Q fever in humans and farm animals in four European countries, 1982 to 2010

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Q fever is a disease of humans, caused by *Coxiella burnetii*, and a large range of animals can be infected. This paper presents a review of the epidemiology of Q fever in humans and farm animals between 1982 and 2010, using case studies from four European countries (Bulgaria, France, Germany and the Netherlands). The Netherlands had a large outbreak between 2007 and 2010, and the other countries a history of Q fever and Q fever research. Within all four countries, the serological prevalence of *C. burnetii* infection and reported incidence of Q fever varies broadly in both farm animals and humans. Proximity to farm animals and contact with infected animals or their birth products have been identified as the most important risk factors for human disease. Intrinsic farm factors, such as production systems and management, influence the number of outbreaks in an area. A number of disease control options have been used in these four countries, including measures to increase diagnostic accuracy and general awareness, and actions to reduce spillover (of infection from farm animals to humans) and human exposure. This study highlights gaps in knowledge, and future research needs.

Introduction

Q fever is a disease of humans [1,2]. The aetiological agent, *Coxiella burnetii*, is a Gram-negative and obligate intracellular bacterium. C. burnetti has also been isolated from a large range of animals including farm animals (e.g. cattle, sheep and goats), wildlife and arthropods [3]. It has a near worldwide distribution.

The febrile illness 'Query fever' (Q fever) was first reported in 1935, among workers in slaughterhouses in Australia [4]. Initial hypotheses about potential exposures and infectious pathways emerged following the development of illness in experimental animals (guinea pigs) via feeding of ticks [5] collected from febrile livestock in Nine Mile, United States. Investigations into cases of atypical pneumonia subsequently revealed the importance of aerosol transmission. Epidemiological linkages with animals were later identified, and infection was found in a broad range of hosts [1,3]. It was initially thought that Q fever was primarily an occupational risk (for people who worked closely with animals) however this was subsequently expanded, with risk groups also including people with a specific health status (pregnancy, cardiac diseases, immune-compromised). Blood donation was identified as a potential source of infection.

In Europe, cases of Q fever in humans were first reported from soldiers in the Balkan region including Bulgaria in 1940 [6], and subsequently in Germany shortly after World War II [2], and in the Netherlands in 1956 [7].

The course of human infection ranges from asymptomatic to severe, but typically results in a mild, self-limiting, influenza-like disease (acute infection). However, some patients develop a more serious chronic infection, including endocarditis and other complicated infections (e.g. vascular or osteoarticular infections). Infection by C. burnetti in pregnancy can also result in spontaneous abortion, premature delivery, low birth weight and the development of chronic C. burnetti infection [8]. The European Union (EU) harmonised Q fever case definition , in use since the year 2003, includes clinical (any person with at least one of the following three symptoms: fever, pneumonia, hepatitis),

laboratory (at least one of the following three diagnostic findings: isolation of *C. burnetii* from a clinical specimen, detection of *C. burnetii* nucleic acid in a clinical specimen, *C. burnetii* specific antibody response (IgG or IgM phase II)) and epidemiological (at least one of the following two epidemiological links: exposure to a common source, animal-to-human transmission) criteria [9].

In domestic ruminants, as in people, C. burnetti infection and Q fever (the disease) are not the same. C. burnetti infection is usually subclinical (i.e. the animal is infected with *C. burnetii* but without clinical signs). Q fever, which develops in a subset of infected animals, presents as late abortion and reproductive disorders [1,2,10,11]. A definitive diagnosis of Q fever in animals is based on the observation of the occurrence of abortions and/or stillbirths, confirmation of the presence of the aetiological agent (i.e. polymerase chain reaction (PCR), isolation, staining, immunofluorescence assay tests are positive) and positive serological findings in the herd [12].

Q fever has generally been associated with transient outbreaks in animals and humans, and sporadic human cases. Prompted by the outbreak of Q fever in the Netherlands that occurred from 2007 to 2010, concerns were raised by the European Commission about factors contributing to the development of large, sustained Q fever outbreaks [2]. The Dutch outbreak was considered to be the largest community outbreak ever recorded [2,13,14], with 4,026 human cases notified between 2007 and 2010 [15-17].

This paper presents a descriptive analysis, comparison and critical appraisal of the epidemiology of Q fever in humans and farm animals, including modes of transmission and control measures, using case studies from four European countries: Bulgaria, France, Germany and the Netherlands.

Methods

This study was conducted as a review of Q fever epidemiology in four European countries. These countries were chosen by experts of a working group of the European Food Safety Authority (EFSA) [2]. The EFSA working group comprised a group of scientists with specialised knowledge and experience on Q fever-related issues, who were assembled to collectively formulate a response to a range of risk-related questions posed by the European Commission. The four countries were chosen based on the following rationale: the Netherlands experienced a large Q fever outbreak between 2007 and 2010, France and Germany are countries in proximity to the Netherlands where Q fever is endemic and where a considerable number of relevant scientific data and publications are available, and Bulgaria is a country with both a long history of Q fever and, as in the Netherlands, with substantial changes in husbandry systems over time. For each of these four countries, information was collected on Q fever in humans and farm animals based on a detailed review of relevant peer reviewed and non-peer reviewed literature. Relevant literature was identified following interrogation of two publication databases, ISI Web of Knowledge and PubMed, using defined qualifiers for infection and disease (*C. burnetii* infection, Q fever), host (humans, farm animals), location (Bulgaria, France, Germany and the Netherlands)) and issue (epidemiology, diagnostics, control, review). The search was limited to literature published from 2005 to 2010, but relating to the period from 1982 to 2010. Additional national literature (both peer reviewed and non-peer reviewed) was obtained by working group members, and complemented with their expert knowledge and opinion, noting that EFSA working group included national experts on these issues from Bulgaria, France, Germany and the Netherlands [2]. Screening of published material was initially conducted by two reviewers of the working group, based on title and abstract, leading, if relevant to the above-mentioned qualifiers, to retrieval of the full paper for consideration in the current review and details available elsewhere [2]. A descriptive analysis was subsequently conducted.

Results

A total of 110 papers were retrieved, based on title and abstract, with 22 being retained, following further evaluation, for the current review.

Farm animals

Seroprevalence

The serological prevalence of *C. burnetii* infection in farm animals varies by host species, geographic area and time (Table 1), whereby it also should be noted that different serological cut-offs were used in different studies. Within-herd prevalence estimates for cattle were up to 20.8% in Bulgaria, 15.0% in France, 19.3% in Germany,, 21.0% in the Netherlands, for goats up to 40.0% in Bulgaria, 88.1% in France, 2.5% in Germany, 7.8% in the Netherlands, and for sheep up to 56.9% in Bulgaria, 20.0% in France, 8.7% in Germany, 3.5% in the Netherlands respectively. Herd prevalence estimates, whereby a herd is considered positive when at least one animal in the herd was serologically-confirmed, were higher than within-herd prevalence. Herd prevalence for cattle was up to 73.0%, in France, and up to 37.0 % in the Netherlands. For goats it was 40.0% in France and 17.8% in the Netherlands while for sheep values of 89.0% in France, and 14.5% in the Netherlands were respectively found. Regional differences were observed: up to four-fold among farm animals in different areas of Bulgaria [18], and higher in some rural German regions [19-21].

Clinical disease

Estimating the Q fever incidence in farm animals is difficult, due to the non-specific nature of disease on the one hand and the multifactorial nature of abortion on the other. Further, it is uncommon for detailed veterinary investigations to occur, including efforts towards

Table 1

Estimated prevalence of *Coxiella burnetii* infection in farm animals, based on studies conducted in Bulgaria, France, Germany and the Netherlands, 1982–2010

Country	Year of study	Number tested		% positive		Test	Reference
		Animals	Herds	Animals	Herds		
Cattle							
BG	1977–1988	20,086	NA	11.8	NA	CFT	[23]
BG	1989–2006	95,737	NA	5.4	NA	CFT	[23]
BG	2002	3,006	NA	8.2	NA	CFT	[23]
BG	2003	3,714	NA	6.5	NA	CFT	[23]
BG	2004	120	NA	20.8[a]	NA	IFA	[32]
BG	2004	3,188	NA	9.7	NA	CFT	[23]
BG	2005	3,026	NA	8.1	NA	CFT	[23]
BG	2006	2,932	NA	10.6	NA	CFT	[23]
DE	1991	1,095	21	11.8	81.0	ELISA	[75]
DE	1992–1993	500 665 383[b] 612	NA 39 33 1	7.6 9.6 19.3 5.6	NA 76.9 78.8 100.0	CFT	[76]
DE	1998	21,196	544	8.0	NA	ELISA	[1]
DE	1996–1997	826	38	14.3[b]	NA	ELISA	[77]
DE	1998–2000	1,167	105	1.4–2.0[b]	NA	ELISA	[78]
FR	NA	NA	NA	1.0–15.0	39–73	NA	[79]
NL	1987	1,160[b]	234	21.0	37.0	ELISA	[80]
NL	2007	2,781[c]	341	16.0	78.6	ELISA	[69]
NL	2007	2,781[c]	341	8.7	56.6	PCR	[69]
Goats							
BG	2002	677	NA	11.8	NA	CFT	[23]
BG	2003	1,044	NA	7.4	NA	CFT	[23]
BG	2004	50	NA	40.0a	NA	IFA	[32]
BG	2004	1,016	NA	21.7	NA	CFT	[23]
BG	2005	832	NA	11.1	NA	CFT	[23]
BG	2006	359	NA	19.2	NA	CFT	[23]
BG	1950–1976	1,417	NA	20.5	NA	CFT	[23]
BG	1977–1988	1,791	NA	10.8	NA	CFT	[23]
BG	1989–2006	54,175	NA	7.6	NA	CFT	[23]
FR	2006	359	NA	36.0	NA	ELISA	[81]
FR	2006	NA	42	88.1	NA	ELISA	[81]
FR	2006	75	NA	65.3[b]	NA	ELISA	[82]
FR	2008	1,057	NA	32.0	NA	ELISA	[81]
FR	2008	42	NA	88.1	NA	ELISA	[81]
FR	NA	NA	NA	2.0–12.0	10–40.0	NA	[79]
DE	1998	278	NA	2.5	NA	ELISA	[1]
NL	1987	498	NA	1.0	NA	ELISA	[80]
NL	2008	3,409	NA	7.8	NA	ELISA	[15]
NL	2008	NA	NA	7.8	17.8	NA	[13]
Sheep							
BG	2002	1,819	NA	12.7	NA	CFT	[23]
BG	2003	1,811	NA	8.3	NA	CFT	[23]
BG	2004	100	NA	21.0a	NA	IFA	[32]
BG	2004	1,258	NA	14.1	NA	CFT	[23]
BG	2005	1,911	NA	15.2	NA	CFT	[23]
BG	2006	1,925	NA	8.4	NA	CFT	[23]
BG	1950–1976	17,088	NA	16.7	NA	CFT	[23]
BG	1977–1988	16,593	NA	18.8	NA	CFT	[23]
BG	1989–2006	99,189	NA	4.8	NA	CFT	[23]
BG	NA–2006	153	NA	56.9[b]	NA	CFT	[62]
DE	NA	NA	95	NA	2.7	NA	[83]
DE	1983–1986	4,337	NA	0.6–4.3	NA	CFT	[40]
DE	1998	1,346	NA	1.3	NA	ELISA	[1]
DE	1999	100	1	57.0	NA	ELISA	[1]
DE	NA	3,460	NA	8.7	NA	ELISA	[84]
FR	NA	NA	NA	0–20.0	0–89.0	NA	[79]
NL	1987	3,603	NA	3.5	NA	ELISA	[80]
NL	2008	12,363	NA	2.4	NA	ELISA	[15]
NL	2008	NA	NA	2.4	14.5	NA	[13]

BG: Bulgaria; CFT: complement fixation test; DE: Germany; ELISA: enzyme-linked immunosorbent assay; FR: France; IFA: indirect immunofluorescence assay; NA: information not available or not specified; NL: Netherlands; PCR: polymerase chain reaction.

[a] Investigation in relation to a human outbreak.

[b] Investigation in relation to clinical signs in the animal population.

[c] Lactating cows.

Table 2

Estimated prevalence of *Coxiella burnetii* infection in people, based on studies conducted in Bulgaria, France, Germany and the Netherlands, 1982–2010

Country	Year of study	Number tested	Sample group	% positive	Test	Reference
BG	1993–2000	14,353	RG	15.0	CFT, MIFT	[23]
BG	1995–1997	224	BD	38.0	MAT, MIFT	[29]
BG	2001–2004	5,207	RG	18.0	CFT, MIFT	[23]
BG	2004	104	HO (PW)	7.7	IFA	[32]
DE	2002	255	HO	22.0	NA	[78]
FR	1982–1990	22,496	RG	23.0	NA	[8]
FR	1988	924	BD	4.0	IFA	[85]
FR	1995	790	BD	1.0	IFA	[1]
FR	1995–1996	785	NA	5.0	IFA	[1]
FR	1996	620	BD	3.0	IFA	[1]
FR	1996	12,716	NA	0.2	IFA	[1]
FR	1996	208	RG	71.0	IFA	[86]
FR	2002–2003	376	RG (PW)	2.6	IFA	[87]
FR	2002–2003	91	RG (CA)	5.5	IFA	[87]
FR	2002–2003	578	HO	14.7	IFA	[87]
NL	1982	222	RG	83.8	NA	[88]
NL	1983	359	BD	24.0	NA	[88]
NL	2006–2007	5,654	GP	2.4	ELISA, IFA	[89]
NL	2007–2009	2,004	HO (PW)	9.1	IFA	[90]
NL	2009	543	BD	12.2	ELISA, IFA	[91]

BD: blood donors; BG: Bulgaria; CA: cardiac abnormalities; CFT: complement fixation test; DE: Germany; ELISA: enzyme-linked immunosorbent assay; FR: France; GP: general population; HO: humans in outbreak areas; IFA: indirect immunofluorescence assay; MAT: microagglutination test; MIFT: microimmunofluorescence test; NA: information not available or not specified; NL: Netherlands; PW: pregnant women; RG: risk group.

laboratory confirmation of the causative agent, following a single abortion in a herd or flock. During the outbreak in the Netherlands between 2007 and 2010, an average of 20% (range of 10–80%) of pregnant goats aborted on affected farms. On two affected sheep farms in the Netherlands, the estimated abortion rate was 5% [13,15]. From 0.5 to 3.8% of abortions in cattle were attributed to *C. burnetii* in surveys in Germany during the period from 1993 to 1996 [2]. Clinical disease (with abortions attributed to *C. burnetii* infection) in five of 21 goat flocks were observed over five years in Deux-Sevres, France [22]. The disease is well recognised among the veterinary community in all four countries, and it has been notifiable in dairy sheep and goats at EU level in Bulgaria, Germany and the Netherlands since 2008. This was not the case in France [2], which may have an influence on the number of cases being reported.

Humans

Seroprevalence

Estimates of prevalence of *C. burnetii* infection, based on serological studies conducted in the four countries since 1982, are presented in Table 2. It should be noted that different serological cut-offs were used in different studies. There is large variability in the overall seroprevalence in the sampled population groups: in the general population, 2.4% in the Netherlands; among blood donors, 1.0 to 4.0% in France, 12.2 to 24.0% in the Netherlands, 22.0% in Germany and 38.0% in Bulgaria; in risk groups 15.0 to 18.0% in Bulgaria (patients presenting with atypical pneumonia and cardio-vascular diseases), 2.6 to 71.0% in France (pregnant woman, patients with cardiac diseases, persons involved in goat breeding, veterinarians; seroprevalence was highest among the latter two groups), 83.8% in the Netherlands (veterinarians dealing with livestock); in humans in outbreak areas, 7.7% in Bulgaria (pregnant women), 9.1% in the Netherlands (pregnant women), 14.7% in France (post epidemic surveillance in outbreak areas among people not considered at higher than normal risk) and 22.0% in Germany (farmers whose livestock experienced abortions).

Clinical disease

In all four countries, Q fever varies considerably in terms of geographic distribution, case numbers and clinical presentation. Disease was notifiable in humans at the national level throughout the full study period (1982–2010) in Bulgaria, Germany and the Netherlands and not in France. Since 2000, Q fever in humans must be monitored and notified within the EU, as required under EU legislation (Commission Decision 2000/96/

Table 3

Reported Q fever outbreaks in the human population in Bulgaria, France, Germany and the Netherlands, 1982–2010

Country: region	Year	Most likely source	Number of cases	Laboratory diagnosis	Reference(s)
DE (former GDR): Suhl, Thuringia	1982–1983	Ruminants	156	CFT	[92] [40]
BG: Knezja, Brenitza, Lazarovo, Enitza (Vratza district)	1984	Ruminants	725	CFT	[23]
BG: Pavlikeni (Veliko Tarnovo district)	1985	Ruminants	544	CFT	[23]
FR: (Martigues, Bouches du Rhône)	1990–1995	Sheep	289	IFA	[1]
DE: Berlin	1992	Sheep	80	CFT	[93,94]
BG: Panagjuriste (Pazardjik district)	1992–1995	Livestock	>1,000	CFT	[23] (for 1993); [29] (for 1992, 1993 and 1995)
DE: Düsseldorf, Nordrhine-Westphalia	1994	Sheep	>18	CFT	[95]
BG: Sopot (Plovdiv district), Troyan (Lovech district), Blagoevgrad, Pleven	1996–2000	Livestock	NA	CFT	[23]
FR: Briançon (Hautes Alpes)	1996	Sheep	29	IFA	[1]
DE: Rollshausen, county of Lohra, Hesse	1996	Sheep	56	ELISA	[96,97]
DE: Baden-Württemberg, not specified	1997	Fallow deer	12	NA	[37]
DE: Dortmund, Nordrhine-Westphalia	1999	Sheep (manure)	82	NA	[1]
FR: Montoison (Drôme)	2000	Goat (manure)	10	NA	[1]
FR: Montoison (Drôme)	2000	Sheep (manure)	5	IFA	[1]
DE: Hochsauerlandkreis Nordrhine-Westphalia, Waldeck-Franckenberg, Hesse	2000–2001	Sheep	75	NA	[98]
DE: Munich, Bavaria	2001	Sheep	3	NA	[98]
BG: Etropole (Sofia district)	2002	Livestock	121	CFT	[23]
FR: Chamonix Valley	2002	Sheep	88	IFA	[1]
DE: Soest, Nordrhine-Westphalia	2003	Sheep	299	ELISA	[99]
DE: Baden-Württemberg	2003	Cattle	8	NA	[100]
BG: Botevgrad (Sofia district)	2004	Sheep, goats	220	IFA, CFT	[32] [23]
DE: Jena, Thuringia	2005	Sheep	331	ELISA	[101]
NL: mainly Noord-Brabant, Limburg, Gelderland, NL	2007–10	Dairy goats	4,026[a]	IFA, CFT, ELISA, PCR	[14-17]
FR: Florac	2007	Sheep	18	NA	[25]
FR: Hautes-Alpes	2008	Livestock	12	IFA	[26]
DE: Lahn-Dill-Kreis, Hesse	2008	Sheep	>46	NA	[102,103]
DE: Aschaffenburg, Bavaria	2008	Sheep	>56	NA	[102]
DE: Paderborn, Westphalia	2009	Sheep	5	NA	[104]
DE: Baden-Würtemberg	2010	NA	235	NA	[2]

BG: Bulgaria; CFT: complement fixation test; DE: Germany; ELISA: enzyme-linked immune-sorbent assay; FR: France; GDR: German Democratic Republic; IFA: indirect immunofluorescence assay; NA: Information not available or not specified; NL: Netherlands; PCR: Polymerase chain reaction.

[a] Includes 168 in 2007, 1,000 in 2008, 2,354 in 2009 and 504 in 2010.

EC, as amended by Decision 2003/534/EC). Earlier reports of sporadic cases and outbreaks in these countries are available (Table 3).

During the period from 1984 to 2006, the number of serologically confirmed cases per outbreak varied between 121 and more than 1,000 in Bulgaria [23]. Outbreaks in Bulgaria have occurred in various geographic areas (including Knezja, Sopot, Etropole, Troyan, Botevgrad) and over several years in a single area (e.g. Panagyurische) (Table 3).

The average annual incidence of Q fever in Germany during 1979 to 1999 was estimated to be 1.1 (0.8–4.1) per million [19]. An estimated total of 200 to 400 human cases were registered as sporadic cases or outbreaks each year from 2007 to 2009 in Germany in the regions of Jena (Thuringia), Göppingen (Baden-Württemberg), Lahn-Dill Kreis (Hesse) and Aschaffenburg (Bavaria), and most frequently from Baden-Württemberg, Hesse and Bavaria [24]. During the period from 2004 to 2009, no significant increase in the number of cases was seen in North Rhine-Westphalia or Lower Saxony, which neighbour the Netherlands.

In the period from 1990 to 1995, an outbreak of Q fever was reported in France (Martigues, near Marseille and Aix-en-Provence, Bouches du Rhône), with 289 human cases [1]. A further 29 cases were reported (Briançon, Hautes Alpes) in 1996 and 15 (Montoison, Drôme) in 2000 [1]. Subsequently, outbreaks have been reported in the Chamonix valley, Haute Savoie in 2002, with 88 human cases [1], in Florac, Lozère in 2007, with 18 cases [25] and in Hautes-Alpes in 2008, with 12 cases [26] (Table 3).

In the Netherlands, annual notifications ranged between one and 32 human cases between 1978 and 2006, with the majority of cases occurring among people with occupational risk (e.g. persons in close contact with farmed animals, including farmers and veterinarians). From May 2007, however, there was a considerable increase in notification of human Q fever cases in the province of Noord-Brabant [27]. *C. burnetii* infection was identified in more than 160 patients presenting during May and June 2007 [14,16,17,28]. In 2008, 1,000 human cases were identified, with a hospitalisation rate of 20.9% [16]. In 2009, 2,354 new Q fever cases were registered in the national infectious disease notification database, with a hospitalisation rate of 19.7%, comparable to the situation in 2008 [16]. In 2010, 504 cases were notified, of which 406 had a known day of onset of illness in 2010, indicating that the peak of the epidemic had been reached in 2009. In this epidemic, most cases were found in the province of Noord-Brabant (Table 3).

Potential risk factors

Proximity to infected animals

Animal proximity and contact with infected animals and/or their contaminated products (e.g. birth products) have been identified as important risk factors for humans in each of the four countries. In most outbreaks, there are reports of spill-over of infection to humans from infected domestic small ruminants, i.e. goats [29,30] or sheep [31]. In contrast, there is no evidence in support of a major contribution of cattle in the history of Q fever in humans in the four study countries. In the Netherlands, living close (<2 km) to a large dairy goat farm where an abortion wave due to *C. burnetii* had occurred was identified as the most important risk factor for human Q fever [30]. The movement of domestic small ruminants through settlements has been linked with a number of outbreaks in Bulgaria (Botevgrad in 2004 [32], Panagyurische in 1992, 1993 and 1995, Kneyzha in 1984 and Pavlikeni in 1985 [29]) and France (Chamonix valley [33]). Sheep shearing is considered an important risk factor in Germany. Infected tick faeces is present in the wool, leading to contamination of dust, and the potential for further spread of the agent through storms and winds [19,31,34]. In some human outbreaks, involvement of other host species has been noted, e.g. contact with contaminated pigeon faeces [35], cats [36] or fallow deer [37].

The outbreaks in Germany and the Netherlands have been associated with urban areas. A large human Q fever cluster in an urban area in the Netherlands in 2008 was clearly linked to a dairy goat farm with more than 400 adult goats. On this farm, an abortion wave due to *C. burnetii* was confirmed, starting a few weeks before the first human cases were seen [30]. In Bulgaria, a number of human outbreaks have involved people without any known occupational hazards, such as employment or place of residence, with agriculture or the processing of animal products [30]. In the Botevgrad outbreak, most patients had no association with goats, sheep or cattle [2]. Proximity should not be seen in isolation, since the geography and landscape may also play a role in the spread of infection [38]. In Bulgaria, France, and Germany, most of the recent 25 outbreaks have occurred in small towns located in valleys close to mountains or semi-mountainous areas with meadows or in regions with specific climatic conditions, in particular dry, windy weather, in Bulgaria (Panagyurische, Sopot, Troyan, Etropole), France (Chamonix valley, Florac) and in Germany (Jena, Thuringia; Göppingen, Baden-Württemberg; Lahn-Dill Kreis, Hesse; Aschaffenburg, Bavaria). The outbreak in the Netherlands contrasts with the geographical features being described here although dry windy weather conditions may have facilitated the spread of the bacterium [13].

Management of the farms and husbandry practices

Intrinsic farm factors, such as production system and management, are believed to influence the number of

outbreaks in an area. In the Netherlands, the introduction of a milk quota system for dairy cattle in 1984 stimulated the development of a dairy goat industry. This subsequently led to an increased number of modern dairy goat farms, many in areas of high human population density, with high numbers of dairy goats on a single farm. In Germany, the production system for sheep meat changed to meet the seasonal demand for mutton. The introduction of new methods of production and synchronisation coincided with peaks of human infections during lambing seasons in spring when sheep flocks were released from winter stables. Since the 1950s, there have been substantial changes in livestock production systems in Bulgaria, from extensive systems to industrial systems and development of small farms [23,39], leading to a substantial reduction in sheep (8 million in 1990, 3 million in 1997) and an increase in goats (430,000 in 1990, 1 million in 1997) [29]. Although *C. burnetii* seroprevalence in farm animals has decreased in Bulgaria in the 2000s comparing with the 1970s and 1980s [23], the prevalence of infection in human risk groups has remained relatively constant (Table 2). Since 1990, there has been a shift in the seasonal presentation of human cases in Bulgaria, concurrent with changes in the seasonal pattern of parturition in goats and sheep [29]. In Bulgaria, cattle herds and sheep flocks tend to be large but are kept separately from the human population, whereas goats are present as multiple small herds within towns. An association between the number of positive animals in a herd and poor management (e.g. introduction of rams of unknown health status for mating, purchase of females of unknown health status, no removing of afterbirth) was noted in Germany [40].

Potential reservoirs of infection in nature

The presence of a natural reservoir in the environment or in wildlife, and spill-over to farm animals, are often considered pre-requisites for endemicity of Q fever in a geographic region. Based on seroprevalence and/or strain isolation, there is evidence of *C. burnetii* infection in a wide variety of host species (domestic livestock, domestic pets, wild mammals, birds and ticks) [23,37,41]. Evidence of *C. burnetii* infection has been found in domestic dogs (seroprevalence of 13%) and cats (26%) in Germany in a study in 1987 [41]. In Bulgaria, 16.8% of ixodic ticks collected between 1993 and 2004 were found to be positive by immunofluorescent haemocytic test [20], and 22 to 26% using other methods [42]. In contrast, low levels of *C. burnetii* DNA in ticks collected between 2006 and2007 have been reported for Thuringia in Germany [43]. Between 2006 and 2010, approximately 3,000 ticks (1,891 questing *Ixodes ricinus* and 1,086 ticks feeding on pets, wildlife and livestock) were tested for the presence of *C. burnetii* DNA in the Netherlands [44]. All ticks were negative, even from high Q fever incidence areas. Only five ticks from one sheep herd tested *Coxiella*-positive and herd was not detected positive after resampling three months later.

Control options

In each of the four countries considered in this review, a range of measures were taken by the competent authorities in response to the disease, as follows:

Measures to increase diagnostic precision and general awareness:

In the Netherlands, the capability for diagnosis of human Q fever had increased substantially in 2008 and 2009, as compared to 2007, the first year of the epidemic [14,16]. Increasing familiarity with the presentation of Q fever in people resulted in more-rapid diagnosis of clinical cases and a lower percentage of hospital admissions. The government-funded Q fever network in Germany [45] was able to transfer diagnostic capability, including cultivation techniques, to two human medical laboratories to address an important gap in diagnostic capability. This network was initiated to promote epidemiological work to identify the risks of Q fever for public health, to develop reasonable counter-measures, to conduct basic research and to raise public awareness. The network relies on a 'One Health' approach among physicians, veterinarians, epidemiologists and software developers. Further, efforts have been made to increase case notification (both in humans and farm animals) and to increase awareness among medical doctors, veterinarians and the broader public, with greater emphasis on timely hospitalisation of patients and optimised medication to reduce life threatening sequelae. Case–control studies and intensive testing carried out during and after the outbreaks in France in 2002 [33] and 2007 [25], and in Bulgaria in 2004 [32], provided more detailed information on the status of affected areas and increased general awareness about the disease.

Measures to reduce human exposure and to reduce spill-over:

A range of temporary ad hoc measures have been used including restrictions on visits to infected farms (the Netherlands during 2007–2010 [2,13-15]), limits to human assembly in high-risk areas [2,32] including the closing of schools in Bulgaria during an outbreak in 2004 [32], the stopping of blood donation in affected areas (France in 2002 and 2007, Germany 2005) [25,31,33], the removal of infected herds/flocks from human settlements (in Bulgaria during 2004) [32], and the introduction of a ban on animal movements (all four countries). Further, good farming practice is recommended, as long term universal measures, particularly for manure [2,13,15,46,47], such as covering and natural composting or ploughing of manure so that no aerosolisation of agents is possible, closed composting with CaO (in the Netherlands) [46,47] or $CaCN_2$ (in France and Germany) [48], and the removal of animal birth and abortion products (all four countries). Other measures have included disinfection of infected premises including paths and general environment of holdings (Bulgaria during 2004) [32], obligatory notification of increased farm animal abortion rate to the local authorities (France, the Netherlands) [2,12,13], the

potential use of veterinary vaccines (France 2009, the Netherlands 2007–2010) [13,49,50] and the implementation of a farm animal breeding ban (the Netherlands 2007–2010 [2,13]).

In the Dutch outbreak between 2007 and 2010, several counter-measures were introduced, following consideration of both national and international (including EFSA) expert opinion. These measures included the development of notification criteria after which Q fever became a notifiable disease in farmed animals, a ban on animal transport especially from infected farms, visitor bans on infected farms, the promotion of general hygiene measures, the implementation of a safe manure management including prevention of aerosolisation, the introduction of a farm animal vaccination programme for small ruminants, testing of bulk milk (milk collected in large quantities from different dairy animals) using a PCR to identify infected herds, and breeding restrictions. The vaccination programme was initiated in October 2008, following special dispensation of a phase I Q fever vaccine (Coxevac, CEVA), through a voluntary scheme involving dairy sheep and dairy goats on farms with more than 50 goats or sheep, pet zoos and nursing farms in a restricted high-risk zone, an area with radius 45 km around the city of Udden. At that time, vaccination was restricted to a limited area, due to a shortage of vaccine. A mandatory vaccination programme was subsequently introduced in an enlarged area including the province of Noord-Brabant, leading to vaccination of dairy sheep and dairy goats prior to 1 January 2010 on farms with more than 50 animals, and on care farms, pet zoos and zoos. Nationwide mandatory vaccination coverage was achieved in 2011, and also included small ruminants attending shows [2]. Culling of more than 50,000 pregnant animals aiming at reducing the shedding of C. brunetii and as a consequence of that, environmental contamination trying to reduce human exposure in 2010, was undertaken on PCR bulk tank milk positive farms followed by a programme of repopulation with fully vaccinated animals originating only from PCR bulk tank milk negative farms. Compensation schemes were available for the farmers when culling was ordered [13,51]. In the Netherlands, a human vaccine (the Australian human vaccine Q-VAX, currently not registered in Europe) was made available in July 2010 to people at risk from chronic Q fever, such as patients with cardiac valve disease, aortic aneurisms, and vascular prostheses [52]. The human vaccination programme commenced in January 2011, after the Q fever outbreak in the Netherlands had subsided [14].

Discussion

This review presents information on the presence of *C. burnetii* during the period from 1982 to 2010 in countries of Europe that differ greatly in terms of animal and human population, livestock density and production systems. The regional presentation of Q fever varies considerably, based on several data sources. Non-standardised serological data are available about the presence of *C. burnetii* in various domestic animal species and wildlife. In addition, severe human outbreaks or epidemic waves have also been described. Information from these four countries illustrates the epidemiological variability of human outbreaks and the considerable range of risk factors involved. Nevertheless, some general patterns emerge which are discussed below, together with areas of uncertainty where further research is justified.

Domestic ruminants are considered the primary reservoir for *C. burnetii* [1,53]. Human cases and outbreaks are attributed to infection in sheep (in Germany) and goats (Bulgaria, France, the Netherlands), but not cattle. We found no evidence in support of a major contribution of cattle in the history of Q fever in the four study countries, even though *C. burnetii* infection can also lead to shedding and abortion in cattle [54]. Abortion in cattle is a less prominent feature of infection compared to sheep and goats [1,49,55]. We speculate that the prominent role of sheep and goats as reservoirs of infection during human outbreaks may be related to the highly seasonal nature of their reproduction cycle, to the larger herd sizes in these species, to differences in management and housing between these species, to the relative importance of shedding and abortions after *C. burnetii* infection, and possibly to species-related differences in the virulence for humans of *C. burnetii*.

Abortions in *C. burnetii*-infected domestic ruminants are accompanied by massive excretion of the bacteria and spread into the environment. This is the most important excretion route of *C. burnetii*, as up to 109 organisms are excreted per gram of placenta tissue [56]. The level of excretion is believed less following the birth of healthy calves, kids or lambs from infected animals [48]. *C. burnetii* has also been detected in faeces, vaginal mucus and milk of infected domestic ruminants [57,58]. In goat herds, in both aborting and non-aborting goats, *C. burnetii* DNA has been detected in faeces, vaginal mucus and/or milk [58]. Also, in cattle, variable excretion via faeces, vaginal mucus and milk has been reported, sometimes independent of an abortion history. Sixty-five per cent of cows seem to shed *C. burnetii* by only one of these routes, with few cows excreting *C. burnetii* by all three routes [55]. Comparison of the three excretion routes in cattle, goats and sheep showed that milk shedding is more frequent in cattle and goats. Ewes shed more and for a longer duration in vaginal mucus than goats [1]. Sheep and goats can both shed *C. burnetii* in subsequent pregnancies [59,60].

An elevated seroprevalence in domestic ruminants has been noted in areas with human outbreaks. In the outbreaks in Etropole (2002) and Botevgrad (2004) in Bulgaria, herd-level seroprevalence ranged from 11.6% to 33.0% (cattle), from 46.6% to 59.5% (sheep) and from 63.3 to almost 100% (goats). In contrast, median herd-level countrywide prevalence was 7.1 to 21.7% [23]. Of 26 sheep, goat and cattle flocks/herds located

within 5 km of an outbreak in Florac (2007) France, 11 were enzyme-linked immunosorbent assay (ELISA)-positive [25,61]. An increase in seroprevalence among ruminant herds in known areas of risk may assist in predicting outbreaks in humans.

Genetic differences of *C. burnetii* strains have been discussed as one reason for differing pathogenicities in guinea pigs and mice when infected with different isolates from domestic ruminants [23,62]. However, it is not clear whether there is a correlation between multilocus variable-number tandem repeat analysis (MLVA) types and virulence. The Dutch outbreak was the first outbreak where detailed investigations were conducted on the genotype of *C. burnetii*. A single MLVA type appeared responsible for the majority of the *C. burnetii*-related abortion on goat farms in the Netherlands [51]. Little is known of MLVA types from other outbreaks. The identified limited genetic diversity in the Netherlands precludes investigation of local transmission pathways and molecular typing methods have to be developed further, including high-resolution genotyping based on whole genome sequencing, to match human, veterinary, and environmental samples.

In the four countries under study, as elsewhere in the world [1,8], there is evidence of widespread exposure to *C. burnetii* in both the human and domestic ruminant populations. However, clinical cases of Q fever in people are generally very rare. As reflected in this review, outbreaks are generally associated with a range of risk factors, including close contact between people and small ruminants, and events (such as abortions) leading to increased shedding of *C. burnetii* in these small ruminant populations. Other factors may also be important, but are not well understood. During the Dutch outbreak, for example, it was suggested that the human population in the Netherlands was more susceptible to disease because seroprevalence was low, the number of animals in the farms was high with consequent assumption of the amount of manure and lochia, with potential of human exposure. The change in management of animals from industrial-type housing to small private farms in Bulgaria was a hypothesis for the observed variation of within-herd prevalence over a period. However, it seems likely that *C. burnetii* infection can be maintained in a wide range of husbandry systems in all four countries. The Dutch outbreak developed in a geographic area without historic Q fever problems. This is different to the recognised pattern, in Bulgaria, France and Germany, of outbreak occurrence and re-occurrence in specific geographic localities. Although a windborne spread appears to have played an important role in the transmission of infection from animals to people, in all four countries. More work is needed to develop a systematic understanding of the risk factors involved and their interactions.

The control measures pursued by different countries were in general targeted at reducing human exposure and spill-over from animal populations to humans. In each of these countries, human cases have generally been linked to exposure to aerosols with high numbers of *C. burnetii* excreted during parturition by infected ruminants. During outbreaks in Bulgaria and France, strategies for prevention and control of Q fever in people were designed, cognisant of the influence of specific conditions [32] on transmission of infection, such as dry weather, wind direction, and the location of human population at risk in a valley with hillside pastures [26]. The main challenges on the control of the disease are linked to the sustainability of measures such as culling or reproduction bans but also the persistence of infection in both animals and the environment. Furthermore there is limited data regarding the effectiveness of different control measures.

Measures that can be applied on-farm to reduce spill-over from farm animals to humans are limited to vaccination and on-farm hygienic measures. There is evidence in support of vaccination being effective in preventing abortions in small ruminants and in reducing the shedding of *C. burnetii* [1,63], although it has been suggested that this must be sustained for at least several years [64,65]. Outbreak vaccination, i.e. vaccinating herds that already are infected [64,66] or otherwise under high infection pressure [65], are each believed to be less effective. The risk of Q fever outbreaks and possibly other zoonotic diseases remains high in relatively small areas such as the province of Noord-Brabant in the Netherlands, with large populations of people (2.4 million) and animals (6.4 million). There remains uncertainty about the effectiveness of control measures other than vaccination. Farm hygienic measurements (such as manure sterilisation/composting and management, disinfection of the paths and ways to the pastures, indoor housing during lambing season, air-filter systems in housings and movement controls) are likely to have limited effectiveness in reducing infection risk. There is incomplete information about either *C. burnetii* survival times in manure and in the general environment, or the period during which surviving bacteria remain a threat for public health. Reports suppose a long lasting period of survival and infectivity, possibly up to two to three years or more [3,67]. Based on information from the Netherlands outbreak between 2007 and 2010, however, we did not find evidence for this. There was a rapid decrease in human cases in 2010, immediately following the last of the abortion storms that occurred in goats in 2009. Similarly, the various outbreaks in Germany and France were single events related to human exposure to small ruminants. In each situation, human cases were limited in time. The inevitable contamination of the environment did not seem to cause an elevation of human cases for a longer period of time.

Concerning the Dutch Q fever outbreak during the period between 2007 and 2010, at least some facts can be ascertained. Seroprevalence among the general population increased from 2.4% before the first outbreak in 2007 to around 12% in the high incidence area

in 2009 (Table 2). During the same period, the number of notified acute Q fever patients decreased from 2,354 in 2009 to 504 in 2010 [14]. Several veterinary measures were implemented in the Netherlands concurrently, making it impossible to establish the relative contribution of each (vaccination, culling, on-farm hygienic measures, or other factors) to this decline in incidence. It should also be noted that the prevalence of *C. burnetii* in an infected herd usually declines over time even if no countermeasures are taken, probably caused by a 'natural' immunisation of susceptible animals (Table 1). However, meaningful scientific data are still missing. The development of a protective and safe vaccine for animals is strongly recommended.

Eradication of Q fever from a herd is not currently straightforward for a range of reasons, including chronic infection in a small number of animals (personal communication, R. Van den Brom, September 2012), the presence of shedding, but test-negative, animals, and the potential for recurrent shedding of the agent [58,59,68]. Reduction of excretion has been reported using a phase 1 *C. burnetii* vaccine for animals, however, this could be affected by herd infection status and the timing of vaccination [63-66]. To minimise human health risks, vaccination of animals may need to be conducted in combination with repeated testing, for example using a PCR on individual milk samples, and the culling of infected animals [69,55,57,64].

In each of the countries under investigation, seroprevalence measured at the individual animal level was lower than herd seroprevalence. In other words, in each herd only a relatively low number of animals seroconverted after contact with *C. burnetii*. This result is somewhat surprising, given the known high rate of infectivity of *C. burnetii* in ruminant populations (Table 1). When *C. burnetii* is introduced on a farm with few pregnant animals (goats), seroconversion is expected mainly in these animals, following birth, because of the strong tropism of the pathogen for placenta trophoblasts [70], although *C. burnetii* is found everywhere in the surroundings. Low within-herd sero-prevalence is also seen with other infections where pathogens also may survive readily outside the host, such as paratuberculosis [71]. The role of differences in individual resistance and cell-mediated response should also be explored.

Epidemiological studies on *C. burnetii* infection and Q fever in humans need to be interpreted with care, given differences in both the underlying epidemiological conditions and the study designs used (including sample size, target groups, serological test, serological cut-off and study purpose). The lack of standardisation between studies was an important constraint in the current work. In most cases, studies have been conducted with biased sub-populations of people, including those with a known risk and elevated levels of *C. burnetii* in animal populations, such as for example, people in the outbreak areas and with potentially compromised health. For these reasons, it can be difficult to draw meaningful conclusions about the underlying seroprevalence of *C. burnetii* in people in these four countries. Further, observed differences over time are difficult to explain, such as the extremely high prevalence in a risk group in the Netherlands in the 1980s in comparison to the much lower prevalence in humans in outbreak area in recent years (February 2006–June 2007) [2,72]. This latter observation may, in part at least, reflect a lack of specificity in earlier testing methods, which relied on in-house immunofluorescent assays and application of a low cut-off for positivity. However, seroprevalence in occupational risk groups in the 1980s and during the 2007 to 2010 outbreak in the Netherlands were comparable [14].

A number of conclusions can be drawn from this review of *C. burnetii* infection and Q fever in people and domestic ruminants in four countries in Europe. In all outbreaks, human contact with sheep and goats, rather than cattle, has been a consistent feature and the most likely source of *C. burnetii* infection. As yet, however, there is insufficient information to enable early prediction of large outbreaks of Q fever in people. Mandatory notification of Q fever in humans is an important surveillance strategy, and has been recommended previously [73], but is yet to be implemented in many countries in the EU [12]. Reporting of *C. burnetii*-related abortion cases in animals is compulsory in some countries [2,12], but interventions by authorities are typically not initiated in sporadic cases. A more systematic use of such data for analysing the dynamics and seasonality of cases and to inform animal owners to take voluntary precautions should be considered. The cooperation and flow of information between veterinary and medical professionals, and vice versa, is critical [2,73], and initiatives to build strong links between authorities involved in the monitoring and control of zoonoses similar to the Human Animal Infection Risk and Surveillance (HAIRS) group in England and Wales [74] are recommended. Much remains unclear about the transmission of C burnetii from animals to humans, about means for early detection of increased risk of outbreaks, the effectiveness of veterinary control measures, and about the best follow-up strategy in territories with repeated outbreaks over several years. Future research should focus on these topics.

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Conflict of interest

None.

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