tested for *Brucella melitensis* monitoring, were also tested for Q fever using an ELISA (Ruminants Serum Q fever LSI Kit, LSI, Lissieu, France). From those samples, 12,363 were of ovine and 3,409 of caprine origin. Based on these blood samples, seroprevalence for sheep in the Netherlands was 2.4% (95% confidence interval [CI]: 2.1–2.7) and for goats 7.8% (95% CI: 6.9–8.7) (van den Brom and Vellema, 2009).

- Dairy sheep and dairy goat farmers were also given the opportunity to test bulk milk samples using a PCR (TaqvetTM *Coxiella burnetii*, TaqMan Quantitative PCR, LSI, Lissieu, France). In total, 306 bulk milk samples were tested and 79 (26%) were positive (van den Brom and Vellema, 2009).

- By 18 February 2010, 73 dairy goat farms and 2 dairy sheep farms, out of the total of 360 dairy goat farms and 40 dairy sheep farms with more than 50 animals in the Netherlands, had been declared infected based on PCR-positive bulk milk testing.

c. Risk factors

The sheep and goat industry in the Netherlands is relatively small with less than one million breeding ewes and a quarter of a million breeding goats (I&R-database, 2009). The total number of registered small ruminant farms is slightly more than 50,000, of which 360 are professional dairy goat farms with over 200 adult goats and 40 are professional dairy sheep farms. All dairy goats are housed throughout the year, except approximately 17,000 goats kept on organic farms. The dairy goat industry started following the introduction of the milk quota system in the dairy cattle industry in 1984. In the ensuing 25 years, total goat milk production has grown from almost zero to over 150 000 tonnes annually (van den Brom and Vellema, 2009).

In retrospect, a large human cluster in an urban area in 2008 could clearly be linked to a dairy goat farm with more than 400 animals with a Q fever related abortion episode a few weeks before the first human cases presented. Persons living within 2 kilometres of the farm had a much higher risk for Q fever than those living more than 5 kilometres away (relative risk 31.1 [95% CI 16.4-59.1])(Schimmer et al., 2010).

There is consensus among public health and veterinary professionals that most of the human Q fever cases are linked to abortion on large dairy goat farms, and to a much lesser extent on dairy sheep farms (van der Hoek et al., 2010). Additional factors such as the role of manure or strain virulence possibly influencing transmission are currently under investigation.

C. burnetii can be typed using by MLVA (Arricau-Bouvery et al., 2006; Svraka et al., 2006). In the Netherlands, in total 14 different MLVA types have been found in 253 clinical samples from dairy goats, and two from dairy cattle. One MLVA type is predominantly present on dairy goat farms with abortion problems due to Q fever and on all goat farms in Noord-Brabant. One goat farm outside Noord-Brabant showed a different MLVA type. The two cattle MLVA types of *C. burnetii* could clearly be distinguished from all goat MLVA types (Roest, 2010).

A large multidisciplinary research programme is being conducted, to generate an improved understanding of Q fever, including *C. burnetii* transmission, to inform improved control options.

d. Control options

Before June 2008, abortion outbreaks were reported on a voluntary basis to the Animal Health Service (GD) and confirmed by immunohistochemistry (Wouda and Dercksen, 1997). Since June 2008, Q fever in small ruminants is notifiable in the Netherlands. Notification criterion for farms with over 100 breeding animals is an abortion wave, defined as an abortion percentage over 5% of all pregnant small ruminants. For smaller holdings, three or more abortions in a 30-day period is used as the basis for notification of authorities.

Because of the consensus between public health and veterinary professionals that most of the human Q fever cases are linked to abortion waves on large dairy goat farms, and to a much lesser extent on dairy sheep farms, interventions have focused on these types of farms. Veterinarians, physicians and the public were informed through targeted mailings, publications and the media.

Since November 2008, all owners of non-pregnant sheep and goats in the area where most of the human cases had occurred in 2008 were given the opportunity to vaccinate their animals on a voluntary basis with a Phase I vaccine containing inactivated *C. burnetii*. The aim of the vaccination was to reduce shedding of *C. burnetii* and thus, environmental contamination, to reduce human exposure. In spring 2009, the Dutch government implemented a compulsory vaccination campaign in the infected area, the province of Noord-Brabant and parts of the provinces of Gelderland, Utrecht and Limburg. This vaccination campaign is compulsory for dairy sheep and dairy goat farms with over 50 animals and for farms with intensive animal–human-contact. Between April and November 2009, approximately 250,000 small ruminants were vaccinated.

In 2010, compulsory vaccination will be applied on a larger scale, and more than 1.5 million doses will be available.

Since February 2009, a stringent hygiene protocol became mandatory for all professional dairy goat and dairy sheep farms in the Netherlands, independent of their Q fever status. The protocol includes some mandatory and some voluntary measures, aiming to preventing environmental contamination. Farmers are obliged to fight against other animal reservoirs, are not allowed to take out manure from their stables for at least one month after the lambing season, are obliged to cover manure during storage and transport and will have to plough it under immediately or after composting it for at least three months. Aborted foetuses and placentas have to be rendered and records of all measures taken have to be kept for at least one year. Farmers are advised to take some voluntary measures to improve general hygiene. They are stimulated to bring in fresh straw every day during the lambing period and to submit aborted foetuses for pathological examination. Farmers are also encouraged not to admit pregnant women, children and elderly people into their stables (van den Brom and Vellema, 2009).

On 1 October 2009, bulk milk monitoring became mandatory on farms with more than 50 dairy goats or dairy sheep, and PCR positive bulk milk has since been used as an additional criterion for veterinary notification of Q fever. The initial frequency of testing each farm every other month was increased to once every two weeks from 14 December 2009 onwards.

In 2010, the number of positive farms is expected to increase towards the peak of the lambing season (March-April). To reverse the trend of the last three years, drastic measures were implemented, including the large-scale culling of pregnant goats on infected farms, which started at the end of December 2009. Table 13 represents an overview of all recent measures taken by the Dutch authorities.

Table 13 : Overview of legislation concerning Q-fever in the Netherlands

Date of implementation	Document code	Measure	Reason
12Jun08	TRCJZ/2008/1622	Q fever notifiable in dairy goat and dairy sheep; Notification when over 5% of abortion within 30 days at farms with more than 100 animals and when over 3 abortions within 30 days at farms smaller than 100 animals	Identification of Q fever positive farms
12Jun08	TRCJZ/2008/1645	Prohibition to take manure out of the stable for 90 days after notification	Avoidance of the spread of Q fever via manure and killing of *C. burnetii* in the manure
		Visitors ban for 90 days after notification	Avoidance of direct contact with humans
16Oct08	TRCJZ/2008/2817	Special dispensation of Coxevac (CEVA) Q fever vaccine to be used in the Netherlands	To start vaccination with a phase I vaccine against Q fever
		Voluntary vaccination in dairy sheep and dairy goats at farms with more than 50 goats or sheep, pet zoos and nursing farms in the restricted 45 km zone (Figure 3)	To prevent abortion and shedding of the bacterium, because of shortage of the vaccine only a small area can be vaccinated
02Feb09	TRCJZ/2009/244	Prohibition to farm more than 50 dairy goats and dairy sheep if not certain hygienic measures are implemented such as, other animal reservoirs control, manure measures, rendering foetuses and placenta's (see text)	To further prevent the spread of *C. burnetii* from the farm toward humans
20Apr09	TRCJZ/2009/1142	Mandatory vaccination of dairy sheep and dairy goats on farms with more than 50 animals, on care farms, pet zoos and zoos in the extended area (Figure 4) before 1 January 2010	Extended prevention of abortion and shedding of *C. burnetii* by dairy goats and dairy sheep
01Oct09	Regulation 40823	Mandatory bulk tank milk monitoring on Q fever every two months	An improved system to detect farms where *C. burnetii* is present
		Prohibition to transport dairy sheep and dairy goats from a positive farm. Vaccinated animals may be transported to positive farms	Prevention of the spread of Q fever by direct transport of animals to negative farms
		Visitors ban at positive farms	Prevention of direct contact with positive animals
09Dec09	Regulation 96744	Ban on increase of the numbers of dairy goats and dairy sheep on a farm	To avoid the risk of spread of Q fever from a farm
		Ban on reproduction of goats	To avoid the risk of spread of Q fever from a farm
14Dec09	Regulation 98748	Mandatory bulk tank milk monitoring for Q fever every two weeks	To enable a more detailed follow up of farms that become positive, thereby allowing measures to be taken as early as possible to reduce the risk of spread of Q fever to humans

16Dec09	Regulation 99604	Prohibition to take the manure out of the stable within 30 days after the ending of the lambing season	To avoid the possible spread of Q fever from the manure
		If manure has to be taken out of the stable, the manure should be stored on the farm for 90 days	To avoid the possible spread of Q fever from the manure and to stimulate composting on the farm to reduce the load of viable Q fever bacteria
16Dec09	Letter to the parliament; VDC 09.2695/CPM	Culling of all pregnant goats and sheep on Q fever positive dairy goat and dairy sheep farms	To reduce the risk of human Q fever in the spring of 2010. No differentiation between infected and non infected positive pregnant animals could be made on the basis of one individual test
18Dec09	Regulation 101785	Prohibition to add sheep or goats to a farm	To avoid enlargement of farms
01Jan10	Regulation 72246	Mandatory vaccination of dairy sheep and dairy goats, on care farms, pet zoos, zoos, on farms with lambs exhibitions, wandering shepherds and in natural reserves nationwide before 2011	To avoid the risk of spread of Q fever from a farm nation wide

e. Implications/lessons learned

Q fever was present in the Netherlands prior to 2005, but few clinical problems were seen. Approximately 20 human cases were seen each year. The reasons for the emergence of clinical problems in the animal population in 2005, and in the human population in 2007, are still unclear. Several hypotheses are suggested, including:

- A steady increase of the dairy goat population since 1984. Goats were kept on large farms with on average more than 600 goats. Q fever animal outbreaks are observed on the larger farms. Population dynamics of infectious diseases can change depending on the size of the farm;
- Intensive goat husbandry in a highly populated area;
- Dry weather conditions and strong winds during and after the lambing season; and
- A change in the virulence of *C. burnetii*.

Research has been initiated to investigate each of these hypotheses.
Evaluation of the effectiveness of individual control options has proved problematic, for a number of reasons:

- It has been hampered by the large number of outbreaks in a relatively small area, and the number of potential sources of infection,
- There has been no observed decline in human cases, despite widespread implementation of draconian measures, including vaccination, strict hygienic measures and the killing of all pregnant goats on bulk milk PCR positive farms, and
- The relationship between animal excretion and human exposure is not always clear. Consequently, there is a need to evaluate options taken in the animal reservoir through changes in the abortion rate or excretion of *C. burnetii.*
- The importance of environmental contamination on human exposure is unclear, and may have been underestimated so far.

- Q fever was first diagnosed in the Netherlands in 1956, and became a notifiable disease in humans in 1978. Between 1978 and 2006, there were an average 17 human cases each year.
- The current Q fever outbreak first emerged in May 2007 and is now the largest community outbreak of Q fever ever recorded. In total, 168 human cases were confirmed in 2007, 1,000 in 2008, 2,357 in 2009, and 237 in the first 10 weeks of 2010. The hospitalization rate was 50% in 2007, 20.9% in 2008 and 19.7% in 2009.
- Since 1984, there has been a very large expansion in dairy goat production, to over 150,000 tonnes of milk annually.
- Q fever was first diagnosed as a cause of abortion on a dairy goat farm in 2005.
- There is consensus among public health and veterinary professionals that most of the human Q fever cases are linked to abortion waves on large dairy goat farms, and to a much lesser extent on dairy sheep farms.
- A large multidisciplinary research programme has commenced, to generate an improved understanding of C. *burnetii* infection and Q fever, and to inform improved control options.
- A broad range of control options have been implemented, including compulsory notification of abortion episodes in small ruminants, blood and bulk milk testing, vaccination, stringent hygiene measures (other animal reservoirs control; manure handling, storage and transport; risk material handling), large-scale culling of pregnant goats. As yet, these measures have not lead to any observed decline in human cases.
- Reasons for the emergence of the current outbreak are unclear, but may be related to the increase in the number of goats and goat farms, changes to the intensity of goat production in highly populated areas, to dry weather conditions and strong winds during and after the lambing/kidding season and to changes in the virulence of C. *burnetii*. Research has been initiated to investigate each of these hypotheses.
- Evaluation of the effectiveness of individual control options has proved problematic.

4. Food safety aspects

4.1. Introduction

The bacterium *Coxiella burnetii*, is the causal agent of Q fever, an acute (on occasion chronic) febrile illness that occurs in humans worldwide. Human clinical Q fever is most commonly seen in isolated or sporadic cases, while mild Q fever cases can often go undiagnosed and therefore unreported.

The disease is usually associated with livestock rearing, dairy farming or employment in abattoirs. In recently described major outbreaks, infected persons were living within a few kilometres of dairy goat farms where an upsurge of abortions was detected (Schimmer et al., 2010). Outbreaks of human Q fever may occur both in rural and urban areas (Schimmer et al., 2008 and 2009; Hawker et al., 1998). Aerosol spread of *C. burnetii* from the affected herds/flocks of domestic animal and their excreta and bedding are considered to be the main route of infection for human cases in such outbreaks. The main factor related to human outbreaks has been the proximity of patients to small ruminants with clinical Q fever, notably abortion, sometimes near densely populated areas. There are correlations, firstly between the density of farming and animal populations (Hatchette et al., 2001) and, secondly, the proximity to residential areas of the affected farming enterprises (Panaiotov et al., 2009). Source attribution of sporadic cases, independent of outbreaks, is less clear.

Most cases of *C. burnetii* infection in animals are asymptomatic. Diagnosis of *C. burnetii* infection in food-production animal populations has often arisen as a sequel to the diagnosis of Q fever in humans. For example, Ganter et al. (2010) reported that when family doctors in Germany identify an outbreak, veterinary officials are informed and steps were then taken to identify the source. In human outbreaks, a causal association between patients and their exposure either direct or indirect, to herds or flocks undergoing abortions and related conditions attributed to *C. burnetii* infection, is often established.

It is acknowledged that occupational and airborne exposures are considered to be the main transmission pathways for *C. burnetii* infection. Transmission by the oral route is documented and has been described as a secondary route of infection (reviews by Cerf and Condron (2006) and Angelakis and Raoult, (2010). This chapter addresses the extent to which food may act as a vehicle for the transmission to humans of the aetiological agent of Q fever, *Coxiella burnetii* and the means of limiting or eliminating exposure to humans by this route.

4.2. Risk factors for foodborne *C. burnetii* infection

Factors to be taken into account when considering food-borne transmission of *C. burnetii* to humans include the characteristics of the bacterium (including dose-response), its occurrence in food and identification of the food types most at risk of exposure, the means of its control along the food chain and, lastly, the susceptibility of the consumer. Also to be considered is the outcome for the exposed host, i.e., whether exposed but not infected, infected but not diseased or exhibiting clinical signs of disease.

Characteristics of the bacterium, *Coxiella burnetti*, that are of relevance to food-borne risk are related to exposure to the small cell variant (SCV or endospore), which is environmentally stable and is the form phagocytosed by macrophages during early infection. The endospore displays a tropism for reproductive organs including the mammary gland, is secreted in the milk of infected animals, both from clinical cases and asymptomatic carriers and is excreted in the detritus of normal births and abortions as well as in the urine and faeces of infected animals. Endospores are extremely resistant to heat, pressure, desiccation and can remain viable for several months in dairy products, meat and meat products, water, aborted foetuses, manure, wool, hay, equipment, and clothing during conditions of high humidity, low temperatures, and no sunlight. Endospores are metabolically inactive and thus remain stable in soil and dust over many years (Frangoulidis, 2010) and can be spread in dust or windborne aerosols for up to 11 miles (18 Km) (Hawker et al., 1998). For example, *C. burnetii* can survive at 4-6°C for 42 months in milk, 12 to 16 months in wool, 120 days in dust, 49 days in dried urine and 30 days in dried sputum (NABC, 2010). *C. burnetii* shows some important physicochemical characteristics including: stability against acids (down to pH 4.5); UV light, pressure (up to 300 MPa); and can survive in 10 % saline for more than six months. *C. burnetii* is killed by exposure (30 minutes) to 5 % H_2O_2, 0.5% hypochlorite, 70% ethanol, and in less than 30 minutes by 5% chloroform or formaldehyde gas (in a 80% humidified environment) or pasteurization, at least 72 °C for 40 seconds (Frangoulidis, 2010; Waag, 2007) or 161 °F/71.7 °C for 15 seconds (Enright et al. (1957a).

Studies in France demonstrated that duration of sheding of *C. burnetii* in sheep, is longer in vaginal mucus, whereas in goats is in milk and in the case of cattle, in milk (Rodolakis et al., 2007). These authors suggested that this could explain why human outbreaks of Q fever are more often related to ovine flocks than to bovine herds. Regarding the recent outbreak in the Netherlands it was concluded to be mainly associated with clinical Q fever in nearby dairy goat flocks and, to a lesser extent, in dairy sheep flocks. In this respect, some 2.4% of the sheep and 7.8% of the goats in the Netherlands were sero-positive by ELISA (Schimmer et al, 2008, 2009; Vellema et al., 2010; Roest et al., 2010). In the area of this recent outbreak and in the rest of the country bulk milk tank (BMT) samples from goat and sheep dairy farms were tested. The normal procedure includes an initial test carried out by the Animal Health Service (AHS, GD) using real-time PCR. If positive this sample is again tested by the Central Veterinary Institute (CVI). If confirmed positive, then a further sample is taken by the Food and Consumer Product Safety Authority (VWA) and if that sample is confirmed as positive the flock/herd is then designated as officially positive for Q fever (Roest et al., 2010). Testing of BMT

samples by PCR from dairy goat and dairy sheep farms in the Netherlands in 2008, showed 30% of the samples positive.

The BMT screening by PCR is undertaken to identify infected flocks of animals; however a positive result is not necessarily an indication that the milk contains infective *C. burnetii* (Vellema et al., 2010). BMT testing of samples is not used for the diagnosis of abortion due to *C. burnetii*, as infected goats and cows without clinical signs can shed *C. burnetii* in their milk (Rodolakis, 2010). Studies in Germany highlighted that 30 out of 50 human Q fever outbreaks were causally linked to sheep farming in the same area (Ganter et al., 2010). The same authors also linked outbreaks in 2003 and 2005 to shedding of *C. burnetii* by one infected sheep in the first outbreak and to a few sheep in the second. During the period 2001 and 2009 Q fever was diagnosed in 1,213 herds with 52% of these occurring in Bavaria and 87% of the cases occurred in cattle. Positive sheep were 7% and 1% in goats.

Because the infective stage of *C. burnetii* is present in aerosols from contaminated premises and in animal manures and excreta especially around the time of birth or abortion, contamination of on-farm milk, of exposed waterways and of fresh produce, particularly those products produced on land with spread contaminated manure and slurries, is unavoidable. Accordingly there is a risk of human exposure to *C. burnetii* from contaminated animal products, water and fresh produce.

There is limited information on the dose response of *C. burnetii* for humans. Generally, it is considered, but not proven, that exposure to aerosols with as low as one bacterium may lead to infection (Harrison et al., 1990; Bayer, 1982; Johnson and Kadull, 1966), a conclusion that supports an earlier estimate of 10 microorganisms or fewer (Tigertt et al., 1961). The exposure dose has been shown to vary inversely with the length of the incubation period (Benson and Tigertt, 1956).

Studies carried out by Enright et al. (1957a) demonstrated that *C burnetii* survived prolonged time in the udder (up to 405 days post-infection), while Moffai et al. (1970) reported that *C. burnetii* can multiply in the udder of cows for at least three years. In a study in Switzerland, screening for the presence of *C. burnetii* in BMT samples from cows, sheep and goats revealed that 17 of 359 (4.7%) of bovine milk samples examined tested positive for *C. burnetii*. In contrast, all 81 ovine and 39 caprine BMT samples tested negative for *C. burnetii* (Fretz et al., 2007). Another study reported shedding of *C. burnetii* in goat milk during the first lactation and of 21 goat kids fed bulk milk from the infected dams during their first lactation period, only three kids were serologically positive (Berri et al., 2005b). The same authors (Berri et al., 2007) also showed excretion in milk and vaginal swabs in the 2nd lambing season (13% and 23% PCR-positive milk samples one and two weeks post parturition respectively). Overall, 17 and 14 females (out of 39) excreted the bacteria into milk at the first and the second kidding season, respectively. Twelve of them shed the bacteria at both kidding seasons.

Both clinical cases and asymptomatic bovine, ovine and caprine carriers excrete the bacterium intermittently throughout lactation in variable numbers, based on studies using PCR.

Along with others, Enright et al. (1957a and b) considered that consumption of contaminated milk may constitute a means of transmission of *C. burnetii* to humans. These latter authors also reported on the numbers of *C. burnetii* in cow's milk and related these to infective doses as measured by intraperitoneal injection of 2 ml. milk in guinea pigs. Eight out of 109 raw milk samples contained viable *burnetii*, and of a further 376 retail milk samples (each sample was from a different creamery and was a composite from different producers supplying the creamery) 14 raw and one pasteurized sample contained *C. burnetii*. The maximum number of bacteria demonstrated in these samples was 1,000 infective guinea pig doses[11]. Milk samples from 137 individual cows in a dairy herd were also examined and 18 samples were found to contain viable *C. burnetii* at different levels. Experimental studies showed that the maximum infective titre in the milk was 10,000 infective guinea pigs doses. As the infective dose was determined after intraperitoneal injection, this should not to be regarded as

[11] An infective guinea pig dose may be defined as the minimum number of *C. burnetii* required to infect a guinea pig by intraperitoneal inoculation. In this study these organisms were always contained in 2 ml of inoculum.

the dose necessary for oral infection. Later studies questioned the origin of the *C. burnetii* strains used, whether they were from one or more animals and whether a single strain or a mixture of strains was used. Other authors report on the dose response for the oral route and stated that moderate doses would not result in clinical disease (Madariaga et al., 2003).

The risk of acquiring meat-borne infection by ingestion of meat carrying *C. burnetii* as a result either of a bacteraemia or contamination at slaughter, is undetermined. In cases involving abattoir workers, it is most likely that infection is via occupational exposure. Moffai et al. (1970) cited reports from different sources that abattoir workers and veterinary practitioners have the highest seroprevalence (between 24-33 %) for *C. burnetii*. The Australian Food Standards, in its Assessment of Microbiological Hazards associated with the Four Main Meat Species (2009), listed Q fever as a hazard as follows: *"Cattle [sheep and goats] may carry pathogens normally associated with handling, which could potentially be transmitted via meat consumption. Notes: Examples include Anthrax (Bacillus anthracis), Melioidosis (Burkolderia pseudomallei) and Q fever (Coxiella burnetii)".*

The Australasian Meat Industry Employees Union raised concern over the approximately 600 cases of Q fever reported nationally each year, with 90% of cases being reported in Queensland, leading to 200 people being hospitalised and three deaths occurring as a result of the disease and the related costs due to disease. There have been no scientific reports that identified the consumption of meat as a source of infection for these workers (AMIEU, 2010). In California, workers at a local meatpacking plant that processed mutton were surveyed and 29% reacted positive to Q fever, with the majority having recently experienced a clinical illness compatible with Q fever (Anonymous, 1986). In a Q fever outbreak in a meat processing plant in Scotland, blood tests confirmed Q fever in 24 patients (HPS, 2006; Donaghy et al., 2006). Other indications that Q fever can arise as an occupational disease were reports that the risk of infection was associated with an occupation in the dairy cow sector: 39 of 359 such persons studied (11%) were seropositive for *C. burnetii* while veterinarians had the highest seropositive rate (36%) (Bosnjak et al., 2009). A similar study among veterinarians in the US found antibodies against *C. burnetii* in 113 (22.2%) of 508 samples tested (Whitney et al., 2009).

C.burnetii was also studied for its potential as a biological weapon in aerosolised form or as a contaminant of food, water, or possibly even postal mail (Madariaga et al., 2003). In that study it was stated that ingestion of "moderate doses" of *C. burnetii* would be unlikely to produce clinical symptoms.

4.3. Milk and dairy products as a source of C. burnetii for the consumer

Milk containing *C. burnetii* is documented by several authors as possible source of oral infection (Marmion et al., 1956; Christie, 1980; Fishbein and Raoult, 1992), and was also documented by EFSA in an earlier opinion (EFSA, 2006). The significance of infection acquired via the oral route remains a subject of discussion (Lorenz et al., 1998) and the role of drinking unpasteurized milk in *C. burnetii* infection remains controversial. Epidemiologic studies suggest that the consumption of contaminated unpasteurized milk has been a mode of exposure to *C burnetii* for humans (Christie, 1980; Fishbein and Raoult, 1992; Benson et al., 1963; Brown et al., 1968; Connolly et al. 1990; Hatchette et al, 2001). Benson et al., (1963) reported that 42 of 120 persons who were routinely drinking unpasteurized milk tested positive for *C. burnetii* in at least one immunological test during the reference period. While none of the positives showed any sign of clinical disease, the presence of Complement Fixation (CF) antibodies was considered to indicate that exposure to *C. burnetii* had occurred. Similar findings on a seroconversion without infection or clinical signs were also reported by Fishbein and Raoult, (1992). In contrast to the findings of Benson et al. (1963), there are also reports on the absence of any immunological response in 34 people drinking unpasteurized milk naturally infected with *C. burnetii* in an area where Q fever was endemic in cattle (Krumbiegel and Wisniewski, 1970). The latter authors suggested that failure to produce response was likely due to a different dosage or to the nature of the *Coxiella* strains present. This difference in strain was also suggested in the recent Q fever outbreak in the Netherlands where typing of the *C. burnetii* bacterium involved in the outbreak revealed that the same strain was isolated from 92% of the samples from the goat farms in the outbreak area. Samples taken from a few cattle were clearly distinguished from the samples taken from the goats. It was

questioned if this strain was more virulent than others. This virulence will now be tested using the Dutch strains and some strains isolated in France (Roest et al., 2010). The above supports the likelihood that infection in occupationally exposed people is due to inhalation of contaminated aerosols rather than consumption of contaminated dairy products. Meanwhile, as stated above, a PCR positive BMT test result is also not in itself conclusive evidence that the said milk contains viable (infective) *C. burnetii* (Vellema et al., 2010).

From the above it is recognised that drinking milk containing *C. burnetii* can result in sero-conversion although it remains unclear as to whether, and if so, to what extent, clinical disease can result from the consumption of milk or dairy products, or of other foods containing *C. burnetii*.

4.4. Heat treatment as a means of controlling C. burnetii in milk and dairy products.

Pasteurization is the main method used to eliminate *C. burnetii* from milk, if present. The Codex Committee on Food Hygiene in 2004 defined pasteurization as follows: "*Pasteurization is a microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. Pasteurization conditions are designed to effectively destroy the organisms Mycobacterium tuberculosis and Coxiella burnetii"* (Anon, 2004). It also describes the current methods in use for pasteurization[12].

Enright et al. (1957) reported that the pasteurisation method then used for cow's milk (*i.e.* 143 °F/61.66 °C for 30 minutes) did not eliminate *C. burnetii* but that heating milk at 145 °F/62.77 °C for 30 minutes inactivated *C. burnetii* in concentrations of 100,000 infective guinea pig doses per 2 ml. Further results of these studies demonstrated that an alternative minimum standard temperature/timing for the pasteurization of milk, namely, 161 °F/71.66 °C for 15 seconds, could be recommended. The latter time/temperature combination is now the current standard for pasteurization. It should be noted that Enright et al. (1957) did not provide details on the origin of the *C. burnetii* strains used in the above study, or whether or not the strains used were from one or more animals or whether a single or mixture of strains was used.

The effect of some variables such as fat content or total solids in milk on the effectiveness of pasteurisation regarding the inactivation of *C. burnetii* in milk of different animal species is lacking. Studies on the inactivation of *C. burnetii* in liquid milk products containing more fat or added sugar or flavouring were reported by Enright (Enright, 1961, cited by Juff and Deeth, 2007). Heat resistance of *C. burnetii* in these products was determined using a laboratory scale heat exchanger and an inoculum of 10,000 infectious guinea pig doses of a strain of *C. burnetii* per ml of product, i.e. an inoculation rate stated as realistic in terms of the levels that can occur in raw milk delivered to a processor. Pasteurisation of cream (up to 40 *per cent* butter fat) and chocolate milk (4% butterfat and 22.5% total solids) at the recommended standards for milk, i.e. 161°F/71.66°C for 15 seconds, was shown to be inadequate to eliminate *C. burnetii* from these products. Increasing the temperature to 166°F/74.4°C for 15 seconds was effective in eliminating *C. burnetii*. Likewise, pasteurisation of ice cream mix at 175°F/79.4°C for 15 seconds was also effective in eliminating *C. burnetii*. Finally, Stumbo (1973, cited by Juff and Deeth, 2007) reported generalised data of $D_{65.6°C}$ = 0.50-0.6 minutes and z values of 4.4-5.5°C for *C. burnetii*.

Data on the effectiveness of heat treatment, as a component of cheese making, in inactivating *C. burnetii* are lacking.

[12] Currently there are two types of pasteurization used: high temperature/short time (HTST) and ultra-high temperature (UHT). There are basically two methods for the HTST type of pasteurization in use- batch and continuous flow. HTST pasteurization processes must be designed so that the milk is heated evenly, and no part of the milk is subject to a shorter time or a lower temperature. To ensure destruction of all pathogenic microorganisms, time and temperature combinations of the process have to be respected. For milk these are 63° C for not less than 30 min. or 72° C for not less than 16 sec.

5. Control options in domestic ruminant populations

5.1. Infectious disease epidemiology (concepts and applications)

A frequently used model to describe the transmission of infectious diseases is the so-called SIR model that assumes that a population consists of susceptible (S), infectious (I) and recovered (R) individuals. The rate at which a susceptible individual (S) becomes infected is determined by the transmission rate parameter β (average number of new infections caused by one infectious individual per unit of time) and the number of infectious individuals present. An infectious individual is considered to have recovered when no longer infectious. It is assumed that recovery occurs at a rate α, which has an expected value equal to the inverse of the duration of the infectious period. From these two events, it follows that the average number of new infections caused by one infectious individual in a totally susceptible population (R_0) equals β/α (Diekmann and Heesterbeek, 2000). R_0 is unambiguously the key parameter of the epidemiological models, because it tells us whether infections can ($R_0>1$) or cannot ($R_0<1$) spread in a fully susceptible population, and thus whether control options such as vaccination are effective in eradicating the pathogen from a population. The equivalent of R_0 in a population housing both Susceptible and Recovered individuals is R_e, which is also determined by the proportion of Recovered individuals in the population.

Applied to Q fever, this means that the transmission of *C. burnetii* infections depends on:

- *The proportion of the population susceptible to infection and the degree of susceptibility of the animals,* which is linked to a range of host factors, including past exposure to the agent, the duration of active and maternally-derived immunity, and population dynamics (recruitment rate into the population, death rate due to infection) (van Dijk, 1994);

- *The proportion of the population infectious and the degree of infectivity*, which is also linked to a range of host factors, including abortion/giving birth, length of the infectious period, active and passive maternally-derived immunity (van Dijk, 1994); and

- *The adequacy of contact between individuals in a population,* which is a composite of three key aspects of infectious disease epidemiology, specifically the number of contacts per unit time, the transmission potential per contact and contact structure within the flock (whom has contact with whom) (Halloran, 1998). Both host and agent factors are important. Host factors include population density, population social structure, management methods (including husbandry and housing), and heterogeneity in transmission arising from factors relating to age, genetics, spatial distribution and behaviour, whereas agent factors include the intrinsic contagiousness of the agent and the mode of transmission. Depending on the infectious agent, vector factors may also be relevant, including, competence or efficiency and mean bites per unit time (van Dijk, 1994).

This theoretical understanding of infectious disease epidemiology can be directly translated to measures to prevent and/or control infection by reducing transmission (within defined populations, such as herds) and spread (between populations). The latter can be regarded as animals of different populations having contact. Consequently, population size plays a role in the latter.

Specifically, there are two broad measures to limit transmission and spread of directly transmissible infections in populations:

- *Reducing the proportion of the population susceptible and decreasing the infectivity of infected individuals,* generally through vaccination. Mass vaccination programmes, which effectively lower R_e, are reliant on both 'vaccine efficacy under field conditions' (degree of reduction of susceptibility and infectivity (both level and duration)) and 'overall coverage rate' (the overall proportion of susceptible animals that are vaccinated). Besides vaccination, a range of measures are routinely used to limit the duration of infectiousness, including (prophylactic and therapeutic) antimicrobial treatment, test and slaughter, etc.

- *Reducing the adequacy of contact between individuals* (animal-animal, animal-human, human-human) through measures to:

    - *Reduce the number of contacts per unit time.* A broad range of measures are commonly applied to limit contact during animal disease control, including quarantine, test and slaughter, livestock movement control, changes to farm management etc

    - *Reduce the transmission potential per contact.* As a feature of the infectious agent, the transmission potential is generally difficult to influence within populations. However, these measures can be very important for reducing the transmission potential per indirect contact between different flocks (hygienic measures).

    - *Reduce the number of different farms in contact.* By limiting the numbers of different farms in contact with each other (trade restrictions), transmission can be reduced.

The above discussion is generic, but provides a robust framework for consideration and assessment of control options for Q fever in animal populations, as outlined in section 5.2.3.

- As with other contagious diseases, the transmission of *C. burnetii* in animal populations depends on:
    - The proportion of the population susceptible to infection, and the degree of susceptibility of the animals,
    - The proportion of the population infectious, and the degree of infectivity, and
    - The adequacy of contact between individuals in a population.
- There are two broad measures to limit transmission and spread of directly transmissible infections in populations, including:
    - Reducing the proportion of the population susceptible and decreasing the infectivity of infected individuals, and
    - Reducing the adequacy of contact between individuals through measures that reduce the number of contacts per unit time, the transmission potential per contact and the number of different farms in contact.
- These concepts in infectious disease epidemiology provide a generic framework to provide a qualitative assessment of available control options for Q fever.

5.2. Available control strategies

5.2.1. The importance of surveillance/monitoring and case ascertainment

Control measures for *C. burnetii* can only be effectively implemented if cases are detected and confirmed. This requires systematic and reliable classification of units/farms as cases. The basis for such a classification is an agreed case definition. Case detection can be based on suspect case reporting (passive surveillance) or screening (active surveillance).

In order to be able to assess the effectiveness and efficiency of an intervention as part of a control effort, surveillance after the implementation of control needs to be implemented. Effective surveillance activities during control programmes typically include case detection, reporting of suspect cases, follow-up investigations and diagnostic confirmation of cases. Surveillance should therefore be an integral part of any control programme. In return, the detected cases will then be subject to control options. Legal basis is required for compulsory control options.

- Control measures for *C. burnetii* can only be effectively implemented if cases are detected and confirmed.
- Effective surveillance activities as part of control programmes typically include case detection, the reporting of suspect cases, follow-up investigations and diagnostic confirmation of cases.

5.2.2. General considerations

The choice of a Q fever control strategy will depend on the overall objective of the control effort. This could, for example, be limited to avoiding severe cases or a focus on "problem" farms or – at the other end of the spectrum – attempt complete eradication of *C. burnetii* infection in the entire population. In order to determine the objective of control at Community level, the appropriate level of protection for other EU MS needs to be discussed. Although the effectiveness of interventions is an important aspect in this, it essentially remains a risk management decision because other factors such as economics also need to be considered.

The objective at regional level will be strongly influenced by the current prevalence of the pathogen/disease. For example, if *C. burnetii* is widely occurring and the majority of farms are affected, an eradication objective is likely to be unrealistic. Whether control will be compulsory or voluntary will also have to be considered. For voluntary control, the issue of herd/animal status certification and implications on movement of animals needs to be discussed.

A prerequisite for any control programme is the ability to classify farms or animals into infected/not-infected or diseased/not-diseased. This will require an agreed case definition which can generally be based on clinical, pathological or laboratory criteria or a combination. The case definition should be of sufficient sensitivity and specificity such that the control objectives can be achieved with the available resources.

Based on the biology and epidemiology of an agent, individual control options may be more or less effective. For example, if alternative transmission pathways exist as is the case for *C. burnetii*, control of only one pathway (e.g. animal trade) may be of reduced effectiveness and may not achieve the desired objective if other pathways such as aerosol transmission persist. While some control strategies may be effective in reducing transmission between farms, they may have severe negative consequences. For example, movement restriction may be a useful approach, but will impact on trade and possibly on animal welfare. The choice of control strategy is limited if wildlife reservoirs are present, which is the case with *C. burnetii*.

Some interventions may be costly or logistically difficult to implement as part of a general control programme. However, there may be special circumstances, such as a large outbreak, that may still justify their implementation. It should therefore be agreed, which events should trigger interventions. Certain interventions – particularly movement restrictions – may also be considered as short-term measures when there are new features of a disease indicating a change in pathogenicity. Such precautionary measures will then allow gaining time to investigate the emerging strain and to adjust the control strategy as required.

The optimal control strategy may not consist of a single approach but may require a combination of several control interventions. A risk-benefit analysis could be conducted in order to assess the justification of control options at Community level.

- The choice of Q fever control strategy will depend on the objective of the overall control effort. This is essentially a risk management decision, based on an understanding of control effectiveness and other factors, including economics.
- At a regional level, the control objective will be strongly influenced by the current prevalence of the pathogen/agent.
- An agreed case definition is a prerequisite of any regional control programme, given the need to classify farms or animal into infected/not-infected or diseased/not diseased,
- The effectiveness of individual control options will be influenced by the biology and epidemiology of the agent. Alternative transmission pathways need to be considered. The choice of control strategy is limited, as with *C. burnetii*, if wildlife reservoirs are present.
- Although some interventions may be costly or logistically difficult, their use may be justified under special circumstances (for example, large outbreaks).
- The optimal control strategy may require a combination of several control interventions. A risk-benefit analysis could be conducted at Community level.

5.2.3. Specific control options

A list of possible control options was elaborated. Some of these control options focus specifically on small ruminants (goats, sheep) as opposed to cattle, given that cattle appears to have a minor role in the spillover of *C. burnetii* infection from domestic ruminants to humans. A brief descriptionof the control optionsis provided in this section. Limitations and an assessment of effectiveness and sustainability of these options are presented in Table 14.

a. Vaccination

Current vaccines used in humans and animals include formalin-killed, whole-cell vaccine preparations (WCV) (Marmion et al., 1990) and chloroform methanol-extracted bacterial residue (CMR) (Williams et al., 1986, 1992; Fries et al., 1993; Waag et al., 1997). The two types of vaccines (WCV and CMR) protect monkeys (*Macaca fascicularis*) against fever and bacteriemia after challenge with an aerosol (Waag et al., 2002). They also induce protection on mice and guinea pigs challenged with virulent phase I *C. burnetii* by aerosol (Waag et al., 1997) or intra-peritoneal injection (Kazar et al., 1995; Zhang et al., 2004, 2007).

In animals, the most effective vaccines are those composed of inactivated whole phase I bacteria. Bacterial shedding in placental tissue and milk was strongly reduced in experimental infection or in natural *C.burnetii* infection of ewes and cows vaccinated by phase I vaccines (Brooks et al., 1986; Sadecky et al., 1975, Sadecky and Brezina, 1977). However, several studies report that, phase I vaccines failed to prevent shedding in milk in naturally infected cows prior to vaccination (Biberstein et al., 1977; Schmeer et al., 1987), highlighting the role of the vaccine in protecting uninfected but not in treating infected animals. Vaccination efficacy will depend on whether immunisation precedes infection *(preventive vaccination)* or not *(outbreak vaccination)*. The efficacy of two commercial vaccines compound of inactivated *C. burnetii* reference strain Nine Mile —a phase I and a phase II vaccine —was compared in pregnant goats experimentally infected with a dose of *C. burnetii* sufficient to cause abortion or premature birth in 85% of the goats in the control group (Arricau-Bouvery et al., 2005). The phase I vaccine prevented abortion, and dramatically reduced the shedding of *C. burnetii* in milk, vaginal mucus, and faeces, thereby reducing both environmental contamination and the risk of transmission to humans. In contrast, the phase II vaccine did not show any difference compared to the control group.
The efficacy of the phase I vaccine was also studied in naturally infected cows (Guatteo et al., 2008). . When vaccinated while not pregnant, an initially non infected animal had a five times lower probability of becoming a shedder than an animal receiving a placebo. An animal which was vaccinated when pregnant had a similar likelihoodlity of becoming shedder as an animal receiving the placebo. The authors hypothesize that pregnancy had an adverse effect on the immune response.

A similar study was carried out in 3 caprine herds on 1701 goats (1081 adults and 620 young animals) (David and de Cremoux ANICAP, 2009[13]). More than 73% of the adults shed *C burnetii* in the vaginal mucus before vaccination while 67.7% of the young did not. 202 young goats and only 2 adults were uninfected before vaccination. As in cows, vaccination of young non-infected goats with phase I vaccine reduced significantly the shedding of *C burnetii* (around 2 logs) in vaginal mucus after parturition.
A bovine herd in which abortions due to Q fever were diagnosed in 2001 was vaccinated with a Phase I vaccine in 2004-2005. Each cow received the vaccine twice at 3 weeks of interval and a yearly booster vaccination between 2 and 5 weeks after calving. The efficacy of the vaccine was assessed by following the shedding of *C. burnetii* in vaginal mucus and milk. For this purpose, qPCR was performed on vaginal mucus and milk the day of calving and 3 days after. The shedding was also assessed 1, 3 and 7 weeks after calving in individual milk samples as well as in bulk milk samples. Four years were needed to stop the shedding, indeed the first year, 40% of the cows shed *C. burnetii* at least by one route, the second year 18% were still shedding and 10% the third (Camuset and Remmy, Word Buiatric Congress Budapest, 2008).

Field and experimental data is lacking on the epidemiology of infection in, and between, infected flocks. For example, R_e and R_h for Q fever in vaccinated flocks are not known. Further, based on current diagnostic tests, it is not possible to serologically distinguish vaccinated and naturally-infected animals.

- In animals, phase I vaccines are much more effective than Phase II vaccines.
- *Preventive vaccination* (immunisation preceding infection) is much more effective than *outbreak vaccination* (infection preceding immunisation).
- Preventive vaccination in goats, using a phase I vaccine, prevents abortion and dramatically reduces the shedding of *C. burnetii* in milk, vaginal mucus and faeces, thereby reducing both environmental contamination and the risk of transmission to humans. In cattle, phase I preventive vaccination substantially reduced shedding.
- Vaccination is a long-term control strategy, particularly in heavily infected herds.
- Field and experimental data is lacking, on the epidemiology of infection in, and between, infected flocks. For example, R_e and R_h for *C. burnetii* infection in vaccinated flocks is not known.
- Based on current diagnostic tests, it is not possible to serologically distinguish vaccinated and naturally-infected animals.

b. Antibiotic treatment

Antibiotic treatment is used effectively in humans to reduce clinical symptoms associated with Q fever. Antibiotic treatment was not demonstrated to be effective in preventing the shedding of bacteria or limiting the duration of bacterial excretion, either in sheep (Astobiza et al., 2009) or goats (Blain, 2007).Consequently, antibiotic treatment is not effective in influencing the epidemiology of infection in animal populations, and cannot be recommended (Berri et al.,2005a). Widespread antibiotic usage should also be avoided to limit the development of antibiotic resistance (WHO, 2001).

- *Antibiotic treatment in animals* is not effective in substantially reducing either the level or duration of bacterial shedding in domestic ruminant populations.

c. Removal of risk material (placentas, aborted foetuses)

[13] In Rodolakis 2010. Q fever in France. Q fever conference, Breda, The Nederlands, 25-26 February.

This strategy concerns the collection and removal of risk material (placentas, aborted foetuses) to specific rendering plants or by other methods of safe disposal. The rationale for this approach is that, in infected flocks, risk material can contain very large numbers (10^9-10^{12}) of *C. burnetii* (Babudieri, 1959). In many farming systems, it may not be possible to retrieve all risk material.

- *The removal of risk material,* such as placentas and aborted fetuses, has been suggested, noting that these materials have the potential to contain very high numbers of *C. burnetii.*

d. Manure management

Animal husbandry systems vary considerably between EU MS. Consequently, there are also considerable differences in methods of manure management, for example: in terms of storage location (on-farm, at a remote site), method of covering (if at all), the addition of lime (or not), industrialized composting, steem treatment of manure. The following systems were assessed for effectiveness:

- Deep litter systems, where goats are kept indoors on straw litters, straw is added regularly and removed only 3-4 times a year. The manure is usually moved to another location in or out of the farm.
- Slurry treated with cyanamide calcium
- Manure composting for a period of time with or without covering
- Untreated slurry is removed and spread onto fields

The goal of manure treatment is to reduce the viable bacterial load of the manure after the production of the manure, either through chemical (Arricau-Bouvery et al., 2001) or heat treatment.

Composting is a manure fermentation process that kills bacteria as temperatures rise. Traditional composting consists in piling manure, between concrete walls and fermentation for 3 months. The inside temp is estimated to be a minimum of 50 C and this process will lead to a minimum of $4.3*10^{-7}$ reduction of Coxiella (based on data extrapolated from experiments in milk pasteurization). Industrial composting consisting of treatment for 1 month for min 50°C and is estimated to lead to a minimum $1.7*10^{-7}$ reduction of Coxiella. Pasteurizing industrial compost at 70°C for 20 min will lead to higher reduction of Coxiella but the degree of reduction is unknown at the moment. A comparison between traditional and industrial composting processes shows that their effectiveness is basically similar (VWA, 2009).

Exact levels of *Coxiella* in the manure and how effectively they are killed in the standard fermentation process cannot be determined to date but research in the topic is currently ongoing.

The level and duration of heat achieved is affected by a range of factors including the level of moisture, the duration of composting and the presence of aerobic conditions (Vellema et al., 2010).

There is a limited understanding of the role of manure in the spillover of infection to humans. In the Netherlands, where manure is often transported off-farm to a remote site, there have been no known human Q fever cases linked to manure transport or remote storage.

- There are differences in systems of *manure management* between EU MS.
- Often either chemical or heat treatment is used to reduce the load of viable bacteria. A range of factors affect the level and duration of heat achieved, including the level of moisture, the duration of composting and the presence of aerobic conditions.

e. Culling of pregnant animals

The culling of pregnant goats/sheep was recently used as a short term control measure spanning the pregnancy/lambing period on dairy goat and sheep farms in the Netherlands. This decision was taken in the face of a rising number of human Q fever cases and of Q fever outbreaks (with abortion) on a number of farms. The rationale for this approach is that infected animals shed bacteria in largest numbers at and shortly following parturition (Arricau-Bouvery et Rordolakis 2005).

- *The culling of pregnant females* would lead to a reduction in bacterial shedding, noting that shedding is greatest from infected animals at or shortly following parturition or abortion.

f. Identifying and culling of shedders

The culling of shedders has been suggested as one possible strategy to control Q fever in animal populations. This strategy relies on a *'test and cull'* approach, to facilitate the identification and culling of infected animals that are excreting *C. burnetii*, thereby reducing the overall prevalence of infection in a herd.

- *The culling of shedders* relies on a 'test and cull' approach.

g. Temporary breeding ban

A temporary breeding ban has been considered on the same premise as that suggested for the culling of pregnant animals. With a temporary breeding ban, the opportunity for bacterial shedding is greatly reduced. As part of the broader control strategy in the Netherlands, the temporary breeding ban is expected to precede subsequent repopulation with vaccinated animals.

- *A temporary breeding ban* would substantially reduce the opportunity for bacterial shedding. This strategy would be expected to precede subsequent repopulation with vaccinated animals.

h. Control of animal movement between farms of differing infection status

The control of animal movement is used as a strategy to prevent and/or control a number of animal diseases. In the context of Q fever, the introduction of infected animals into naïve herds could be avoided if there were control of movement of animals of defined status between herds of defined status. However, this strategy is reliant on the reliable classification of animals and herds (for example, a farm-level certification system including testing scheme, cut offs, status definition etc). Given the diagnostic quality of currently available diagnostic tests, however, considerable uncertainty is likely to remain as to the true status of an animal or herd. Further, alternative transmission pathways may persist, and reduce the effectiveness of this intervention.

- *The control of animal movement between farms of differing infection status* might be considered as a method to prevent the introduction of infected animals into naïve herds, but only if there were a reliable means to classify animals and herds.

i. Stand still measures

This is a temporary movement restriction applied at a regional level or in a zone that is defined for disease control purposes. It is typically applied during a major disease outbreak with the objective to eliminate transmission related to trade, and to gain time. A stand still is normally combined with other interventions such as outbreak investigation, tracing, stamping-out, testing, outbreak vaccination and cleaning and disinfection. Note that the status of herds inside and outside the zones would need to be defined, for example through systematic investigations, including testing. It is assumed that all units outside the zone are negative. The presence of positive wildlife is a challenge for defining zones, but examples exist how to deal with this, for example, from classical swine fever.

- *Stand still measures* are typically applied during an outbreak, with the objective to eliminate transmission related to trade, and to gain time.

j. Changes to farm characteristics (number of animals/farm size, farm location)

This control option concerns a number of potential changes to farm characteristics, including the number of animals per farm/farm size, and farm location:

- *Number of animals/farm size:* According to the mass action principle, given all things being equal (such as density of animals within the flock), the transmission within a population is independent of the number of animals in the population (R_0 does not increase as population size increases; Bouma et al., 1995). However, as farm size increases, the probability of introduction of a pathogen increases. The reason is that more contacts from outside the farm take place (for example, more replacement stock and more feed is introduced into a farm). In addition, as farm size increases, more animals can become infected, and consequently the infectious load produced in a large farm exceeds that of a smaller farm. In addition, large farms will also have more outbound contacts than smaller farms (for example, more animals will leave the farm, more milk is delivered). The above reasoning assumes that all other things are equal between large and small farms (such as bio security measures, trading policy, etc.), which might not be realistic if larger farms were to take more risk-reducing measures than smaller ones as for example closed stables.

- *Farm location:* Proximity to goat farms has been reported as a significant risk factor for human infection (Schimmer et al., 2010.). Given that neighbourhood spread is important in many animal diseases, density-dependent infection is also likely for the spread of *C. burnetii* between farms (such as, for example, has also been shown for avian influenza, CSF, FMD, etc.; Boender et al., 2007a, 2007b, 2008). To date, however, no published information about Q fever is available. As a consequence, give all things equal, increasing the distance between farms will decrease transmission and increasing distance to human dwellings will reduce exposure of humans. Unfortunately, we cannot yet suggest an optimal distance between farms, or between a farm and a human dwelling. The reason is the lack of quantitative data of between-flock transmission and its relation with other factors such as flock size and bio security measures (see above). Such data are of great importance, because of the highly non-linear nature of the transmission process.

- *Changes to farm characteristics* have been raised as potential methods of prevention/control of Q fever, including:
  - *Changes in animal numbers/farm size.* All other things being equal, R_0 does not increase with increasing population size. However, introduction probability is likely to increase with increasing farm size, because there is greater opportunity for contact outside the farm. Further, the infectious load on large infected farms will be greater than on small infected farms.
  - *Changes in farm location.* All other things being equal, between-herd spread will decrease with increasing distance between farms. Similarly, the risk of human exposure will decrease with increasing distance from human dwellings. However, the optimal distance between farms, or between farms and humans dwellings, is currently not known. This could be estimated once quantitative data were available of between-flock transmission, and its relation with other factors such as flock size and biosecurity measures.

k. Control of other animal reservoirs of infection

A range of other animal species, apart from cattle, sheep and goats, can become infected with *C. burnetii* (Stoker and Marmion, 1955; Webster et al., 1995; Martinov et al., 2007a). However, their role in the epidemiology of *C. burnetii* in domestic ruminants is not completely understood. For this reason, the impact of this control measure is uncertain.

- *The role of other animal reservoirs of infection* in the epidemiology of *C. burnetii* infection in domestic ruminants is not completely understood. The impact of this control measure is uncertain.

l. A segregated lambing/kidding area

A segregated lambing/kidding area has been proposed, as a means to reduce within-herd transmission of *C. burnetii.* The rationale for this approach is that infected animals shed bacteria in largest numbers at and shortly following parturition (Arricau-Bouvery et. al., 2001)

- *A segregated lambing/kidding area* has the potential to reduce within-herd transmission, noting that shedding is greatest from infected animals at or shortly following parturition.

m. Shearing management

A number of Q fever outbreaks in humans have been associated with shearing of sheep (Hellenbrand et al., 2001; Hellenbrand et al., 2005).Sheep wool can become heavily contaminated with tick faeces (1g can contain up to 10^{12} bacteria) and infected birth products. Dust containing *C. burnetii* is produced during shearing, and bacterial DNA can be found in the air of barns where sheep have been shorn (Schulz et al., 2005). In such situations, shearing personnel should wear protective filter masks and the fleece should be kept wet or even disinfected. Shearing should be done in closed barns.

- A number of Q fever outbreaks in humans have been associated with sheep shearing.
- Wool can become heavily contaminated with *C. burnetii*, through contact with tick faeces and infected birth products.

n. Tick control

Some authors consider ticks as an important reservoir of infection (Eldridge and Edman, 2000, Stoker and Marmion, 1955; Martinov, 2007a). Consequently, tick control intervention may play a role in limiting a potential reservoir.

- Ticks may be a reservoir for the infection, and therefore tick control intervention can play a role .

o. Stamping out

*S*tamping out (the slaughter of all animals on defined, infected farms) may be warranted in emergency situations where public health is at risk. This would lead to an immediate reduction in transmission from highly focal problem herds. By definition, stamping out will prevent further shedding from infected animals, however, it will not directly address problems of environmental contamination. We are unaware of any published information about the effectiveness of stamping out to control Q fever outbreaks.

- *Stamping out* (the slaughter of all animals on defined, infected farms) may be warranted in emergency situations where public health is at risk.

p. General biosecurity including visitor ban

Biosecurity refers to hygienic measures and protocols that are applied on farms to limit the introduction and spread of infectious diseases of animals. To be effective, these measures and

protocols are applicable to all people who enter or leave a premise where farm animals are kept. General guides for good biosecurity practice are available (DEFRA, 2008[14]). These measures will reduce, but not eliminate, the risk of *C. burnetii* introduction on to non-infected farms. A visitor ban will reduce the number of humans in direct contact with infected animals, but may have limited impact on methods of indirect contact (for example, through wind-borne spread). We are unaware of any published information about the effectiveness of general biosecurity to control the zoonotic impact of Q fever outbreaks.

- *Biosecurity* refers to hygienic measures and protocols that are applied on farms to limit the introduction and spread of infectious diseases of animals.
- *A visitor ban* will reduce the number of humans in direct contact with infected animals, but may have limited impact on methods of indirect contact (for example, through wind-borne spread).

5.2.4. Effectiveness and sustainability of potential control options

An assessment of effectiveness and sustainability of individual control options for *C. burnetii* infection in domestic ruminant populations is presented in Table 14, in relation to impact on animal health (reducing within-herd transmission, limiting spread to other farms) and public health (reducing spillover to humans). This assessment was conducted using expert opinion, as outlined in Section 1.2.3.

[14] http://www.defra.gov.uk/foodfarm/farmanimal/diseases/documents/biosecurity_guidance.pdf (accessed 12/4/10)

- For almost all of the control options, there is a medium to high level of uncertainty associated with estimates of control effectiveness. Little published information is available, and estimates of effectiveness are primarily based on expert opinion, derived from observation rather than rigorous scientific assessment.
- The relative importance of specific transmission pathways for *C. burnetii* is not known. In the absence of such information, it has not been possible to accurately estimate the effectiveness of individual control options.
- It is likely that control methods would need to be used in combination, given that:
  - No control measure is likely to be completely effective in limiting within-herd transmission, between-herd spread and spillover to humans, and
  - Within-herd transmission, between-herd spread and spillover to humans are each likely to involve more than one transmission pathway.
- Based on currently available information and expert opinion, it is likely that there is considerable variation in the effectiveness of different control options. The most effective control options include:
  - *To limit within-herd transmission:* preventive vaccination, culling of pregnant animals, breeding ban
  - *To limit between-herd spread:* preventive vaccination, culling of pregnant females, breeding ban, manure management [treating slurry with cyanamide calcium, deep litter composting]
  - *To limit spillover to humans:* preventive vaccination, culling of pregnant females, breeding ban, manure management [treating slurry with cyanamide calcium, deep litter composting]
- Vaccination is a long-term control option; effectiveness may not be observed in the short-term.
- Many of the control options are consistent with good farming practice and could be of value in reducing the risk of future outbreaks
- Many control options are unlikely to be sustainable in the medium-to-long term, for a broad range of reasons.Some control options are best suited for use in the face of an outbreak.
- A number of methods could be considered to limit the risk of introduction of *C. burnetii* onto uninfected farms, including biosecurity, control of animal movement and manure management.
- Control measures to address proximity between humans and small ruminants, particularly around the time of lambing/kidding, would be expected to meaningfully contribute to a reduction in spillover from animal populations to humans.

Table 14: Effectiveness, sustainability of potential control options for *C burnetii* infection in domestic ruminant populations

Control option	Effectiveness/Uncertainty						Sustainability[c]	Limitations
	Relating to animal health				Relating to public health (reducing spillover from farmed animal populations to humans)			
	Reducing within-herd transmission[a]		Limiting spread to other farms[a]		Effectiveness[a]	Uncertainty[b]		
	Effectiveness[a]	Uncertainty[b]	Effectiveness[a]	Uncertainty[b]				
Preventive vaccination (immunisation preceding infection)	High	Medium	High	Medium	High	Medium	High	In animals, phase I vaccines are much more effective than Phase II vaccines. Effectiveness will depend of the use of vaccination in all farms in a region, and the combined use of other measures. There is a need for a sufficient vaccine supply. Cost may become a limitation.
Culling of pregnant animals	High	High	High	High	High	High	Low[d]	Extremely high costs including political, financial (both for restocking and production loss) and social. Culling of pregnant healthy animals is a real ethical (and in some countries legal) problem. Leads to the generation of biological waste. Impact may not be observed in a short medium term, as a result of environmental contamination. Non-pregnant animals can also shed *C. burnetii*.
Manure management *[slurry treated with cyanamide calcium]*	Low	High	High	Medium	High	Medium	High	
Manure management *[deep litter systems, manure composting for 3 m]*	None	High	High	High	High	High	High	Financial costs associated with storage. Potential opportunity costs (constraints on time to sell or spread).
Temporary breeding ban	High/	High	High	High	High	High	Low[d]	With goats: Financial costs, particularly reduced production (noting that goats can milk continually for 2-2½ years). Costs will increase greatly if temporary breeding ban is extended. With sheep: Financial costs are much greater in sheep than goats (as extended lactation is not possible)
Identifying and culling of shedders	Low	Medium	Low	Medium	Low	Medium	Low[d]	Shedders are difficult to identify, given current diagnostic methods.

Control of animal movement between farms of differing infection status	Low	High	Medium	High	Low	High	Low[d]	Strategy is only possible if the infection status of each herd is known, i.e. requires mass testing/screening of all herds participating in trade. Likely to be more effective in limiting long distance spread. Requires identification and certification of animals and herds, and a legal basis if it is to be compulsory. There may be a substantial financial cost .
Stand still	Low	High	Medium	High	Low	High	Low[d]	Not well suited to Q fever, noting that *C. burnetii* infection is widespread. May be logistically difficult to implement, but provided the legal basis is available and the consequences are justifiable, Financial cost (loss of benefits from trade). Strategy is only possible if the infection status of regions or zones is known. Requires certification of animals and a legal basis. Likely to be more effective in limiting long distance spread.
Control of other animal reservoirs of infection	Low	High	Low	High	Low	High	High	The specific effectiveness against Q-fever is difficult to be measured The activities should be part of the routine prophylactic measures but is difficult to estimate threshold or acceptable level of the applied measures.
Tick control	Low	Medium	Low	Medium	Low	Medium	High	The potential for environmental pollution and tick resistance, the need for special authorization.
Changes to farm characteristics *[number of animals/farm size, farm location]*	Low	High	Medium	High	Medium	High	High	The threshold (*number of animals/farm size)* is not known, but will depend on both proximity to humans and farm infra-structure (eg closed stables may lead to increased within-herd transmission but reduced potential for spillover). Very high initial costs.
Wool shearing management	Low	High	Low	High	Medium	High	High	It is not clear how to decontaminate or dispose of *Coxiella* contaminated wool. Effectiveness will be related to distance between farms.
Outbreak vaccination (infection preceding immunisation)	Medium	Medium	Medium	Medium	Medium	Medium	High[d]	In animals, phase I vaccines are much more effective than Phase II vaccines. Impact may not be observed in a short-medium term as a result of environmental contamination. Requires sufficient vaccine supply.
Removal of risk material (placentas, aborted foetuses)	Medium	High	Medium	High	Medium	High	High	Creates biological waste. Feasibility will depend on the farming system. It is unlikely that there will be complete removal in any system.
A segregated lambing/ kidding area	Medium	High	Medium	High	Medium	High	High	Depends on the effective of separation. There are cost implications.
Stamping out	None		Medium	High	Medium	High	Low[d]	Depends on the level of existing environmental contamination. The policy for repopulation needs to be established

Visitor ban	None	-	Low	High	Medium	High	High	
Manure management *[untreated slurry, subsequent land spreading]*	Low	High	-	-	-	-	High	Effectiveness at limiting spread to other farms and reducing spillover to humans depends on subsequent land spreading method and possible treatment.
Antibiotic treatment	None	Low	None	Low	None	Low	Low	The treatment is not effective in substantially reducing either the level or duration of bacterial shedding in domestic ruminant populations, and could induce antibiotic resistance.

a. Effectiveness was assessed as follows:
- *High*, very effective;
- *Medium*, moderately effective;
- *Low*, very limited effect;
- *None*, no effect.

b. The level of uncertainty was considered:
- *High:* Scarce or no data available; evidence provided in unpublished reports, or few observations and personal communications, and/or authors' or experts' conclusions vary considerably
- *Medium:* Some or only incomplete data available; evidence provided in small number of references; authors' or experts' conclusions vary, or limited evidence from field observations, or solid and complete data available from other species which can be extrapolated to the species being considered
- *Low:* Solid and complete data available; strong evidence in multiple references with most authors coming to the same conclusions, or considerable and consistent experience from field observations.

c. The scale of sustainability included:
- *High*, sustainable in the long-term;
- *Medium*, sustainable only in the short-to-medium term;
- *Low,* sustainable only in the short term.

d. Particularly suited for outbreak response

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

TOR 1: To assess the significance of the occurrence of Q fever in the EU Member States for a better understanding of the scale and distribution of the disease and infection (with the focus on farm animals and humans):

Based on available evidence,

- *Coxiella burnetii* infection is endemic in domestic ruminants *(cattle, sheep, goats)* in most, if not all, EU MS, although there is considerable uncertainty about estimates of prevalence and incidence.

- Although infection in domestic ruminants is common, disease is rare. The overall impact of *C. burnetii* infection on the health of domestic ruminants in EU MS is limited. However, the impact on individual goat farms during an abortion episode can be significant.

- *C. burnetii* infection in humans is present in most, if not all, EU MS, although there is considerable uncertainty about estimates of prevalence and incidence.

- Q fever is a zoonotic disease with limited public health impact within the EU, although in certain epidemiological circumstances and for particular risk groups the health impact can be significant. Many cases of Q fever in humans are unrelated to occupational exposure. As yet, the Q fever burden of disease in humans has not been determined.

TOR 2: To assess the risk factors for Q fever occurrence and persistence in animal husbandry, and the related risks for humans, taking into account at least the presence and density of susceptible livestock and the type of husbandry in which they are kept:

Based on available evidence,

- *C. burnetii* infection is endemic in domestic ruminants throughout Europe. Therefore, it seems likely that infection can be maintained in a wide range of husbandry systems.

- There is considerable uncertainty about the relative importance of risk factors for maintenance of *C. burnetii* infection in domestic ruminant populations, and for spillover from domestic ruminants to humans. Further, for most of these risk factors, causality has not been established. There is also an incomplete understanding of the transmission pathways for either maintenance or spillover.

- Maintenance of *C. burnetii* infection within a farm might be favoured by persistently infected domestic ruminants, other animal reservoirs of infection, ticks, husbandry practices that favour within-herd transmission and/or environmental contamination.

- Currently, there is no clear evidence of an association between bacterial genotypes/isolates and virulence. Further there is no clear evidence of species specificity. The importance of bacterial genotype/isolate on maintenance of infection and disease progression is uncertain.

- Among EU MS, there are some differences in the risk factors associated with spillover of *C. burnetii* infection from domestic ruminants to humans. Nonetheless, some risk factors are common, including:

    - an association between human infection and small ruminants (sheep and goats),

- an indication of proximity between animals and human populations, particularly in association with parturition in animals (and to abortions, in the case of goats), and
- specific climatic conditions, in particular dry, windy weather.

- In humans, the risk of exposure to *C. burnetii* is increased in several circumstances, either:
  - Following close contact with animals infected with *C. burnetii*, or
  - Following community-based exposure, caused by an elevation of *C. burnetii* in the wider environment following release and dissemination from infected animal hosts.
- There is no conclusive evidence in support of a link between an increased density of animals and/or farms and spillover of *C. burnetii* from infected farms to humans. However, such a link would be consistent with the consequences of increased pathogen pressure.
- The factors leading to outbreaks of Q fever in the human population are not fully understood. Risk factors are likely to interact, and the importance of any single risk factor may be difficult to evaluate.

Related to food safety[15]

The widespread distribution of *C. burnetii* in food producing animals and its occurrence in the milk supply has called into question the role of food as a vehicle for the transmission of this zoonotic bacterium to humans. The conclusions reached in this Opinion address this concern.

- *C. burnetii* infection in occupationally or otherwise exposed people is mainly due to inhalation of infected aerosols rather than consumption of contaminated food (e.g. contaminated milk and dairy products)
- *C. burnetii* is excreted in milk of infected animals (cattle, sheep and goats) for variable periods during lactation irrespective of whether these are showing clinical signs or not.
- In addition, milk can be contaminated with *C. burnetii* by faecal materials or from sites of infection in the periparturient and/or lactating animal
- Contaminated milk and milk products can be considered as a source of exposure to *C. burnetii* for humans; however, no comprehensive data are available on the numbers of *C. burnetii* contained in milk.
- There are epidemiological indications that consumption of milk and/or milk products containing *C. burnetii* has been associated with sero-conversion in humans. However, no data are available on the dose-response for human infection through food.
- There is no conclusive evidence that the consumption of milk and milk products containing *C. burnetii* has resulted in clinical Q fever in humans.
- No data are available with reference to differences in the pathogenicity for humans of the different *C. burnetii* strains acquired through food.
- Consumption of raw milk and raw milk products represent a relatively greater risk of human exposure to *C. burnetii* than the consumption of both milk and dairy products made with milk that has undergone appropriate heat treatment.

[15] The conclusions related to food safety were adopted by BIOHAZ Panel on 22 April, 2010.

- There are no data available regarding the potential of transmission of C. burnetii infection to humans through consumption of meat, meat products or fresh produce.

TOR 3: To assess the effectiveness and efficiency of disease control options such as vaccination, pharmaceutical treatments, establishing animal movement restrictions, the culling of animals, etcetera.

- For almost all of the assessed control options, there is a medium to high level of uncertainty associated with estimates of control effectiveness. Little published information on control effectiveness is available, and estimates of effectiveness are primarily based on expert opinion, derived from field observation and experience, rather than rigorous scientific assessment.

- It is likely that individual control options would need to be used in combination, given that:

    - Within-herd transmission, between-herd spread and spillover to humans are each likely to involve more than one transmission pathway, and

    - No control measure is likely to be completely effective in limiting within-herd transmission, between-herd spread and spillover to humans.

- There is variation in the sustainability of the assessed control options.

- A number of long-term control options were identified, including preventive vaccination, manure management (based on systems that effectively reduce bacterial load, and therefore environmental contamination), changes to farm characteristics, wool shearing management, segregated lambing/kidding area, removal of risk material, visitor ban, control of other animal reservoirs and tick control. Each of these options, except for the last two, relate specifically to small ruminants. These options are ranked according to effectiveness, as assessed by expert opinion, in reducing spillover from domestic ruminants to humans.

- Several options were not considered sustainable for long-term control, but may have a role in the face of an outbreak, including the culling of pregnant animals, a temporary breeding ban, stamping out, identification and culling of shedders, control of animal movements and stand still. Each of these measures relate specifically to small ruminants. These options are ranked according to effectiveness, as assessed by expert opinion, in reducing spillover from domestic ruminants to humans.

- *C. burnetii* is highly resistant in the environment; consequently, persistent environmental contamination is a matter of concern.

- Vaccination can be used both to reduce the risk of future outbreaks (preventive vaccination) and in the face of an outbreak (outbreak vaccination), noting that:

    - Preventive vaccination is more effective than outbreak vaccination,

    - Phase I is more effective than phase II vaccination,

    - Vaccination is more effective in non-infected than infected animals,

    - Effectiveness may not be observed in the short-term.

RECOMMENDATIONS

TOR 1: To assess the significance of the occurrence of Q fever in the EU Member States for a better understanding of the scale and distribution of the disease and infection (with the focus on farm animals and humans):

- Harmonization of diagnostics and agreed case definitions are needed.
- PCR is suitable as a method for the routine diagnosis of *C. burnetii* infection in domestic ruminants, keeping in mind its strengths and weaknesses
- Improved diagnostics are needed:
    - To substantiate freedom from infection, and
    - In situations when a test-and-cull strategy needs to be implemented.
- Further work is needed to better understand the pathogenesis of Q fever in different animal species to facilitate the development of improved diagnosis to distinguish:
    - Antibodies to phase I and phase II antigens, and
    - Vaccinated and naturally infected animals.
- There is a need for harmonized field and laboratory data collection about *C. burnetii* infection in animals in EU MS, to allow comparison of prevalence/incidence estimates over time and between countries.
- There is a need to strengthen systems to promote rapid identification and reporting of Q fever in animals (abortion episodes).
- Consideration should be given for support of early information exchange between veterinarians and public health counterparts regarding potential events with zoonotic potential, including Q fever.
- Further studies on the estimation of Q fever burden of disease in humans are needed.

TOR 2: To assess the risk factors for Q fever occurrence and persistence in animal husbandry, and the related risks for humans, taking into account at least the presence and density of susceptible livestock and the type of husbandry in which they are kept:

- Prevalence and incidence studies in domestic ruminants should focus on small ruminants, rather than cattle, to provide a clearer picture of the risk of exposure for humans.
- Further investigations and research are needed to:
    - Clarify the relationship between genotype and virulence, the molecular basis for virulence, and the relationship between mice models and virulence studies in domestic ruminants,
    - Determine the host specificity of different bacterial isolates,
    - Identify factors influencing the maintenance of infection, including an improved understanding of transmission pathways,
    - Quantify the number of bacteria excreted under different conditions,

- Quantify survival of *C. burnetii* in the environment,
- Clarify the role of environmental contamination and climatic factors in the spillover of infection from animals to humans, and
- Clarify the trade off between farm density and farm size in the maintenance and spillover of infection.

TOR 3: To assess the effectiveness and efficiency of disease control options such as vaccination, pharmaceutical treatments, establishing animal movement restrictions, the culling of animals, etcetera.

- Future investigations are needed to objectively assess the effectiveness of control options for *C. burnetii* infection in domestic ruminant populations. Assessment should focus on relevant epidemiological parameters, including rates of within-herd transmission, between-herd spread and spillover from animal populations to humans.
- Control measures should generally be used in combination, given that:
  - Within-herd transmission, between-herd spread and spillover to humans are each likely to involve more than one transmission pathway, and
  - No control measure is likely to be completely effective in limiting within-herd transmission, between-herd spread and spillover to humans.
- Longer-term measures to control *C. burnetii* infection in domestic ruminants should be considered in those situations where the public health risk is considered unacceptable. Control measures where effectiveness at reducing spillover was assessed as either high or medium include preventive vaccination, appropriate manure management, changes to farm characteristics, wool shearing management, segregated lambing/kidding area, removal of risk material and visitor ban.
- Control measures to address proximity between humans and small ruminants, particularly around the time of lambing/kidding, would be expected to meaningfully contribute to a reduction in spillover from animal populations to humans.
- There is a need to build awareness among farmers and veterinarians of *C. burnetii* infection in farmed ruminants, including risk factors for spillover from domestic ruminant populations to humans.
- Short term measures such as the culling of pregnant animals, a temporary breeding ban, stamping out, identification and culling of shedders, control of animal movements and stand still could be used to reduce shedding from infected animals. However, other measures (including preventive vaccination, manure management, wool shearing management, segregated lambing/kidding area, removal of risk material) should be considered to reduce human exposure through environmental contamination.
- Vaccination should be considered as a long-term control measure. In order to better assess vaccine efficacy, it is recommended that field and experimental data are gathered, to improve our understanding of the epidemiology of *C. burnetii* infection in, and between, infected flocks, both prior to and following vaccination.
- Antibiotic treatment is not effective in substantially reducing either the level or duration of bacterial shedding in domestic ruminant populations; therefore the use of antibiotics cannot be recommended.

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APPENDICES

APPENDIX A - LITERATURE SEARCH

ISI search

Qualifiers

Disease:
Q fever OR Q fever or *Coxiella* OR C *burnetii* OR *burnetii* OR Query fever OR Nine Mile OR Balkan grippe

AND

Host:
Cow$ OR Cattle OR Bovine OR Calf$ OR Bull$ OR Heifer$ OR Livestock OR Ruminant$ OR Herd$ OR Sheep OR Ram$ OR Lamb$ Or Flock$ OR Ovis OR Ovine OR Ewe OR Goat OR Capra OR Kid OR billy goat OR he-goat OR farm animals OR farm Or Domestic or host$
Time frame - Year of publication: 2005 - 2010
409 *reports from ISI 4 /2/2010*

AND

Location:
Europe OR Europa OR EU OR Austria OR Belgium OR Bulgaria OR Cyprus OR Denmark OR Germany OR Netherlands Or France OR United Kingdom OR UK OR Ireland OR Spain OR Portugal OR Luxembourg OR Switzerland OR Italy Or Greece OR Czech republic OR Slovakia OR Slovenia OR Hungary OR Romania OR Poland OR Sweden OR Finland OR Latvia Or Lithuania Or Estonia Or Norway OR Iceland Or Switzerland Or Turkey Or Croatia Or Macedonia Or Kosovo Or Albania Or Bosnia OR Montenegro Or Yugoslavia or Serbia
139 *reports from ISI 4/2/2010*

PubMed search

Title/abstract
Q fever" or Q fever or *Coxiella* or "C *burnetii*" or *burnetii* or "Query fever" or "Nine Mile" or Balkan grippe) AND (Cow$ OR Cattle OR Bovine OR Calf$ OR Bull$ OR Heifer$ OR Livestock OR Ruminant$ OR Herd$ OR Sheep OR Ram$ OR Lamb$ Or Flock$ OR Ovis OR Ovine OR Ewe OR Goat OR Capra OR Kid OR billy goat OR he-goat OR "farm animals" OR farm Or Domestic or host$) AND (Europe OR Europa OR EU OR Austria OR Belgium OR Bulgaria OR Cyprus OR Denmark OR Germany OR Netherlands Or France OR United Kingdom OR UK OR Ireland OR Spain OR Portugal OR Luxembourg OR Switzerland OR Italy Or Greece OR Czech republic OR Slovakia OR Slovenia OR Hungary OR Romania OR Poland OR Sweden OR Finland OR Latvia Or Lithuania Or Estonia Or Norway OR Iceland Or Switzerland Or Turkey Or Croatia Or Macedonia Or Kosovo Or Albania Or Bosnia OR Montenegro Or Yugoslavia or Serbia
Time frame : 2005 - 2010
434 *reports from PubMed 4 /2/2010*
119 *reports from ISI 4 /2/2010*

Combine results of ISI and PubMed search 138 + 119 = 257
Automatic removal of the duplicates
220 combined result
Manual removal of the duplicates
183

The search sensitivity was verified by comparison with a set of records provided by the WG of experts.

Relevance criteria

The title and abstracts were screened independently for relevance by 2 reviewers following the criteria described below:

Criterion	included	excluded
Language publication	English abstract and text available in English, French, Spanish, Dutch, German	Abstract not available or text not in English, French, Spanish, Dutch, German
Concerns epidemiological aspects of *C.burnetii* infection in humans and or animals	yes	no
Concerns diagnostic of *C.burnetii*infection	yes	no
Concerns control options for *C.burnetii* infection in animals	yes	no
Constitutes a review paper including epidemiological aspects of *C.burnetii* infection in humans and or animals	yes	no

Disagreements were discussed by the 2 reviewers. When no consensus was possible the record was included for review of the full text.

Relevant:110
Not relevant : 59

A second relevance screening of titles and abstracts was conducted based on the below criteria leading to the inclusion of the records in 5 groups.

Groups	Criterion	included	excluded	Results*
1	Primary research that provides information on Q fever epidemiological aspects.	yes	no	77
2	Primary research that provides information on diagnostic of *C.burnetii* infection	yes	no	23
3	Primary research that concerns control options for *C.burnetii* infection in animals	yes	no	7
4	Constitutes a review paper including epidemiological aspects of *C.burnetii* infection in humans and or animals	yes	no	9
5	Concerns food safety aspects	yes	no	4

*Some records were included in more than 1 category

Data extraction

Group 1 records full text were classified also in terms of study design and sampling strategy

Data was extracted from 22 records; results are presented in Table 4 – Animal seroprevalence

APPENDIX B - *AD HOC* EFSA CONSULTATION (2010)

In order to collect Q fever occurrence data from EU MS and neighbour countries a email and questionnaire was send by EFSA (see bellow) on the 1/2/2010.The request was distributed to both the Animal Health and Welfare network and the Zoonosis task force and deadline of 10/2/2010 was established. Replies were received from 24 of 27 EU MS plus Norway.

"Dear Task Force member and Animal Health network member,

I am contacting you about your latest data on Q fever in animals. This disease is now topical due to the Q fever outbreak in the Netherlands.

The European Commission has sent EFSA a mandate (see attached) asking for scientific advice about Q fever, specifically on the significance of the occurrence of Q fever in the EU Member States, the risk factors related to Q fever infection in animals and possible control options.
EFSA's Animal Health and Welfare panel (AHAW) is taking the lead in this mandate and they will prepare a scientific opinion on the Q fever to address Commission's questions. The opinion has a tight time line, it should be provided by 30 April 2010.

The AHAW working group on Q fever is collecting available information at Member States level to describe the current Q fever situation in animals within the EU, specifically data is required to address the questions below:

- *Number of farm-level outbreaks (clinical disease) where Q fever was confirmed*
- *Number of animal-level tests conducted [surveillance, clinical investigation], number of positive*
- *Whether there is a programme of active Q fever surveillance [representative, non-representative sampling]: % farms seropositive, % animals seropositive*

To answer these questions we would like to ask if you could provide ***data on Q fever in animals in 2009*** *by filling in the excel worksheet* ***QFEVER*** *. If you have additional data on testing for Coxiella burnetii could you report it in the worksheet* ***OtherData****. Additionally could you report your* ***cattle, sheep and goat populations*** *for 2009 (and for 2008 and 2007 if you have not previously provided this).*

Please note that AHAW working group already has received the data reported on Q fever and animal populations by the Member States in the framework of the annual zoonoses reporting (Directive 2003/99/EC) for the years 2005-2008. Thus, there is no need to report this data again.

We would be most grateful if you could send the data to us by the ***10th FEBRUARY 2010****. The formal reporting of the Q fever in animals results in the framework of the zoonoses reporting (Directive 2003/99/EC) will take place later, by end May through the zoonoses reporting web application, as usual.*

You can send your information to us by e-mail to qfever@efsa.europa.eu and we would ask you to use the attached excel file.

This e-mail is sent both to the members of Task Force of Zoonoses Data Collection and of AHAW network members. We would kindly ask you to coordinate together at the national level regarding this request.

Thank you in advance for your support and collaboration! In case you have any questions, do not hesitate to contact us (ana.afonso@efsa.europa.eu or milen.georgiev@efsa.europa.eu)

With the best regards"

Total Tested: Please provide the total number of animal-level tests, and the total number of these that were positive, *Coxiella burnetii* (Q fever) in Goats, Sheep and Cattle in 2009 in your country

Animal species	Source of information	Animals tested	Animals positive for *Coxiella burnetii*	Herds tested	Herds positive for *Coxiella burnetii*
Cattle					
Sheep					
Goats					

Clinical Investigations: 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 tested and the number of herds testing positive in response to clinical investigations to allow an assessment of the number of outbreaks occuring in your country in 2009.

Animal species	Sampling Stage	Test Type	Source of information	Animals tested	Animals positive for *Coxiella burnetii*	Herds tested	Herds positive for *Coxiella burnetii*
Cattle							
Sheep							
Goats							

Monitoring & Surveillance: Please provide the results of testing for *Coxiella burnetii* (Q fever) in the context of surveillance, monitoring and national surveys. 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 in 2009.

Animal species	Sampling Stage	Sampling Context	Test Type	Source of information	Animals tested	Animals positive for *Coxiella burnetii*	Herds tested	Herds positive for *Coxiella burnetii*
Cattle								
Sheep								
Goats								

APPENDIX C - INFECTION AND DISEASE INFORMATION

Table 15 : Number of laboratory tests conducted in European countries for *C. burnetii* in domestic ruminants and number (%) positive, by species, based on data collected in the EFSA/ECDC zoonoses database about 2006-2008 and collected from *ad hoc* EFSA consultation (2010) about data in 2009

CATTLE													
	Animal population in 2008	2006			2007			2008			2009		
Country		No test	No pos	%	No test	No pos	%	No test	No pos	%	No test	No pos	%
Austria	1 997 209	863	72	8.3	1070	16	1.5	1147	13	1.1	926	31	3.4
Belgium	2 618 040	166	4	2.4	220	73	33.2	314	25	8.0	1692	301	17.8
Bulgaria	584 810	.	.	.	3366	368	10.9	249	27	10.8	3353	161	4.8
Cyprus	*55 600										NA	NA	NA
Czech Republic	1 443 640										0	0	0
Denmark	1 598 038	236	59	25.0	.	.	.	.	.	.	205	33	16.1
Estonia	236 681										0	0	0
Finland	915 345	.	.	.	.	.	.	.	.	.	25	0	0
France	19 366 182	.	.	.	7	0	0.0	.	.	.	NA	NA	NA
Germany	12 987 543	11397	998	8.8	6936	742	10.7	11866	1270	10.7	NA	152	NA
Greece	768 890	.	.	.	.	.	.	.	.	.	2	1	50
Hungary	790 036	510	33	6.5	536	40	7.5	4	0	0.0	584	36	6.2
Ireland	6 232 527	.	.	.	.	.	.	.	.	.	283	23	8.1
Italy	5 967 760	552	86	15.6	464	114	24.6	1743	320	18.4	NR	NR	NR
Latvia	380 363										NR	NR	NR
Lithuania	731 250										NA	NA	NA
Luxembourg	196 300	.	.	.	.	.	.	.	.	.	23	0	0
Malta	16 836										NR	NR	NR
Netherlands	3 890 195	.	.	.	1062	1	0.1	1201	5	0.4	NA	NA	NA
Poland	6 080 517	51	0	0.0	91	2	2.2	1130	453	40.1	369	20	5.4
Portugal	1 478 774	170	0	0.0	147	6	4.1	.	.	.	1	0	0
Romania	*2 683 600										NR	NR	NR
Slovakia	504 797	7334	373	5.1	7587	224	3.0	5786	281	4.9	NR	NR	NR
Slovenia	469 983	.	.	.	.	.	.	1305	59	4.5	415	17	4.1
Spain	5 554 871	.	.	.	.	.	.	.	.	.	198	60	30.3
Sweden	1 559 725	.	.	.	.	.	.	.	.	.	33	1	3.0
United Kingdom	10 106 985	.	.	.	.	1	.	.	2	.	1373	2	0.14
United Kingdom (N.I.)		.	.	.	.	.	.	5182	323	6.2	NA	NA	NA
Norway		.	.	.	.	.	.	.	.	.	68	0	0
Switzerland		.	.	.	.	.	.	2660	64	2.4	NR	NR	NR

SHEEP													
Country	Animal population in 2008	2006			2007			2008			2009		
		No test	No pos	%	No test	No pos	%	No test	No pos	%	No test	No pos	%

Austria	389 379	78	18	23.1	9	0	0.0	27	0	0.0	34	0	0
Belgium	205 624	4	0	0.0	1	0	0.0	2	0	0.0	1	0	0
Bulgaria	1 293 909	.	.	.	3410	381	11.2	820	41	5.0	1709	116	6.8
Cyprus	267 300*										NA	NA	NA
Czech Republic	197 823										0	0	0
Denmark	173 131										61**	0	0
Estonia	64 087										0	0	0
Finland	122 218										NA	NA	NA
France	7 715 207	.	.	.	330	133	40.3	.	.	.	NA	NA	NA
Germany	2 537 791	1425	96	6.7	527	31	5.9	1880	194	10.3	NA	40	NA
Greece	5 550 390	.	.	.	202	41	20.3	30	8	26.7	35	1	2.9
Hungary	1 114 681	70	3	4.3	27	2	7.4	4	0	0.0	82	3	3.66
Ireland	3 422 900*										NA	NA	NA
Italy	7 385 812	1856	182	9.8	903	150	16.6	9	0	0.0	NR	NR	NR
Latvia	66 801										NR	NR	NR
Lithuania	51 327										NA	NA	NA
Luxembourg	8 100*										NA	NA	NA
Malta	12 942										NR	NR	NR
Netherlands	1 212 956	.	.	.	144	0	0.0	129	13	10.1	NA	NA	NA
Poland	310 689	.	.	.	1	.	.	.	.	.	0	0	0
Portugal	3 144 600*	55	1	1.8	75	0	0.0	727	64	8.8	15	0	0
Romania	8 881 600*										NR	NR	NR
Slovakia	372 039	3200	19	0.6	3758	3	0.1	1476	0	0.0	NR	NR	NR
Slovenia	138 958										4669**	155	3.3
Spain	22 439 727	.	.	.	.	.	.	.	.	.	27	12	44.4
Sweden	508 921										0	0	0
United Kingdom	33 131 009	.	.	.	.	.	.	.	2	.	1709	0	0
United Kingdom (N.I.)		.	.	.	.	.	.	1022	126	12.3	NA	NA	NA
Norway		.	.	.	.	.	.	.	.	.	627	0	0
Switzerland		.	.	.	.	.	.	141	2	1.4	NR	NR	NR

GOATS													
Country	**Animal population in 2008**	**2006**			**2007**			**2008**			**2009**		
		No test	**No pos**	**%**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	77 655	20	6	30.0	5	0	0.0	109	11	10.1	90	2	2.2
Belgium	48 379	2	0	0.0	.	.	.	2	0	0.0			
Bulgaria	238 157	.	.	.	.	.	.	25	3	12.0	774	58	7.49
Cyprus	318 400*										NA	NA	NA
Czech Republic	23 459										0	0	0
Denmark	23 142										61**	0	0
Estonia	2 166										0	0	0
Finland	5 918										NA	NA	NA
France	1 267 198	.	.	.	110	33	30.0	.	.	.	551	82	14.9

Germany	190 000*	227	17	7.5	190	20	10.5	499	78	15.6	NA	24	NA
Greece	2 715 177	.	.	.	114	17	14.9	.	.	.	5	4	80
Hungary	66 000*	50	1	2.0	76	0	0.0	14	0	0.0	62	1	1.61
Ireland	7 800*										NA	NA	NA
Italy	951 158	291	15	5.2	141	13	9.2	11	2	18.2	NR	NR	NR
Latvia	12 973										NR	NR	NR
Lithuania	7 386										NA	NA	NA
Luxembourg	2 900										NA	NA	NA
Malta	6 402										NR	NR	NR
Netherlands	354 878	.	.	.	74	7	9.5	160	51	31.9		58***	
Poland	37 238	.	.	.	.	.	.	.	.	.	1	0	0
Portugal	495 900*	7	4	57.1	24	5	20.8	5	3	60.0	37	0	0
Romania	898 300*										NR	NR	NR
Slovakia	7 775	176	0	0.0	227	0	0.0	130	2	1.5	NR	NR	NR
Slovenia	24 228										4669**	155	3.3
Spain	2 877 611	.	.	.	.	.	.	.	.	.	131	82	62.6
Sweden	5 509	.	.	.	.	.	.	.	.	.	3	0	0
United Kingdom	96 156	.	.	.	.	3	.	.	1	.	19	1	5.3
United Kingdom (N.I.)		.	.	.	.	.	.	54	5	9.3	NA	NA	NA
Norway		.	.	.	.	.	.	.	.	.	31	0	0
Switzerland		.	.	.	.	.	.	139	9	6.5	NR	NR	NR

NA – information not available
NR – no reply to the consultation in 2010
*Data source - EUROSTAT
** sheep and goats (not specified exact number per species)
*** herd level data

Table 16 : Details of tests conducted in European countries during 2009 as part of a clinical investigation for *C. burnetii* in domestic ruminants, by species and country, based on data collected from *ad hoc* EFSA consultation (2010)

CATTLE								
			Animals			**Herds**		
Country	**Sampling stage**	**Test**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	at farm-blood	CF	926	31	3.3	.	26	.
Belgium	at farm-blood	ELISA	680	58	8.5	.	.	.
	at farm-milk	PCR	16	6	37.5	.	.	.
	at farm-organ/tissue	PCR	125	23	18.4	.	.	.
Bulgaria	NA							
Cyprus	NA							
Czech Republic	NA							
Denmark	placenta or fetus material from abortions	FISH technique	14	1	7.1	.	.	.
Estonia			0	0	0			
Finland	at farm-blood	ELISA	25	0	0.0	.	.	.
France	NA							
Germany	NA							

Greece	at farm-blood	ELISA	2	1	50.0	1	1	100.0
Hungary	at farm-blood	CF	453	34	7.5	.	19	.
	at farm-organ/tissue	immunohistochemical test	131	2	1.5	.	2	.
Ireland	at farm-blood	ELISA	4	4	100.0	1	1	100.0
Italy	NR							
Latvia	NR							
Lithuania	NA							
Luxembourg	at farm-organ/tissue	PCR	23	0	0.0	8	0	0.0
Malta	NR							
Netherlands	at farm-organ/tissue	PCR				NA	0	0
Poland		CF	113	20	17.7	8	2	25.0
Portugal	at farm-organ/tissue	PCR	1	0	0.0	1	0	0.0
Romania	NR							
Slovakia	NR							
Slovenia	NA							
Spain	at farm-blood	ELISA	198	60	30.3	8	2	25.0
Sweden	at farm-bulk milk	ELISA	0	0	.	2	0	0.0
	at farm-individual blood	ELISA	16	1	6.3	2	1	50.0
	at farm-individual milk	PCR	1	0	0.0	1	0	0.0
	at farm-organ/tissue	PCR	1	0	0.0	1	0	0.0
United Kingdom	at farm-organ/tissue	CF	1373	2	0.1	874	2	0.2
Norway	at farm-blood		1	0	0.0	1	0	0.0
Switzerland	NR							

SHEEP								
			Animals			**Herds**		
Country	**Sampling stage**	**Test**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	at farm-blood	CF	34	0	0.0	.	0	.
Belgium	at farm-organ/tissue	PCR	1	0	0.0	.	.	.
Bulgaria	NA							
Cyprus	NA							
Czech Republic	NA							
Denmark	NA							
Estonia			0	0	0			
Finland	NA							
France	NA							
Germany	NA							
Greece	at farm-blood	ELISA	35	1	2.9	4	1	25
Hungary	at farm-blood	CF	40	0	0.0	.	0	.
	at farm-organ/tissue	immunohistochemical test	42	3	7.1	.	2	.
Ireland	NA							
Italy	NR							
Latvia	NR							
Lithuania	NA							
Luxembourg	NA							
Malta	NR							
Netherlands	at farm-organ/tissue	PCR				NA	0	0
Poland	NA							
Portugal	at farm-organ/tissue	PCR	15	0	0.0	.	0	.
Romania	NR							
Slovakia	NR							
Slovenia	NA							

Spain	at farm-blood	Indirect Immunofluorescence assay (IFA)	27	12	44.4	2	1	50
Sweden	NA							
United Kingdom	at farm-organ/tissue	CF	1709	0	0.0	816	0	0
Norway	at farm-blood	ELISA	25	0	0.0	3	0	0
Switzerland	NR							

GOATS								
			Animals			**Herds**		
Country	**Sampling stage**	**Test**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	at farm-blood	CF	90	2	2.2	.	2	.
Belgium	NA							
Bulgaria	NA							
Cyprus	NA							
Czech Republic	NA							
Denmark	NA							
Estonia			0	0	0			
Finland	NA							
France	NA							
Germany	NA							
Greece	at farm-blood	ELISA	5	4	80.0	1	1	100.0
Hungary	at farm-blood	CF	60	0	0.0	.	0	.
	at farm-organ/tissue	immunohistochemical test	2	1	50.0	.	1	.
Ireland	NA							
Italy	NR							
Latvia	NR							
Lithuania	NA							
Luxembourg	NA							
Malta	NR							
Netherlands	at farm-organ/tissue	PCR				NA	6	NA
Poland		CF	1	0	0.0	1	0	0.0
Portugal			37	0	0.0	.	0	.
Romania	NR							
Slovakia	NR							
Slovenia	NA							
Spain	at farm-blood	Indirect Immunofluorescence assay (IFA)	131	82	62.6	4	3	75.0
Sweden	at autopsy blood	ELISA	3	0	0.0	1	0	0.0
United Kingdom	at farm-organ/tissue	CF	19	1	5.3	15	1	6.7
Norway	NA							
Switzerland	NR							

Indirect Immunofluorescence assay (IFA), Enzyme Linked Immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR), Complement fixation (CF)

* Investigation in relation to clinical symptoms in the population (animals)

NA – information not available; NR – no reply to the consultation in 2010

Table 17 : Details of tests conducted in European countries during 2009 as part of a monitoring and/or surveillance activities for *C. burnetii* in domestic ruminants, by species and country, based on data collected from *ad hoc* EFSA consultation (2010)

CATTLE								
			Animals			Herds		
Country	**Sampling stage**	**Test**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	NA							
Belgium	at farm-bulk milk	ELISA	.	.	.	1043	727	69.7
	at farm-bulk milk	PCR	.	.	.	159	37	23.3
	at farm-milk	ELISA	871	214	24.6	364	270	74.2
Bulgaria		CF	3353	161	4.8	168	67	39.9
Cyprus	NA							
Czech Republic			0	0	0			
Denmark	NA							
Estonia			0	0	0			
Finland	at farm-bulk milk	ELISA	.	.	.	1871	2	0.2
France	NA							
Germany	NA							
Greece	NA							
Hungary			0	0	0			
Ireland	at farm-blood	ELISA	279	19	6.8	202	19	9.4
Italy	NR							
Latvia	NR							
Lithuania	NA							
Luxembourg	NA							
Malta	NR							
Netherlands						0	0	0
Poland		CF	256	0	0.0	11	0	0.0
Portugal	NA							
Romania	NR							
Slovakia	NR							
Slovenia	at farm-blood	ELISA	415	17	4.1	76	9	11.8
Spain	NA							
Sweden	at AI station-blood	CF	14	0	0.0	.	.	.
United Kingdom	NA							
Norway	at farm-blood	ELISA	67	0	0.0	45	0	0.0
Switzerland	NR							

SHEEP								
			Animals			Herds		
Country	**Sampling stage**	**Test**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	**NA**							
Belgium	**NA**							
Bulgaria		CF	1709	116	6.8	76	40	52.6
Cyprus	NA							
Czech Republic			0	0	0			
Denmark	NA							
Estonia			0	0	0			
Finland								
France	NA							
Germany	NA							
Greece	NA							
Hungary			0	0	0			

Ireland	NA							
Italy	NR							
Latvia	NR							
Lithuania	NA							
Luxembourg	NA							
Malta	NR							
Netherlands	at farm-milk	PCR				40**	0	0
Poland	NA							
Portugal	NA							
Romania	NR							
Slovakia	NR							
Slovenia	NA							
Spain	NA							
Sweden	NA							
United Kingdom	at farm-blood	ELISA	5737	53	0.9	.	.	.
Norway	at farm-blood	ELISA	602	0	0.0	121	0	0.0
Switzerland	NR							

GOATS								
			Animals			**Herds**		
Country	**Sampling stage**	**Test**	**No test**	**No pos**	**%**	**No test**	**No pos**	**%**
Austria	**NA**							
Belgium	**NA**							
Bulgaria		CF	774	58	7.5	51	26	51
Cyprus	NA							
Czech Republic			0	0	0			
Denmark	NA							
Estonia			0	0	0			
Finland								
France	blood	ELISA	402	72	17.9	10	8	80
	vaginal swab	PCR	149	10	6.7	10	2	20
Germany	NA							
Greece	NA							
Hungary			0	0	0			
Ireland	NA							
Italy	NR							
Latvia	NR							
Lithuania	NA							
Luxembourg	at farm-milk	PCR	.	.	.	2	1	50
Malta	NR							
Netherlands	at farm-milk	PCR				360	58	16.1
Poland	NA							
Portugal	NA							
Romania	NR							
Slovakia	NR							
Slovenia	NA							
Spain	NA							
Sweden	NA							
United Kingdom	at farm-blood	ELISA	513	4	0.8	.	.	.
Norway	at farm-blood	ELISA	31	0	0.0	1	0	0
	at farm-bulk milk	ELISA	.	.	.	348	0	0
Switzerland	NR							

Indirect Immunofluorescence assay (IFA), Enzyme Linked Immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR), Complement fixation Test (CFT)

* Investigation in relation to clinical symptoms in the population (animals)

[a]The data was extracted from a EFSA *ad hoc* consultation (2010) and relate to data collection classifiers Surveillance-objective sampling
** herd level data
NA – information not available; NR – no reply to the consultation in 2010

Table 18: Selected number of human outbreaks of Q fever in Germany

Year	Area	No of cases	Source	Risk factors	Comments	Reference
2010	Baden-Würtemberg	235		being a farmer or waste control worker; seeing rodents regularly during work; close and direct contact to animals e.g. goats, cattle and (pet) rats; living in sight of a goat or sheep stable; sheep densitiy; increasing age	seroprevalence study and risk factor analysis; three years study	Brockmann et al., in press
2009	Paderborn, Westphalia	5	sheep	handling of pregnant animal, abortion	aquired during animal experiment (obstetrics); other earlier cases have been described for the university of Giessen, Hesse	Henning et al., 2009
2008	Aschaffenburg, Bavaria	>56	sheep	living near infected herds	15% children and youngsters	RKI, 2008
01/2008 to 3/2008	Lahn-Dill-Kreis, Hesse	>46	sheep	living or working near infected herds; walking over grazing grounds	16% children and youngsters; 26 of 30 tested sheep had an active *C.burnetii* infection; 5 sheep were 'positive' for *C. burnetii*	RKI, 2008 Hamann et al., 2009
07/2005 to 08/2005	Jena, Thuringia	>331	sheep	living near the grazing, grounds (400m); hot and dry weather; male gender; age of 25-64 years	Case definition: a. CFT Phase II antigen titer $\geq$ 1:80 or ELISA Phase II IgM positive b. fever $\geq$ 38.5°C, myalgy or headache lasting longer than 1 day during 13.06. and 28.07.	Gilsdorf et al., 2008 RKI, 2006
2003	Baden-Württemberg Not specified	8	cattle	calving; contact to stable	family members visiting the farm; calf died; ELISA and culture positive	RKI, 2004
2003	Soest, Nordrhine-Westphalia	299	sheep	contact to super spreading ewe and contaminated pen at 4th.05.; duration of contact; age (children had an attack rate of 3%, adults of >20%); high seroprevalence and positive PCR results in sheep flocks; susceptibility of human population; no correlation: wind, tick bites, consumption of unpasteurised goat or sheep	farmers' market; 25% of the animals of the herd tested positive in ELISA; case definition: symptom onset 4th.05. to 3rd.June, exclusion criterion: negative	Porten et al., 2006 RKI, 2003

				cheese, pregnancy, heart valve	IgM titer for Phase II antigens; shortest onset of disease 2 days; 25% hospitalised, no deaths 4 of 11 pregnant women were infected without further complications	
07/2001 till 08/2001	Munich, Bavaria	3	sheep	exposure to contaminated dust	movie team: working in a barn with wind generators for more than 10h; 75% of sheep tested serological positive, *Coxiella* positive placenta was found; 17 further human cases in neighbouring areas found	RKI, 2002
12/2000 till 05/2001	Hochsauerlandkreis Nordrhine-Westphalia; Waldeck-Francken berg, Hesse	75	sheep	contaminated dust	serological positive animals in herds	RKI, 2001
05/1999 till 08/1999	Dortmund, Nordrhine-Westphalia	82	sheep manure	exposure to farm, faeces and contaminated manure; steady wind; duration time of exposure; contact to dogs and cats; smoking cigarettes; no correlation: type of outdoor activity, precipitation and temperature, exposure to meadows with grazing sheep, consumption of unpasteurised goat or sheep cheese (another farm nearby located), contact to other farm animals or pigeons, tick bites, consumption of raw milk	700 sheep; lambing within a barn on farm; manure was stored in lambing barn till 3rd till 15th of May then displaced on fields nearby; 57% of herd animals were ELISA positive in serology;	Reintjes et al., 2000
1997	Baden-Württemberg not specified	12	fallow deer	contact	abortions, abnormal fetuses, high mortality (50%) of offspring in a herd of 71 fallow deer (*Cervus dama*); attack rate of 92% in humans	RKI, 1997
01/1996 to 06/1996	Rollshausen, county of Lohra,	56	sheep	contact to sheep, walking near by sheep farm; outdoor lambing; dry season; no correlation: drinking	75% of sheep tested were seropositive; small	Lyytikäinen et al., 1997

	Hesse			raw milk, tick bites, contact with other domestic animals, living on a farm with cattle, sheep, or goats, outdoor leisure activities	town with 300 inhabitants	Lyytikäinen et al., 1998
1994	Düsseldorf, Nordrhine-Westphalia	>18	sheep	hot and dry weather; wind; age (young persons being affected: average 38 years)		Schulze et al., 1996
4/1992 to 5/1992	Berlin	80	sheep	direct or indirect contact to a clinically diseased sheep herd at an animal hospital wind and dry season	contact to diseased animals during aid or section; contact to animal transporting van; contact to personnel of the hospital; serological and culture positive herd	Schneider et al., 1993 Molle et al., 1995b
1982/1983	Suhl, Thuringia, GDR	156	various ruminant species	direct or indirect contact	the only reported outbreak in the GDR	Stelzner et al., 1986 Kramer, 1990

Table 19: *C. burnetii* infection in animals -Germany

location	Year	Animals /herds	Tests	Prevalence animals/herds % / %	Comments	Reference
Lower Saxony	nd	nd / 95	Nd, seroprevalence	2.7 / nd	Prevalence of the agent:0.7	Runge and Ganter, 2008
Baden-Würtemberg	nd	3.460 sheep /nd	CFT >1:10 ELISA >0.4	0 to 1.4 (total: 0.5)/ nd 0.9 to 10.2 (total: 8.7) / nd	Samples collected in the four districts of BW; correlation CFT and ELISA 90.5%	Sting et al., 2004
Northerrn Baden-Württemberg	1998-2000	1.167 cattle / 105	ELISA antigen capture ELISA	1.4 to 2 / nd	Q fever correlated with abortion; herds with fertility problems	Sting et al., 2002
Baden-Würtemberg	1996/97	826 cattle / 38	CFT antigen capture ELISA	6.7 / nd 8.5 / nd	In th group fertility problems: 14.3% were positive with the Ag-ELISA versus 0.6% without problems; in CFT 9.0% versus 3.4%	Sting et al., 2000
Hannover, Lower Saxony	1992/93	500 cattle (random) / nd 665 cattle /	CFT >1:10	7.6 / nd 9.6 / 76.9 19.3 / 78.8 5.6 / 100	Correlation with herd size	Wittenbrink et al., 1994

		39 383 (fertility problems)/ 33 612 bulls / 1				
Southern Bavaria	1991	1.095 cattle / 21	ELISA	11.8 / 81.0	Survey also on reservoir animals	Rehacek et al., 1993
Arnsberg, Northrhine-Westphalia	1989/ 90	3.500 cattle / 155	ELISA, IFT >1:8	13.3 / 57.4 (within herd 24.7) 12.9 / 53.5 (within herd 21.0)	Correlation coefficient was 0.79; prevalence increased with herd size	Klemt and Krauss, 1991
Northern Bavaria		3.884 cattle/ 246	CFT	7.6 / 30.0	prevalence increased with herd size: big farms 61.1% and 9.5% of animals positive; small farms: 12.1% and 3.1% of animals positive	Roth and Bauer, 1986
Stuttgart, Baden-Würtemberg		2.109 cattle / 125 Farms with fertility problems: 1.064 cattle/ 208	CFT >1:5 CFT >1:10	8 / 35.2 24.4 / 48.1	describes eradication strategies	Woernle and Müller, 1986
Thuringia, GDR	1980ties	7.580 cattle / 17	CFT	19.4 /100		Lange et al., 1992
Thuringia, GDR	1980-1989	4.337 sheep / nd	CFT >1:20	1.1 / 47.0	Correlation to herd size and bad mangement	Lange et al., 1992

GLOSSARY AND ABBREVIATIONS

Ab	antibodies
CD	Council Decision
CFT	Complement fixation test
DIVA	Differentiate Infected from Vaccinated Animals
EC	European Commission
ECDC	European Center for Disease Prevention and Control
EFSA	European Food safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
FITC	Fluorescein isothiocyanate
IFA	Indirect Fluorescent Antibody
IHC	Imunohistochemistry
LCV	Large cell variant
MAT	Micro agglutination test
MIFT	Microimmunofluorescence test
MLVA	Multiple Loci VNTR Analysis
MS	Member states
MST	Multispacer sequence typing
NRL	National Reference Laboratory
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
SCV	Small cell variant
SDC	Small dense cell
ToR	Terms of reference
VNTR	Variable Number of Tandem Repeats
WG	Working group

Burden of disease - the impact of a health problem in an area measured by financial cost, mortality, morbidity, or other indicators.

Monitoring - system of collecting, analysing and disseminating data on the occurrence of disease, infectious agents and contamination. As opposed to surveillance, no active control options are taken when positive cases are detected (Dir. 2003/99).

Positive result - situation stating when the sample is considered to be positive for the agent.

Prevalence - the proportion of existing cases in a population at the specified time of testing.

Sample - set composed of one or several units or a portion of matter selected by different means in a population or in an important quantity of matter, which is intended to provide information on a given characteristic of the studied population or matter and to provide a basis for a decision concerning the population or matter in question or concerning the process which has produced it (Reg. (EC) No 2073/2005).

Sampling strategy - planned procedure for selecting samples from a population and for conducting the sampling in order to obtain the information needed.

Sampling unit - the unit which the specimens taken represent and which is considered either infected (contaminated) or not, based on the analyses result. For animal data, the sampling unit may be "*Animal*", "*Flock"*, "*Herd"*, "*Holding*" or "*Slaughter batch"*; for food data, the sampling unit might be "*Single"* or "*Batch"*.

Surveillance - a close and continuous observation for the purpose of control. As opposed to monitoring, control options are taken when positive cases are detected. This type of programme does often but not always have a defined target for diseases / contamination reduction.

Survey - study involving a sample of units selected from a well-delineated population. This (target) population is the entire set of units to which findings of the survey are to be extrapolated. The units to examine are to be selected randomly (Rothman, 1986 and Noordhuizen et al., 2001).

Suspect sampling following a recent case - unplanned selection of a sample, where the individual units are selected based on the recent judgement and experience regarding the occurrence of infection or contamination in the population, lot, or sampling frame, *e.g.* earlier positive samples. The samples obtained from this procedure are not randomly extracted.