Impact factor 6.15

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 18 | Weekly issue 08 | 21 February 2013

Rapid communications

Imported dengue virus serotype 1 from Madeira to Finland 2012 2
by E Huhtamo, EM Korhonen, O Vapalahti

Surveillance and outbreak reports

Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012 6
by U Buchholz, MA Müller, A Nitsche, A Sanewski, N Wevering, T Bauer-Balci, F Bonin, C Drosten, B Schweiger, T Wolff, D Muth, B Meyer, S Buda, G Krause, L Schaade, W Haas

Review articles

Q fever in humans and farm animals in four European countries, 1982 to 2010 13
by M Georgiev , A Afonso, H Neubauer, H Needham, R Thiéry, A Rodolakis, HJ Roest, KD Stärk, JA Stegeman, P Vellema, W van der Hoek, SJ More

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Imported dengue virus serotype 1 from Madeira to Finland 2012

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Citation style for this article:
Huhtamo E, Korhonen EM, Vapalahti O. Imported dengue virus serotype 1 from Madeira to Finland 2012. Euro Surveill. 2013;18(8):pii=20405. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20405

Article submitted on 06 February 2013 / published on 21 February 2013

Imported dengue cases originating from the Madeiran outbreak are increasingly reported. In 2012 five Finnish travellers returning from Madeira were diagnosed with dengue fever. Viral sequence data was obtained from two patients. The partial C-preM sequences (399 and 396 bp respectively) were found similar to that of an autochthonous case from Madeira. The partial E-gene sequence (933 bp) which was identical among the two patients grouped phylogenetically with South American strains of dengue virus serotype 1.

Between August and December 2012, the hospital district of Helsinki and Uusimaa laboratory (HUSLAB) which solely performs dengue diagnostics in Finland, received samples from 16 patients with clinically suspected dengue fever and who had visited the Autonomous Region of Madeira, Portugal, where an outbreak of dengue fever is taking place since the beginning of October 2012 [1,2].

Confirmation of dengue virus infection

The serum samples of the patients were analysed by dengue non-structural protein (NS)1 antigen (Ag) strip test (BioRad), IgM-enzyme immunoassay (EIA) (Focus Technologies) and an in-house IgG immunofluorescence assay (IFA) using dengue virus (DENV)-3 infected Vero E6 cells as antigens. The dengue diagnosis was confirmed in five patients by serological tests. Three of five serologically-confirmed patients were also positive for NS1 Ag.

The earliest available samples of the five serologically-confirmed dengue patients were studied by reverse transcription-polymerase chain reaction (RT-PCR) to obtain information of the infective virus strain. RNA was extracted from serum samples using QIAamp viral RNA mini kit (Qiagen) according to manufacturer's instructions. The RNA was used as a template in dengue typing RT-PCR [3] and in an RT-PCR amplifying a partial sequence of the envelope gene (E-gene) [4]. RT-PCR products were obtained from two patients, for which the typing RT-PCR product size corresponded to DENV serotype 1 (DENV-1) [3].

The obtained RT-PCR products were purified enzymatically using ExoSAP-IT (USB laboratories) and directly sequenced (in case of E-gene, using DENV-1 E-gene specific primers). Primer sequences are available from the authors upon request). The obtained sequences of the E-gene (933 bp) from the two patients were identical to one another and the sequence information was deposited in GenBank under accession number: KC567679. Sequences from the capsid-pre-membrane (C-preM) region [3] derived from the first patient (399 bp, GenBank accession number: KC616348) and the second patient (396 bp, GenBank accession number: KC616349) differed from each other by one nucleotide.

The sequences from the patients were compared to available DENV sequences in GenBank database and the basic local alignment search tool (BLAST) results confirmed that the infective virus was DENV-1. This finding was in line with the typing RT-PCR results and with previous reports [1,5,6].

The two RT-PCR positive patients had returned from Madeira in November 2012. The first patient was a female in her 50s, who was IgG negative but NS1 Ag and IgM positive in the first sample (used for RT-PCR), and IgG seroconverted after one week with an IgG titre of 80 (IFA). The second patient was a man in his 60s, whose first available sample was positive for IgM and NS1 Ag, and borderline positive in the IgG test, but for whom a sample collected five days later had an IgG titre of 1,280 (IFA). The symptoms of both patients could be regarded as typical for dengue fever including fever (≥38°C), headache, muscle pains, rash and gastrointestinal symptoms.

Phylogenetic analysis of the viral sequence derived from the patients

All of the four known serotypes of DENV are further genetically categorised into several distinct genotypes. Two different ways of categorising DENV-1 strains have been used: into three [7-9] or into five different genotypes [10-12]. The DENV-1 viruses include a sylvatic strain originating from Malaysia (designated as sylvatic genotype or genotype III). In the five genotype

classification, some strains originating from Thailand in the 1950s and 1960s are designated as genotype II.

All the rest of the epidemic strains can be divided into three main groups corresponding to a three genotype classification. These groups include: (i) mainly Asian strains and some east African strains designated as genotype I (or Asian I genotype in the five genotype classification), (ii) strains originating mainly from the west Pacific islands and Australia designated as genotype II (or South Pacific genotype IV in the five genotype classification), and (iii) strains originating from Africa, some Asian strains and all strains originating from the Americas designated as genotype III (or American/African genotype V in the five genotype classification). To identify dengue virus strain(s) responsible for a given outbreak, characterisation of the infecting virus by sero- and genotyping is needed. Although dengue viruses currently have a global circulation, their genetic characterisation likely provides clues of their origins.

The E-gene sequence derived from the patients was aligned with sequences sharing highest nucleotide homologies in GenBank and with a global collection of representatives of different recent epidemic DENV-1 genotypes using multiple sequence comparison by log expectation (MUSCLE) (www.ebi.ac.uk/Tools/msa/muscle/) and a neighbour-joining phylogenetic tree was estimated with the maximum composite likelihood method using the programme MEGA (www.megasoftware.net/) (Figure).

The results suggested that the Madeiran strain represented the American/African genotype (designated as genotype III or V). In the phylogenetic tree, the Madeiran strain was clustered together with strains from Colombia and Venezuela. The highest nucleotide similarities were shared between the Madeiran strain and a strain from Colombia from 2008 (99.9%). The Madeiran strain had a single nucleotide synonymous substitution (T®C) in comparison to the Colombian strain at position 2,040 (GenBank accession number: GQ868570).

Additional sequences from the C-preM region [3] were also compared to Genbank sequences and to the available sequence of the autochthonous case from Madeira that became recently available [6]. The sequence from the first patient was identical to the sequence from the autochthonous case within the alignable region of 385 bp, and the sequence from the second patient had a single nucleotide change (position 82, GenBank accession number: KC248375). The BLAST search results for the C-preM sequences were in line with those of the E-gene sequence, showing highest sequence homologies to strains from South America.

Discussion and conclusion

Our results are in line with previous reports [1,5,6], and all data thus far suggests that possibly a single strain of DENV-1 likely originating from South America caused the Madeiran epidemic, although more sequence data, e.g. complete genome data and sequences from more patients of the outbreak would be needed to confirm these findings.

Interestingly, DENV-1 was also identified in 2010 from autochthonous cases in southern France [13] and in Croatia [14,15]. The available sequences from Croatia [15] did not represent the same region as our sequence, and thus could not be included in our analysis. The analysis of the sequences from Croatia was based on short E-NS1 junction sequences (222 bp). The results suggested that the Croatian DENV-1 also represented genotype III (a genotype also designated as American/African genotype V), but grouped together with strains from India [15]. As the Croatian sequences were associated with strains from India rather than strains from South America, it seems unlikely that the Croatian strains would be closely related to the strain circulating in Madeira. However, more comprehensive sequence data would be needed from the autochthonous cases found previously in Europe for comparisons.

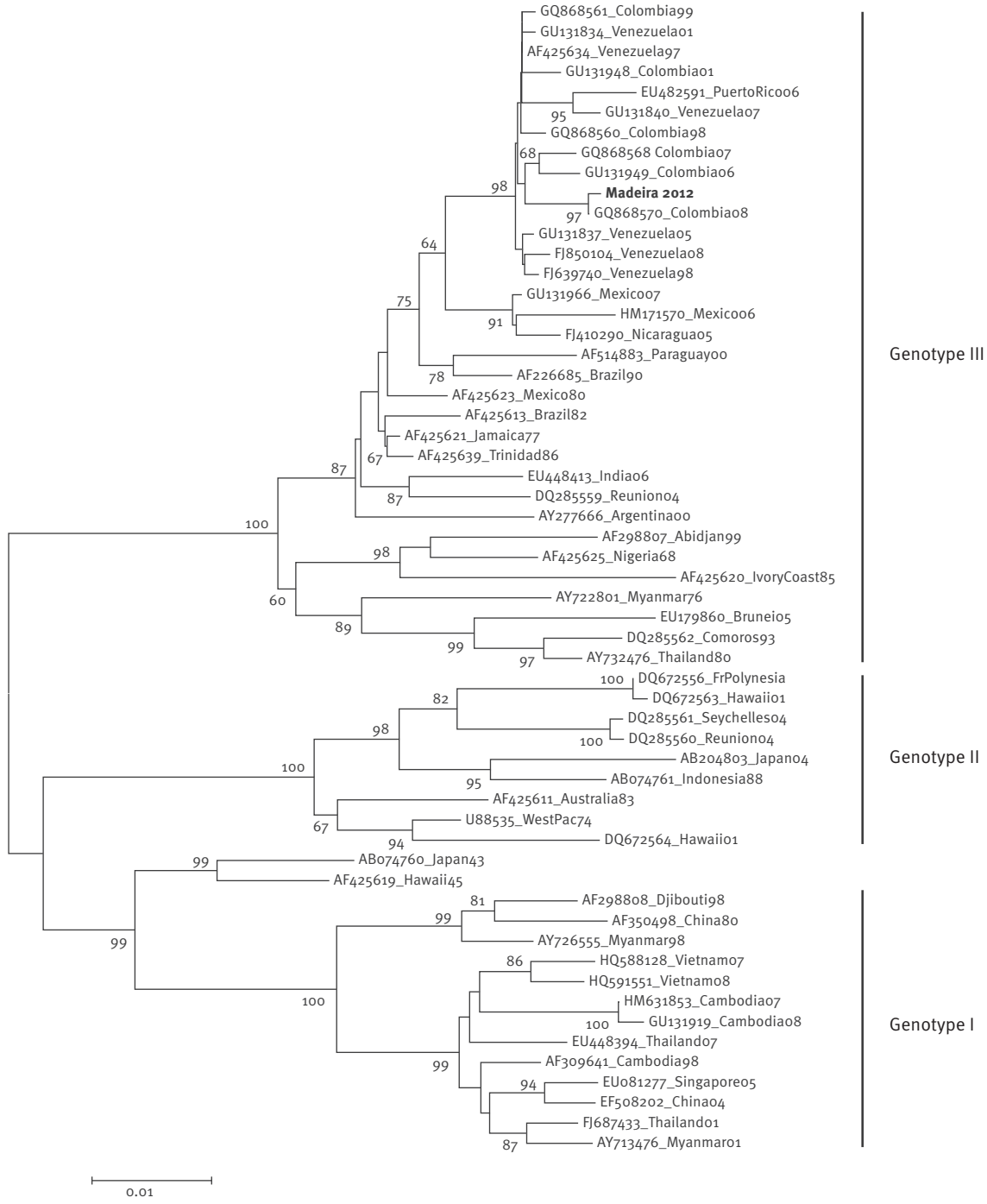
Aside from the autochthonous cases of dengue fever in southern France [13] and Croatia [14], the Madeiran event is the first epidemic of dengue fever in Europe since 1928, when Greece was affected by an outbreak [16,17]. The 2012 Madeiran epidemic has had a great impact on local residents, but has also affected visitors of the island. The latest available data from February 2013 reports that 78 visitors from European countries got infected in Madeira [18]. These countries, listed according to decreasing number of cases, include the United Kingdom, Germany, mainland Portugal, Finland, Sweden, and France, followed by Denmark, Austria and Norway, and finally Croatia, Slovenia, Spain and Switzerland. The number of imported cases in Europe is however likely to be an underestimate. Also the actual number of persons in Finland, who got infected by DENV in Madeira may be higher than the number reported here as the travel history information was not available for all the suspect dengue patients whose samples were tested at the HUSLAB.

Whereas the risks for possible endemic transmission of dengue virus (DENV) in Europe have been mainly associated with the spreading of *Stegomyia albopicta (=Aedes albopictus)* [13,14], interestingly, the vector associated with the Madeiran outbreak was reported to be *Stegomyia aegypti (=Aedes aegypti)* [1] as was the 1928 outbreak in Greece [16,17] (mosquito nomenclature according to Reinert et al., 2009 [19]).

Similarly to Madeira, it has been suggested that island locations that are major tourist attractions such as Mauritius [20] may be at elevated risk for dengue introduction via travellers if suitable vectors are available. Although significant efforts have been taken to map and collect the information of the relevant mosquito vectors of mosquito-transmitted diseases in Europe

Figure

Phylogenetic analysis of partial envelope gene sequence derived from two imported dengue cases from Madeira to Finland, 2012



The tree, determined by neighbour-joining method, was based on partial (933 bp) envelope gene sequences of dengue viruses of serotype 1. Bootstrap support values >60% are shown at the nodes. The scale bar represents nucleotide substitutions per site. The GenBank accession numbers of the sequences used as well as the country of origin and year are shown on the tree. The strain from the case imported from Madeira in 2012 is indicated in bold and clusters with strains of genotype III (a genotype also designated as American/African genotype V).

by e.g. the European Centre for Disease Prevention and Control (ECDC) VBORNET, this information is not available from all locations. For example, according to ECDC VBORNET mosquito maps [21] no information is currently available on competent dengue vectors, *(Stegomyia albopicta (=Aedes albopictus)* or *Stegomyia aegypti (=Aedes aegypti))* for some locations situated relatively closely to Madeira, such as the Canary Islands. The Madeiran outbreak highlights the importance for obtaining up-to-date information of the vector species distribution in Europe and urges the need for planning countermeasures and surveillance at virological, medical and environmental levels [22].

Although the Madeiran dengue outbreak was widely reported in the international news, dengue may still not be generally suspected in intra-European travellers. The possible endemisation of dengue in Europe highlights the need for diagnostic preparedness and awareness of clinicians to suspect and recognise dengue originating from within Europe. We conclude that the detailed molecular characterisation of the DENV strains from travellers, autochthonous cases and outbreaks are needed for studying the molecular epidemiology and transmission chains of dengue virus strains in Europe.

Acknowledgements

The authors thank Dr. Anssi Reponen and Dr. Timo Tuominen for providing patient information and HUSLAB for financial support.

Conflict of interest

None declared.

Authors' contributions

All authors contributed to the study design, interpretation of the results, writing of the manuscript and approved the final version. EMK performed RNA extractions and RT-PCR tests. EH performed the NS1 antigen tests, sequence analysis and wrote the first version of the manuscript. OV collected the diagnostic and clinical data.

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Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012

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Citation style for this article:
Buchholz U, Müller MA, Nitsche A, Sanewski A, Wevering N, Bauer-Balci T, Bonin F, Drosten C, Schweiger B, Wolff T, Muth D, Meyer B, Buda S, Krause G, Schaade L, Haas W. Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012. Euro Surveill. 2013;18(8):pii=20406. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20406

Article submitted on 24 January 2013 / published on 21 February 2013

On 24 October 2012, a patient with acute respiratory distress syndrome of unknown origin and symptom onset on 5 October was transferred from Qatar to a specialist lung clinic in Germany. Late diagnosis on 20 November of an infection with the novel Coronavirus (NCoV) resulted in potential exposure of a considerable number of healthcare workers. Using a questionnaire we asked 123 identified contacts (120 hospital and three out-of-hospital contacts) about exposure to the patient. Eighty-five contacts provided blood for a serological test using a two-stage approach with an initial immunofluorescence assay as screening test, followed by recombinant immunofluorescence assays and a NCoV-specific serum neutralisation test. Of 123 identified contacts nine had performed aerosol-generating procedures within the third or fourth week of illness, using personal protective equipment rarely or never, and two of these developed acute respiratory illness. Serology was negative for all nine. Further 76 hospital contacts also tested negative, including two sera initially reactive in the screening test. The contact investigation ruled out transmission to contacts after illness day 20. Our two-stage approach for serological testing may be used as a template for similar situations.

Introduction

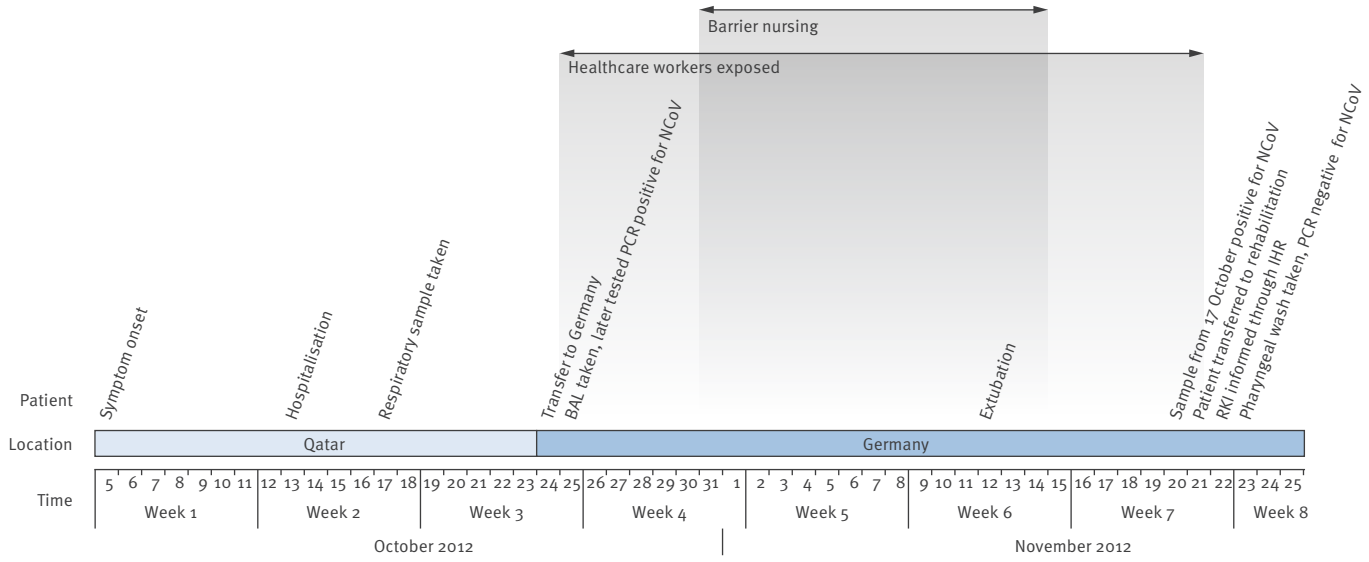
A novel human coronavirus (NCoV) has recently emerged in the Arabian Peninsula. The first two reported cases infected by the novel agent, then provisionally termed hCoV-EMC, occurred in June and September 2012, respectively [1-3]. As of 18 February 2013, a total of 12 cases have been confirmed by WHO [4], including five deaths. Among five cases reported from the Kingdom of Saudi Arabia, three were part of a family cluster. Two further cases were linked to probable exposure in Qatar, and two cases were confirmed retrospectively, by diagnostic of respiratory specimens, from Jordan with disease onset in April 2012. The latter were part of a cluster of 11 patients with acute respiratory symptoms linked to a hospital [5]. The most recent three cases identified constitute another cluster that occurred in the United Kingdom (UK) in January to February 2013 [4]. The index case in this cluster is a UK citizen with travel history to Saudi-Arabia and Pakistan before symptom onset [5]. Two of his family members who had not travelled outside the UK and became ill were most likely infected through person-to-person transmission. While one of them had an underlying disease and died, the other presented with milder, influenza-like illness symptoms only.

Because of the long period, 10 months, over which the cases occurred, the source and transmission patterns of the virus remain elusive. Hypotheses include a predominance of zoonotic acquisitions with little potential for human-to-human transmission [5], widespread and unnoticed occurrence of clinically mild infections, and finally the possibility of an early-stage epidemic caused by a highly pathogenic novel human virus.

Because of the potential of human-to-human transmission in the hospital outbreak in Jordan and the family clusters, as well as the observed severity of disease, current recommendations regarding protective measures rely on experiences with severe acute respiratory syndrome (SARS) in 2003 [6]. The first of the two Qatari patients was treated in the UK where, under strict isolation measures, no secondary cases occurred. Investigations by polymerase chain reaction (PCR) of 10 healthcare workers (HCW) who had cared for the patient and subsequently developed mild respiratory disease yielded no evidence of infection [7]. However, to date, published investigations of individuals with proven exposure to NCoV have not presented

Figure

Timeline of disease of novel coronavirus case and possible exposure of healthcare workers, Germany October–November 2012



BAL: bronchoalveolar lavage; IHR: International Health Regulations; NCoV: novel coronavirus; PCR: polymerase chain reaction; RKI: Robert Koch Institute.

a strategy how to identify retrospectively infections in a large group of (contact) persons through serological testing.

On 22 November 2012, the Robert Koch Institute in Berlin, Germany, was informed according to the International Health Regulations [8] about a case of NCoV infection in a Quatari patient in his forties, treated in Germany (Figure). After an acute onset of symptoms on 5 October, he had been admitted to a hospital in Doha, Qatar, on 13 October, where he developed respiratory failure requiring ventilation, and was reported to have had temporary renal impairment. On 24 October, he was transferred to a specialist lung hospital in Essen, Germany. A respiratory sample had been taken in Qatar on 17 October. After some delay due to difficulties with the shipment of specimens, the sample tested positive for NCoV in a laboratory in the UK. The result was consequently communicated by the UK Health Protection Agency to the World Health Organization (WHO) on 21 November 2012. Until that date the hospital in Essen had not considered NCoV in the differential diagnoses for the patient. Only routine personal protection of HCW and no specific measures of respiratory protection had been followed during the whole course of treatment in the intensive care unit (ICU). After weeks of mechanical ventilation in ICU, the patient was discharged on 21 November. A lag time of four weeks between patient transfer and laboratory confirmation of the NCoV infection resulted in potential exposure of a considerable number of HCW in Germany. Here we report on an interview with the patient asking for potential sources of infection, the investigation of individuals exposed to the patient, virological investigation of respiratory samples from the patient as well as an approach used to test retrospectively a large number of contacts.

Methods

Patient interview and samples for laboratory investigation

After the patient had recovered he was interviewed in person. The interview was conducted in Arabic with the help of an interpreter. It was targeted at potential modes of acquisition of the infection. The questionnaire contained questions about the early course of disease, social status, living conditions, profession, hobbies and regular activities, exposure to animals, eating habits, and contacts with individuals with respiratory illness in the 10 days before his illness onset.

We searched for stored respiratory and blood samples at the hospital laboratory that were still available to be tested for NCoV and identified a specimen that originated from a bronchioalveolar lavage (BAL) done on 25 October, illness day 20 (i.e. late third illness week), as well as a serum sample from the same day. In addition, on 23 November (eighth illness week), we took a pharyngeal wash and a serum sample from the patient after he was discharged and had started his rehabilitation program on 21 November. Both respiratory samples were tested by real-time reverse-transcription (RT)-PCR. The first sample was also subjected to virus isolation in LLC-MK2 cells.

Contact investigation

Contact persons were identified based on electronic procedures registration, supplemented by a targeted request to HCW to report contact with the patient while in ICU (and during the transport to the hospital). The electronic registration of procedures requires that any person performing a task in the patient's room must sign in, sign out and document which procedure was conducted. Using a standardised questionnaire, information was collected about the time of the first contact, types of contact, closest distance to the patient, frequency of using a surgical face mask when in contact with the patient, and occurrence of acute respiratory illness (ARI) up to ten days after the last contact with the patient. No information was collected on the duration or frequency of contact.

Consenting individuals gave blood for serological testing on one of three dates (3, 7 or 14 December). The median interval from first patient contact to venipuncture was 39 days (range: 13–50 days). Contacts were considered at high risk if they had their first contact with the patient at the beginning of his stay in ICU, i.e. at the end of the patient´s third or fourth week of illness, if they had conducted an aerosol-generating procedure, such as suctioning the intubated patient or performing a BAL, and if they had rarely or never used surgical face masks while caring for the patient.

Laboratory methods

Nucleic acid detection was performed by RT-PCR as described previously [9,10] after viral RNA was extracted from 300 µl of bronchioalveolar lavage using the MagAttract Viral RNA Kit M48 (Qiagen GmbH, Hilden, Germany).

Serological testing was performed in a two-stage approach. As a first step, screening for antibodies reactive to NCoV was done by indirect immunofluorescence assay (IFA) as described previously [10]. Preliminary evaluation of IFA on 50 sera from blood donors yielded no reactivity. For resolution of reactive results, IFA was done on Vero B4 cells expressing recombinant spike (S) and nucleocapsid (N) proteins of NCoV, SARS-CoV, hCoV-OC43, and hCoV-NL63. Details of procedures for recombinant IFA are outlined in Corman et al. [10]. For serum neutralisation tests (SNT), Vero B4 cells were grown to subconfluence in 24 well plates. Preincubation involved 25 plaque-forming units of NCoV in 100 µl of medium, mixed 1:1 with patient sera prediluted in medium as indicated. The starting dilution was 1:8. After 1 h incubation at 37 °C, each well was infected for 1 h at 37 °C using the total 200 µl preincubation reaction. Supernatants were removed and overlaid with Avicell resin exactly as described by Herzog et al. [11]. Assays were terminated and stained after three days.

Statistical tests

Comparison of frequency distributions were done using Fisher's exact test.

Ethical clearance and data protection

The contact investigation was carried out based on legal requirements of the Protection against Infection Act of Germany [12] and the International Health Regulations [8], and was led by the local health authorities. After information about the investigation and its aims, contacts signed a consent form if they agreed with the analysis of blood samples. All questionnaires and samples were fully anonymised before analysis.

Results

Patient interview

The patient reported to live in Doha, Qatar. He used to be a heavy smoker (2 to 3 packs of cigarettes per day), but denied smoking waterpipe or chewing qat. Disease onset was rapid, with initial symptoms including fever (40 °C), cough, runny nose, and shortness of breath. Subjective weakness was pronounced. After the first two days of illness he improved a little but deteriorated again, and was finally admitted to hospital on day eight of illness because of increasing dyspnoea. He reported no subjective symptoms of renal impairment such as foamy urine, reduced urine output, or back pain. He had not travelled and had no known contact with any other reported cases of NCoV infection. The patient owned a camel and goat farm and reported a large number of casual contacts (approx. 50 persons per day) on a regular basis. He remembered that before his disease onset some goats were ill and had fever. He did not have direct contact with the goats or any other animals especially falcons or bats, but said he had eaten goat meat. He also reported to have had contact with one of his animal caretakers who was ill with severe cough and was hospitalised. Other than the animal caretaker, he did not remember persons with severe respiratory illnesses in his wider or closer social environment.

Patient samples

Virus detection in the initial sample from illness day 20 and preliminary serological investigations have been described by Corman et al. [10]. Isolation of virus in cell culture failed. Serological testing yielded an IgM titre against NCoV of 1:1,000 and an IgG titre of 1:10,000 at day 20 (week three) of illness. At week eight of illness the IgG titre was still at 1:10,000 while the IgM titre had already decreased to 1:100. SNT titres against NCoV were 1:640 at week three and 1:640 at week eight of illness. The pharyngeal wash sample taken on 23 November 2012 (week eight of illness) tested negative by real-time RT-PCR.

Contact investigation

We identified 120 hospital and three out-of-hospital contacts, including the interpreter of the patient. Protective measures were largely limited to HCW wearing gloves and gowns when providing intimate care and use of surgical face masks during suctioning. From 31 October until 4 November (illness weeks five and six), the patient was isolated using barrier nursing due to

a concurrent *Pseudomonas aeruginosa* infection. This included use of surgical masks only. Among the 120 hospital contacts the largest group were nurses (n=59; 49%), followed by physicians (n=26; 22%) and laboratory technicians (n=15; 13%) (Table 1). Median time from first contact to venipuncture was 39 days (range: 13–50 days).

Eighty-five (69%) of all respondents reported contact at a distance of less than or equal to 2 m, 14 (11%) of more than 2 m, and 24 (20%) of unknown distance to the patient. Frequency of ARI by week of first contact differed significantly among the groups (Table 1). However, there was no trend in the ARI proportion over time: eight of 33 contacts with first exposure during illness weeks three or four experienced ARI within 10 days of last contact; five of nine contacts with first exposure during the patient's fifth week of illness; and none of 14 with first contact during week six of illness developed ARI.

Among 81 contacts reporting exposure within 2 m, 21 had ARI compared to none of 14 with contact of more than 2 m (p value; 0.04) (Table 1). Among those with first exposure in week three or four of illness of the patient, the proportion of contacts with ARI was not significantly different between those considered to be at high risk and the remaining contacts (p value, 0.87) (Table 1). Thirteen HCW had contact to the patient in weeks three or four of illness, had contact within 2 m to the patient and had worn surgical face masks rarely or never. Among these, nine were high-risk contacts, including one nurse who assisted in a bronchoscopy on 25 October. All nine provided a blood sample. The median time after last contact with the patient for these nine HCW was 32 days (range: 13–46 days). No sample was reactive by IFA.

Of the remaining 76 blood samples, one serum showed reactivity for IgM even at dilutions up to 1:100. This titre could be resolved as a cross-reacting recent infection with hCoV-NL63 by IFA using recombinant S and N proteins from major hCoVs (Table 2), as well as absence of NCoV-specific neutralising antibodies. Another serum showed indeterminate IgG-reactivity in a 1:10 dilution. Specific anti-NCoV antibodies were ruled out by recombinant IFA, indicating earlier infection with hCoV-OC43 and hCoV-NL63, as well as absence of any significant titre in SNT (Table 2).

Discussion

Here we describe a case and contact investigation of a laboratory-confirmed patient with NCoV infection for whom the suspicion of this possible aetiology had not been discussed with the treating hospital upon admission of the patient. The patient still tested PCR-positive late in his third week of illness. Despite this we concluded from the laboratory findings that his infectiousness was then absent or very low. While at that time no consistent dedicated personal protective measures had been applied by HCW caring for the patient, our public health investigation did not show infection in any of the 85 serologically tested contact persons, mainly HCW. The conducted serological two-stage approach was an effective method of screening a large number of contact persons for infection.

For initial risk assessment, after the information in November about the cause of the patient's disease, it was important to know if he had been potentially infectious at the time of arrival at the hospital in Germany in October. One stored respiratory sample taken at the time of admission, yielded clear, albeit very low quantities of NCoV RNA in the range of 66.5 to 100 copies per mL [10]. Attempts to isolate virus from this sample were unsuccessful. Even though the sample had been stored for prolonged time under less-than-optimal conditions, these combined RT-PCR and cell culture data suggested absent or very low infectiousness at the time of admission. Negative RT-PCR four weeks later, just after discharge from hospital, suggested the patient had cleared the virus, and no further respiratory precautions were necessary upon admission to the rehabilitation centre.

Nevertheless, anxiety and lack of any other epidemiological data made it necessary to gauge rapidly the significance of some cases of ARI experienced in HCW who had been in contact with the patient. Our data yielded no direct correlation of ARI rates with time of exposure. In particular, those contacts considered at highest risk had no more ARI than other contacts who also had their first contact with the patient during the third or fourth illness week.

In the context of a retrospective contact investigation, our two-staged serological approach proved effective in ruling out any NCoV infections among contacts including those who developed acute respiratory disease. Preliminary screening using a generic serological test provides a reliable result for negative samples. Hereafter only positive or indeterminate results need to be further scrutinised using the described methods.

During two interviews that the patient kindly agreed to, we explored a wide spectrum of factors that he might have been exposed to. Even though NCoV is genetically similar to bat coronaviruses [1,13,14], other animals may serve as (intermediate) host as well. While our patient denied contact to bats, he remembered ill goats among the animals on his farm. Albarrak et al. reported that the first Saudi case was exposed to farm animals, but the first Qatari patient and the second Saudi patient were not [15]. Although our patient reported no direct contact with his animals, one animal caretaker working for him was ill with cough and might have been an intermediate link in the chain of infection.

Coronaviruses do infect ruminants such as goats [16] and thus goats could be considered as a possible source of origin for the novel virus, particularly in

Table 1

Profession, type of contact, occurrence of acute respiratory illness and serological results in contacts of case of novel coronavirus infection, Germany October–November 2012

Variable	N	N (with information)	%	P value[a]
Contacts	123	123	100	–
Hospital staff	120	123	98	–
Out-of-hospital persons	3	123	2	–
Response to questionnaire	110	123	89	–
Acute respiratory infection	24	104	23	–
Provided blood sample	85	123	69	–
Interval between first contact and venipuncture (n=48)	median: 39 days (range: 13–50)			–
Serology for antibodies against NCoV				
Positive	0	85	0	–
Negative	85	85	100	–
Professional group among hospital staff				
Nursing staff	59	120	49	–
Physicians	26	120	22	–
Laboratory technicians	15	120	13	–
Physician and team assistants	13	120	11	–
Physiotherapists	4	120	3	–
House maintenance	4	120	3	–
Cleaning staff	2	120	2	–
Contact distance to patients				
≤2 metres	85	123	69	–
>2 metres	14	123	11	–
Unknown	24	123	20	–
First contact in the 3rd or 4th week of patient's illness				
Yes	36	123	29	–
Later or unknown	87	123	71	–
ARI by contact distance to patients				
≤2 metres	21	81	26	0.04
> 2 metres	0	14	0	
ARI by week of first contact				
3rd/4th illness week	8	33	24	<0.01
5th illness week	5	9	56	
6th illness week or later	0	17	0	
ARI in those exposed in 3rd or 4th week, by risk level				
High risk, i.e. performing aerosol-generating procedures, face mask rarely/not worn	2	9	22	0.87
All others	6	24	25	
High-risk contacts[b]				
who provided blood	9	9	100	–
Interval between last contact and venipuncture (n=9)	median: 27 days (range: 12–46)			–

ARI: acute respiratory illness; NCoV: novel coronavirus.

[a] Based on Fisher's exact test.

[b] A high-risk contact is a contact who had contact in the (3rd or) 4th week of the patient's illness, performed aerosol-generating procedures and wore face mask rarely or not at all.

Table 2

Cross-reactivity test on contact persons and of case of novel coronavirus infection (at week 3 and week 8 of illness) with recombinant spike and nucleocapsid indirect fluorescence antibody test[a], Germany October–November 2012

Virus	Antibody type	Contact 1	Contact 2	Patient (week 3)	Patient (week 8)	Negative control[b]
NCoV						
Spike	IgM[c]	+/–	+/–	>1:320[d]	>1:320	–
	IgG	–	–	>1:320	>1:320	–
Nucleocapsid	IgM	–	1:20	ND	–	–
	IgG	–	+/–	ND	+	–
SARS-CoV						
Spike	IgM	+/–	–	ND	+	–
	IgG	–	–	ND	–	–
Nucleocapsid	IgM	–	–	ND	–	–
	IgG	–	–	ND	–	++
hCoV-OC43						
Spike	IgM	+/– / +	–	+	+	–
	IgG	++	>1:80	++	++	+++
Nucleocapsid	IgM	–	–	+/–	+/–	–
	IgG	+/– / +	+/-	+/– / +	+/– / +	+
hCoV-NL63						
Spike	IgM	1:80	+/–	1:80	>1:320	–
	IgG	>1:320	1:20	>1:320	>1:320	–
Nucleocapsid	IgM	–.	–	ND	–	–
	IgG	++	+/–	ND	+/– / +	–

CoV: Corona virus; hCov: human coronavirus; NCoV: novel coronavirus; ND: not done; SARS: severe acute respiratory syndrome.

[a] All sera were applied in a 1:20 dilution and rated from negative (–), intermediate (+/–) to positive (+ until +++).

[b] A non-patient contact negative-control serum.

[c] IgG depleted.

[d] Titres (selective) were determined by serial dilutions in a range of 1:20 to 1:640.

the geographical and cultural context of our patient. Recent experimental studies have found that NCoV can infect and replicate in cells of various species including humans, swine, monkeys and bats, suggesting a more promiscuous host specificity compared to other human coronaviruses such as SARS CoV [17]. Susceptibility of goat cells was not tested, but it cannot be excluded that NCoV might infect this species as well.

Especially hospitals with ICU, specialist lung hospitals and similar facilities should consider NCoV in patients with severe respiratory disease of unknown aetiology. These patients should be tested for the novel virus as well as pathogens causing illnesses that need to be considered for differential diagnosis in severe lung disease. Full personal protective equipment such as recommended for handling patients with SARS, including N95 masks independent of the procedure performed, should be used by HCW in such cases, and responsible public health agencies should be informed timely. In general, it is prudent that HCW in contact with any patient with a severe respiratory illness of unknown origin apply droplet precautions. Should patients with suspected NCoV infections be transferred for special treatment it is important to fully inform the receiving hospital. Public health management recommendations should be further informed through future research that include the route, amount and duration of virus shedding. In addition, more information is needed on the ability of the virus to transmit from person to person.

Our investigation has some important limitations. We have not obtained a questionnaire and blood from all contacts of this patient. Nevertheless, response rate was high and information on contacts with the highest risk for infection was complete. Available information on the interval between exposure and venipuncture could only be approximated because contacts were exposed over more than one day. In our study we used the day of first contact because the patient was likely most infectious at this point in time. Theoretically, seroconversion may have occurred in some after contacts had provided blood. However, the need to rapidly evaluate the situation urged us to commence the contact investigation immediately. A further limitation is that the patient's negative result of virus isolation could have been due to the long storage time of the

sample – in contrast to our favoured hypothesis of low RNA concentration.

In spite of this, we believe that it is fair to conclude the patient's infectiousness on illness day 20 was absent or very low. Our contact investigation has found no evidence of infection among hospital or out-of-hospital contacts. Our two-staged approach to serological screening where a first-line testing is done by full-virus IFA and supplemented by confirmatory recombinant IFA and SNT should provide a template for similar investigations in the future. Finally, if patients suspected to be infected with NCoV are to be transferred for specialised treatment, receiving hospitals need to be informed so that appropriate infection control measures can be implemented.

Acknowledgements

We thank Kerstin Prahm and Aryna Zanuzdana for support with data entry and validation, Sami Marzougi and Ghassan Moussa for their kind interpreting assistance, Annette Jurke for being the contact point at the state health department, Mareen Adam, Susi Hafemann, Ingrid Zadow and Julia Hinzmann for excellent technical assistance regarding PCR analyses and Petra Kreher and Anette Teichmann for serology test development and testing of sera. We thank Ron Fouchier of Erasmus Medical Centre for providing the virus isolate.

Work at the University of Bonn was supported by the European Union FP7 project EMPERIE (contract number 223498), ANTIGONE (contract number 278976), the German Research Foundation (DFG grant DR 772/3-1, KA1241/18-1), as well as the German Ministry of Education and Research (BMBF SARS II).

Conflict of interest

None declared.

Authors' contributions

UB: assisted in designing the study, analysed data, wrote manuscript. MAM: performed laboratory testing, read and revised manuscript. AN: performed laboratory testing, read and revised manuscript. AS: collected data, read and revised manuscript. NW: collected data, read and revised manuscript. TBB: collected data, read and revised manuscript. FB: collected data, read and revised manuscript. CD: assisted in designing the study, performed laboratory testing, read and revised manuscript. BS: performed laboratory testing, read and revised manuscript. TW: performed laboratory testing, read and revised manuscript. DM: performed laboratory testing, read and revised manuscript. BM: performed laboratory testing, read and revised manuscript. SB: assisted in designing the study, read and revised the manuscript. GK: assisted in designing the study, read and revised the manuscript. LS: assisted in designing the study, read and revised the manuscript. WH: assisted in designing the study, co-ordinated the study, read and revised the manuscript.

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

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Citation style for this article:
Georgiev M, Afonso A, Neubauer H, Needham H, Thiéry R, Rodolakis A, Roest HJ, Stärk KD, Stegeman JA, Vellema P, van der Hoek W, More SJ. Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro Surveill. 2013;18(8):pii=20407. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20407

Article submitted on 20 April 2012 / published on 21 February 2013

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.

Acknowledgements

This review was conducted as part of an opinion on Q fever by the Animal Health and Welfare (AHAW) Panel of the European Food Safety Authority. The authors acknowledge the input from panel members during the development of this review.

Conflict of interest

None.

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