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Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*

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Abstract

Background: *Coxiella burnetii*, the bacterium causing Q fever, is an obligate intracellular biosafety level 3 agent. Detection and quantification of these bacteria with conventional methods is time consuming and dangerous. During the last years, several PCR based diagnostic assays were developed to detect *C. burnetii* DNA in cell cultures and clinical samples. We developed and evaluated TaqMan-based real-time PCR assays that targeted the singular *icd* (isocitrate dehydrogenase) gene and the transposase of the *IS1111a* element present in multiple copies in the *C. burnetii* genome.

Results: To evaluate the precision of the *icd* and *IS1111* real-time PCR assays, we performed different PCR runs with independent DNA dilutions of the *C. burnetii* Nine Mile RSA493 strain. The results showed very low variability, indicating efficient reproducibility of both assays. Using probit analysis, we determined that the minimal number of genome equivalents per reaction that could be detected with a 95% probability was 10 for the *icd* marker and 6.5 for the *IS* marker. Plasmid standards with cloned *icd* and *IS1111* fragments were used to establish standard curves which were linear over a range from 10 to 10^7 starting plasmid copy numbers. We were able to quantify cell numbers of a diluted, heat-inactivated *Coxiella* isolate with a detection limit of 17 *C. burnetii* particles per reaction. Real-time PCR targeting both markers was performed with DNA of 75 different *C. burnetii* isolates originating from all over the world. Using this approach, the number of *IS1111* elements in the genome of the Nine Mile strain was determined to be 23, close to 20, the number revealed by genome sequencing. In other isolates, the number of *IS1111* elements varied widely (between seven and 110) and seemed to be very high in some isolates.

Conclusion: We validated TaqMan-based real-time PCR assays targeting the *icd* and *IS1111* markers of *C. burnetii*. The assays were shown to be specific, highly sensitive and efficiently reproducible. Cell numbers in dilutions of a *C. burnetii* isolate were reliably quantified. PCR quantification suggested a high variability of the number of *IS1111* elements in different *C. burnetii* isolates, which may be useful for further phylogenetic studies.

Background

Coxiella burnetii is the causative agent of Q fever, a zoonosis that occurs worldwide and infects a variety of different animals, including domestic mammals like cattle and sheep. Whereas animals in general show no clinical signs of infection except occasional abortions, *C. burnetii* can cause serious illness in humans, where infections usually occur via aerosols. Acute disease often presents as a self-limiting influenza-like illness with fever and headaches, but severe cases with atypical pneumonia or hepatitis may occur. The disease can become chronic with life-threatening endocarditis as the most frequent clinical manifestation that requires long lasting antibiotic treatment [1]. Although an obligate intracellular organsim, the bacterium is very resistant to environmental conditions due to extracellular spore-like forms, and even a single organism can produce disease. Because of its widespread availability, environmental stability and low infective dose, *C. burnetii* is considered a potential bioterrorist agent and is classified as a group B agent by the Centers for Disease Control and Prevention in Atlanta, USA [2].

C. burnetii is a slow growing bacterium that can be cultivated in embryonated eggs or eukaryotic cell culture, which is time consuming and must be performed in biosafety level 3 laboratories. Antigen detection of bacteria by capture ELISA or direct immunofluorescence is difficult and has relatively high detection limits. Therefore, diagnosis is still mainly based on serological methods like indirect immunofluorescence, complement fixation or ELISA, with the disadvantage of delayed diagnosis because specific antibodies appear only one to two weeks after infection [3].

During the last years, several PCR based diagnostic assays were developed to detect *C. burnetii* DNA in cell cultures and clinical samples. These assays used conventional PCR [4-8], nested PCR [9-12] or real-time PCR conditions with LightCycler [13-15], SYBR Green [16] or TaqMan chemistry [17]. The target sequences of the assays originated from singular chromosomal genes like *com1* or *htpB*, on plasmids (QpH1, QpRS) or on the transposase gene of insertion element *IS1111* [18] that is present in 20 copies in the genome of the *C. burnetii* Nine Mile RSA493 strain [19]. Due to the multicopy number of the *IS1111* element, the corresponding PCR is very sensitive. However, quantification of cells cannot be performed based on PCR of the *IS* element, because the numbers of *IS1111* elements present in different *Coxiella* isolates are not known.

The prerequisite for a diagnostic PCR is a target sequence that is specific for *C. burnetii* to exclude false positive results with other organisms and that is conserved and present in all *C. burnetii* isolates to prevent false negative reactions. The PCR assays mentioned before were usually evaluated with relatively small numbers of characterized isolates or with uncharacterized clinical samples, though it should be noted that most importance was attached on sensitivity of the assay, whereas the suitability of the assays for a great panel of different isolates was less relevant.

The *icd* gene for the isocitrate dehydrogenase was sequenced in 19 strains and shown to be conserved [20]. We used a fragment of this gene as target for real-time TaqMan PCR based on TaqMan chemistry. In addition, we performed a real-time PCR assay based on a fragment of the transposase gene of the *IS* element *IS1111a*. Both assays were validated for specificity and sensitivity, and suitability of the *icd* assay for the quantification of *Coxiella* cell numbers was shown. As the exact number of *IS* elements is only known for the sequenced genome of the Nine Mile strain, we examined the number of *IS1111* elements per genome, or per *icd* copy, respectively, in a large panel of *Coxiella* isolates of worldwide origin.

Results and discussion

Evaluation of the specificity of the real-time PCR assays

To determine whether false positive reactions occurred in real-time PCR assays with the *icd* and *IS1111* markers, PCR was performed with DNA of the bacterial species listed in the Methods section. Based on the sequence of its 16S rRNA, *C. burnetii* is classified into the order Legionellales, with *Legionella* spp. and *Francisella* spp. as nearest phylogenetic neighbours [1]. Both for these related species and for all other species tested, the PCR was negative, confirming the specificity of both targets.

Determination of precision and detection limit of the assays

Based on the measured DNA concentration (29 ng/µl) and the length of the published sequence of the *C. burnetii* Nine Mile genome (1,995,275 bp), the theoretical number of genome equivalents (GE) was calculated to be 1.3×10^7 GE per µl. This corresponds to 2.6×10^8 *IS1111* elements per µl for the Nine Mile strain (20 per genome). To determine the precision of the *icd* and *IS* real-time PCR assays, C_t (threshold cycle) values for eight replicates of tenfold dilutions of purified *C. burnetii* Nine Mile genomic DNA were measured (Table 2). The results represent independent dilution series and different PCR runs. The mean C_t values, standard deviation, and percent CV (coefficient of variation) were calculated for each dilution. The results showed low variability, with CVs ranging from 1.3 to 1.9 % for the *icd* target and 1.1 to 1.6 % for the *IS* target, indicating efficient reproducibility of both assays. Standard curves drawn from the copy numbers and mean C_t values shown in Table 2 had slopes of -3.687 for the *icd* curve and -3.527 for the *IS* curve (data not shown), indicating PCR efficiencies of approximately 90 % for both

Table 1: Characteristics of *C. burnetii* isolates used in this study

C. burnetii Isolate	Restriction Group [23,24]	Geographical Origin
Nine Mile RSA493	I	USA
Balaceanu	I	Romania
Hardthof	I	Germany
Bernard	I	France
CS 1	I	Slovak Republic
CS 3	I	Slovak Republic
CS 4	I	Slovak Republic
CS 5	I	Slovak Republic
CS 6	I	Slovak Republic
CS 7	I	Slovak Republic
CS 8	I	Slovak Republic
CS 9	I	Slovak Republic
CS 10	I	Slovak Republic
CS 11	I	Slovak Republic
CS Dayer	I	Slovak Republic
CS L 35	I	Slovak Republic
CS Poland	I	Poland
J 1	I	Japan
J 3	I	Japan
J 27	I	Japan
Priscilla Q177	IV	USA
Scurry Q217	V	USA
CS S	V	USA
Dugway 5J108-111	VI	USA
Z 3027	VI	Germany
Z 3205a	VI	Germany
Z 3205b	VI	Germany
Z 3351	VI	Germany
Z 3568	VI	Germany
Z 3749	VI	Germany
Z 257	VI	Germany
Boren	I	USA
CS 48	I	Slovak Republic
CS II/Ia	I	Slovak Republic
CS F	I	Slovak Republic
CS Ixodes	I	Russia
CS SI	I	Russia
Florian	I	Slovak Republic
Frankfurt	I	Germany
München	I	Germany
Henzerling	I	Italy
RT 1	I	North Western Russia
RT 3	I	North Western Russia
Gbud	I	Slovak Republic
Geier	I	Romania
Andelfingen	2	Switzerland
Herzberg	2	Greece
CS Z 57	2	Slovak Republic
S 1	2	Sweden
S 4	2	Sweden
Soyta	2	Switzerland
Utvinis	2	Romania
Stanica	2	Romania
Z 3478	2	Germany
Z 3574	2	Germany
Z 4313	2	Germany
Z 4485	2	Germany
Z-104	2	Germany
Z 3464	4	Germany

Table 1: Characteristics of *C. burnetii* isolates used in this study *(Continued)*

Z 3567	5	Germany
Brustel	6	France
Z 2534	6	Austria
Z 3055	6	Germany
Z 2775	7	Germany
Brasov	8	Romania
Namibia	9	Namibia
Schperling	11	Kirgisia
Ouaret	12	France
Jaquemot	13	France
Campoy	13	France
Pallier	14	France
Lombardi	15	France
Raphael	16	France
Butin	16	France
Z 349-36/94	unknown	Germany

targets ($E = 10^{-1/s} - 1$, where E is the run efficiency and s is the slope of the standard curve).

Determination of the detection limit by probit analysis was performed with DNA of the *C. burnetii* Nine Mile strain. For the singular *icd* marker, detection of 100 to 0.75 GE/reaction was tested by PCR. For the *IS1111* marker, where 20 copies are expected per genome, lower concentrations from 25 to 0.2 GE/reaction, or 500 to 4 copies of the *IS1111* element, respectively, were tested. Each PCR was repeated three times with eight replicates for each concentration. The minimal number of genome equivalents per reaction that could be detected with a 95 % probability by real-time PCR was 10 when the *icd* marker was used (Fig. 1). With the *IS1111* marker, 6.5 genome equivalents per reaction were detected with 95 % probability (Fig. 1), corresponding to 130 copies of the target gene. Detection of lower *IS1111* copy numbers was possible, as mentioned below for plasmid standards, but less reproducible. PCR products of the *icd* and *IS1111* assays were analysed on agarose gels and showed the expected single bands of 76 bp and 295 bp, respectively.

Quantification using plasmid standard curves

Tenfold serial dilutions of plasmids with cloned *icd* and *IS1111* fragments were used to establish standard curves for each PCR run. For both markers, the quantification was linear over a range of 10 to 10^7 starting plasmid copy numbers, and the detection limit was ten copies per reaction (data not shown).

To assess whether the number of *icd* and *IS1111* copies per genome could be sufficiently calculated by using standard curves derived from plasmid standards, PCR assays for both targets were performed with tenfold serial dilutions of *C. burnetii* Nine Mile DNA and plasmid standards. The results are shown in Table 3. Especially for lower DNA concentrations, the theoretical numbers of *icd* and *IS1111* copies (calculated from genome size and DNA concentration as shown before) corresponded quite well to the respective copy numbers determined experimentally.

Determination of Coxiella *cell numbers by real-time PCR*

The cell numbers of purified *Coxiella* isolates can be determined by Gimenez stain. To assess whether the cell densities quanitified by real-time PCR were comparable, we

Table 2: Summary of eight different PCR runs performed on eight separate DNA dilution series of the *C. burnetii* Nine Mile RSA493 strain

icd marker				*IS1111* marker			
No. of copies/µl	Mean C_t	SD[a]	CV[b] (%)	No. of copies/µl	Mean C_t	SD[a]	CV[b] (%)
1.3×10^7	15.81	0.23	1.5	2.6×10^8	15.70	0.25	1.6
1.3×10^6	18.64	0.25	1.3	2.6×10^7	17.63	0.29	1.6
1.3×10^5	22.08	0.33	1.5	2.6×10^6	21.37	0.30	1.4
1.3×10^4	25.93	0.46	1.8	2.6×10^5	25.15	0.27	1.1
1.3×10^3	29.63	0.58	1.9	2.6×10^4	28.76	0.36	1.2
1.3×10^2	33.51	0.60	1.8	2.6×10^3	32.52	0.48	1.5
1.3×10^1	37.79	0.49	1.3	2.6×10^2	36.39	0.51	1.4

[a]SD, standard deviation of eight replicates
[b]CV, coefficient of variation

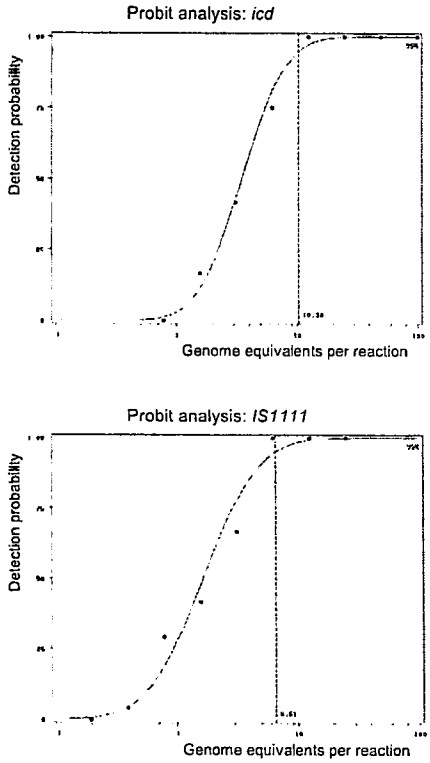

Figure 1
Determination of detection limits for the *icd* and *IS1111* assays. The graphs show curves determined by probit analysis for real-time PCR assays targeting the *icd* and *IS1111a* sequences of *C. burnetii*. With the respective targets, 10 and 6.5 genome equivalents per reaction can be detected with a probability of 95 %.

performed PCR reactions of heat inactivated isolates targeting the *icd* marker without previous DNA extraction. An exponential dilution series was made from heat inactivated particles of the Nine Mile isolate containing 4.2 × 10^9 particles per ml, and 1 µl of each dilution was applied per PCR reaction. Cell numbers were quantified using standard curves derived from diluted plasmid standards. The results are shown in Table 4. Given that only one copy of the chromosome is present per bacterial cell, which can be expected for a slow growing bacterium like *Coxiella*, the number of genome equivalents based on *icd* quantification should be comparable to the number of bacteria. Indeed, the *icd* quantity correlated well with the numbers of coxiellae determined microscopically. The detection limit for real-time PCR was 17 particles per reaction, which is in good agreement with the detection limit for purified *Coxiella* DNA and far below the particle number that can be quantified microscopically.

Determination of the number of IS elements in 75 different Coxiella *isolates*

Although the measured *icd* and *IS1111* copy numbers shown in Table 3 exceeded the calculated numbers in some cases, the number of *IS1111* elements per genome (i.e., per *icd* copy) varied between 13 and 17 for different DNA concentrations, which is close to the published number of 20 *IS1111* elements for the Nine Mile isolate. Therefore, with this assay, DNA samples of 75 isolates of *C. burnetii* from all over the world were assessed for presence of the *icd* and *IS* markers and the numbers of *IS1111* elements per genome were calculated. Quantification of *icd* and *IS* markers was based on standard curves obtained from diluted plasmids. Each DNA sample was tested in duplicate in three independent PCR runs targeting both markers except for DNA from the Nine Mile isolate, where six runs were performed.

In a recent study where Q fever patients were examined 12 years after infection, the *IS1111* element could not be amplified, whereas PCR for other targets was positive [21]. Our results indicated that all isolates contained both the *icd* and the *IS1111* markers. Different PCR runs resulted in discrepancies of the measured quantities and accordingly, different values and standard deviations for the number of *IS1111* elements per genome equivalent were obtained (data not shown). For the Nine Mile RSA493 strain the number of *IS* elements was determined to be 23 (± 3.43), which is in good agreement with the number revealed by sequencing. The mean number of *IS1111* elements per genome varied between seven (isolate J 3) and 110 (isolate Z2534), and between 10 and 30 for the majority of isolates. In French isolates of the related restriction groups 12 to 16 (Table 1), however, the number of *IS1111* elements was found to be above 30, being highest in strain "Raphael" (around 95). All isolates of restriction group I had numbers below 30 insertion elements, so that for these isolates a correlation of the number of *IS1111* elements with the restriction group seems likely. In other restriction groups, however, the number of *IS1111* elements was highly variable. Although the standard deviations were very high for some values, our data suggest that the number of *IS1111* elements can vary widely between different *C. burnetii* isolates and some isolates seem to contain a very high number of *IS1111* elements. To further confirm our real-time PCR based quantification, Southern blot analyses should be performed.

Insertion sequences play a major role in determining band pattern differences between isolates produced by methods such as PFGE (pulsed-field gel electrophoresis)

Table 3: PCR quantification of DNA dilutions of the *C. burnetii* Nine Mile RSA493 strain. The measurements were performed in duplicate; mean values are shown.

	Calculated Values[a]		Measured Values[b]		
DNA conc [pg/µl]	*icd*	*IS1111*	*icd*	*IS1111*	*IS* per genome[c]
2900	1.3×10^6	2.6×10^7	4.5×10^6	5.9×10^7	13.1
290	1.3×10^5	2.6×10^6	5.2×10^5	8.1×10^6	15.6
29	1.3×10^4	2.6×10^5	3.9×10^4	5.0×10^5	12.8
2.9	1.3×10^3	2.6×10^4	2.8×10^3	3.5×10^4	12.5
0.29	1.3×10^2	2.6×10^3	2.3×10^2	2.6×10^3	11.3
2.9×10^{-2}	1.3×10^1	2.6×10^2	1.5×10^1	2.6×10^2	17.3

[a]Number of target copies based on DNA concentration and genome length.
[b]Number of target copies based on PCR quantification using plasmid standards.
[c]Calculated as *IS1111* measured per *icd* measured.

in many bacterial species [22]. *C. burnetii* expresses a low degree of genetic heterogeneity among strains by DNA-DNA hybridization. However, *Not* I restriction of total DNA followed by PFGE resulted in the characterization of 20 restriction groups among 80 *C. burnetii* isolates collected worldwide, as indicated in Table 1[1,23,24]. Typing *C. burnetii* based on restriction fragment length polymorphisms of the locations of the *IS1111* element, like published for the insertion sequence *IS100* of *Yersinia pestis* [25], may add to the elucidation of the phylogenetic relationship of *Coxiella* isolates. Moreover, the insertion sites of *IS1111* could be examined by inverse PCR or by a recently described technique, the so called vectorette PCR [26].

So far, our data are too incomplete for judgements on clinical outcome, namely, to find any correlation between the number of *IS1111* elements and the virulence of an isolate. Nevertheless, it is tempting to speculate that an increased number of *IS* elements in the genome of an isolate could have a deteriorating effect on its fitness, because essential genes might be interrupted by the insertion sequences.

Table 4: Comparison of microscopical and PCR-based determination of *Coxiella* cell numbers. The PCR measurements were performed in duplicate; mean values are shown.

Coxiella particles per µl	
Determined microscopically[a]	Quantified by PCR[b]
4.2×10^6	5.00×10^6
4.2×10^5	3.05×10^5
4.2×10^4	3.15×10^4
4.2×10^3	2.45×10^3
4.2×10^2	1.45×10^2
4.2×10^1	1.70×10^1
4.2×10^0	0

[a]Cell numbers were only counted from undiluted sample.
[b]Real-time PCR targeting the *icd* marker.

Conclusion

We validated TaqMan-based real-time PCR assays targeting the singular *icd* gene and the transposase of the *IS1111a* element present in multiple copies in the genome of *C. burnetii*. The assays were evaluated with a variety of other bacterial species and shown to be specific for *C. burnetii*. Dilution series of *C. burnetii* DNA and of plasmids with cloned *icd* and *IS1111* inserts demonstrated the sensitivity of the assays. Less than 10 genome equivalents per reaction were reproducibly detected. Using the *icd* marker, cell numbers of *C. burnetii* isolates were quantified also at very low cell concentrations. As a first approximation, the combination of both assays was useful to assess the numbers of *IS1111* elements in 75 *C. burnetii* isolates from all over the world. Our data indicate that the numbers of this insertion element in the different isolates seem to be highly variable. The differences in the content of *IS1111* elements might be of importance for further phylogentic analyses of *C. burnetii* isolates.

Methods

Bacterial strains and growth conditions

The *C. burnetii* isolates used in this study are shown in Table 1. *C. burnetii* bacteria were grown in Buffalo green monkey cell cultures and isolated as described [7]. To determine bacterial concentrations, a defined volume of a diluted suspension was fixed on a slide and stained by the Gimenez method. Bacteria were counted and the concentration of the suspension was calculated.

The following DNA samples from other bacterial species were used as negative controls for PCR: *Legionella pneumophila* (ATCC 33152, JR32 and 130b), *Francisella tularensis* ssp. *novicida* (ATCC 15482) and ssp. *tularensis* (Schu4), *Bacillus subtilis* (DSM 347), *Bacillus anthracis* (UD III-7), *Bacillus cereus* (DSM 31), *Bacillus thuringiensis* (DSM 350), *Bacillus megaterium* (DSM 90), *Bacillus licheniformis* (DSM 13), *Staphylococcus aureus* (DSM 20231), *Streptococcus equi* (ATCC 9528), *Pseudomonas putida* (ATCC 12633), *Pseu-*

domonas aeruginosa (ATCC 9027), *Pseudomonas fluorescens* (ATCC 49838), *Burkholderia mallei* (RR0053), *Burkholderia pseudomallei* (ATCC 23343), *Burkholderia stabilis* (CCUG 34168), *Burkholderia multivorans* (CCUG 37240), *Yersinia enterocolitica* (O:8 Ye/80), *Yersinia pseudotuberculosis* (DSM 8992), *Yersinia pestis* (Kim), *Brucella melitensis* biotype 1 (16M Weybridge), *Brucella abortus* biotype 1 (544 Weybridge), *Brucella suis* biotype 1 (1330 Weybridge), *Brucella ovis* biotype 1 (63/290 Weybridge), *Klebsiella oxytoca* (CCUG 15788), *Serratia marcescens*, *Proteus mirabilis*, and *Escherichia coli* (DSM 30083). The DNA preparations of *L. pneumophila* were kind gifts from Dr. A. Flieger (NG 5, Robert Koch-Institut).

DNA extraction

C. burnetii isolates were mixed with an equal volume of ATL Tissue Lysis Buffer (Qiagen, Hilden, Germany) and heat inactivated (90°C, 20 min). DNA was extracted from 400 µl of this suspension according to the protocol for Gram-negative bacteria of the DNeasy Tissue Kit (Qiagen) and eluted in 100 µl of AE buffer.

Primers and probes for real-time PCR

The *icd* assay targets a 76 bp fragment of the *C. burnetii icd* gene.

Primers:

forward, icd-439F = CGTTATTTTACGGGTGTGCCA (439–459)

reverse, icd-514R = CAGAATTTTCGCGGAAAATCA (494–514)

TaqMan probe:

icd-464TM = FAM-CATATTCACCTTTTCAGGCGTTTT-GACCGT-TAMRA-T (464–492).

The numbers in brackets show the positions based on the GenBank accession no. AF146284.

The *IS1111* assay targets a 295 bp fragment of the transposase gene of the *C. burnetii IS1111a* element.

Primers:

forward, Cox-F = GTCTTAAGGTGGGCTGCGTG (219–238)

reverse, Cox-R = CCCCGAATCTCATTGATCAGC (493–513)

TaqMan probe:

Cox-TM = FAM-AGCGAACCATTGGTATCGGACGTT-TAMRA-TATGG (259–287).

The numbers in brackets show the positions based on the GenBank accession no. M80806.

All sequences are given in 5'-3' orientation. Primers and probes were designed using the Primer Express software (Applied Biosystems, Darmstadt, Germany) and purchased from TIB Molbiol (Berlin, Germany).

Preparation of plasmid standards

The target sequences were amplified by conventional PCR using DNA from *C. burnetii* Nine Mile RSA493 strain as template and with the same primers as for real-time PCR in the case of the *IS1111* marker and with primers icd-418F (5'-TATGTTTGCCTTAGGCCCGT) and icd-818R (5'-AAGGGCTTTGCTCCAAATTC) in the case of the *icd* marker, for which a 401 bp long amplicon was obtained. Plasmid standards with cloned (TOPO TA Cloning System, Invitrogen, Karlsruhe, Germany) and sequenced inserts were generated by GenExpress (Berlin, Germany). Plasmid preparations were quantified spectrophotometrically, and plasmid copy numbers were calculated. Dilutions of the plasmids were used in real-time PCR reactions to prepare standard curves for quantification of the initial copy numbers.

PCR assay conditions

Real-time PCR reaction mix consisted of 6.25 µl of Universal Master Mix (Applied Biosystems, Darmstadt, Germany) containing dNUTPs, $MgCl_2$, reaction buffer and AmpliTaq Gold DNA polymerase, 300 nM of each primer and 100 nM of fluorescence-labeled TaqMan probe. For most assays, water was added to a final volume of 24 µl, and 1 µl of purified template DNA or heat inactivated *C. burnetii* isolate was used as template. For determination of the *IS1111* copy numbers in the 75 *C. burnetii* isolates, water was added to a final volume of 15 µl, and 10 µl of 10-fold dilutions of the DNA were used as templates to minimise pipetting errors. All real-time PCR reactions were performed in duplicate in a 7700 Sequence Detection System (Applied Biosystems) as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles at 15 s 95°C and 30 s at 60°C. Data were analyzed with the corresponding software.

Probit analysis

The number of *C. burnetii* Nine Mile genome equivalents (GE) with a genome size of 1,995,275 bp in a DNA preparation with a concentration of 29 ng/µl was calculated to be 1.3×10^7 GE/µl. To determine the number of GE that can be detected with a probability of 95 %, eight replicates of serial DNA dilutions from 100 GE/reaction to 0.75 GE/reaction for *icd* or 25 GE/reaction to 0.2 GE/reaction for

IS1111 were tested in independent PCR reactions performed by different persons. The reaction volume was 1 µl. Each PCR gave a positive or negative result at the concentration tested. The detection probability was obtained by plotting the proportion of positive PCRs observed against the genome equivalents. Statistical analysis was performed using the SAS version 9.1 software.

Authors' contributions

SRK designed and coordinated the study, drafted the manuscript and participated in performing real-time PCR assays. JT and GB were responsible for the cultivation of the *C. burnetii* isolates, participated in the design of the study and helped to draft the manuscript. HE participated in the design of the study, the evaluation of the PCR assays and helped to draft the manuscript. TF isolated *C. burnetii* DNA and participated in performing PCR assays. SL participated in DNA isolation and evaluation of the PCR assays. BA participated in the design of the study, provided technical and financial support, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Q fever cases in Gloucestershire

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Details of six cases of Q fever in Cheltenham were provided. The dates given ranged from 15th May 2007 until 12th June 2007 and were, in each case, the date each person reported themselves as being ill. Since the disease has an incubation period of 2 to 4 weeks, and assuming that each person reported themselves as being ill immediately, this gives an infection period for the cluster of cases between 17th April 2007 and 29th May 2007 (i.e. 4 weeks prior to the first reported case up until 2 weeks prior to the last reported case).

Wind data at a height of 10 m for Cheltenham was obtained from the Met Office's numerical weather prediction model. The meteorological data has a horizontal resolution of approximately 12 km and so is not able to fully capture the local flow within the urban area. Figure 1 shows the wind rose produced from the numerical weather prediction data over the period 12Z on 17/04/07 until 12Z on 29/05/07. The wind rose shows the proportion of winds over this period categorised by wind direction and wind speed. Wind direction is given as the direction from which the wind is coming from (hence a south-westerly wind will transport airborne substances in a north-easterly direction). The wind rose shows that the predominant wind directions during the period were south-westerly and north-easterly which is consistent with the Q fever cases lying along a north-east to south-west line. However, the wind rose also shows that winds came from the whole range of directions during the infection period.

Observations of wind speed and direction were also available from the meteorological observing site at Pershore. Figure 2 shows the wind rose produced using observational wind data from this location for the same period (12Z 17/04/07 – 12Z 29/05/07). The observational wind rose shows a similar pattern to that of the numerical weather prediction data wind rose. The predominant wind directions were south-westerly and north-easterly, but, during the period of infection, all wind directions were observed.

The Met Office's atmospheric dispersion model, NAME, was run backwards from Cheltenham to identify possible source regions. Figure 3 shows the history of air arriving at Cheltenham (marked using a black cross) within the infection window of 17/04/07 to 29/05/07. The locations of the abattoirs (as provided) are marked using red diamonds. The contour colours can be interpreted as representing the proportion of air arriving at Cheltenham within the infection time period, which has come from that map point. (Note that darker colours (purple / blue) represent a higher proportion than lighter colours (green).) The effects of the predominant wind directions are clearly evident in Figure 3 with air more likely to have arrived at Cheltenham on a north-easterly or south-westerly track. However, Figure 3 does not rule out sources from any direction since it shows that air has come for all possible directions during this period in agreement with the wind roses in Figures 1 and 2.

NAME was also run for two specific cases on the northern and southern geographical extent of the outbreak region: (i) the case reported on 04/06/07, and (ii) the case reported on the 12/06/07. With an incubation period of 2 to 4 weeks this gives an infection window of (i) 07/05/07 – 21/05/07 and (ii) 15/05/07 – 29/05/07, respectively. NAME was run backwards, in each case, to determine the air history for air arriving at each location during the respective infection periods. Figures 4 and 5 show the NAME air history maps for the 04/06/07 case and the 12/06/07 case, respectively. Again we see that none of the abattoirs shown can be ruled out as potential sources since air has come from all of these locations to the location of the case during the respective infection window.

Conclusions

The predominant wind directions during the identified infection period are consistent with the linear locations of the Q fever cases. This suggests that the mode of transmission may well have been airborne. However, due to the variation in wind direction over the period in question coupled with the short scale of the airborne transmission, results from the NAME modelling are inconclusive in highlighting a potential source since none of the abattoirs suggested can be ruled out as potential sources.

Notes
The horizontal resolution of the numerical weather prediction data used in Figure 1 and by NAME (Figures 3, 4 and 5) is 12 km and therefore will not represent the local flow fully within the urban conurbation of Cheltenham.

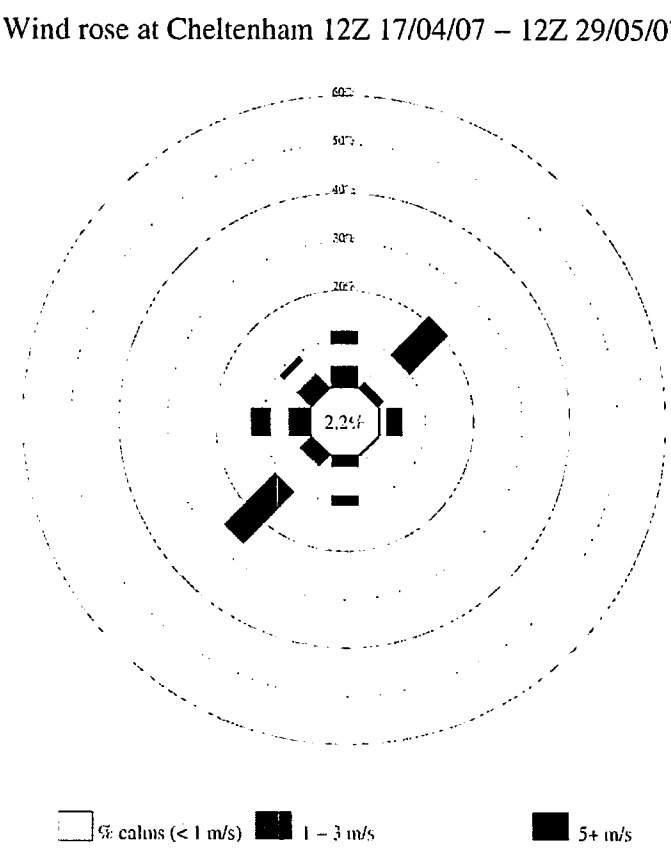

Figure 1: Wind rose of numerical weather prediction 10 m wind data for Cheltenham during the period 12Z 17/04/07 until 12Z 29/05/07

Wind rose at Pershore 12Z 17/04/07 – 12Z 29/05/07

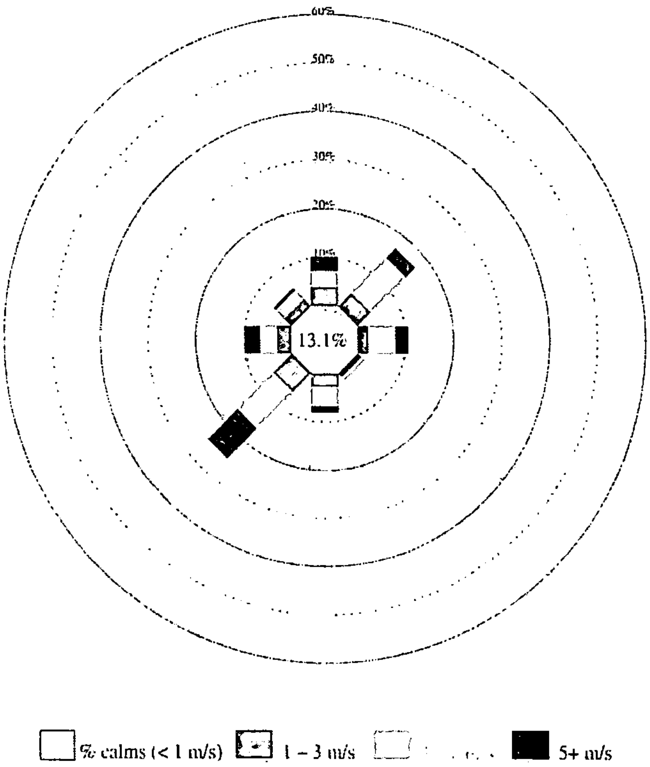

Figure 2: Wind rose of observational 10 m wind data for Pershore during the period 12Z 17/04/07 until 12Z 29/05/07

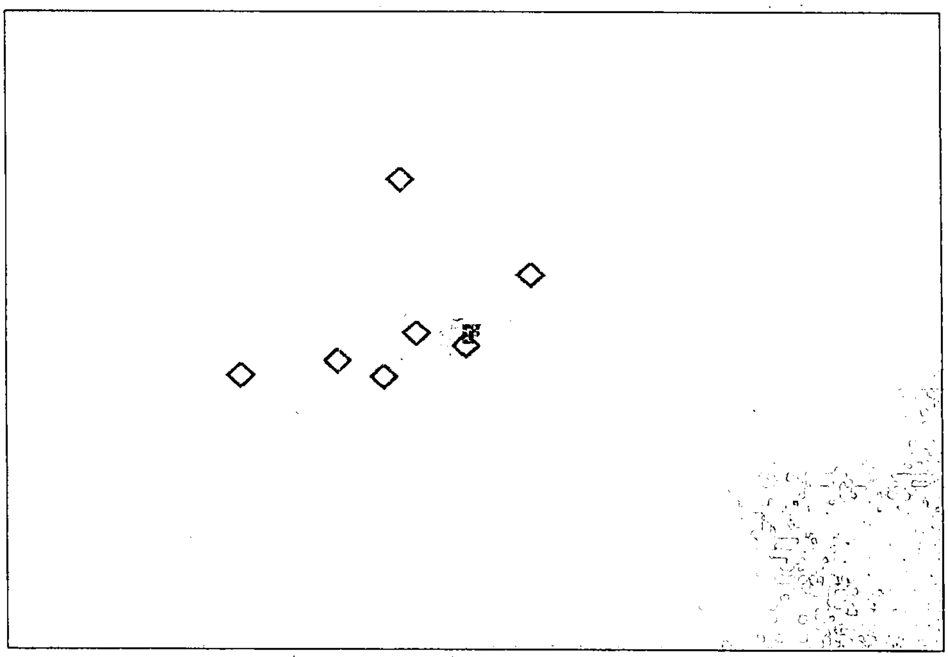

Figure 3: NAME air history map showing where air arriving at Cheltenham between 17/04/07 and

29/05/07 has come from. (Red diamonds mark location of abattoirs)

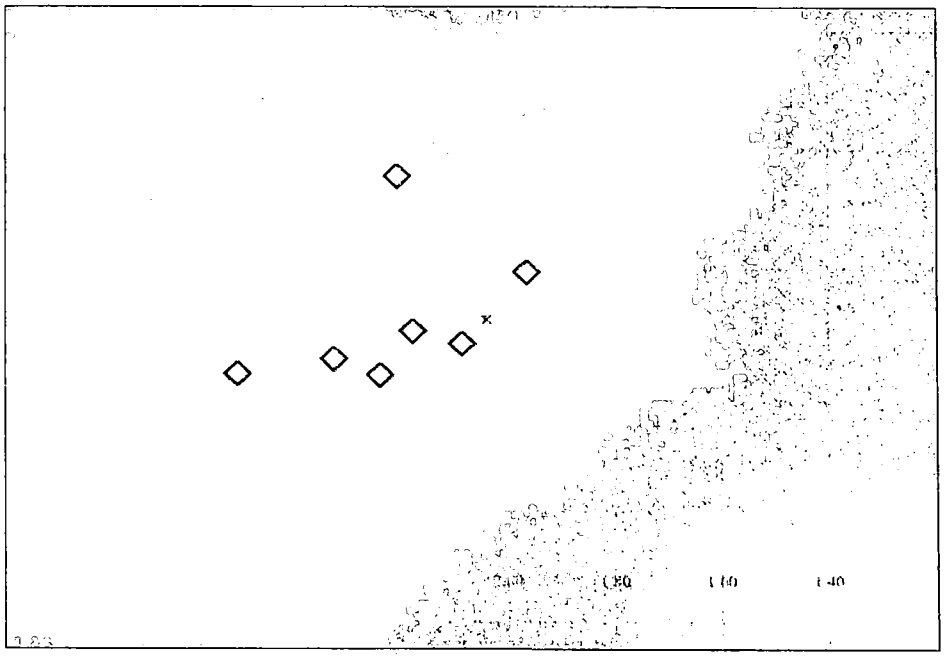

Figure 3: NAME air history map showing where air arriving at the location of the 04/06/07 case during the infection period between 07/05/07 and 21/05/07 has come from. (Red diamonds mark locations of abattoirs)

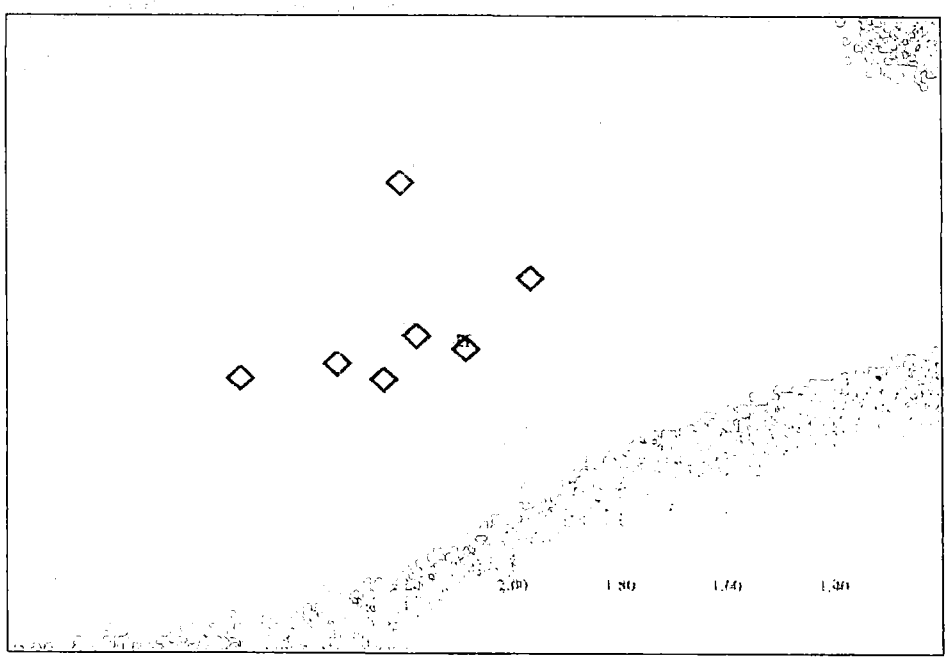

Figure 5: NAME air history map showing where air arriving at the location of the 12/06/07 case during the infection period between 15/05/07 and 29/05/07 has come from. (Red diamonds mark locations of abattoirs)

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ORIGINAL INVESTIGATION

Q Fever During Pregnancy

Diagnosis, Treatment, and Follow-up

Didier Raoult, MD, PhD; Florence Fenollar, MD; Andreas Stein, MD, PhD

Background: Q fever, caused by *Coxiella burnetii*, may result in abortions, premature deliveries, and stillbirths in infected pregnant women.

Objective: To evaluate the best treatment strategy for Q fever during pregnancy.

Methods: We evaluated the prognosis of 17 pregnant women who developed Q fever with and without co-trimoxazole (trimethoprim-sulfamethoxazole) treatment.

Results: The outcome of the pregnancy was found to depend on the trimester. Abortions occurred in 7 of 7 insufficiently treated patients infected during the first trimester vs 1 of 5 patients infected later. Co-trimoxazole given until delivery protected against abortion (0/4) but not against the development of chronic infections, and it did not significantly reduce the colonization of the placenta (2/4 vs 4/4).

Conclusions: Our results show that *C burnetii* infections cause abortion and that women who develop Q fever while pregnant should be treated with co-trimoxazole for the duration of pregnancy, specifically when infected during the first trimester.

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Q FEVER IS A zoonosis caused by *Coxiella burnetii* and occurs worldwide. Although the organism may infect mammals, birds, and arthropods,[1] domestic animals and pets are the most frequent sources of human infections. Q fever is usually acquired by inhalation of aerosols from parturient fluids or the placenta of infected animals.[2] In female animals, *C burnetii* infections are often chronic and have been associated with abortions in sheep,[3] goats,[4] and mice,[5] and low birth weight and infertility in cattle[6] and mice.[5] Acute infections result in granuloma formation in infected viscera, and IgM and IgG antibodies develop mainly against the laboratory-derived avirulent form of *C burnetii* (phase II). In some people, the infection is not controlled by the immune response or by granuloma formation, and very high antibody levels of IgG and IgA types develop, which are directed against both the virulent (phase I) and the avirulent forms (phase II) of *C burnetii*.[2]

Previously there were few data on Q fever in pregnant women.[7] Five new cases have recently been reported and 21 others reviewed from the literature[8-19]; subsequently other cases have been described.[20] This work has shown that Q fever, when contracted during pregnancy, can result in abortions or neonatal deaths (9 cases, 38%), premature births, low birth weight (8 cases, 33%), or no abnormalities (7 cases, 29%). In some cases, pregnancy was found to be associated with the development of chronic infections and relapses.

We have now collected additional data on 17 patients who contracted Q fever while pregnant and who were treated and followed up as we have proposed. We herein report our findings.

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RESULTS

We were involved in the diagnosis and follow-up of 17 pregnant women who developed Q fever (**Table**), with 15 of the women having been examined and followed up by one of us (D.R. or A.S.). From 1 to 4 cases were diagnosed each year of the study, with 13 patients coming from Marseille or the vicinity, 2 from other locations in France, 1 from Africa, and 1 from Iceland. Eleven patients developed Q fever during the first trimester of their pregnancy, 3 during the second, and 3 during the third trimester. Two patients had a heart murmur. In 1 patient the murmur

PATIENTS AND METHODS

PATIENT CHARACTERISTICS

As a reference center for the diagnosis and study of rickettsial diseases, our laboratory regularly receives specimens from France and internationally for the diagnosis of Q fever.[21] This enabled us to identify pregnant women who presented with unexplained fever and/or abortion and were tested for Q fever. The physician in charge of each patient completed a questionnaire to provide data on epidemiologic and clinical features. Age, permanent address, and occupation were recorded and the presence of valvular disease noted. Other infectious diseases were excluded on the basis of negative blood cultures and lack of serologic evidence of evolutive infection with *Toxoplasma gondii*, rubella virus, cytomegalovirus, human immunodeficiency virus, hepatitis B, influenza viruses, parvovirus B19, adenovirus, *Chlamydia* species, and *Mycoplasma pneumoniae*. The administration of antibiotics and the length of treatment were also recorded. Finally, the outcome of the pregnancy was categorized as abortion, premature delivery (<36 weeks), low birth weight (<3 kg), or normal outcome.

SEROLOGIC PROCEDURES

Indirect immunofluorescent antibody tests were carried out as described previously.[22] As cutoff values, titers of 200 or higher anti–phase II IgG and 50 or higher anti–phase II IgM were required for the diagnosis of acute Q fever, and titers of 1600 or higher anti–phase I IgG for the diagnosis of chronic Q fever. On collection, placental tissue samples were frozen and stored at −80°C until tested for the presence of *C burnetii* by polymerase chain reaction and culture as previously reported.[22]

TREATMENT AND FOLLOW-UP

Beginning in 1996, women developing Q fever while pregnant were treated with co-trimoxazole (320 mg of trimethoprim in combination with 1600 mg of sulfamethoxazole) until term. Serologic testing was performed each month during the pregnancy, and after delivery patients with serologic evidence of chronic disease were treated with a combination of doxycycline (200 mg/d) and hydroxychloroquine (600 mg/d) for 1 year. The hydroxychloroquine dose was adapted to obtain a drug plasma level of 1 ±0.20 µg/mL. Every 6 months, a specific ophthalmologic examination was performed to detect intraretinal accumulation of hydroxychloroquine.[23] A clinical follow-up was performed each month to observe compliance, tolerance, and efficiency of the treatment. Data were analyzed using the Fisher exact test; $P<.05$ was considered significant.

had been detected before the pregnancy and followed rheumatic fever. The second patient had mitral insufficiency, which was first identified in our study but was not investigated further.

None of the pregnancies were normal. In 8 cases the fetus died, and in 9 cases delivery was premature or there was a low birth weight. Co-trimoxazole was administered throughout pregnancy to 4 patients, for 6 months to 1 patient, and for 3 weeks to another. The drug was not administered to the remaining 11 patients. In the untreated patients who became infected in the first trimester, 6 of the 6 aborted compared with 1 of 5 who became infected during the second or the third trimester ($P=.01$). One patient treated from the 8th to the 21st week aborted during the 24th week. *Coxiella burnetii* was found in both placenta and fetus.[9]

Seven of the patients seroconverted during the study, and 12 had serologic profiles consistent with chronic infections; 12 of the 14 women who had Q fever in the 2 first trimesters developed chronic infections. Of the 2 patients infected in the first trimester who did not develop chronic infections, one aborted soon after the diagnosis was made (patient 7, Table) and the other was treated with co-trimoxazole for the remaining 6 months of her pregnancy (patient 17). Her placenta was found to be negative for *C burnetii* by culture and polymerase chain reaction.

Nine patients with chronic infections were given doxycycline and hydroxychloroquine for 18 months. Subsequent pregnancies[9] occurred in 7 patients and were normal. One patient completed only 3 months of treatment and had a normal pregnancy 1 year later. A patient who did not receive this treatment was given co-trimoxazole for the duration of a subsequent normal pregnancy.

There were no abortions in 4 women treated with long-term co-trimoxazole, but abortions occurred in 8 of 11 untreated women and in 1 treated for only 3 weeks ($P=.01$). During the first trimester, all untreated women aborted (7/7) compared with none of the 4 who were treated ($P<.01$). During the second and third trimesters, no differences were observed, and only 1 woman infected during the second trimester experienced a fetal death.

Coxiella burnetii was detected by culture and/or polymerase chain reaction in the placentas of all 4 women who were not treated and in 2 of 4 of those treated with co-trimoxazole. Long-term treatment started during the first trimester did not prevent the development of chronic infections: 4 of the 5 treated patients and 8 of the 9 untreated patients developed high anti–phase I titers.

COMMENT

Q fever is a therapeutic challenge because *C burnetii* is an intracellular bacterium that lives in an acidic vacuole, which may protect it from the bacteriocidal effect of antibiotics.[24] Several antibiotics, however, have bacteriostatic effects on the organism, including tetracyclines, rifampin, co-trimoxazole, and fluoroquinolones.[25] The only effective bacteriocidal regimen in vitro is the concurrent use of doxycycline and chloroquine. Chloroquine affects intracellular pH, and when it is present at a concentration of 1 µg/mL the pH of the phagolysosome increases from 4.8 to 5.7, which restores the bacteriocidal effect of doxycycline.

Cases of Q Fever During Pregnancy

Patient No./ Age, y/Year of Diagnosis	Place of Diagnosis	Trimester of Infection	Serologic Findings	Duration of Co-trimoxazole Treatment	Pregnancy Outcome	Isolation/PCR From Placenta	Treatment for Chronic Infection	Subsequent Pregnancy (Years After Q Fever)
1/30/1991	M	2	C	None	Prematurity	NA	No	No
2/26/1992	M	1	O, A, C	3 wk	Abortion	Yes	Yes	Yes (4)
3/32/1993	M	3	A	None	Prematurity	NA	No	NA
4/32/1994	M	1	C	None	Abortion	Yes	Yes	Yes (3 and 6)
5/39/1994	F	1	O, A, C	None	Abortion	Yes	No	Yes (1)
6/22/1995	M	3	A	None	Prematurity	NA	No	NA
7/34/1995	M	1	O, A	None	Abortion	NA	No	No
8/29/1996	M	1	O, A, C	Full pregnancy	Prematurity	Negative	Yes	Yes (2)
9/24/1997	M	2	C	None	Abortion	NA	Yes	Yes (2 and 4)
10/26/1997	M	1	O, A, C	None	Abortion	Yes	Yes	Yes (2 and 3)
11/35/1997	M	1	A, C	None	Abortion	NA	Yes†	Yes (2)
12/23/1999	M	2	A, C	Full pregnancy	Low birth weight	Yes	Yes	No
13/36/1999	Iceland	1	C	Full pregnancy	Prematurity	NA	Yes	No
14/35/2000	M	3	A	None	Prematurity	NA	No	No
15/23/2000	M	1	O, A, C	Full pregnancy	Prematurity	Yes	Yes	No
16/36/2000	Rwanda	1	C	None	Abortion	NA	Yes	No
17/42/2000	F	1	O, A	6 mo	Prematurity	Negative	No	No

*M indicates Marseille and vicinity; F, France except Marseille and vicinity; O, seroconversion; A, acute profile (IgM anti-phase I titer ≥50; IgG anti-phase II titer ≥200); C, chronic profile (IgG anti-phases I and II titers ≥1600; IgA anti-phases I and II titer ≥100); PCR, polymerase chain reaction; and NA, not available.
†Interruption by the patient after 3 months.

Chronic infections with *C burnetii* develop only in some individuals whose immune system is unable to control the organism.[2] A good indicator of such chronic infections is a high level of antibodies to the phase I stage of *C burnetii*.[21] Patients with existing valvular or vascular diseases are at particular risk of developing chronic infections, as are immunocompromised patients and pregnant women.

As in other mammals that become infected with *C burnetii*, in pregnant women the bacteria colonize and multiply in the uterus, mammary glands, and placenta.[7] Chronic infections develop if the woman is pregnant at the time of the primary infection, and this could be related to lack of an appropriate immune control. Apparently, women who have acute Q fever before they become pregnant do not have increased risks of abortion or premature delivery. There are few data, however, on the effects of Q fever contracted during pregnancy.

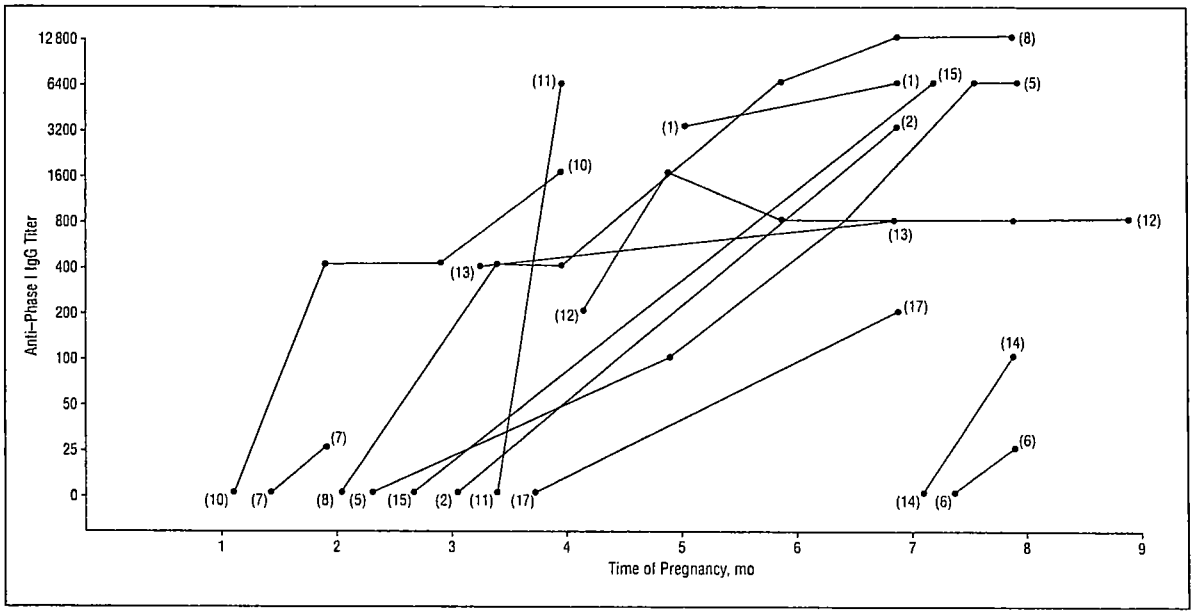
In our study we found abnormalities in all the pregnancies associated with acute Q fever. Fetal death occurred in two thirds of the untreated patients we studied, and one third gave birth prematurely. Our finding of abortions occurring in all untreated or incompletely treated pregnant women shows for the first time that a primary infection with *C burnetii* during the first trimester of pregnancy is a specific risk for abortion. We have previously isolated organisms directly from fetal tissues to show that fetal death is caused by infection.[9] Teratogenicity has not been associated with *C burnetii* infections,[7] and our findings show that specific therapy is indicated to attempt to save the fetus in pregnant women who develop Q fever during the first trimester.

Doxycycline and quinolones are contraindicated during pregnancy. Co-trimoxazole and rifampin may be used with caution, but they are not bacteriocidal. Case 2[9] showed us that short-term treatment was unable to prevent abortion, so we tested long-term therapy. We have used co-trimoxazole to treat pregnant women with acute Q fever since 1996, and this prevented fetal death in 5 of the women and prevented infection of the placenta in 2 of 4 women. Although all 5 treated women gave birth early, and all of their babies had low birth weights, we believe that treatment with co-trimoxazole should be recommended routinely.

In our study, most patients infected with *C burnetii* during the first 6 months of pregnancy developed chronic Q fever, regardless of treatment (**Figure**). The major factor influencing the development of chronic Q fever seemed to be the duration of the infection during pregnancy. Of the 5 patients who did not develop chronic infections, 1 had an early abortion, 1 received long-term treatment (patient 17), and 3 were infected only late in their pregnancies.

We used the treatment recommended for Q fever endocarditis on our chronically infected patients to prevent the possible development of endocarditis, as may occur in mice,[5] and to prevent recurrent abortions.[8] After this treatment, all pregnancies were normal. *Coxiella burnetii* has been isolated from the milk of women in several studies,[7] and we believe that breastfeeding should not be recommended for women who have had Q fever during their pregnancy. The presence of *C burnetii* has been reported in the placenta of asymptomatic women, but the significance of this has yet to be determined.[26]

In conclusion, our study has confirmed that Q fever acquired during pregnancy is a serious disease. Infections with *C burnetii* in the first trimester frequently result in abortion, while those occurring in the second trimester result in prematurity. Long-term co-trimoxazole treatment prevents abortion and neonatal death but not the development of chronic infections. Treatment of patients with chronic Q fever using doxycy-



Anti–*Coxiella burnetii* IgG phase I serologic evolution for 13 patients during pregnancy. Numbers in parentheses indicate patient number (see Table). The shaded area indicates the titer range for chronic Q fever.

cline and hydroxychloroquine for a year after their pregnancy resulted in the elimination of *C burnetii*, and subsequent pregnancies were normal.

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72.E

Q fever in Europe

In order to give a European overview, members of the editorial board of *Eurosurveillance* were asked a few questions about the surveillance of Q fever in the countries they represent and the possible occurrence of similar outbreaks in recent years.

We received reports from Belgium, Denmark, England and Wales, France, Germany, Ireland, Italy, the Netherlands, Portugal, Scotland, and Spain. Four representatives gave additional comments.

Belgium and the Netherlands have two surveillance systems: mandatory notification by physicians and a laboratory reporting system. In Belgium, cases of Q fever are included in notifications of rickettsiosis. England and Wales, Scotland, and Spain collect data on Q fever only through laboratory reports of *Coxiella burnetii* infections. Germany has a specific mandatory notification system for Q fever. Italy and Portugal have a mandatory notification system for all rickettsial infections including Q fever (in Italy thyphus exanthematicus and in Portugal Boutonneuse fever are notified separately). Ireland, Denmark, and France have no reporting scheme for Q fever. In Denmark, however, requests for serological tests are collated at a national laboratory and in France they are centralised at a national reference centre for rickettsiosis.

Only a few cases of Q fever occurred each year in Belgium, Denmark, Ireland, the Netherlands, Portugal, and Scotland, and no outbreaks similar to those described in this issue were identified in recent years. In England and Wales, four outbreaks were reported (June to August 1981, 29 cases in Gwent (1); April to June 1983, 25 cases in Oxfordshire (2); Spring 1989, 147 cases in the West Midlands (3); March 1992, 4 cases (4)). In Spain, between 1990 to 1996, ten outbreaks were reported among which three were quite large (14, 11, and 48 cases). In Italy, an outbreak occurred in the Veneto region (summer and autumn 1993, 58 cases (5)). In Germany since 1990, 27 to 100 cases are reported each year and two outbreaks occurred in recent years (1992, 80 cases in Berlin; 1993, 121 cases in Hessen).

Some additional information was received about possible modes of transmission. Two Q fever outbreaks in England and Wales and one in Italy were investigated using case control studies (1,3,5). In Gwent, Wales, the likeliest explanation was that farm vehicles spread contaminated straw, manure, or dust, and residents of the affected area became infected by inhaling infected dust particles. In the West Midlands, the geographical distribution of cases in an urban area close to many farms where lambs and calves were being born suggested that infected aerosols from parturient sheep were spread by the wind. In the Veneto region, Italy, the case control study showed a significant association with exposure to flocks of sheep. Three flocks of sheep, which passed through the outbreak area between late May and early June in the annual transmigration, were shown to be infected. Birth products were not incriminated since this outbreak began months after the birthing season. Contamination of soil through infected urine and faeces and dry season may have played a role in airborne transmission. In Spain, cases in most outbreaks were suspected to have been infected by contact with contaminated livestock: in 1990, 14 cases were found among workers in a slaughterhouse, and in 1992, 48 cases were found in a military community that camped in a cowshed. In Germany, two outbreaks

were investigated; in 1992 one occurred in a Berlin research facility where sheep were kept and the other in 1993 in a rural area in Hessen. In both outbreaks infected sheep were suspected to be the source of the outbreaks.

Comments

K de Schrijver (Gezondheidsinspectie, Antwerpen, Belgium): Four cases were identified by the laboratory system in 1995. The low level of reporting may be explained by the fact that symptoms of Q fever are quite non specific and clinicians rarely request serological tests for this illness.

R Pebody (writing for England and Wales, EPIET fellow currently based at the National Public Health Institute, Helsinki, Finland): Large outbreaks of Q fever have rarely been detected. The source of infection often remains speculative. Although both of the outbreaks detected in England and Wales were thought to be related to the inhalation of infected particles from nearby areas, the evidence was inconclusive. In the German outbreak reported here the combination of the descriptive epidemiology and a cohort study, positive coxiella serology in the sheep flock, and appropriate weather conditions provided strong evidence that the outbreak was related to airborne transmission of sheep birth products from a neighbouring farm. This article is an important addition to documenting the epidemiology of *Coxiella burnetii* infection. Coxiellosis is often asymptomatic in farm animals, but can cause considerable morbidity in humans. Control and eradication measures require a multidisciplinary approach from farmers, veterinarians, and public and environmental health workers (6).

M Esveld (*Infectieziekten Bulletin*, The Netherlands): In some human cases no relation with "classical" sources can be found and possible new sources must be sought. In a serological study of dogs and cats, 13.2% (91/688) of dogs and 10.4% (46/441) of cats were found to be positive for specific antibodies against *C. burnetii* (7). This implies that cats and dogs may be a source of infection. Special attention to hygiene during parturition may be needed.

G Salamina (Istituto Superiore di Sanità, Italy): In Italy the case definition for rickettsiosis used for surveillance purposes specifies both clinical symptoms and serological confirmation. Therefore, it would be possible, in theory, to identify cases of Q fever among notifications of rickettsiosis to the Ministry of Health. However to date this information has not been published.

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Surveillance report

Outbreak of Q fever in Lohra-Rollshausen, Germany, spring 1996

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Introduction

Q fever is an acute (and sometimes chronic) febrile illness caused by the rickettsial organism *Coxiella burnetii*. The commonest animal reservoirs for *C. burnetii* are cattle, sheep, and goats. Infected animals shed the organisms, which resist desiccation, in birth products particularly. Humans are infected mainly by inhalation of contaminated dust and aerosols (1). In May 1996, the Health Department of Marburg-Biedenkopf in Hessen was informed of a cluster of patients with high and persistent fever in Rollshausen and surrounding towns. Serological testing of some patients by the local health authorities suggested that these people had Q fever.

In July 1996, the Robert Koch Institute was invited to help investigate this cluster in order to ascertain whether an outbreak of Q fever had occurred in Rollshausen and to assess potential risk factors for Q fever among the cases.

Background

Rollshausen is a rural town with about 300 inhabitants. Five surrounding towns are located 1 to 3 km from Rollshausen. Two flocks of sheep were farmed near Rollshausen before the outbreak. One flock, of more than 1000 sheep, was kept on a farm north west of Rollshausen from October 1995 to May 1996. A glut of lambs were born in December 1995 and January 1996 both indoors and outdoors. In May 1996, the flock was moved to a summer pasture more than 10 km away. The second flock, with about 20 sheep, has been kept north east of Rollshausen since 1995.

Methods

During the initial epidemiological investigation, we contacted the family doctors serving the area to identify all those who had sought medical care for possible Q fever since January 1996. We also reviewed the medical records of all patients

admitted to local hospitals with possible Q fever. During July 1996, we attempted to retest all of these patients for *C. burnetii* antibody and administer a questionnaire, which we also used in a cohort study of Rollshausen residents, described below.

To determine the extent of the outbreak and risk factors for illness, we conducted a retrospective cohort study of all Rollshausen residents aged 15 years and over. A self-administered questionnaire, to gather information about the onset, duration and types of symptoms since 1 January 1996, age, sex, occupation, livestock exposure, consumption of raw milk, tick bites, and outdoor activities from each eligible person in Rollshausen, was distributed on 10 July. Blood was taken for *C. burnetii* antibody from all willing residents the next day.

Two case definitions were used. To meet the clinical case definition, patients had to have had fever > or equal 39°C lasting >2 days and at least 3 of the following symptoms (chills, sweats, severe headache, cough, aching muscles/joints, back pain, fatigue or feeling ill) after 1 January 1996. The laboratory case definition was fulfilled by the detection of IgM against *C. burnetii* antibodies in serum. A person was considered to have had *C. burnetii* infection if they met either case definition.

The laboratory of the "Institut für Hygiene und Infektions Krankheiten der Tiere der Justus-Liebig-Universität Gießen" tested for *C. burnetii* antibody using by an enzyme linked immunosorbent assay (ELISA). Human serum specimens were tested both for IgG and IgM antibodies; IgG and IgM were not distinguished in animal specimens.

Twenty serum specimens from the large sheep flock and 12 from other animals obtained in May were tested for *C. burnetii* antibody by ELISA. Nine sheep of the smaller flock were also tested for *C. burnetii* antibody. Weather reports for the region of Lohra-Rollshausen from 1992-96 were obtained from the "Deutscher Wetterdienst/Klima und Umweltberatung".

Results

One hundred and ninety-three (81%) of the 239 eligible residents completed the questionnaire, 35 (18%) of whom met the clinical case definition. Half of the eligible residents (120) gave blood to be tested for *C. burnetii* antibody and 35 (29%) met the laboratory case definition. Two hundred of the eligible residents had either given blood or completed the questionnaire, 49 (25%) of whom met either the clinical or laboratory case definition. The 49 cases were geographically distributed throughout Rollshausen. Attack rates (AR) were similar for males (24%) and females (25%). The ARs did not differ significantly by age group: 15-19 years (13%), 20-39 years (26%), 40-59 years (30%), and > or equal 60 years (16%).

The commonest symptoms reported by the 49 persons who met either of the two case definitions were fatigue (39), fever (38), feeling ill (37), and chills (35). All those who met the clinical case definition contacted family doctors and four were admitted to hospital for a median of 11 days (range 7-18) with radiological evidence of pneumonia. Twenty-seven of the 35 who met the clinical case definition had become ill between March and May (Figure 1). Serum specimens were available from 25 of the 35 patients, in 21 of which IgM *C. burnetii* antibody was detected. Of the 35 persons who met the laboratory case definition, 21 met the clinical case definition,

eight had some symptoms but did not meet the clinical case definition, and six were asymptomatic.

Outbreak of Q fever in Germany

Two weeks ago Eurosurveillance Weekly reported on three cases of Q fever in the United Kingdom, which affected farm workers helping to cull cattle during an outbreak of foot and mouth disease in the country (1). Elsewhere in Europe, Q fever has been associated with sheep flocks passing populated areas (1). A prolonged outbreak in Germany between December 2000 and May 2001 was recently reported in Germany's national surveillance bulletin (2). The outbreak affected three adjacent county districts in the German state of North-Rhine Westphalia, and sheep were implicated as its likely source.

Altogether 73 cases were notified to the Robert Koch-Institut (RKI) in Berlin by the district where the first outbreak occurred; five of these did not have clinical symptoms. The epidemic peaked from 30 January to 17 February 2001 (week 5-7); after 1 May the number of new cases had dropped to one or two per week. Veterinary investigations of blood specimens showed that several flocks of sheep in the district were the likely source of infection. The likely transmission route was inhaled dust containing Coxiella burnetii from contaminated afterbirth that had been deposited during the lambing season. Local physicians and the public were kept informed by the health authorities and the media. There was a potential risk of transmission to a large number of people attending an open air theatre festival season in the town of Hallenberg, an annual event taking place between June and September, but no additional cases were notified subsequently. As a result of strict preventive measures imposed on sheep farmers in the area, after the end of the lambing season the number of notified cases decreased; the last case was notified at the end of May (week 21).

Two neighbouring country districts reported 25 cases and two cases of Q fever, respectively, to RKI in weeks 7-24, most of whom lived in close proximity to the first district. Preventive measures were imposed on sheep farmers, and the public and local physicians were informed by the local authorities in both these districts. No further cases have been reported since week 24.

In a special meeting on 9 May, which was attended by experts and representatives from authorities at a federal, state, and local level from all three affected districts, it was decided that, although the current risk of transmission was low, it could not be excluded altogether, and the following measures were imposed on sheep farmers in the region.

- Lambing must take place inside a building located outside a residential area. The doors have to be kept shut until after the birth and after disposal of the contaminated straw.

- Ewes and newborn lambs have to be kept indoors for a minimum of 14 days after the birth.

- Afterbirths and stillbirths must be collected in closed sealed containers and have to be disposed of through a disposal unit for animal carcasses. After collection of the body parts, the containers have to be cleaned and disinfected immediately.

- Contaminated straw and dung must be piled up, covered with a strong plastic cover, secured against wind damage, and kept untouched for two years, after which it may be used as fertiliser.

- Shearing is allowed only in buildings outside a residential area. The staff must wear protective masks. The wool has to be kept in an enclosed room until collection.

- After shearing, the sheep have to be treated with a chemical bath to eliminate ticks.

- Grazing areas must not be closer than 700 metres to residential or industrial areas (except for Hallenberg, but no details are available with regard to the limit there).

These measures take into account the transmission routes and aim at preventing contact of the population with contaminated afterbirth of sheep. It may be advisable to extend the time ewes and lambs are kept indoors to a total of eight weeks. Humans should also avoid exposure to dust from the fleece, which may be contaminated by excretions of ticks, which are a known host for C. burnetii.

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Q fever outbreak in Botevgrad, Bulgaria: May-June 2004

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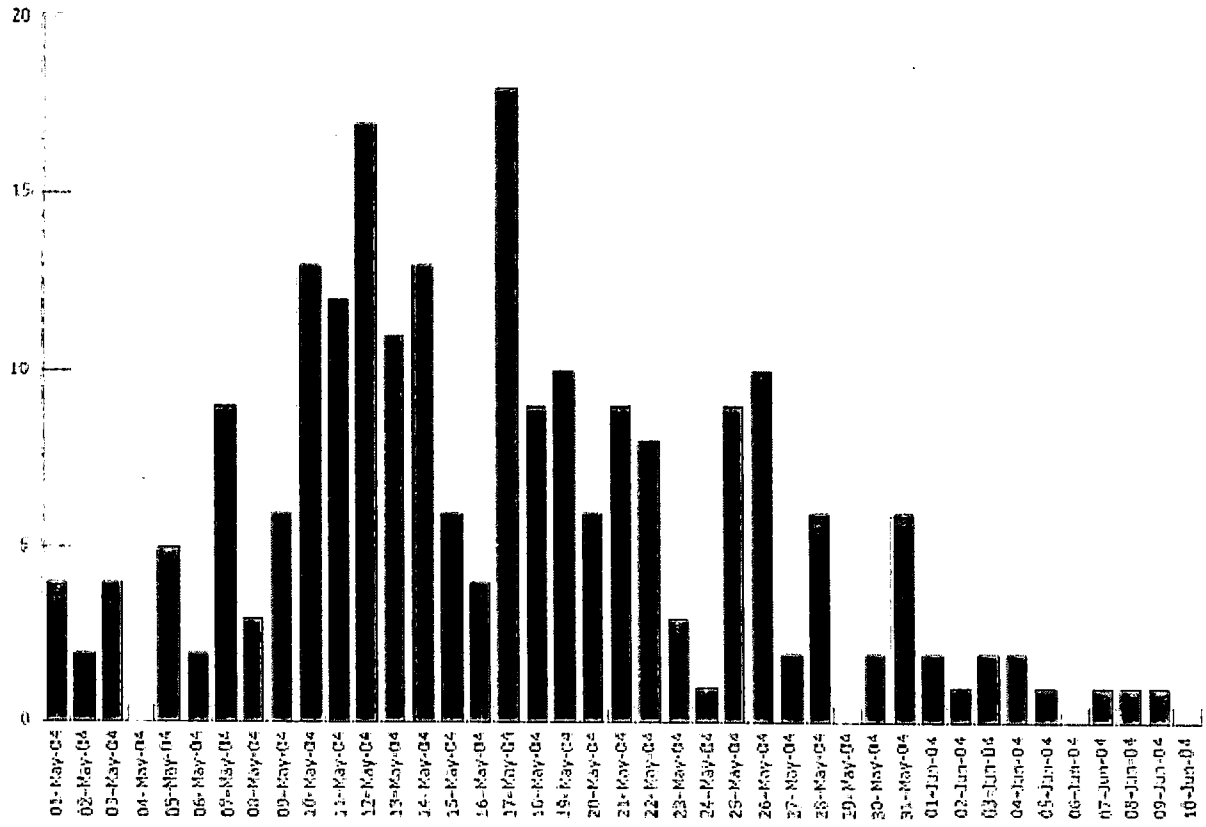
Q fever is a widespread zoonosis in Bulgaria, caused by *Coxiella burnetii*. The major route of transmission from animals to humans is by infected aerosols. Over the past decade, the number of both sporadic cases and outbreaks in Bulgaria has increased. This may be associated with changes in livestock breeding on goat, sheep and cattle farms, as livestock are the usual sources of *C. burnetii* outbreaks in humans. During the 1990s, economic and social changes led to a decrease of larger cattle herds and sheep flocks in rural areas and an increase of the number of cattle kept on small farms, and this has intensified contact between farm animals and people.

Investigation

In early May 2004, an increase was noted in the number of pneumonia cases in patients attending a clinic in Botevgrad (population 28 000, situated 60 km northeast of the capital Sofia). At first, these cases, diagnosed as atypical pneumonia, did not have Q fever in the differential diagnosis and were not thought to be associated with any outbreak. On 11 May, the Hygiene and Epidemiological Inspectorate (HEI) was informed of a cluster of cases of atypical pneumonia. On 12 May, an epidemiological and clinical investigation was started, and common characteristics suggested Q fever. Two days later, the first positive serological results were obtained with antibodies to phase II *C. burnetii* antigen in hospitalised patients.

Immediately the HEI, together with the veterinary and municipality authorities, implemented preventive measures to stop the outbreak in Botevgrad. The public was informed through mass media of the risk of the disease, the route and prevention of transmission, as well as the need to properly dispose of all animal birth products (including aborted foetuses), to restrict access to barns and animals and to use protective clothing during contact with animals. Proper decontamination of surfaces with disinfectants and not drinking unpasteurised milk was recommended. Despite this, the number of patients continued to grow, because of the large number of people already infected who were incubating the disease. Between 1 May and 9 June the number of patients admitted to hospital that were diagnosed with atypical pneumonia in Botevgrad reached 220 (Figure).

Figure. Pneumonia cases during Q fever outbreak in Botevgrad, by admission date to hospital, May-June 2004.



The diagnosis of atypical pneumonia of hospitalised patients was made based on characteristic clinical, laboratory and x-ray data. The first 48 hospitalised patients were questioned and clinically examined by the investigation team.

Results

The ratio of infected men to women was 3 to 2. Of patients admitted to hospital, 72% were between 22 and 60 years old. Diagnostic titres of antibodies for phase II *C. burnetii* antigen were found in 91 people.

Forty-eight of the 220 patients admitted to hospital were investigated. Seventy-five percent (36) were male and 25% (12) were female. The frequency of symptoms is shown in the table:

Table. Clinical findings in 48 hospitalized patients with Q-fever

Symptom	Number of patients	Overall percentage (%)
Fever	48	100
Chills	36	75
Sweats	46	96
Headache	24	50
Arthralgia	7	15
Myalgia	7	15
Loss of appetite	12	25
Nausea	18	38
Chest pain	9	19

Cough	25	52
Dyspnea	2	4
X-ray changes	47	98

Laboratory analyses detected leucopenia (white blood cell<3.5x109/l) in 33% (16), elevated erythrocyte sedimentation rate in 65% (31) and mild elevation of aminotransferase activity in 29% (14).

During the investigation, patients often reported being in a dust storm, which occurred at the beginning of May and probably covered the whole town. Only a few patients reported direct contact with animals.

Discussion

The apparent reason for the outbreak of atypical pneumonia due to *C. burnetii* was the inhalation of infected aerosols. The occurrence of the dust storm supports the infected aerosol hypothesis.
The large number of infected domestic animals (nearly 40% of goats investigated were found to carry *C burnetii*) in the town may have been the cause. The character of pneumonic illnesses during May implies a point source.

A comparatively large number of general practitioners in Bulgaria are not acquainted with the clinical features and epidemiology of Q fever, which may have led to delays in diagnosis and treatment. This may also have delayed notification of Q fever to the HEI, hence the late implementation of preventive measures This slow reaction by the health authorities emphasises the necessity of enhancement of the epidemiological surveillance in Bulgaria.

The early diagnosis of Q fever in risk regions can be helped by epidemiological data on morbidity due to influenza-like illnesses and atypical pneumonia. In such conditions, physicians must treat with appropriate antibiotics before serological confirmation of the diagnosis of Q fever.

To ensure improved prevention of Q fever in Bulgaria, there is a need to amend legislation concerning livestock breeding in populated areas, and introduce preventive measures. The public health, veterinary and municipal authorities must work together to educate the population about the basic principles of Q fever prevention which includes restricting contact between people and cattle and improving infection control in the places where animals are bred.

Acknowledgements: epidemiological investigation was assisted by staff from the Medical University of Sofia.

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Q fever outbreak in the Chamonix Valley, France, summer 2002

At the end of July 2002, the Direction Départementale des Affaires Sanitaires et Sociales (DDASS) was informed of several dozen patients who had consulted their general practitioners (GPs) in Chamonix with fever, myalgia, and severe headaches. Most of the patients had serum transaminases 2-3 times above normal level. Most patients recovered spontaneously after 5-10 days. Several patients were hospitalised.

In mid-August the diagnosis of Q fever was confirmed for 10 patients by the presence of Q fever phase II IgM antibodies > 25 in serum.

An epidemiological investigation was carried out by the Cellule Interrégionale d'Epidémiologie de Lyon (CIRE), the Centre National de Référence des Rickettsies (CNR), the Institut de Veille Sanitaire (InVS), and the DDASS, to identify the mode of transmission and the source of the outbreak, and to implement appropriate control measures.

Cases were identified through the GPs of the Chamonix valley, the local hospital, the medical laboratory in the valley and the CNR.

Case definitions used in the investigation:

- Possible Q fever: individual residing in, or visiting Chamonix valley since June 2002 and having presented after 20 June with fever >39 degrees C, accompanied by at least two of the following symptoms: myalgia, nausea, chills.

- Probable Q fever: a possible case with an increase of transaminases AST and ALT, above normal.

- Confirmed Q fever: a possible or probable case with positive serology for Q fever (phase II IgG >/= 100 and phase II IgM at >/=25).

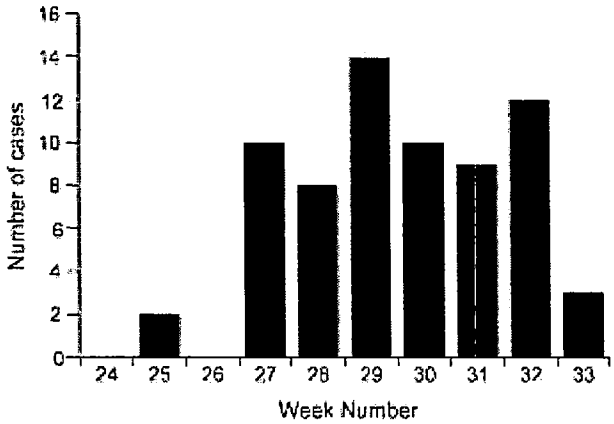
Up to 30 August, 79 cases were identified: 25 possible, 32 probable and 22 confirmed cases. All were adults between 17 and 92 years of age. The M:F ratio was 1.8:1. Eleven individuals required hospital treatment.

Place of residence was known for 71 cases. Fifty seven (80%) live in the commune of Chamonix, 11 (15%) live in another town in the valley, or close to the valley, and 3 are French tourists.

An exploratory study carried out on 19 confirmed cases suggested airborne contamination from one or several excretory herds (sheep, goats or cattle), possibly moving to summer pastures, or from manure spreading areas. None of the cases has a high-risk profession for Q fever infection (for example, stock breeder, abattoir technician, etc.). During the exposure period, four individuals had close contact with ruminants, four had eaten cheese made from unpasteurised milk, of which one only had eaten locally produced cheese. None had drunk unpasteurised milk. Seven had domestic animals. The cases questioned did not attend an event involving animals. Neither is there an abattoir or knacker's yard in the valley.

The epidemic curve, which is not complete for week 33 and after, due to the delay in getting the onset dates, suggests a persistent source of contamination.

Figure: Cases (possible, probable and confirmed) of Q fever by week of onset of symptoms, Q fever outbreak, Chamonix Valley, 2002.



A case control study is in progress to determine risk factors and transmission methods (movement, consumption of unpasteurised milk, direct or indirect contact with manure or animal herds, participation in an event involving indirect or direct contact with animals, etc.). In parallel, information is being gathered on the animal herds present in the valley since June 2002. These enquiries should further clarify the hypothesis on the source of contamination and allow us to put control measures in place.

Recommendations to the public

Since June 2002, residents and visitors of the Chamonix valley may have been exposed to Coxiella burnetii, the agent causing Q fever.

Consequently, the Ministry of Health recommends that persons belonging to high-risk groups (pregnant women, persons with valvular cardiac disease, or immunosuppressed persons) who were resident or who stayed in the Chamonix valley between June and the present time consult a doctor to get a diagnostic test, and, if necessary, suitable medical treatment.

An alert was sent to the European national health authorities via the European surveillance network for alert action on 27 August 2002.

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Occupational exposure risk for Q fever and other zoonoses among those working on control to the foot and mouth disease epidemic in the United Kingdom

Q fever has been identified in three people who worked on a number of farms to help with the culling of cattle as a result of the national foot and mouth disease outbreak. Two presented with an influenza-like illness with onsets on 6 and 11 May 2001, and the third joined the two co-primary cases on one farm. All three have been confirmed as having positive serology by IgM enzyme linked immunosorbent assay. This also serves as a reminder of other potential occupational zoonotic risks that may be associated with the outbreak of foot and mouth disease.

Q fever is an acute (and sometimes chronic) febrile illness caused by the rickettsial organism Coxiella burnetii. The commonest animal reservoirs for C. burnetii are cattle, sheep, and goats. The organism is found in placental tissues and birth fluids, and in the milk, urine, and faeces of infected animals. Human infection usually occurs by inhalation of infected dust or from exposure to amniotic fluid or placenta where they are present in high quantities. Infection may also be acquired by inhalation of aerosols from the environment (soil, straw and wool) which become contaminated when these animals give birth. Exposure to C. burnetii is common in farm workers (1). The organisms are resistant to heat and drying and the infectious dose is thought to be low.

In humans, infection is characterised by a self limiting flu-like illness or pneumonia but in chronic cases endocarditis is the main syndrome (2). Osteomyelitis, infections of vascular grafts, or aneurysms and infections during pregnancy have also been reported.

The geographic distribution of Q fever is wide and C. burnetii is found in virtually every country in the world, except New Zealand (3), although, until recently, some parts of northern Europe were thought to be free of Q fever, and occasional cases were attributed to infection overseas. Recent evidence now suggests that C. burnetii infection is now endemic in Sweden and Poland (4).

In many countries, Q fever is not a notifiable disease, and it may be difficult to ascertain how many cases occur; furthermore, the source of infection often remains speculative. In Europe, surveillance of Q fever varies with the surveillance systems in place in each country (5). Large outbreaks of Q fever involving people without occupational exposure have rarely been reported. Indirect exposure to sheep flocks passing populated areas was shown to be a determinant of the outbreaks in Switzerland (6) and northern Italy (7), and in Britain, urban outbreaks have been associated with the passage of farm vehicles containing contaminated straw and manure (8) or to windborne spread from farmland near an urban area (9).

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Bichat guidelines for the clinical management of Q fever and bioterrorism-related Q fever

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Q fever is a zoonotic disease caused by *Coxiella burnetii*. Its interest as a potential biological weapon stems from the fact that an aerosol of very few organisms could infect humans. Another route of transmission of *C. burnetii* could be through adding it to the food supply. Nevertheless, *C. burnetii* is considered to be one of the less suitable candidate agents for use in a bioterrorist attack; the incubation is long, many infections are inapparent and the mortality is low. In the case of an intentional release of *C. burnetii* by a terrorist, clinical presentation would be similar to naturally occurring disease. It may be asymptomatic, acute, normally accompanied by pneumonia or hepatitis, or chronic, usually manifested as endocarditis. Most cases of acute Q fever are asymptomatic and resolve spontaneously without specific treatment. Nevertheless, treatment can shorten the duration of illness and decrease the risk of complications such as endocarditis. Post-exposure prophylaxis is recommended after the exposure in the case of a bioterrorist attack.

Urban outbreak of Q fever, Briançon, France, March to June 1996

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Q fever is an ubiquitous zoonosis caused by the rickettsial organism *Coxiella burnetii* (1). Both sporadic cases and epidemics occur in areas where sheep and goats are bred. The main route of transmission is by inhalation of aerosols from the environment (soil, straw, wool) which become contaminated when sheep, goats, or cattle give birth (1). In this report, we describe an urban outbreak of Q fever that occurred between March and May 1996 in Briançon, a town of 12 000 inhabitants in a breeding area in the Hautes Alpes.

Method

The outbreak was recognised when ten cases of acute Q fever had been admitted to the General Hospital of Briançon. Given the unusual urban location and the spatio-temporal clustering of these cases over three months (March, April, May 1996) in Briançon, it was decided to carry out an epidemiological investigation.

Cases were defined as residents of Briançon with clinical symptoms suggestive of Q fever between March and June 1996, associated with the detection of antibodies against *C. burnetii* by indirect immunofluorescence (IIF) test phase 2 (IgG > 200 and IgM > 50). Patients who were tested two months after becoming ill were included as cases in the absence of IgM if IgG >800 (phase 2 by IIF). Serological tests for Q fever were performed by the National Reference Centre (NRC) for rickettsiosis.

Cases were actively sought at of the hospital of Briançon (among inpatients or blood donors at the transfusion centre) from serological screening tests performed by the NRC for rickettsiosis, a local analysis laboratory, and the virology laboratory of the University Hospital of Grenoble. Clinical and descriptive data were obtained from patients' medical records.

An initial field visit to Briançon and the surrounding area and early interviews with cases focused attention on the area of a slaughterhouse in Briançon between March and June 1996. The potential for airborne transmission existed: sheep and goat

slaughtering increased in March, there was straw and manure on the slaughterhouse soil, and helicopters created dust when they flew over from a heliport near to the slaughterhouse.

A case control study was conducted between 25 June and 5 July 1996 to test the hypothesis of airborne transmission by contaminated dust aerosols from the slaughterhouse. Two controls for each case were sought by visiting residents aged 18 to 60 years in areas where cases lived. Controls were excluded if they reported clinical symptoms compatible with Q fever between March and June 1996. Serological tests for Q fever were offered to each control and performed on finger prick blood specimens collected on blotting paper. Positive controls with total Ig (IgM + IgG) phase 2 (>50) were excluded.

In addition to sociodemographic information and previous history, cases and controls were interviewed using a standardised questionnaire about their exposure to different animals (goats, sheep, and cows), the slaughter area, and manure, their consumption of raw milk, raw meat, and raw cultivated mushrooms, and on their degree of contact with goats, sheep, or cattle. To test the hypothesis, a semiquantitative scale of exposure to air from the slaughterhouse (nil, low, moderate, or high) was developed, integrating the subject's mode of exposure (walking, cycling, driving with open or closed windows near the slaughterhouse), the duration of exposure (frequency of journeys, stopping, and work in the area) and detailed location in relation to the slaughterhouse (maps with names of roads indicated were attached to the questionnaire).

The relation between exposures and the occurrence of Q fever was evaluated by the odds ratio (OR) and its 95% confidence interval. Multiple logistic regression was then used to assess the independent contribution of each variable associated to Q fever in univariate analysis.

Results

Twenty-nine cases were identified, 12 of whom were admitted to hospital. Cases arose between 30 March and 15 June 1996 with a maximum number of cases in the week from 15 to 21 April (figure 1). Twenty-six cases reported fever (>38.5°C, often up to 40°C), 22 headache, 25 myalgia, and 20 arthralgia. Most of the cases admitted to hospital had increased levels of hepatic transaminases and moderate thrombocytopenia. Pneumonia was diagnosed for only one patient who had a history of asthma. All patients lived in the urban area of Briançon. The age distribution of patients was 18 to 60 years (average 35 years) and 26 were male; no cases were detected among farm workers.

Q fever

CDC Contact Information for Q Fever Questions: 1-800-CDC-INFO (1-800-232-4636)
For comprehensive CDC information about bioterrorism and related issues, please visit http://www.bt.cdc.gov.

Overview

Q fever is a zoonotic disease caused by *Coxiella burnetii,* a species of bacteria that is distributed globally. In 1999, Q fever became a notifiable disease in the United States but reporting is not required in many other countries. Because the disease is underreported, scientists cannot reliably assess how many cases of Q fever have actually occurred worldwide. Many human infections are inapparent.

Cattle, sheep, and goats are the primary reservoirs of *C. burnetii.* Infection has been noted in a wide variety of other animals, including other species of livestock and in domesticated pets. *Coxiella burnetii* does not usually cause clinical disease in these animals, although abortion in goats and sheep has been linked to *C. burnetii* infection. Organisms are excreted in milk, urine, and feces of infected animals. Most importantly, during birthing the organisms are shed in high numbers within the amniotic fluids and the placenta. The organisms are resistant to heat, drying, and many common disinfectants. These features enable the bacteria to survive for long periods in the environment. Infection of humans usually occurs by inhalation of these organisms from air that contains airborne barnyard dust contaminated by dried placental material, birth fluids, and excreta of infected herd animals. Humans are often very susceptible to the disease, and very few organisms may be required to cause infection.

Ingestion of contaminated milk, followed by regurgitation and inspiration of the contaminated food, is a less common mode of transmission. Other modes of transmission to humans, including tick bites and human to human transmission, are rare.

Signs and Symptoms in Humans

Only about one-half of all people infected with *C. burnetii* show signs of clinical illness. Most acute cases of Q fever begin with sudden onset of one or more of the following: high fevers (up to 104-105° F), severe headache, general malaise, myalgia, confusion, sore throat, chills, sweats, non-productive cough, nausea, vomiting, diarrhea,

abdominal pain, and chest pain. Fever usually lasts for 1 to 2 weeks. Weight loss can occur and persist for some time. Thirty to fifty percent of patients with a symptomatic infection will develop pneumonia. Additionally, a majority of patients have abnormal results on liver function tests and some will develop hepatitis. In general, most patients will recover to good health within several months without any treatment. Only 1%-2% of people with acute Q fever die of the disease.

Chronic Q fever, characterized by infection that persists for more than 6 months is uncommon but is a much more serious disease. Patients who have had acute Q fever may develop the chronic form as soon as 1 year or as long as 20 years after initial infection. A serious complication of chronic Q fever is endocarditis, generally involving the aortic heart valves, less commonly the mitral valve. Most patients who develop chronic Q fever have pre-existing valvular heart disease or have a history of vascular graft. Transplant recipients, patients with cancer, and those with chronic kidney disease are also at risk of developing chronic Q fever. As many as 65% of persons with chronic Q fever may die of the disease.

The incubation period for Q fever varies depending on the number of organisms that initially infect the patient. Infection with greater numbers of organisms will result in shorter incubation periods. Most patients become ill within 2-3 weeks after exposure. Those who recover fully from infection may possess lifelong immunity against re-infection.

Diagnosis

Because the signs and symptoms of Q fever are not specific to this disease, it is difficult to make an accurate diagnosis without appropriate laboratory testing. Results from some types of routine laboratory tests in the appropriate clinical and epidemiologic settings may suggest a diagnosis of Q fever. For example, a platelet count may be suggestive because persons with Q fever may show a transient thrombocytopenia. Confirming a diagnosis of Q fever requires serologic testing to detect the presence of antibodies to *Coxiella burnetii* antigens. In most laboratories, the indirect immunofluorescence assay (IFA) is the most dependable and widely used method. *Coxiella burnetii* may also be identified in infected tissues by using immunohistochemical staining and DNA detection methods.

Coxiella burnetii exists in two antigenic phases called phase I and phase II. This antigenic difference is important in diagnosis. In acute cases of Q fever, the antibody level to phase II is usually higher than that to phase I, often by several orders of magnitude, and generally is first detected during the second week of illness. In chronic Q fever, the reverse situation is true. Antibodies to phase I antigens of *C. burnetii* generally require longer to appear and indicate continued exposure to the bacteria. Thus, high levels of antibody to phase I in later specimens in combination with constant or falling levels of phase II antibodies and other signs of inflammatory disease suggest chronic Q fever. Antibodies to phase I and II antigens have been known to persist for months or years after initial infection.

Recent studies have shown that greater accuracy in the diagnosis of Q fever can be

achieved by looking at specific levels of classes of antibodies other than IgG, namely IgA and IgM. Combined detection of IgM and IgA in addition to IgG improves the specificity of the assays and provides better accuracy in diagnosis. IgM levels are helpful in the determination of a recent infection. In acute Q fever, patients will have IgG antibodies to phase II and IgM antibodies to phases I and II. Increased IgG and IgA antibodies to phase I are often indicative of Q fever endocarditis.

Treatment

Doxycycline is the treatment of choice for acute Q fever. Antibiotic treatment is most effective when initiated within the first 3 days of illness. A dose of 100 mg of doxycycline taken orally twice daily for 15-21 days is a frequently prescribed therapy. Quinolone antibiotics have demonstrated good in vitro activity against *C. burnetii* and may be considered by the physician. Therapy should be started again if the disease relapses.

Chronic Q fever endocarditis is much more difficult to treat effectively and often requires the use of multiple drugs. Two different treatment protocols have been evaluated: 1) doxycycline in combination with quinolones for at least 4 years and 2) doxycycline in combination with hydroxychloroquine for 1.5 to 3 years. The second therapy leads to fewer relapses, but requires routine eye exams to detect accumulation of chloroquine. Surgery to remove damaged valves may be required for some cases of *C. burnetii* endocarditis.

Prevention

In the United States, Q fever outbreaks have resulted mainly from occupational exposure involving veterinarians, meat processing plant workers, sheep and dairy workers, livestock farmers, and researchers at facilities housing sheep. Prevention and control efforts should be directed primarily toward these groups and environments.

The following measures should be used in the prevention and control of Q fever:

- Educate the public on sources of infection.
- Appropriately dispose of placenta, birth products, fetal membranes, and aborted fetuses at facilities housing sheep and goats.
- Restrict access to barns and laboratories used in housing potentially infected animals.
- Use only pasteurized milk and milk products.
- Use appropriate procedures for bagging, autoclaving, and washing of

laboratory clothing.

- Vaccinate (where possible) individuals engaged in research with pregnant sheep or live *C. burnetii.*

- Quarantine imported animals.

- Ensure that holding facilities for sheep should be located away from populated areas. Animals should be routinely tested for antibodies to *C. burnetii,* and measures should be implemented to prevent airflow to other occupied areas.

- Counsel persons at highest risk for developing chronic Q fever, especially persons with pre-existing cardiac valvular disease or individuals with vascular grafts.

A vaccine for Q fever has been developed and has successfully protected humans in occupational settings in Australia. However, this vaccine is not commercially available in the United States. Persons wishing to be vaccinated should first have a skin test to determine a history of previous exposure. Individuals who have previously been exposed to *C. burnetii* should not receive the vaccine because severe reactions, localized to the area of the injected vaccine, may occur. A vaccine for use in animals has also been developed, but it is not available in the United States.

Significance for Bioterrorism

Coxiella burnetii is a highly infectious agent that is rather resistant to heat and drying. It can become airborne and inhaled by humans. A single *C. burnetii* organism may cause disease in a susceptible person. This agent could be developed for use in biological warfare and is considered a potential terrorist threat.

Arricau-Bouvery N., Souriau A., Bodier C., Dufour P., Rousset E., Rodolakis A. (2005) "Effect of vaccination with phase I and phase II Coxiella burnetii vaccines in pregnant goats". Vaccine 23:4392-4402.

Vaccine. 2005 Aug 15;23(35):4392-402. Related Articles, Links

Effect of vaccination with phase I and phase II Coxiella burnetii vaccines in pregnant goats.

Arricau-Bouvery N, Souriau A, Bodier C, Dufour P, Rousset E, Rodolakis A.

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Livestock is considered to be the major "source" of human Q fever. The efficacy of two currently available vaccines (Coxevac, phase I, CEVA Sante Animale and Chlamyvax FQ, phase II, MERIAL) against Coxiella excretion was investigated in terms of risks to human health. Two months before mating, 17 goats were vaccinated subcutaneously against Coxiella burnetii with an inactivated phase I vaccine and 16 goats were vaccinated with an inactivated phase II Coxiella mixed with Chlamydophila abortus vaccine. Fourteen goats were left unvaccinated. At 84 days of gestation, the goats were subcutaneously challenged with 10(4) bacteria of C. burnetii strain CbC1. Phase I vaccine was effective and dramatically reduced both abortion and excretion of bacteria in the milk, vaginal mucus and feces. In contrast, the phase II vaccine did not affect the course of the disease or excretion.
PV: lage aantallen, historie bekend? Rol van farmaceut (CEVA?)

1: Vet Rec. 2005 Apr 23;156(17):548-9.

Progression of Q fever and Coxiella burnetii shedding in milk after an outbreak of enzootic abortion in a goat herd.

Berri M, Rousset E, Hechard C, Champion JL, Dufour P, Russo P, Rodolakis A.

INRA Tours-Nouzilly, Pathologie Infectieuse et Immunologie, 37380 Nouzilly, France.

Vet Microbiol. 2004 Apr 19;99(3-4):301-5. Related Articles, Links

FULL-TEXT ARTICLE

Occurrence, distribution, and role in abortion of Coxiella burnetii in sheep and goats in Sardinia, Italy.

Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, Tola S.

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Between 1999 and 2002, 9349 sera and 517 aborted samples (422 foetuses and 95 placenta) were analysed from 675 sheep and 82 goat farms distributed all over the island of Sardinia. After abortion notification, sera collected at random from adult animals were examined to detect antibodies specific to Coxiella burnetii by ELISA, whereas foetuses and placenta were analysed by PCR assay. Specific IgG antibodies were detected in 255 (38%) sheep farms and in 39 (47%) goat herds whereas 40 ovine (10%) and 3 (6%) caprine foetuses were C. burnetii PCR-positive. Although C. burnetii DNA was amplified from different types of tissues, placenta was the tissue with the highest detection rate. Seroprevalence analysis indicates that C. burnetii distribution in sheep and goats is very high, but PCR results demonstrate that C. burnetii has a relatively low role in abortion, especially in goats.

PMID: 15066733 [PubMed - indexed for MEDLINE]

Vet Res. 2003 Jul-Aug;34(4):423-33. Related Articles, Links

Experimental Coxiella burnetii infection in pregnant goats: excretion routes.

Arricau Bouvery N, Souriau A, Lechopier P, Rodolakis A.

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Q fever is a widespread zoonosis caused by Coxiella burnetii. Infected animals, shedding bacteria by different routes, constitute contamination sources for humans and the environment. To study Coxiella excretion, pregnant goats were inoculated by the subcutaneous route in a site localized just in front of the shoulder at 90 days of gestation with 3 doses of bacteria (10(8), 10(6) or 10(4) i.d.). All the goats aborted whatever the dose used. Coxiella were found by PCR and immunofluorescence tests in all placentas and in several organs of at least one fetus per goat. At abortion, all the goats excreted bacteria in vaginal discharges up to 14 days and in milk samples up to 52 days. A few goats excreted Coxiella in their feces before abortion, and all goats, excreted bacteria in their feces after abortion. Antibody titers against Coxiella increased from 21 days post inoculation to the end of the experiment. For a Q fever diagnostic, detection by PCR and immunofluorescence tests of Coxiella in parturition products and vaginal secretions at abortion should be preferred to serological tests.

PMID: 12911859 [PubMed - indexed for MEDLINE]

1: Vector Borne Zoonotic Dis. 2003 Spring;3(1):11-5. Related Articles, Links

Natural history of Q fever in goats.

Hatchette T, Campbell N, Hudson R, Raoult D, Marrie TJ.

The Department of Medicine, Dalhousie University, Halifax, Nova Scotia.

During the spring of 1999, an outbreak of Q fever resulted in 30 abortions among 174 (17%) goats in a caprine cooperative in Newfoundland. The intent of this study was to determine the natural history of Coxiella burnetii infection in goats. Twenty-four goats on one farm were followed through the next two kidding seasons following the Q fever outbreak. Antibodies to phases I and II C. burnetii were determined using an indirect immunofluorescence assay and samples of placentas were cultured for C. burnetii and polymerase chain reaction was used to identify C. burnetii DNA. Phase I antibody was present in high levels and was maintained over the study period, while phase II antibody levels declined to the seronegative range in 60% of the infected goats. Molecular studies suggest that excretion of C. burnetii in the placenta of infected goats seems to be limited to the next kidding season following an outbreak. We therefore conclude that C. burnetii infection in goats seems to be limited to two kidding seasons. Phase I antibody levels are maintained, while phase II antibody levels decline.

Can Vet J. 2002 May;43(5):363-4. Related Articles, Links

Seroprevalence of Coxiella burnetii in selected populations of domestic ruminants in Newfoundland.

Hatchette T, Campbell N, Whitney H, Hudson R, Marrie TJ.

Department of Medicine, Dalhousie University, Halifax, Nova Scotia.

The seroprevalence of Coxiella burnetii among cattle, sheep, and goats in Newfoundland was determined by microimmunofluorescence. Seropositivity to phase II antigen increased in sheep from 3.1% in 1997 to 23.5% in 1999-2000 ($P < 0.001$). Cows (24%) had antibodies to phase I antigen; goats (15.6%) had antibodies to phase II antigen. Seroprevalence of C. burnetii is increasing among sheep.

1: J Vet Diagn Invest. 2001 May;13(3):265-70.

Causes of caprine abortion: diagnostic assessment of 211 cases (1991-1998).

Moeller RB Jr.

California Animal Health and Food Safety Laboratory System, University of California, Tulare 93274, USA.

In an 8-year period, 1991-1998, 217 accessions of caprine abortions were submitted to the California Veterinary Diagnostic Laboratory System. Of these 217 submissions, 211 were suitable for examination in this study (6 had insufficient data). Infectious agents as the cause of abortions were found in 37% of the cases: bacterial agents were identified in 30.5%, viral agents in 2%, fungal agents in 0.5%, and protozoal agents in 4% of the cases submitted. The most common causes of abortions were Chlamydia psittaci and Coxiella burnetii infection, which accounted for 23% of all goat abortions. Mineral deficiencies were observed in 4%, fetal anomalies accounted for 3%, and leukoencephalomalacia of the brain (probable oxygen deprivation) accounted for 3% of the submissions. No diagnosis was made in 112 of the 211 submissions (53%). No lesions were noted in 104 of the submissions (49%). The other 8 submissions (4%) had histologic lesions suggestive of a bacterial agent; however, no infectious agents were identified in these cases.

1: Emerg Infect Dis. 2001 May-Jun;7(3):413-9. Related Articles, Links

Goat-associated Q fever: a new disease in Newfoundland.

Hatchette TF, Hudson RC, Schlech WF, Campbell NA, Hatchette JE, Ratnam S, Raoult D, Donovan C, Marrie TJ.

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In the spring of 1999 in rural Newfoundland, abortions in goats were associated with illness in goat workers. An epidemiologic investigation and a serologic survey were conducted in April 1999 to determine the number of infections, nature of illness, and risk factors for infection. Thirty-seven percent of the outbreak cohort had antibody titers to phase II Coxiella burnetii antigen >1:64, suggesting recent infection. The predominant clinical manifestation of Q fever was an acute febrile illness. Independent risk factors for infection included contact with goat placenta, smoking tobacco, and eating cheese made from pasteurized goat milk. This outbreak raises questions about management of such outbreaks, interprovincial sale and movement of domestic ungulates, and the need for discussion between public health practitioners and the dairy industry on control of this highly infectious organism.

1: Am J Trop Med Hyg. 1992 Jul;47(1):35-40. Full Text Am J Trop Med Hyg Links

A cluster of Coxiella burnetii infections associated with exposure to vaccinated goats and their unpasteurized dairy products.

Fishbein DB, Raoult D.

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An outbreak of Q fever occurred among patients and staff of a psychiatric institution in southern France. Some of the patients and staff left the institution daily to work on a farm where goats were raised for raw milk and cheese production. The goats had all been vaccinated annually with a commercial vaccine containing phase II Coxiella burnetii antigen. A serologic survey revealed that 40 (66%) of the 61 patients and staff had elevated titers to C. burnetii. Seropositive persons were more likely to report an acute illness (P = 0.001), fever (P = 0.04), weakness (P = 0.04), arthralgia (P = 0.04), and headaches (P = 0.06) in the preceding year than were seronegative persons. Seropositivity rates were significantly higher among persons who worked on the farm and consumed unpasteurized milk products (69% [22 of 32]; P = 0.007), those who only had worked on the farm (75% [9 of 12]; P = 0.009), and those who only had consumed unpasteurized milk products (75% [9 of 12]; P = 0.009), compared with those who had not worked with the goats or consumed unpasteurized milk products (0 of 5). Despite vaccination against Q fever, no antibodies to C. burnetii were detectable in 17 (59%) of 29 goats. All 12 seropositive goats had antibodies to both phase I and phase II antigens, indicating that they were naturally infected, and two of three goats examined were shedding C. burnetii in their milk. Vaccination of this herd did not prevent the outbreak and might have increased shedding of C. burnetii in the dairy products.

1: Tidsskr Nor Laegeforen. 1997 Nov 10;117(27):3937-40.

[Q-fever imported into Norway]

[Article in Norwegian]

Jensenius M, **Maeland A**, **Kvale D**, **Farstad IN**, **Vene S**, **Bruu AL**.

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Q fever is an important zoonosis that occurs throughout the world. In contrast to most other European countries, there has been no evidence of endemic Q fever in Norway up to now. The disease is caused by Coxiella burnetii, a rickettsia-like bacterium. Humans are infected mainly by inhalation of contaminated aerosols from cattle, sheep and goats. Clinical manifestations are protean, ranging from asymptomatic infection to life-threatening endocarditis. In this article we present the first four cases of serological proven acute Q fever imported into Norway. The patients were Norwegian tourists who had visited Bhutan, the Canary Islands, and Morocco. Two patients had fever with maculopapular exanthema, one had pneumonia, and one had biopsy-proven granulomatous hepatitis. Three were treated with tetracyclines. All four patients recovered well.

CSIRO PUBLISHING
1: N S W Public Health Bull. 2007 Jul-Aug;18(7-8):133-6. FULL TEXT ARTICLE Links

A review of the efficacy of human Q fever vaccine registered in Australia.

Chiu CK, Durrheim DN.

NSW Public Health Officer Training Program, NSW Department of Health, Australia.

BACKGROUND: Q fever remains an important occupational zoonosis in rural Australia. Although Q fever vaccine is recommended in high-risk occupational groups, its availability has been limited in recent years. METHOD: A literature review of the efficacy of the human Q fever vaccine registered in Australia was conducted. RESULTS: Seven relevant vaccine efficacy studies were identified but no large double-blind, randomised, placebo-controlled studies have been conducted. Vaccine efficacy has ranged from 83-100% but limitations of study designs hamper a precise estimate of vaccine efficacy. CONCLUSION: Despite the shortcomings of efficacy studies, the Q fever vaccine available in Australia has considerable protective benefit in established high-risk environments, particularly of an occupational nature.

1: Res Vet Sci. 2007 Aug;83(1):47-52. Epub 2006 Dec 21. FULL TEXT ARTICLE Links

Goats may experience reproductive failures and shed Coxiella burnetii at two successive parturitions after a Q fever infection.

Berri M, Rousset E, Champion JL, Russo P, Rodolakis A.

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Q fever is a zoonosis caused by the obligate intracellular bacterium, Coxiella burnetii. Aborting domestic ruminants are the main source of human infection. In January 2003, an abortion episode occurred in a dairy caprine herd where 18/60 (30%) goats experienced reproductive problems: 4/60 (7%) aborted and 14/60 (23%) had stillbirths. Serological screening for abortion-related infectious diseases suggested Q fever. The diagnosis of C. burnetii infection was confirmed with PCR based on the occurrence of C. burnetii shedding into vaginal mucus, faeces and colostrums taken after kidding from the affected animals. The pregnancy following this episode resulted in one abortion and four stillbirths; three of those goats had already experienced reproductive failure during the previous kidding season. The seroprevalence of C. burnetii infection and the bacteria shedding were investigated using both ELISA and PCR assays, respectively, during the course of the initial and subsequent kidding seasons. Serological testing, performed on the whole herd 6 weeks after the abortion episode, showed 48/60 (80%) of ELISA positive goats. PCR assay performed on both vaginal swab and milk samples showed that the bacterium was shed for almost four months after the outbreak. C. burnetii DNA was also amplified from vaginal swab and milk samples taken from goats after the second kidding season. Furthermore, the bacteria were found into 14 vaginal swabs and 12 milk samples taken from infected females at both kidding seasons.

1: Vet Res. 2007 Nov-Dec;38(6):849-60. Epub 2007 Sep 20. Free Text Article at www.edpsciences.org Links

Coxiella burnetii shedding by dairy cows.

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While shedding routes of Coxiella burnetii are identified, the characteristics of Coxiella shedding are still widely unknown, especially in dairy cattle. However, this information is crucial to assess the natural course of Coxiella burnetii infection within a herd and then to elaborate strategies to limit the risks of transmission between animals and to humans. The present study aimed at (i) describing the characteristics of Coxiella burnetii shedding by dairy cows (in milk, vaginal mucus, faeces) in five infected dairy herds, and at (ii) investigating the possible relationships between shedding patterns and serological responses. A total of 145 cows were included in a follow-up consisting of seven concomitant samplings of milk, vaginal mucus, faeces and blood (Day 0, D7, D14, D21, D28, D63, D90). Detection and quantification of Coxiella burnetii titres were performed in milk, vaginal mucus and faeces samples using real-time PCR assay, while antibodies against Coxiella were detected using an ELISA technique. For a given shedding route, and a given periodicity (weekly or monthly), cows were gathered into different shedding kinetic patterns according to the sequence of PCR responses. Distribution of estimated titres in Coxiella burnetii was described according to shedding kinetic patterns. Coxiella burnetii shedding was found scarcely and sporadically in faeces. Vaginal mucus shedding concerned almost 50% of the cows studied and was found intermittently or sporadically, depending on the periodicity considered. Almost 40% of cows were detected as milk shedders, with two predominant shedding patterns: persistent and sporadic, regardless of the sampling periodicity. Significantly higher estimated titres in Coxiella burnetii were observed in cows with persistent shedding patterns suggesting the existence of heavy shedder cows. These latter cows were mostly, persistently highly-seropositive, suggesting that repeated serological testings could be a reliable tool to screen heavy shedders, before using PCR assays.

1: Vet Res. 2008 May-Jun;39(3):23. Epub 2008 Feb 7. Free Text Article at www.edpsciences.org Links

Association between Coxiella burnetii shedding in milk and subclinical mastitis in dairy cattle.

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The objective of this research was to explore the potential association between Coxiella burnetii shedding in milk and chronic subclinical mastitis in dairy cattle. In two separate studies, we identified an association between PCR-based detection of C. burnetii in milk and chronic subclinical mastitis in lactating dairy cows. These studies were conducted in a commercial dairy herd where there was ongoing intensive monitoring of subclinical mastitis by aerobic bacteriology, but no prior knowledge or management of C. burnetii infections. In a case-control study, quarter level C. burnetii status determined by real-time quantitative PCR (RT-qPCR) was strongly associated with chronic subclinical mastitis as measured by milk somatic cell counts. In a subsequent cross sectional study, 147 (45%) of 325 lactating cows were positive for C. burnetii by RT-qPCR of composite milk samples. In a generalized linear model, accounting for the effect of covariates including aerobic intramammary infection status, C. burnetii PCR status was a significant predictor of linear somatic cell count score. In agreement with a small number of previous reports, this research provides evidence that there may be mammary gland specific manifestations of C. burnetii infections in dairy cattle.

1: BMC Infect Dis. 2006 Oct 6;6:147.

A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany.

Porten K, Rissland J, Tigges A, Broll S, Hopp W, Lunemann M, van Treeck U, Kimmig P, Brockmann SO, Wagner-Wiening C, Hellenbrand W, Buchholz U.

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BACKGROUND: In May 2003 the Soest County Health Department was informed of an unusually large number of patients hospitalized with atypical pneumonia. METHODS: In exploratory interviews patients mentioned having visited a farmers' market where a sheep had lambed. Serologic testing confirmed the diagnosis of Q fever. We asked local health departments in Germany to identify notified Q fever patients who had visited the farmers market. To investigate risk factors for infection we conducted a case control study (cases were Q fever patients, controls were randomly selected Soest citizens) and a cohort study among vendors at the market. The sheep exhibited at the market, the herd from which it originated as well as sheep from herds held in the vicinity of Soest were tested for Coxiella burnetii (C. burnetii). RESULTS: A total of 299 reported Q fever cases was linked to this outbreak. The mean incubation period was 21 days, with an interquartile range of 16-24 days. The case control study identified close proximity to and stopping for at least a few seconds at the sheep's pen as significant risk factors. Vendors within approximately 6 meters of the sheep's pen were at increased risk for disease compared to those located farther away. Wind played no significant role. The clinical attack rate of adults and children was estimated as 20% and 3%, respectively, 25% of cases were hospitalized. The ewe that had lambed as well as 25% of its herd tested positive for C. burnetii antibodies. CONCLUSION: Due to its size and point source nature this outbreak permitted assessment of fundamental, but seldom studied epidemiological parameters. As a consequence of this outbreak, it was recommended that pregnant sheep not be displayed in public during the 3rd trimester and to test animals in petting zoos regularly for C. burnetii.

1: J Comp Pathol. 2006 Aug-Oct;135(2-3):108-15. Links

Experimental Coxiella burnetii infection in pregnant goats: a histopathological and immunohistochemical study.

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Pregnant goats were inoculated subcutaneosly with Coxiella burnetii and the course of infection was studied. Abortion in the last third of pregnancy occurred in all infected animals. Tissues from the placenta and other organs were studied before and after abortion by immunohistochemistry and PCR analysis. After infection, mild lesions were observed in several maternal organs, mainly the mammary gland but also the lung and the liver. The trophoblast cells of the choriallantoic membrane were the first target cells of the placenta; there was, however, a substantial delay between initial infection and placental colonization. In the last weeks of pregnancy, just before abortion, massive bacterial multiplication was detected in the placenta. In this stage of infection a necrotic and suppurative placentitis separated the fetal trophoblast cells from maternal syncytial epithelium. Vasculitis was observed in the fetal mesenchyme. A strong maternal T-cell response was detected in the inter-placentomal areas but not in the placentomes, where only neutrophils and smaller numbers of macrophages were associated with the lesions. Neither lesions nor C. burnetii DNA were found in maternal organs in animals maintained until day 120 post-abortion.

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ik heb je in totaal zes keer een mail met bijlagen gestuurd. Het zou kunnen zijn dat enkele bijlages te groot zijn omdat ik enkele als niet verstuurd terug heb gekregen. Zou je aan kunnen geven welke mails je hebt gehad?

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SHORT COMMUNICATION

Serological diagnosis and follow-up of asymptomatic and acute Q fever infections

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Abstract

During an outbreak of Q fever at a farmer's market in Soest (North Rhine-Westphalia, Germany) in 2003, we examined 263 serum samples of presumably infected persons for Q fever antibodies. One hundred and seventy-one of these patients were tested positive for acute Q fever infection. Furthermore, 29 persons of certain risk groups like pregnant women ($n = 11$) or patients with valvular heart disease ($n = 18$) were examined. Among these, in four pregnant women and two heart patients an acute but asymptomatic infection could be diagnosed. With 30 patients we performed a serological follow-up for 8–60 weeks. In our study, phase 2 (PH2)-IgM antibodies as a marker for acute infection were present in all 30 patients 3–4 weeks after onset of clinical signs and disappeared 3–4 months later. Six weeks to three months after clinical manifestation, all patients developed PH1-IgG antibodies in low levels with no clinical signs of chronic Q fever. Three patients, including one pregnant woman showed high-level titres and were treated for chronic Q fever. Eleven patients with low PH1-IgG antibodies and all three patients with high titres developed IgA antibodies from 10 weeks after clinical manifestation; therefore PH1-IgA cannot be used as the only serological marker for chronic Q fever. Chronic infections were indicated only by a continuous increase of PH1 antibodies and a high persistence of PH2-IgG. We therefore conclude that the exclusion of chronic Q fever infection by a single serological examination cannot be done. At least three consecutive tests should be performed, that is 3, 6, and 9 months after initial Q fever infection.

Keywords: Q fever; *Coxiella burnetii*; Pregnant women; Chronic Q fever; Acute Q fever

Introduction

Q fever, a tick-borne zoonotic disease caused by *Coxiella burnetii* (Rickettsiaceae), primarily affects sheep and cattle (Babudieri, 1953; Aitken et al., 1987). The animals become infected by bites of the hard ticks *Dermacentor marginatus* and/or *D. reticulatus*. Transmission to humans is usually air-borne and associated with contact to infected animals' birth products, infectious tick faeces in animal fur, or contaminated dung and straw. Organisms of the infectious agent are spore-shaped corpuscles (small cell variants) which are extremely contagious and highly resistant against environmental extremes (Schliesser, 1991) and various disinfectants.

Acute Q fever often remains undiagnosed. Only 30% of the patients develop an influenza-like illness (Tissot Dupont et al., 1992), sometimes complicated with hepatitis, atypical pneumonia, or myopericarditis. In the majority of the cases (70%), Q fever infection occurs without clinical signs. Women with an acute Q fever infection during pregnancy have a high risk of abortion (Raoult et al., 2002) and a high risk of becoming

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chronically infected. Many years after initial exposure, chronic Q fever can become evident (Edlinger, 1987). Chronic Q fever is characterised by high phase 1 (PH1-IgG) and phase 2 (PH2-IgG) antibodies. Patients may develop endocarditis (Maurin and Raoult, 1999; Fenollar and Raoult, 2001), a life-threatening complication.

C. burnetii undergoes a host-dependent variation and appears in two different forms, phase I and phase II form (Stocker and Fiset, 1956). The serological diagnosis of acute Q fever can be performed by various tests with a high specificity and sensitivity. Acute infection is diagnosed by PH2-IgM antibodies followed by PH2-IgG. PH1-specific antibodies (IgG, IgA) are considered markers of chronic infection. An ELISA-technique is usually used for qualitative tests (Péter et al., 1987). The serological profile in the follow-up of patients is determined by indirect fluorescent antibody tests (Peacock et al., 1983).

This paper describes a follow-up study of persons infected with *C. burnetii* during a Q fever outbreak that occurred at a farmer's market in Soest (North Rhine-Westphalia) in 2003 (Anonymous, 2003). We were involved in our function as consulting laboratory in epidemiological investigations and diagnostic procedures.

Materials and methods

Selection and classification of patients

During the mentioned outbreak of Q fever, all serum samples of persons with contact to the farmer's market at the questionable time were examined by the consulting laboratory, altogether 263 samples.

Pregnant women or patients with valvular heart defects have a high risk to develop chronic Q fever followed by abortion or heart failure, respectively. The Robert Koch Institute (the German Centres for Disease Control) called upon internists and gynaecologists to send serum samples to the consulting laboratory from these risk groups who had been at the farmer's market during the outbreak. Eleven pregnant women and 18 patients with artificial heart valves were examined.

Serological testing

The serological diagnosis of acute Q fever was done by PH2-IgM-ELISA (Virion/Serion). The serum samples were pretreated with rheumatoid factor-absorbent to avoid false-positive results. In the follow-up study, the presence of antibodies to *C. burnetii* PH1 and PH2 antigens was detected using an indirect immunofluorescence test kit (BIOS, Germany).

Results

We tested 171 out of 263 submitted serum samples positive for acute Q fever by PH2-IgM antibodies. Thirty patients could be followed up for 8–60 weeks (Figs. 1 and 2). PH2-IgM antibodies were present in all these patients 3–4 weeks after clinical manifestation and disappeared 3–4 months later. Six to thirteen weeks after onset of clinical signs, all patients had developed PH1-IgG antibodies in low levels (<1:512) with no signs of chronic Q fever. Three patients showed high PH1-IgG antibody titres and were therefore treated for chronic Q fever. Eleven patients with low PH1-IgG antibody titres (<1:512) and all three patients with high titres (>1:512) developed IgA antibodies 10 weeks post-infection.

In the investigation of the risk groups, pregnant woman and cardiological patients, the serological constellation in 4 of 11 pregnant women and 2 of 18 cardiological patients pointed at a Q fever infection.

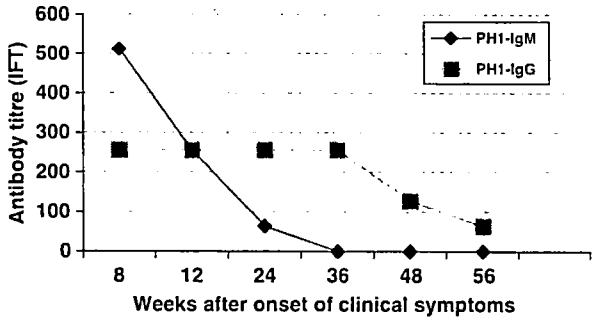

Fig. 1. Serological profile of acute Q-fever: PH1-IgG (30 follow-ups).

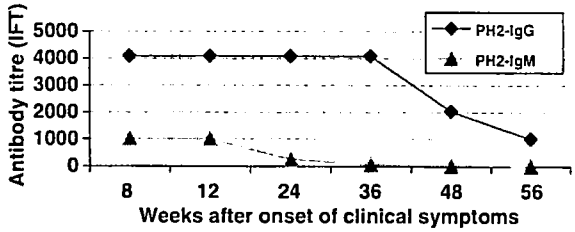

Fig. 2. Serological profile of acute Q-fever: PH2-IgG (30 follow-ups).

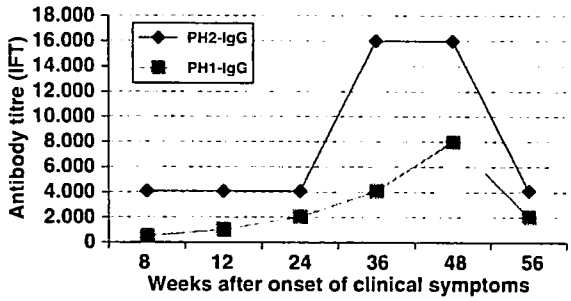

Fig. 3. Serological profile of chronic Q fever: follow-up of a pregnant woman.

In the follow-up study, one pregnant woman developed chronic Q fever. The antibody titres had increased to high levels 36 weeks p.i. (PH1-IgG 1:4000, PH2-IgG 1:16,000) (Fig. 3).

Discussion

The results of the present study highlight the importance of a serological diagnosis and the follow-up of acute Q fever infections. The presence of PH1-IgG antibodies after acute infection alone does not prove that a Q fever infection has become chronic. Chronic infections are indicated only by a continuous increase of PH1 antibodies and a high persistence of PH2-IgG. Also, PH1-IgA cannot be used as the only serological marker for chronic Q fever because IgA antibodies occur in many cases without clinical signs of chronic infection. It is therefore not possible to exclude a chronic infection by only one single serological follow-up test. At least three consecutive tests have to be performed, after 3, 6, and 9 months.

In the context of a Q fever outbreak, it is necessary that the offices of public health call upon internists and gynaecologists to examine risk groups like pregnant woman and patients with valvular heart defects, which had contact with the source of infection independently of the development of any clinical symptoms.

The accomplished study points out the great significance of Q fever infection for pregnant women. Pregnant women and patients with valvular heart defects in rural areas with contact to sheep or cattle should be informed about the risk of Q fever infection. To assess the importance of Q fever in endemic regions, the seroprevalence of Q fever in the population of these regions shall be examined in further studies.

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Cowdria ruminantium

Significance and Habitats. Agent of heartwater in cows, sheep and goats (ALEXANDER 1931).

	Blood Survival time in h
In carefully defibrinated blood stored at ..... room temperature	12–38 but not 72
Desiccation	
In defibrinated blood dried at room temperature over concentrated H_2SO_4 in 16 h or at 37° C over anhydrous calcium chloride	0

(ALEXANDER 1931). Vide p. 722.

	Ticks
C. ruminantium is transmitted by *Amblyomma gemma, A. hebraeum, A. pomposum* and *A. variegatum* (MITSCHERLICH and WAGENER 1970).	

Coxiella burnetii

Significance and Habitats. Agent of Q fever in man. Susceptible are such wild animals as mice, rats, gerbils, marmots, hares, bandicoots and certain species of wild birds, domestic animals such as cows, sheep, goats, dogs, horses, donkeys, camels, mules and possibly also domestic birds. Monkeys are markedly resistant to the agent (ZDRODOVSKII and GOLINEVICH 1960).

	Air
C. burnetii was isolated from 771 l of air in a shed where a group of goats was kept (LENNETTE and WELSH 1951 quoted from ZDRODOVSKII and GOLINEVICH 1960). Air-borne infection is thought to have been the cause of: Two outbreaks of Q fever in Amarillo and Chicago among persons connected with meat-packing plants. The source of infection was considered to be infected cattle at Amarillo and infected calves or sheep at Chicago. An outbreak of Q fever occurred among Allied troops in northern Italy in 1944–1945. Many of the soldiers had used hay for bedding and others were billeted in dusty quarters which also housed cattle and sheep or had sheltered goats. An outbreak of Q fever during World War II among five squadrons of a Bomb Group which had been stationed at the Grottaglie air base in southern Italy. Sheep and goats were pastured near the air base (LENNETTE et al. 1949). Infection in man may occur from primary aerosols generated during parturition of infected sheep or cows. Infection may also be acquired from secondary aerosols, i.e., contaminated dust, hides, fleece, etc., since desiccation of infected birth fluids and placenta, as well as of other secretions or excretions, gives rise to an environment in which aerosols of infective dust can be created. The infection chain may be envisioned as one in which the organisms are air-borne from the reproductive tract of one animal to the respiratory tract of another (RIVERS and HORSFALL 1959). Transmission by means of inhalation of infected dust or particulate matter in the air is postulated to be the cause of	

outbreaks of Q fever in different laboratories (ROBBINS and RUSTIGIAN 1946; Commission on acute respiratory diseases, Fort Bragg, North Carolina 1946). Vide p. 725.

	Blood Survival time in d
Infectious coagulated blood of guinea pigs was stored under Vaseline in tubes at ..... room temperature	280 but not 330

(COMBIESCO and DUMITRESCO 1949).

Desiccation	
Infectious blood of guinea pigs was dried and stored at ..... room temperature	182

(PARKER et al. 1949 quoted from WEYER 1949/50). Vide p. 722.

	Buffer	
Dried infectious egg material was suspended in phosphate buffers of different pH values in the ratio of 1:10.000 which corresponded to 10^5–10^6 infective doses for chick embryos and guinea pigs. Samples were stored at different temperatures.		Survival time in d
In buffer of pH 5.0 stored at	4– 6° C	240 but not 270
	15–20° C	10 but not 60
	34–36° C	5 but not 10
in buffer of pH 6.0 stored at	4– 6° C	360 but not 480
	15–20° C	90 but not 150
	34–36° C	< 60
in buffer of pH 8.0 stored at	4– 6° C	750 but not 930
	15–20° C	240 but not 360
	34–36° C	90 but not 150
in buffer of pH 8.0 with 10% glycerol stored at	4– 6° C	1,260 (n.f.t.)
	15–20° C	360 but not 480
	34–36° C	90 but not 150

(IGNATOVICH 1959a). Vide p. 728.

	Carcass and organs Survival time in d
In an infectious liver emulsion of a guinea pig and stored at ..... 5° C	24–92 but not 123

(DERRICK 1937).

Desiccation	
In an infectious mouse spleen which was dried at 32° C and then pulverized	60

(WEYER 1949/50). Vide p. 722.

	Survival time in min
Moist Heat Resistance	
A 10% suspension of infectious mouse spleens heated in test tubes in a water bath at ..... 55° C	30 (n.f.t.)
60° C	60 (n.f.t.)
65° C	< 15

(KIRBERGER 1951/52). Vide p. 710.

Culture

		Survival time
In a cell-free culture medium kept at	28° C	at least 109 d

(Cox 1939 quoted from Derrick 1953).

Dust

C. burnetii was isolated from dust of places where infected cows and sheep stayed (De Lay et al. 1950), of a stable harboring infected goats (Lennette and Welsh, 1951), of a maize bag and of a hayloft (Bingel et al. 1952) of the clothes of a shepherd (Marmion and Stoker 1956) and of cow sheds (Bektemirow et al. 1956; all authors quoted from Weyer 1959).

In Chiaravalle in Italy 100 persons developed Q fever during the winter of 1949. 80% of the patients lived, or had shops opening onto, the two roads used daily by a flock of contaminated sheep going to and returning from pasture (Babudieri 1953).

2-ml amounts of a suspension of an egg culture of *C. burnetii* (Greta strain) diluted in the ratio of 1:10,000 and containing 10^5–10^6 embryo infective doses were placed on sterilized weighed 1-g samples of wood sawdust. The samples were dried in a vacuum of 2–7 Pa and stored at a relative humidity of 53 to 68% at

	Survival time in d
4–6° C	60 but not 120
15° C	<30
36° C	<30

(Ignatovich 1959b). Vide p. 725.

Fats

Butter

	Survival time
In butter made from unpasteurized cream and stored refrigerated	41 d

(Jellison et al. 1948 quoted from Derrick 1953). Vide p. 728.

Insects

Beetles

Larvae of the meal beetle (*Tenebrio molitor*) can be experimentally infected (Weyer 1952/53).

Bugs

	Survival time
C. burnetii was isolated from specimens of *Cimex lectularius* after a natural infection for	134 d
and from the feces of the bugs stored at room temperature for	49 d (n.f.t.)

The organism is able to multiply in the bugs (Weyer 1952/53).

Cockroaches

C. burnetii was isolated from the intestine of the cockroach (*Phyllodromia germanica*) after an infectious meal for	3 d

(Weyer 1952/53).

Fleas

Specimens of *Nosopsyllus fasciatus* and *Leptopsylla segnis* can be experimentally infected. Fleas do not appear to play an important role in the epidemiology of Q fever (Weyer 1952/53).

Flies

C. burnetii was isolated from larvae and adults of *Musca domestica*. The organisms disappear at metamorphosis. Infected flies can act as carriers of the agent for	ca. 1 month

Attempts to transfer infection from infected to normal guinea pigs by houseflies failed. The role of flies in spreading Q fever is probably not of great importance (Philip 1948; Weyer 1952/53; Babudieri 1953).

Lice

	Survival time
The agent was isolated from specimens of *Pediculus humanus corporis* (Giroud 1950, Giroud and Jadin 1950, Jadin 1951; all authors quoted from Weyer 1952/53). *C. burnetii* multiplies in specimens of this *Pediculus* species after an experimental infection and is excreted with the feces of these insects for	24 d

It seems to be possible that lice transmit *C. burnetii* in nature (Weyer 1952/53).

Louse Flies

C. burnetii was isolated from specimens of *Hippobosca equina*, *Melophagus ovinus* (Combiescu 1957 quoted from Weyer 1952/53) and *Ornithomyia biloba* (Syručeк and Raška 1956).

Reduviids

Triatoma infestans is able to transmit the disease from infected to normal guinea pigs if the suction act of the insect is interrupted (Philip 1948). Larvae of *Rhodnius prolixus* may be infected (Weyer 1952/53).

Meat

		Survival time
In contaminated meat stored in the	refrigerator	30 d

(Wilson and Miles 1964).

Milk

C. burnetii was isolated from raw milk samples of four dairies in Southern California (Huebner et al. 1948).

		Survival time in d
Naturally infected goat's milk was stored at	4° C	90

(Sidky 1950 quoted from Reusse 1960).

In experimentally contaminated sterile milk stored at	room temperature	125
	4° C	273

(Lerche 1966).

In skim milk inoculated with dried egg material containing *C. burnetii* at a dilution of 10^{-4}, which corresponded to 10^5–10^6 infective doses for chick embryos and guinea pigs and stored at	4–6° C	1,260 (n.f.t.)
	15–20° C	720 (n.f.t.)
	34–36° C	<180 d

(Ignatovich 1959a).

Moist Heat Resistance

Samples of skim milk were inoculated with *C. burnetii* organisms in different densities and placed in thin-walled ampules, which were flame-sealed and submerged in a water bath where the samples were heated to the desired temperature in less than 3 min. Samples inoculated with

		Survival time in min
1,000 cells ml^{-1} and stored at	60.6° C	30 (n.f.t.)
	61.1° C	30
	61.7° C	<30
10,000 cells ml^{-1} and stored at	61.7° C	30
	62.2° C	<30
100,000 cells ml^{-1} and stored at	61.7° C	30 (n.f.t.)
	62.2° C	<30

(Enright et al. 1953).

A 0.1% suspension of heavily infected yolk sacs in sterile skim milk was distributed in 6- to 8-ml amounts in 10-ml lyophile ampules which were sealed with a gas flame and then completely immersed in a water bath. Two strains were examined.

		Survival time
Strain AD25 heated at	63° C	40 min (n.f.t.)
	65° C	30 min
strain Henzerling heated at	60° C	40 min (n.f.t.)
	63° C	30 but not 40 min

(Ransom and Huebner 1951).

A 5% suspension of heavily infected yolk sacs in sterile milk was heated at

	62° C	30 min (n.f.t.)
	70° C	60 s (n.f.t.)
	71° C	40 but not 80 s
	75° C	80 s
	80° C	14 s
	85° C	28 s
	100° C	<7 s

(Bingel and Engelhardt 1952).

In artificially contaminated milk heated at	61.5° C	30 min (n.f.t.)
	61.9° C	<30 min
	70-70.6° C	15 s
	71.6° C	<15 s

(Marmion et al. 1951 quoted from Zdrodvoskii and Golinevich 1960).

		D value in min
In contaminated milk heated at	65.6° C	0.50–0.60

z value 4.5–5.6° C

(Enright et al. 1956 quoted from Stumbo 1973).

2-ml samples of milk contaminated with 100,000 infective guinea pig doses of *C. burnetii* were subjected to different temperatures for different times. The following table gives some selected time-temperature points on the regression lines derived from laboratory data. The maximum times of survival and minimum times of destruction from which the regression lines were derived include the factor representing the lethal effect occurring during the heating-up and cooling-down periods expressed in equivalents of the times at the holding temperature.

Temperature °C	50% end point	Minimum time of destruction	Minimum time of destruction plus 2 sigmas
61.7	29.39 min	33.02 min	46.03 min
62.8	16.29 min	18.31 min	25.42 min
71.1	11.7 s	13.2 s	20.4 s
71.7	8.7 s	9.8 s	15.4 s
72.2	6.5 s	7.3 s	11.6 s

(Enright et al. 1956; 1957). Vide p. 710.

Milk products

Cheese

Cottage cheese was prepared from cow's milk which had been contaminated with *C. burnetii* in such a way that it was still pathogenic for guinea pigs at a dilution of 1:100 — Survival time 42 d (n.f.t.)

(Šipka 1958). Vide p. 728.

Milk, Fermented

	Survival time in h
In milk which had gone sour	<24
in kefir	24 but not 48
in whey	24 but not 48

(Lerche 1966). Vide p. 729.

Mites

C. burnetii was isolated from the mites *Dermanyssus passerinus*, *Haemolaelaps megaventralis*, *Steatonyssus viator* (Thiel 1974) and *Leeuwenhoekia major* (Weyer 1959). Adult mites of the species *Lyponyssus hacoti* are able to transmit the agent from mouse to mouse (Weyer 1952/53). *C. burnetii* also was isolated from nine species of Gamasidae, which lived on birds and wild animals in Kasachstan (Zemskaja et al. 1968 quoted from Thiel et al. 1977).

Soil

2-ml quantities of a suspension of a diluted (10^{-4}) egg culture of *C. burnetii* (Greta strain) containing 10^5–10^6 embryo infective doses were placed on sterilized weighed amounts of 8 g of clay and 8 g of sand. The samples were dried in a vacuum of 2–7 Pa and then stored at a relative humidity of 53 to 68% at

	Survival time in d
4–6° C	210 but not 270
15–20° C	120
34–36° C	<60

(Ignatovich 1959b). Vide p. 732.

Sputum

Desiccation — Survival time

In dried sputum — 30 d

(Beer 1974a). Vide p. 722.

Textiles

2-ml quantities of a suspension of a diluted (10^{-4}) egg culture of *C. burnetii* (Greta strain) containing 10^5–10^6 embryo infective doses were placed on sterilized weighed amounts of 0.4 g of wool. The cloth hangings with the organisms placed on them were dried in a vacuum of 3–7 Pa and then kept at a relative humidity of 53 to 68% at ....

	Survival time in d
4–6° C	365 but not 480
15–20° C	210 but not 270
34–36° C	30–60

(Ignatovich 1959 b).

C. burnetii was isolated from a wool sample of a sheep which voided an infected placenta. The sample contained $10^{3.25}$ guinea pig infective doses g^{-1} (Stoker et al. 1955).

C. burnetii was isolated from wool fleece samples of infected sheep taken 14–30 d post partum and from a wool tag taken 7 d post partum (Albinati et al. 1955).

Six cases of Q fever occurred in laundry employees who handled contaminated clothing from the Rocky Mountain Laboratory (Oliphant et al. 1949). An outbreak of the disease was observed among workers of a carpet factory, which received wool from a place where the infection was endemic (Kulagin 1955 quoted from Zdrodovskii and Golinevich 1960). An outbreak of the disease was observed among workers of a plush factory. Atomized feces of infected ticks was suggested to be the cause of the infections (Gmitter 1956). An epidemic of Q fever occurred in a wool- and hair-processing plant. It was believed that the agent was introduced into the plant with bales of wool or hair (Sigel et al. 1948). An epidemic of Q fever in a cotton factory in Slovakia occurred when working with imported cotton from which the agent was isolated (Bardos et al. 1957). There were four cases of Q fever in the household of a calf buyer in southern California whose clothes, often soiled with calf excreta, were brought into the house (Beck et al. 1949). A woman in northern California who had an inapparent infection laundered her husband's clothing, which was frequently soiled with the excreta of sheep and cattle (Clark et al. 1951). Another instance of indirect infection was that of a husband and wife who housed a laboratory worker. This worker handled infected eggs, but showed no sign of disease himself. The most reasonable explanation for the two infections was that *C. burnetii* was carried from the laboratory to home on clothing, shoes, hands or hair (Beeman 1950 quoted from Derrick 1953). This train of thought may be pursued further. Why cannot men whose clothing is contaminated carry infection into the shops and offices that they visit and thereby infect the air that shop assistants and office workers inhale? This may explain some cryptic infections of bartenders, barbers and other indoor workers who have had no direct contact with livestock (Welsh 1951 quoted from Derrick 1953). Vide p. 738.

Ticks

The following species of thicks were kound naturally (+) or could be experimentally infected with *C. burnetii:*

Amblyomma americanum+, *A. cayennense, A. maculatum, A. nutalli, A. paulopunctatum, A. splendidum, A. triguttatum, A. variegatum*+,
Aponomma excornatum, A. halli,
Argas persicus, A. reflexus,
Boophilus annulatus, B. australis, B. decoloratus, B. microplus,
Dermacentor andersoni+, *D. daghestanicus, D. marginatus, D. occidentalis*+, *D. pavlovskyi, D. pictus,*
Haemaphysalis bispinosa, H. humerosa+, *H. leachi*+, *H. leporis-palustris*+, *H. punctata,*
Hyalomma anatolicum, H. detritum, H. dromedarii+, *H. excavatum*+, *H. excavatum lusitanicum*+, *H. impressum-near-planum, H. marginatum*+, *H. mauretanicum*+, *H. plumbeum, H. rufipes glabrata, H. savignyi*+, *H. scupense,*
Ixodes crenulatus, I. dentatus+, *I. holocyclus, I. lividus, I. persulcatus, I. ricinus*+,
Ornithodorus coriaceus, O. erraticus, O. gurneyi, O. hermsi, O. lahorensis, O. moubata+, *O. parkeri, O. talaje, O. tartakovski, O. turicata,*
Otobius megnini+,
Rhipicephalus bursa+, *R. cuspidatus, R. sanguineus*+, *R. simus, R. turanicus.*

Transovarial transmission occurs in the species *Amblyomma cajennense, Dermacentor andersoni* and *Ornithodorus moubata.*

C. burnetii remained infective in specimens of

	Survival time
Ornithodorus gurneyi for	539 d
Ornithodorus hermsi for	979 d
Ornithodorus moubata for	726 d
Ornithodorus turicata for	1,001 d

(Blanc and Brumeau 1949; Weyer 1949a; Schloßberger and Langbein 1952; Weyer 1952/53; Derrick 1953; Hengel et al. 1954; Stoker and Marmion 1955; Karulin 1960 quoted from Thiel et al. 1977; Thiel 1974; Mitschierlich and Wagener 1970; Liebisch et al. 1978).

	Survival time
In the feces of ticks	65–85 d

(Sidky 1950).

Desiccation

In the feces of *Dermacentor andersoni* dried at 22° C and 77% relative humidity and stored at room temperature	586 d

(Philip 1948). Vide p. 722.

Ticks thus provide a high-titer geographically wide-spread reservoir of *C. burnetii.* Their hosts assist them, either by taking part in a tick–animal–tick cycle of transmission or, where transovarial passage occurs in the tick, simply as accessories necessary for their nourishment. The hosts also, by providing transport, ensure wide dissemination of infection. In spite of this importance, ticks have very little direct association with human infection (Derrick 1953).

Urine

Desiccation

	Survival time
In dried urine of guinea pigs	49 d

(Reusse 1960). Vide p. 722.

Water

C. burnetii was isolated from open pond water possibly contaminated by animal excreta (Yaday and Seth 1980).

Tap water and distilled water samples were contaminated with dried egg material containing *C. burnetii* at a dilution of 10^{-4}, which corre-

sponded to 10^5–10^6 infective doses for chick embryos and guinea pigs, and stored at different temperatures.

		Survival time in d
In tap water stored at	4–6° C	900 but not 1.080
	15–20° C	90 but not 150
	34–36° C	30 but not 60
in distilled water stored at	4–6° C	630 but not 720
	15–20° C	60 but not 150
	34–36° C	< 60

(IGNATOVICH 1959a).

In contaminated tap water stored at ..... 20–22° C — Survival time 160 d

(KULAGIN and SILITSCH 1956 quoted from REUSSE 1960). Vide p. 738.

Dermatophilus congolensis

Significance and Habitats. Etiological agent of streptotrichosis, an exudative, pustular dermatitis of worldwide distribution, affecting mainly cattle, sheep and horses, but also goats and other domesticated and feral mammals and occasionally humans (GORDON 1974).

Growth Limits. Temperature and hydrogen ion concentration: Vide Table 1.

Culture

		Survival time
In nutrient broth cultures stored at	room temperature	4 years
		4 months

(ODUYE 1976).

Moist Heat Resistance

Pasteur pipettes were charged with 1-ml samples of 3-d-old serum broth cultures and submerged in a water bath at different temperatures. Two strains were tested at

	Survival time
60° C	4 h (n.f.t.)
65° C	5 min

(WEBER and SCHLIESSER 1971). Vide p. 710.

Exudate

Desiccation — Survival time

In dried crusts of infected hides of sheep ..... months, even years

(HART 1976). Vide p. 722.

Insects

Flies

D. congolensis was transmitted from infected to normal rabbits by the stable fly *Stomoxys calcitrans* and the house fly *Musca domestica*. Mechanical disruption of the host's skin by the feeding fly was not necessary for transmission. Moistening of both the lesions on donor rabbits and fly-feeding sites on recipient rabbits enhanced fly transmission. *S. calcitrans* transmitted the infection for periods up to 24 h after feeding on an infected rabbit (RICHARD and PIER 1966).

Soil

10-g quantities of heat sterilized soil samples of different pH values were inoculated with 2 drops of a suspension of zoospores and stored at ..... 17–20° C

	Survival time in d
In soil samples with a final pH of 5.0	7–12
5.1	5–15
6.3–6.4	21
7.0	50–77
7.7	96

10-g lots of eight soils were mixed each with 0.1-g quantities of scab material. The bottles were shaken to disperse the scab particles through the soil. 4 ml of de-ionized water was added to one bottle of each soil and another was left dry. Bottles were stored in the dark at ..4° C.

In samples of wet soil	9 but not 21
in samples of dry soil	120 (n.f.t.)

The infectivity of the samples was tested on the back of sheep. *D. congolensis* is apparently unable to multiply in soil (ROBERTS 1963b). Vide p. 732.

Ticks

D. congolensis was experimentally transmitted from cattle to a rabbit by *Amblyomma variegatum*. On bovine ears, swabbed with cultures of *C. congolensis*, tick bites set up persistent lesions which failed to spread in conditions of high humidity. The common "paint brush" lesions on backs of cattle were different from those produced by tick bites, and it is suggested that the former may result from biting flies (MACADAM 1962).

Desulfotomaculum nigrificans syn. *Clostridium nigrificans*

Significance and Habitats. Is important in pasteurization of foods; cause of "sulfur stinker" spoilage; pathogenicity unknown (GILLESPY and THORPE 1968; STUMBO 1973).

Growth Limits. Temperature, hydrogen ion concentration: Vide Table 1.

Food

Spoilage has been recorded for canned peas, corn, mushrooms and creamed rice, all in the pH range of 5.9–6.4 (GILLESPY and THORPE 1968).

Moist Heat Resistance — D value

Approximate range of heat resistance in canned foods at ..... 121.1° C — 2.0–3.0 min

(STUMBO 1973). Vide p. 710.

Edwardsiella tarda

Significance and Habitats. *E. tarda* is probably a normal intestinal inhabitant of snakes. The organism has been isolated occasionally from the stool of humans with diarrhea or those in good health, from the urine of man, in several instances also from cattle and pigs and a great variety of wild animals. *E. tarda* was also found in water samples (WHITE et al. 1973; SAKAZAKI 1974a).

Vide Table 115, p. 729. — **Honey**

73 C

Role of Sex, Age, Previous Valve Lesion, and Pregnancy in the Clinical Expression and Outcome of Q Fever after a Large Outbreak

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Background. Q fever is a zoonosis caused by *Coxiella burnetii*. After a large outbreak occurred in the Chamonix Valley in the French Alps in 2002, an extensive surveillance was conducted, to describe the variations in the clinical expression of acute Q fever according to host factors, as well as to monitor the risk of evolution of acute Q fever to chronic Q fever in patients at risk.

Methods. Three groups of patients with risk factors for evolution of acute Q fever to chronic Q fever were considered: 376 pregnant women, 19 immunocompromised patients, and 91 patients with valvular or vascular abnormalities. A group of 578 people without risk factors for evolution of acute Q fever to chronic Q fever was also tested. Diagnosis of Q fever was based on serologic testing by immunofluorescence assay.

Results. Between 30 August 2002 and 31 July 2003, a total of 1946 serum samples obtained from 1064 persons were tested. A total of 101 patients (9.3%) had acute Q fever diagnosed, and 5 patients (0.5%) had chronic Q fever diagnosed. A diagnosis of acute Q fever was established for 11 pregnant women (2.6% of 379 pregnancies), 5 patients with valvular disease (5.5%), and 85 people without risk factors (14.7%) (71 [27.9%] of 254 symptomatic patients and 14 [4.3%] of 324 asymptomatic patients). A new pregnancy in a woman with negative results of serologic tests for Q fever exposes the woman to a new risk for acute Q fever able to evolve to chronic Q fever. The rates of clinical expression were 90.6% in adult men, 75% in adult women, and 33.3% in children, and they were significantly lower (9.1%) in pregnant women. Evolution to chronic Q fever was observed in 5 patients.

Conclusion. This study emphasizes the importance of active surveillance in postepidemic conditions, especially among patients at risk, as well as the importance of systematic serologic testing during pregnancy.

Q fever is a worldwide zoonosis due to *Coxiella burnetii*, an obligate intracellular bacterium. The main characteristic of Q fever is its clinical polymorphism [1]. In acute cases, the most common clinical syndromes are fever, granulomatous hepatitis, and pneumonia. In chronic cases, endocarditis is the main syndrome [1]. Osteomyelitis, infections of vascular grafts or aneurysms, and infections occurring during pregnancy [2] are also reported. The usual reservoirs for *C. burnetii* are cattle, sheep, and goats [3], which shed the bacterium in urine, feces, milk, and birth products [1]. Infected pets, such as cats [4], rabbits [1], and dogs [5], have been sources of outbreaks in humans [3]. Human infection mainly occurs after inhalation of contaminated aerosols. The diagnosis of Q fever is based on specific serologic findings. The reference technique is indirect immunofluorescence assay [6].

Various factors influence the clinical expression and outcome of the disease [7]. *C. burnetii* itself plays a role in the intensity of the clinical expression of acute Q fever: in animal models, the Nine Mile strain is associated with more active disease than is the Priscilla strain [8]. Some genotypes are rarely isolated from patients with acute infections, but all have been found in patients with chronic endocarditis [9]. The route of infection and inoculum size are also involved [10, 11]. However, the main factors able to influence the clinical expression of acute Q fever, as well as its evolution to

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chronic Q fever, are host factors [7]: immunocompromised patients present more often with pneumonia, and they are likely to experience relapse. Approximately 40% of persons with acute Q fever and valvular disease, which may be mild or undiagnosed but is present before the onset of acute Q fever, will develop chronic endocarditis. Acute Q fever in pregnant women, whether symptomatic or not, may result in abortion, prematurity, or a low birth weight. Q fever may evolve to chronic Q fever after delivery and may be associated with recurrence of miscarriage [12]. Age is a risk factor: at comparable exposure levels and seroconversion rates, symptomatic Q fever is 5 times more likely to be diagnosed in people ≥15 years of age than in younger people. [7]. Sex is also a risk factor: at comparable levels of exposure and seroprevalence, the ratio of male to female subjects is 2.45 among adults in France. The predisposition for infection in men is explained by the protective role of female sex hormones [13].

During the summer of 2002, a large outbreak of Q fever occurred in the Chamonix Valley in the French Alps. Because of the large proportion of asymptomatic cases, and because of the high risk of chronicity among at-risk patients (i.e., pregnant women, patients with a valvular or vascular abnormality, and immunocompromised patients), we decided to perform serologic surveillance for 1 year. The goals of this surveillance were (1) to describe the variations in the clinical expression of acute Q fever depending on host factors, and (2) to monitor the risk of evolution of acute Q fever to chronic Q fever in patients at risk.

MATERIALS AND METHODS

The Chamonix Valley. Chamonix is a city (altitude, 1050 m) located at the foot of Mont Blanc in the French Alps. Its population is 9829 inhabitants. The valley is 27 km long, with a south-north orientation. The population of the whole valley includes 12,927 inhabitants (49.4% of whom are male, and 50.6% of whom are female) and thousands of tourists.

Surveillance modalities. As soon as the outbreak was investigated, the surveillance was started. All the physicians were informed about Q fever and the outbreak investigation. Several groups of patients were considered. Systematic serologic testing was proposed for patients known to be at risk for developing chronic Q fever—that is, pregnant women, patients with any valvular or vascular abnormality, and immunocompromised patients (those with HIV infection or cancer and those receiving corticosteroid therapy). Serologic testing for Q fever was also performed for patients with clinical symptoms evocative of Q fever (e.g., fever with or without headaches, flulike syndrome, myalgia, arthralgia, and liver involvement). Moreover, any person wishing to be tested for any reason could be sampled, and any physician could recommend serologic testing if he or she believed it was necessary.

Blood samples were drawn in private laboratories, and every couple of days, they were sent to the National Reference Center for Rickettsial Diseases in Marseille, France. The epidemiological and clinical data that were collected and sent to the National Center for Rickettsial Diseases included data on sex, date of birth, term of pregnancy, symptomatology (and date of onset), the physician, and hospitalization.

During the case-control study, 111 control subjects were tested. These control subjects were randomly selected from nonfebrile persons who were present in the Chamonix Valley during the period of the outbreak exposure. Thus, all of them can be considered to be asymptomatic.

Serologic tests. Because of the frequency of asymptomatic infections (60%) [1], which lead to such patients having similar risks of evolution to chronic Q fever, the case definitions were based on results of serologic tests. Indirect immunofluorescence assays were performed as described elsewhere [6]. Under the usual diagnostic conditions, in our hands, a serum sample is considered to be diagnostic of evolutive Q fever (acute or chronic) when the phase II IgG titer is ≥200 and the phase II IgM titer is ≥50, or when seroconversion has been noted. In the context of an epidemic, when the positive predictive value was increased, it was important to enhance the sensitivity of serologic testing. Therefore, a cutoff value that was 1 dilution lower (i.e., a phase II IgG titer ≥100 and/or a phase II IgM titer ≥25) was considered for the diagnosis of acute cases. A diagnosis of chronic Q fever is considered when the phase I IgG titer is ≥800 [6].

Statistical analysis. Frequencies of qualitative data were compared using Pearson's χ^2 test or Fisher's exact test with the use of EpiInfo software, version 6.04fr (Centers for Disease Control and Prevention and EpiConcept). A difference was considered to be statistically significant when $P < .05$.

RESULTS

Physician participation. A total of 73 different physicians sent to the laboratory serum samples that were obtained from their patients. Twenty-three local physicians had testing performed for >10 patients. The 2 obstetricians sent serum samples obtained from 219 and 101 pregnant women; the cardiologist sent serum samples obtained from 40 patients; and the 9 most active general practitioners sent serum samples obtained from 31 to 53 patients.

From 30 August 2002 through 31 July 2003, a total of 1946 serum samples obtained from 1089 persons underwent testing. A total of 101 cases of acute Q fever (9.3%) and 5 cases of chronic Q fever (0.5%) were diagnosed (table 1).

Pregnant women. A total of 891 serum samples, obtained from 376 pregnant women, were tested during pregnancy. Of the women, 350 had ongoing pregnancies, 11 had testing per-

Table 1. Summary of patient groups and results of serologic testing for *Coxiella burnetii* after an outbreak of Q fever in the Chamonix Valley in the French Alps, August 2002 through July 2003.

Patient group, by presentation or risk factor	Serum samples tested, no.	No. (%) of patients			
		All	With acute Q fever	With chronic Q fever	With residual antibodies
All	1907	1067[a]	101 (9.3)	5 (0.5)	59 (5.4)
Pregnancy	891	376[b]	11 (2.6)	1 (0.3)	21 (5.5)
Cardiac abnormality	177	91	5 (5.5)	1 (1.1)	7 (7.7)
Immunodeficiency	27	19	0	0	0
No known risk factor	812	578	85 (14.7)	3 (0.5)	31 (5.4)
Symptomatic	...	254	71 (27.9)	3 (1.2)	11 (4.3)
Asymptomatic	...	324	14 (4.3)	0	20 (6.2)

[a] Total includes 379 pregnancies (among 376 women).
[b] The 376 pregnant women had a total of 379 pregnancies; therefore, 379 was used as the denominator in the calculation of percentages of pregnant patients.

formed shortly after normal delivery, and 18 had testing performed after spontaneous abortion. A total of 379 pregnancies were considered, because 2 women had 2 normal consecutive pregnancies during the surveillance and 1 woman had a normal pregnancy after abortion. The mean age (± SD) of these women was 31 ± 7 years. One hundred eighty-eight women had a single serum sample tested, 77 women had 2 serum samples tested, and 111 women had ≥3 serum samples tested.

The results of serologic tests were negative for 343 (90.5%) of 379 pregnancies, for 317 (90.6%) of 350 ongoing pregnancies, and for 18 (100%) of 18 abortions, as well as for 8 (72.7%) of 11 women after delivery. Acute Q fever was diagnosed in 11 women (2.6% of 379 pregnancies), one of whom later developed a profile of chronic infection. The results also showed residual antibodies (phase II IgG without IgM) in association with 21 (5.5%) of 379 pregnancies (in 19 [5.4%] of 350 ongoing pregnancies and in 2 [18.2%] of 11 women after delivery). For 4 pregnancies, the data were not sufficient to draw conclusions (i.e., a single serum sample had IgG and/or IgM titers below the cutoff value).

Most (371 of 376) of these pregnant women were asymptomatic, whereas 5 (1.3%) had fever, 1 complained of headache, and 1 had fatigue. One woman who had testing performed had a suspected diagnosis of pericarditis. Four of these symptomatic women had negative test results.

All 11 women who had positive test results were treated according to our protocols (with trimethoprim-sulfamethoxazole, 160 mg/800 mg twice daily until delivery [12]) and were carefully monitored. One of them seroconverted and then exhibited antibodies typical of chronic infection at month 8 of pregnancy. After delivery, this patient received treatment, as for any patient with chronic Q fever, according to our protocols (i.e., doxycycline plus hydroxychloroquine sulfate [14]).

Persons with cardiac involvement. A total of 177 serum samples that were obtained from 91 persons with cardiac abnormalities were tested. Seventy-four patients had a known valvular abnormality, 6 had cardiopathy, 1 had myocarditis, 1 had pericarditis, and 3 had a coronary vascular graft. The 6 remaining persons had a nonspecified "cardiologic problem."

The mean age (± SD) of the study participants with cardiac involvement was 65 ± 17 years of age. The ratio of male to female subjects was 0.91 (43 male and 47 female subjects). Sixty-two persons had a single serum sample tested, 14 persons had 2 serum samples tested, and 15 persons had ≥3 serum samples tested.

A total of 5 persons (5.5%) had acute Q fever diagnosed; one of these persons later experienced endocarditis. Seven persons had residual antibodies. Most (74 of 91) of the patients were asymptomatic. Of the 6 patients who experienced fever, 2 had negative test results, 3 were considered to have acute Q fever (one case of which became chronic), and 1 had residual antibodies.

Of the 5 patients who had acute Q fever diagnosed, 4 could be treated with doxycycline plus hydroxychloroquine sulfate and monitored for 1 year according to our protocols. Unfortunately, and as expected, the patient who was not treated later had a chronic form of Q fever develop.

Persons with immunodeficiencies. A total of 27 serum samples obtained from 19 persons (5 male and 14 female subjects; mean age [± SD], 58 ± 20 years) were tested. The reported causes of immune suppression were as follows: solid or hematologic cancer ($n = 14$), immunosuppressive treatment ($n = 2$), HIV infection ($n = 2$), and an unspecified cause ($n = 1$). Four (21.1%) of 19 patients were symptomatic; 3 of the 4 patients had fever (which was associated with nausea in one patient and with sore throat in 1 patient). One patient complained of fatigue. None of these patients had positive test results.

Patients without known risk factors. A total of 812 serum samples were obtained from this group of 578 patients and were tested; 461 (79.8%) of these patients had negative test

Table 2. Comparisons of the prevalence of Q fever, by patient group and sex, in the Chamonix Valley in the French Alps, August 2002 through July 2003.

Patient group, by presentation and risk factor	Patients with Q fever, *n/N* (%)[a]		*P*
	Male	Female	
No known risk factor	51/259 (19.7)	34/319 (10.5)	.002
Symptomatic	49/142 (34.5)	24/112 (21.4)	.02
Asymptomatic	2/117 (1.7)	10/207 (4.8)	.15
Cardiac abnormality	3/43 (6.9)	2/47 (4.2)	.57
Immunodeficiency	0/5 (0)	0/14 (0)	NA
All	54/307 (17.6)	36/380 (9.5)	.002

[a] Data are no. of patients with Q fever/total no. of patients tested (% of patients with Q fever).

results, 85 (14.7%) had acute Q fever, and 31 (5.4%) had residual antibodies. Three patients with acute Q fever were later found to have antibodies compatible with the presence of chronic infection. Data were not sufficient to draw conclusions for one patient.

One group of 254 patients had a clinical presentation compatible with a diagnosis of acute Q fever. Of these patients, 169 (66.5%) had negative test results, 11 (4.3%) had residual antibodies, and 71 (27.9%) had acute Q fever. Three of these 71 patients had Q fever that eventually become chronic. The first of these 3 patients was a 36-year-old man who was symptomatic in July 2002 and who was considered to have endocarditis because he had an aortic insufficiency, with no vegetation noted on ultrasound examination. The second patient was a 78-year-old woman who had a history of pulmonary tuberculosis. She was symptomatic in July 2002. Findings of cardiac ultrasonography were normal. A thoracic CT scan demonstrated a residual pulmonary opacity. The third patient was a 44-year-old man who was symptomatic in July 2002 and who experienced relapse in August 2002. His first serum sample (obtained on 13 August 2002) had a negative test result, and the result for his second sample (obtained on 31 December 2002) demonstrated a chronic profile. No clinical and evolutive data were available.

The second group of 324 patients was asymptomatic. Of those patients, 290 (89.5%) had negative rest results, 14 (4.3%) had recently been exposed to *C. burnetii,* and 20 (6.2%) had received a positive serologic test result, although it was below the cutoff for diagnosis of evolutive disease.

When we compare these 2 groups by use of the χ^2 test, the rate of positive results was higher among the group of symptomatic patients (27.9%) than among the group of asymptomatic patients (4.3%) (OR, 8.59; 95% CI, 4.6–16.9; P <.001).

Serum samples for which data were not available. Thirty-six serum samples for which data were not available were sent to the laboratory. All samples tested negative, except for those obtained from 3 patients who exhibited low titers of phase II IgG, without IgM.

Control subjects. Of the 111 asymptomatic control subjects who underwent testing, 1 (0.9%) had serologic titers (phase II IgG, 50; phase II IgM, 25) corresponding to our definition of acute Q fever.

Comparisons between groups. For this comparison, we considered the groups as previously defined: pregnant women ($n = 11$); patients with cardiac involvement ($n = 5$); patients with immunodeficiency ($n = 0$); and patients without known risk factors, who were known as "other" patients ($n = 85$); and control subjects ($n = 1$). We considered the patients who met the case definition to be symptomatic.

Under these conditions, pregnant women were less often symptomatic (1 of 11 such patients was symptomatic) than were patients with cardiac involvement (3 of 5 such patients were symptomatic) (OR, 0.07; 95% CI, 0–1.61; $P = .029$). They were also less often symptomatic than the other patients (71 of 85 such patients were symptomatic) (OR, 0.02; 95% CI, 0–0.17; $P < .001$). Pregnant women were also less often symptomatic than nonpregnant women (48 of 54 nonpregnant women with a diagnosis of acute Q fever [in other groups]) (OR, 0; 95% CI, 0–0.07; $P < .001$).

Incidence of acute Q fever, by sex, age, and patient group. Table 2 shows the comparison of cases of acute Q fever among the previously defined patient groups, according to sex. A difference was found when all patients were compared: more cases of acute Q fever were diagnosed among 307 male subjects (17.6%) than among 380 female subjects (9.5%) (OR, 0.4; 95% CI, 0.14–1.08; $P = .002$). A difference was also noted in the group of subjects without known risk factors: more cases were diagnosed among 259 males (19.7%) than among 319 females (10.5%) (OR, 2.06; 95% CI, 1.25–3.39; $P = .002$). In the subgroup of symptomatic patients, more cases were also diagnosed among 142 males (34.5%) than among 112 females (21.4%) (OR, 1.9; 95% CI, 1.06–3.58; $P = .02$).

Of the group of people without known risk factors, 23 children ≤15 years of age were tested. Of these children, 11 were boys and 12 were girls. Nine (5 boys and 4 girls) reported symptoms that included fever. Three (13.04%) of the 23 children had acute Q fever diagnosed, and 2 of these children were symptomatic (i.e., they had fever).

Table 3 summarizes the data on age and sex for patients with serologically defined cases of acute Q fever: pregnant women were the patients who were less often symptomatic (9.1%), whereas the rate of symptomatic cases was slightly higher (33.3%) among children and was much higher among adult nonpregnant females and males >14 years of age (75% and 90.6%, respectively). The statistical comparison shows a significant difference between pregnant women and adult males (OR, 96; 95% CI, 9.1–4244; $P < .001$), between pregnant women

Table 3. Comparisons between pregnant women, children, nonpregnant females, and males, in terms of Q fever seroprevalence and symptomatic cases.

Patient group	No. (%) of patients		
	Tested	With acute Q fever	Who were symptomatic
Persons >14 years of age			
Males	293	53	48 (90.6)
Nonpregnant females	360	32	24 (75)
Children[a] ≤14 years of age	23	3	1 (33.3)
Pregnant women	379	11	1 (9.1)

[a] Both sexes.

and nonpregnant women (OR, 40; 95% CI, 4.2–1787; $P < .001$), and between adult males and children (OR, 19.2; 95% CI, 0.78–1154; $P = .038$). The small number of cases did not permit demonstration of the differences between pregnant women and children ($P = .09$), between males >14 years of age and nonpregnant women ($P = .054$), and between adults and children ($P = .07$).

DISCUSSION

Knowledge regarding Q fever in humans has widely increased during the past decade, mainly in terms of understanding its pathophysiology [7, 8], the clinical presentations of acute [1, 15–17] and chronic cases [7], and the specific populations at risk for chronic infection (e.g., people with valvular disease, pregnant women [18], immunocompromised hosts [1, 15], and children [19, 20]). Diagnosis of Q fever has been improved in terms of molecular testing, by use of real-time PCR [21, 22]. Treatment strategies, including drug testing and monitoring, have been proposed for acute [1] and chronic [14] cases, as well as for specific hosts, such as pregnant women [12] or patients with valvular damages.

To our knowledge, the present study reports, for the very first time, active serologic surveillance of Q fever in humans, for 1 year after a large outbreak that occurred in a tourist area and for which there was no removable source of *C. burnetii*. This surveillance has demonstrated its feasibility. The efficiency of the surveillance was also demonstrated, because a total of 101 patients with acute Q fever and 5 with chronic Q fever had Q fever diagnosed and then were monitored. Moreover, this is the first outbreak in which asymptomatic, acute, and chronic infections were diagnosed. We also confirmed that 1 patient can have acute Q fever evolve to chronic Q fever.

In terms of knowledge of Q fever, we have been able to show a difference in the clinical expression of Q fever, according to sex and age: although not statistically significant, because of the small number of cases, a higher rate of symptomatic cases may be confirmed among adult males (91%) than among females (75%), as described in most of epidemiological studies [1, 7], and as explained in animal models by the protective role of 17β-estradiol, the adult female hormone, which could also explain why the sex ratio is biased only after puberty [13]. Maltezou et al. [19, 20] have shown that the clinical expression of Q fever in children <14 years of age was much lower than that in adults. Again, although nonsignificant, clinical expression was 2.6 times lower in children (33%) than in adults of both sexes (85%). We were able to show a significant difference between men and children.

We demonstrated that pregnant women were less symptomatic than other women and other patients. Q fever during pregnancy was known to be likely asymptomatic [12], which was the case in our series, because only 1 of the 11 pregnant women who had Q fever diagnosed was symptomatic. The conditions of the surveillance among pregnant women (involving 2 concerned obstetricians who tested all pregnant women they monitored) can be considered to be the conditions of a survey. Under these conditions, 11 cases have been diagnosed among 379 nonselected pregnancies (with 1 case developing per 34 pregnancies), which is much more common, under this epidemic conditions, than was estimated in Martigues, France, an area of high endemicity (where 1 case developed per 415 pregnancies) [23].

The present study also let us observe an evolution from acute to chronic Q fever in 5 patients: a pregnant women and a patient with valvular abnormalities (who both refused the antibiotic prophylaxis), as well as 3 patients without known risk factors. The necessity of systematic follow-up of acute cases after such an outbreak has recently been emphasized [24], as has the importance of the detection of minimal valvular diseases among patients with acute Q fever [25].

In conclusion, to our knowledge, this was the very first time that such a surveillance was conducted during and after the epidemic, showing its feasibility and usefulness. Thus, under epidemic conditions (when >5 grouped cases are noted within 1 month), our advice is to test (once) any person considered to be at risk (as defined above). This surveillance was especially active among pregnant women, demonstrating that such women are less symptomatic than any other patients with acute Q fever. This enhances the importance of systematic testing during pregnancy in areas where Q fever is prevalent: under standard conditions, a pregnant woman should be tested when febrile or after any abnormal delivery. Under epidemic conditions, or in populations with frequent animal contacts, our advice is to test any pregnant woman. Those with positive test results should be treated and monitored. Pregnant women with negative test results should be tested every month until delivery.

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Q fever: een overzicht

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Overzichtsartikel

Samenvatting

Q fever is een zoönose. De infectie komt wereldwijd voor bij vele diersoorten en de mens en wordt veroorzaakt door de gramnegatieve bacterie *Coxiella burnetii*. Herkauwers worden beschouwd als de belangrijkste besmettingsbron voor de mens. De belangrijkste besmettingsroute bij de mens is het inhaleren van besmette fijne partikeltjes.
Abortus is het belangrijkste klinische verschijnsel bij herkauwers. Tijdens en na de abortus scheidt een dier grote hoeveelheden bacteriën uit via de placenta en andere vaginale excreta. De kiem komt ook voor in melk en mest. Na uitscheiding kan de kiem lang overleven in de buitenlucht en over grote afstanden verspreid worden. Zowel in Nederland als in meerdere andere landen is de seroprevalentie bij rundvee vrij hoog. Een infectie kan worden gediagnosticeerd door het aantonen van antistoffen tegen de bacterie of door het aantonen van de bacterie zelf door middel van een PCR-techniek. Het effect van het toedienen van antibiotica en vaccins voor therapeutische of preventieve doeleinden is nog onduidelijk. Tot op heden zijn er wereldwijd nog geen effectieve bestrijdingsprogramma's ontwikkeld.

Summary

Q fever: an overview
Q fever, a zoonosis caused by the gram-negative bacterium Coxiella burnetii, *occurs worldwide and affects both humans and animals. Ruminants are considered to be the main source of infection of humans, with the main route of infection being through inhalation of the organism of fine-particle aerosols. Abortion is the main clinical sign in ruminants. During and after abortion, large quantities of the bacterium are shed via the placenta and other vaginal secretions. The bacterium may also be present in faeces and milk. The bacterium can survive for a long time in the environment after shedding and can be spread over long distances.*
Seroprevalence among cattle is rather high in the Netherlands and in many other countries. Infection is diagnosed by detecting antibodies against the bacterium or the bacterium itself by means of a PCR method. The efficacy of using antibiotics or vaccines for treatment or prevention of the disease in cattle is still unclear, and there are currently no effective disease control programmes.

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Introductie

Q fever, ook wel Q-koorts genoemd, is een wereldwijd voorkomende infectie bij mens en dier. Het is een zoönose, die veroorzaakt wordt door de bacterie *Coxiella burnetii*. De letter Q komt of van het woord 'Query', wat vraagteken betekent (de verwekker van de ziekte was namelijk lang onbekend), of van Queensland (Australië), waar de ziekte in 1935 voor het eerst is beschreven bij slachthuispersoneel (20). Deze ziekteuitbraak is nader onderzocht door Burnet. Min of meer tegelijkertijd werd de kiem ook gevonden in de Verenigde Staten door Cox, die de bacterie isoleerde uit teken. Cox en Burnet zijn door de naamgeving van de bacterie verbonden gebleven.
De bacterie wordt wereldwijd gevonden, met uitzondering van in Nieuw-Zeeland (65). Het reservoir is de dierenwereld, waarbij de bacterie in zeer veel diersoorten is gevonden: wilde dieren, gedomesticeerde dieren, diverse vogels, zoogdieren en allerlei insecten. Bij besmetting van (wilde) dieren spelen teken mogelijk een rol (44, 58), maar dit wordt betwijfeld door andere onderzoekers (47, 52).
In dit artikel zal vooral aandacht worden besteed aan Q fever bij het rund. Daarnaast zullen een aantal belangrijke aspecten van de ziekte bij de mens en andere diersoorten, met name bij schapen en geiten, worden beschreven.

Bacterie

Coxiella burnetii is een obligaat intracellulaire, gramnegatieve bacterie. Eerder werd de kiem geschaard onder de Rickettsiae; nu behoort deze tot de orde van de Legionellae, in de familie Coxiellaceae waarvan *Coxiella burnetii* het enige species is.
De bacterie heeft twee vormen, namelijk een 'small cell'-variant (SCV) en een 'large cell'-variant (LCV). Deze vormen zijn te onderscheiden met behulp van elektronenmicroscopie. De SCV is zeer resistent tegen chemische invloeden, uitdroging, hoge en lage temperaturen en wordt soms ook wel 'spore' genoemd. De SCV is zeer stabiel in aërosolen (in ingedroogde dierlijke cellen) en is zeer infectieus. De SCV wordt bij entree in een gastheer (dier of mens) gefagocyteerd door macrofagen en vormt zich vervolgens in de fagocyt om tot LCV. Als LCV vermeerdert de kiem zich in de fagocyten en blijft daar persistent aanwezig.
Coxiella burnetii heeft twee antigene fasen: fase I en fase II. Wanneer de bacterie direct vanuit een dier of mens wordt onderzocht, is deze in fase I; wanneer de bacterie enkele malen gekweekt is op celcultuur of bebroede kippeneieren, is deze in fase II.
Deze antigene variatie heeft zijn oorsprong in een verandering in de LPS-laag (lipo-polysaccharidelaag) van de bacterie. In fase I is de LPS-laag compleet en zorgt er onder meer voor dat de immuunglobulinen van de gastheer gehinderd worden om te binden aan de oppervlakte-eiwitten van de bacterie, daarmee een effectieve afweerreactie verstorend. In fase I is *Coxiella burnetii* zeer infectieus en virulent. In fase II heeft het LPS een andere suikersamenstelling en is het LPS korter. Hierdoor krijgen immuunglobulinen de kans de bacte-

rie te naderen en onschadelijk te maken. Deze variatie in antigene fasen (fase I en fase II) is analoog aan 'smooth'- en 'rough'-varianten van andere gramnegatieve bacteriën. Dit is van belang in de diagnostiek en de vaccinbereiding (23, 48, 65). *Coxiella burnetii*-isolaten zijn genetisch sterk homogeen, maar met restrictie-enzymtechnieken kunnen wel verschillende stammen worden onderscheiden.

Ziekte bij de mens

Humane infecties zijn afkomstig uit het dierreservoir. Schapen, geiten en runderen worden gezien als de belangrijkste bron voor humane infecties (48). Ook katten, honden en knaagdieren zijn mogelijke bronnen voor humane infecties (23, 37, 49). Infecties van mens op mens zijn zeldzaam (23). De belangrijkste porte d'entree bij de mens is het respiratoire slijmvlies of de conjunctiva en waarschijnlijk is ook het intestinale slijmvlies een mogelijke route. Na entree volgt een hematogene verspreiding en een systemische infectie. Bij de mens is de infectie meestal zelflimiterend. Waarschijnlijk persisteert de bacterie soms in het baarmoederslijmvlies en in melkklieren (26). *C. burnetii* is, net als bij dieren, ook bij mensen in placenta en moedermelk aangetoond (52).
De ziekteverschijnselen bij de mens zijn divers. Een groot deel van de infecties gaat symptoomloos voorbij. Wanneer er wel verschijnselen optreden, bestaan ze in de acute fase uit de volgende griepachtige symptomen: langdurig (zeven tot tien dagen) hoge koorts, hoofdpijn, spierpijn, geen eetlust, misselijkheid, braken, diarree, hoesten en pijn op de borst (24, 48, 52). Een atypische pneumonie of leverontsteking wordt vaak gezien. De incubatietijd is twee tot vier weken, soms zelfs zes weken. Wanneer de infectie chronisch verloopt, kan een endocarditis ontstaan. Dit kan jaren na de oorspronkelijke infectie nog manifest worden. Andere verschijnselen zijn abortus, doodgeboorte (48) en chronische vermoeidheid (26, 48). Meestal herstellen mensen met Q fever. Wanneer de infectie chronisch is geworden, sterft echter 1 tot 11 procent (23).
In diverse landen zijn grote uitbraken van Q fever beschreven. Zes werknemers van een vleesverwerkende fabriek in Schotland moesten worden opgenomen in het ziekenhuis (43). In Frankrijk is ook een uitbraak beschreven die gerelateerd was aan een slachthuis (17). Bij een uitbraak in een school in Groot-Brittannië was de infectie waarschijnlijk afkomstig van vijf geiten die werden gehouden op school (34). In een Frans onderzoek werden humane gevallen geassocieerd met een mistralwind die een maand eerder had plaatsgevonden in een periode kort na de aanvang van het lammerseizoen (60).
In Nederland is de ziekte bij mensen meldingsplichtig. Tot 2007 werden jaarlijks circa twintig gevallen gemeld. Algemeen wordt verwacht dat dit een sterke onderschatting is van het werkelijke aantal ziektegevallen, vooral omdat de symptomen zo divers zijn en de diagnostiek gecompliceerd is (24). In 2007 werd echter een groter aantal humane infecties vastgesteld.
Besmettingsbronnen voor humane infecties zijn moeilijk te vinden omdat de kiem over grote afstanden met de wind kan worden verspreid, zeer resistent is en daarom lang kan overleven. Hierdoor is het mogelijk dat mensen besmet worden zonder direct contact met dieren (24, 62). Het drinken van gepasteuriseerde melk wordt niet beschouwd als een bron van besmetting voor de mens (18).
Mensen met Q fever worden behandeld met antibiotica. Daarbij wordt onderscheid gemaakt tussen de behandelingen van de acute en de chronische fase. In de acute fase wordt een kuur van veertien dagen doxycycline aanbevolen. Bij chronische infecties moeten antibiotica gedurende perioden van anderhalf tot drie jaar worden genomen (24).

Klinische verschijnselen bij herkauwers

C. burnetii dringt vooral via de luchtwegen binnen. Bij dieren zijn (in tegenstelling tot bij de mens) primaire hart- of longinfecties slechts zelden vastgesteld, behalve na experimentele infecties (50). In de chronische fase zijn bij dieren geen klinische afwijkingen beschreven.
Indien er klinische verschijnselen optreden bij allerlei diersoorten, is abortus in een gevorderd stadium van de dracht het belangrijkste symptoom (65). Naast abortus kunnen doodgeboorten, aan de nageboorte staan, baarmoederontsteking en onvruchtbaarheid mogelijk als klinische verschijnselen optreden. Placentitis is het meest karakteristieke kenmerk van Q fever. De placenta blijkt leerachtig en verdikt en kan grote hoeveelheden witgelig exsudaat bevatten aan de randen van de cotyledonen en ook tussen de cotyledonen. Soms kan het exsudaat roodbruin van kleur zijn. Kleine stolsels en vaatwandontsteking kunnen gezien worden bij histologisch onderzoek. Bij de geaborteerde kalveren en geiten- en schapenlammeren is pneumonie waargenomen. Meestal zijn de afwijkingen in de geaborteerde foeten niet specifiek (45).
In diverse landen is onderzoek gedaan naar het voorkomen van *C. burnetii* bij runderen met vruchtbaarheidsproblemen. In Italië hadden geaborteerde runderen ten opzichte van 'at random' onderzochte runderen significant vaker Q fever-antistoffen (14).
In een ander Italiaans onderzoek was twaalf procent van de 138 onderzochte, geaborteerde runderfoetussen PCR positief (47). Soms wordt Q fever beschreven als mogelijke oorzaak van terugkomers (56), in een ander onderzoek is dat niet bevestigd (57). In een Japans onderzoek zijn 61 uterusswabs van melkkoeien met vruchtbaarheidsproblemen onderzocht op de aanwezigheid van *C. burnetii* en bij 21 procent werd de kiem aangetoond met PCR (31). In een onderzoek van Tainturier (59) is *C. burnetii* beschreven als mogelijke oorzaak van metritis.
Tot op heden is in Nederland de diagnose Q fever op basis van immunohistochemisch onderzoek van de placenta nog niet gesteld bij verwerpende runderen (66).
Infectie van drachtige geiten en schapen kan abortus veroorzaken. Daarnaast kunnen lammeren slap geboren worden (11). In Sardinië (Italië) was 10 procent van 372 onderzochte schapenfoetussen en 6 procent van vijftig onderzochte geitenfoetussen PCR-positief (41).
Na een natuurlijke infectie treden de meeste vruchtbaarheidsproblemen op tijdens het eerste aflammerseizoen (12). Echter, Hatchette et al. (27) beschreven dat geiten ook chronisch geïnfecteerd kunnen worden, waarbij de uitscheiding van de kiem aangetoond werd tot twee aflammerseizoenen na de infectie.
In Nederland is op basis van immuunhistochemisch onderzoek van nageboorten van geaborteerde schapen en geiten de diagnose Q fever gesteld (66).

Diagnostiek

De diagnostiek berust op het aantonen van antistoffen tegen *C. burnetii* of uit het aantonen van het agens in weefsels, secreta of excreta.

Bij herkauwers zijn antistoffen tegen *C. burnetii* aan te tonen met een Complement Bindingsreactie (CBR), zoals deze bijvoorbeeld wordt gebruikt in het internationale handelsverkeer (in Nederland uitgevoerd door CIDC). De CBR heeft een matige gevoeligheid en na infectie duurt het vrij lang alvorens de test antistoffen aantoont (23). Er zijn diverse ELISA-testen beschikbaar en deze zijn meestal gebaseerd op het aantonen van IgG tegen een combinatie van Fase I en II antigenen. Een beperkte vergelijking van testen is beschreven door Schmeer (54) en Behymer et al. (9). Dit zijn gedateerde studies en de huidige ELISA-testen zijn daarin niet onderzocht. Dieren blijven na infectie waarschijnlijk maanden tot jaren seropositief (11, 48).

De detectie van het agens kan worden uitgevoerd met behulp van moleculair biologische technieken, zoals PCR en 'in situ' hybridisatie, immuunhistochemie of kweek in celcultuur. Recent zijn PCR-methoden beschreven en gevalideerd om het genoom van de bacterie aan te tonen (23, 29). Deze zijn routinematig in gebruik in diverse landen (Italië, Frankrijk). De meeste PCR-testen zijn gebaseerd op het vermeerderen en detecteren van een conservatief deel van het genoom, het zogenaamde transposonlike element (Trans PCR). Dit kan met een enkelvoudige PCR (10) of een gevoeliger 'nested' PCR (47). Omdat *C. burnetii* celgebonden is, heeft een PCR in serum waarschijnlijk weinig waarde (door de afwezigheid van cellen), wellicht met uitzondering van de acute fase van de infectie (48). Ook zijn real time PCR-methodes beschreven, die ook kwantitatief gebruikt kunnen worden (26, 35). In melk kan de PCR worden gecombineerd met immunomagnetische separatiemethoden (16, 42) of silica bindingsmethoden (40) om ook kleine aantallen kiemen op te sporen. De PCR kan met enige aanpassing ook worden toegepast op faeces (11), paraffinecoupes van weefsel (67), vaginaal slijm (vaginale swabs) en faeces. Voor verworpen foeten wordt aangeraden om hersenen en lever te testen met PCR (47).

Met behulp van immuunhistochemische kleuring kan de bacterie worden aangetoond in weefsel. Deze techniek wordt uitgevoerd bij placenta's van verworpen vruchten, onder andere door GD in Nederland (66). Deense onderzoekers hebben recentelijk met succes 'in situ' hybridisatie gebruikt voor het aantonen van *C. burnetii* in placentaweefsel (33). Voor het isoleren van de bacterie door middel van kweek in celcultuur is een speciaal toegerust laboratorium (biosafety level 3) nodig. De kweekmethode wordt in Nederland noch bij de veterinaire noch bij de humane diagnostiek toegepast.

Uitscheiding van de kiem

C. burnetii wordt door een dier op verschillende manieren uitgescheiden. De afgelopen decennia zijn hierover meerdere onderzoeken uitgevoerd. De resultaten hiervan zijn moeilijk te vergelijken, met name omdat in diezelfde periode de diagnostische methoden sterk zijn veranderd. De laatste jaren is bij veel onderzoeken gebruikgemaakt van de PCR-techniek.

Excreta waarmee de kiem door herkauwers wordt uitgescheiden, zijn:

- *Placenta.* De bacterie vermeerdert zich sterk in de placenta en wordt via de nageboorte en vruchtwater uitgescheiden (50). De hoeveelheid kiemen, die via de placenta wordt uitgescheiden, kan erg groot zijn. Waarden van meer dan 10^9 kiemen zijn bij de ooi vastgesteld (6).
- *Foetus.* Bij een onderzoek van geaborteerde runderen was 12 procent van de onderzochte foetussen PCR-positief op *C. burnetii* (47).
- *Vaginale excreta.* In een onderzoek van To et al. (61) werd *C. burnetii* aangetoond in 21 procent van de 61 koeien met verminderde vruchtbaarheid in de vaginale excreta. Ook bij schapen kan de kiem met PCR worden aangetoond in vaginale swabs (10). Na kunstmatig opgewekte abortus bij geiten, scheidden deze tot veertien dagen na de abortus bacteriën uit via vaginale excreta (4).
- *Melk.* In een studie in de VS werden koeien van een besmet koppel gevolgd waarbij gedurende enkele weken bij circa de helft van de koeien met PCR *C. burnetii* werd aangetoond in tankmelk (35). In Japan is met PCR in supermarktmelk het genoom van *C. burnetii* aangetoond, maar dit bleek bij inspuiting in muizen niet meer infectieus (30). In andere studies is de kiem gekweekt uit rauwe melk (21, 40, 53). De uitscheiding kan intermitterend optreden en de uitscheidingsduur varieert (11). Na kunstmatig opgewekte abortus bij geiten scheidden deze tot 52 dagen na de abortus bacteriën uit via de melk (4).
- *Faeces.* In het onderzoek van Guatteo et al. (25) werd bij veertig procent van de zestig besmette runderen met PCR de kiem aangetoond in de faeces. Na experimentele besmetting van geiten scheidden alle dieren de kiem uit en deze uitscheiding duurde gemiddeld veertig dagen (4).
- *Sperma.* In een Pools onderzoek is *C. burnetii* aangetoond in het sperma van seropositieve stieren (38). Dit lijkt echter geen belangrijke transmissieroute (45).

Geïnfecteerde runderen kunnen de kiem in één of meerdere excreta uitscheiden. In Frankrijk is bij zestig runderen die PCR-positief waren in faeces en/of melk en/of vaginale uitvloeiïng, nagegaan in welke mate ze positief waren in de drie genoemde excreta (25). Van de zestig runderen bleek slechts 7 procent positief te zijn in alle drie excreta, 15 procent positief in twee van de drie excreta en 78 procent was PCR-positief in één van de drie excreta.

Prevalentie

De eerste meldingen van Q fever zijn afkomstig van Australische en Amerikaanse onderzoekers. Vervolgens is de kiem wereldwijd in andere landen aangetoond bij diverse diersoorten.

Nederland

In totaal zijn 1160 sera, afkomstig van 234 melkveebedrijven met ademhalingsklachten bij het rundvee, in 1987 onderzocht op Q fever door middel van een indirecte Elisa (op basis van fase II antigeen) (32). De seroprevalentie op dierniveau was 21 procent. Op 37 procent van de bedrijven werd tenminste één seropositief rund gevonden. In hetzelfde onderzoek was 3,5 procent van 3603 schapen seropositief, terwijl slechts één geitenserum positief werd gevonden (n= 498).

Het percentage seropositieve runderen dat in Nederland voor exportonderzoek werd onderzocht door middel van de CBR, steeg in de periode 1994 tot 1997 van 0 procent tot 8 procent (1). Het aantal onderzochte monsters steeg in diezelfde periode van 290 tot 3018.

In het winterseizoen 2005 tot 2006 is de tankmelk van 344 aselect gekozen bedrijven onderzocht op Q fever met een indirecte antistoffen-ELISA (met een combinatie van fase I en II antigenen). Daarbij was 57 procent van de tankmelkmonsters positief. Op basis van de testeigenschappen is berekend dat op 35 procent van de bedrijven minstens 30 procent van

de aanwezige runderen besmet is. Verdeeld over de verschillende categorieën tankmelkuitslagen (negatief tot hoog positief) zijn 96 bedrijven geselecteerd, waarvan per bedrijf 25 runderen van tenminste drie jaar oud individueel serologisch zijn onderzocht. De prevalentie bij deze koeien was 32,7 procent. Op 20 procent van de individueel onderzochte bedrijven was het percentage seropositieve runderen kleiner dan 10 procent, op 20 procent van deze bedrijven was meer dan 50 procent van de runderen seropositief (51).

Andere landen

In een Italiaans onderzoek zijn in totaal 1188 runderen serologisch onderzocht met behulp van een indirecte immunofluorescentietest. De seroprevalentie was 14,4 procent (15). De seroprevalentie in de regio Campania (Italië) is berekend op 14 procent. Bij schapen was de seroprevalentie 12 procent, bij geiten 6 procent (16). In Oost-Turkije waren de seroprevalenties bij runderen en schapen 5,8 procent en 10,5 procent. Het percentage seropositieve rundveebedrijven was 35 procent, bij de schapenbedrijven was dit 45 procent (19).
Gedurende de jaren 2001 tot 2003 zijn van 316 bedrijven in de Verenigde Staten tankmelkmonsters onderzocht door middel van de PCR-techniek (35). De monsters zijn niet aselect genomen. De prevalentie over de drie jaren was gemiddeld 94,3 procent. Tankmelkmonsters afkomstig van 373 'at random' geselecteerde bedrijven uit Wales en Engeland zijn onderzocht met behulp van een ELISA (46). Daarbij was 21 procent van de monsters positief op antistoffen.
In Frankrijk zijn verschillende serologische studies uitgevoerd bij runderen, schapen en geiten. Mede omdat de selectie van dieren en de wijze van diagnostiek verschillend was, is er een wijde 'range' van de prevalenties. De uiterste waarden van de prevalenties op dierniveau zijn bij rundvee, schapen en geiten respectievelijk 1 tot 15 procent, 0 tot 20 procent en 2 tot 12 procent; op koppelniveau zijn deze waarden 39 tot 73 procent, 0 tot 89 procent en 10 tot 40 procent (22).
In Duitsland is een toenemend aantal besmette bedrijven met herkauwers per jaar gemeld, van gemiddeld 71 bedrijven per jaar in de zeventiger jaren, tot 303 bedrijven per jaar in de negentiger jaren (28). Hierbij moet wel vermeld worden dat Q fever bij dieren in Duitsland meldingsplichtig is.

Therapie en preventie

Therapeutisch en preventief zijn er enkele (mogelijke) opties: (a) vaccinatie, (b) antibiotica, (c) opsporen en afvoeren van besmette dieren en (d) algemene maatregelen.

Vaccinatie

Vaccins tegen Q fever kunnen in twee groepen worden verdeeld, namelijk in vaccins die gebaseerd zijn op de Fase I of Fase II van de bacterie. Beide groepen vaccins kunnen gemaakt zijn van hele bacteriën of bacteriefracties. De resultaten van het toepassen van de vaccins waren niet altijd hetzelfde. Daarbij werden er soms ernstige reacties op de injectieplaats waargenomen (2).
Mogelijk kan door vaccineren worden voorkomen dat herkauwers na infectie klinische verschijnselen gaan vertonen en wordt gezorgd dat ze geen of een sterk verlaagd aantal kiemen gaan uitscheiden. Na vaccinatie met een Fase I-vaccin is nagegaan of deze invloed had op de mate van uitscheiding van *C. burnetii* in de melk van runderen (13). Bij de niet gevaccineerde dieren bleek na besmetting 24 procent uitscheider te zijn, bij de gevaccineerde runderen was dit 1 procent. Een vermindering van de uitscheiding is ook aangetoond in een ander onderzoek, waarbij tevens een bescherming tegen het optreden van abortus werd aangegeven. Na vaccinatie bleven de titers gedurende ten minste twintig maanden vier keer hoger dan bij niet gevaccineerde runderen (7). Bij runderen zijn Fase I- en Fase II-vaccins nog niet met elkaar vergeleken. Verwacht mag worden dat, net als bij geiten, Fase I-vaccins het meest effectief zijn (22). Bij een vaccinatieproef bij geiten verminderde een Fase I-vaccin zowel het aantal abortusgevallen als de excretie van kiemen in de melk sterk. Een Fase II-vaccin beïnvloedde het percentage abortus en de excretie in de melk niet (5).

Antibiotica

C. burnetii is in vitro gevoelig voor meerdere antibiotica, onder andere tetracyclines en macroliden. Het is in de praktijk erg moeilijk om de effectiviteit in vivo te meten, met name omdat het erg moeilijk is om de kiemen te kweken en te tellen. Mogelijk kan hier een kwantitatieve PCR-methode worden ingezet, maar daarmee worden ook dode kiemen gedetecteerd.
Bij de behandeling met antibiotica wordt het injecteren van langwerkende oxytetracyclines als de beste aanpak beschouwd, hoewel moet worden betwijfeld of daarmee de uitscheiding via placenta (64), vaginale uitvloeiing (11) en melk (22) voldoende wordt tegengegaan. Bij kleine herkauwers lijkt behandeling met antibiotica op besmette bedrijven nog niet erg effectief (66). Wanneer bij herkauwers antibiotica alleen in het acute stadium effectief zijn (zoals bij mensen), dan is adequate vroegtijdige diagnostiek nodig.
Er zijn nauwelijks studies beschreven over het effect van antibioticumbehandelingen. Een orale behandeling met een dosis van 8 mg/kg/dag chloortetracycline gedurende dertig dagen is getest bij twee drachtige, *C. burnetii* uitscheidende, koeien (8). Bij één rund stopte de uitscheiding in de melk na de tweede week van behandelen, bij de andere koe werd de uitscheiding intermitterend.
In Frankrijk wordt momenteel geadviseerd, indien men besluit om op bedrijven met veel abortussen antibiotica toe te passen, tijdens de laatste maand van de dracht twee injecties met 20 mg/kg langwerkend oxytetracycline toe te dienen met een interval van twee weken. Voor het terugdringen van uitscheiding in de melk zou een vergelijkbaar schema kunnen worden toegepast op het moment dat de dieren worden drooggezet. Het effect van dit behandelingsschema is nog onbekend en mogelijk zal dit schema in Frankrijk worden aangepast als de resultaten van nieuwe onderzoeken bekend worden (22).

Opsporen en afvoeren besmette dieren

Het opsporen en afvoeren van besmette, uitscheidende dieren is een van de maatregelen om overdracht van kiemen zowel binnen als tussen bedrijven te reduceren of te voorkomen. Directe of aërogene overdracht tijdens de geboorte of abortus speelt een belangrijke rol bij de besmetting van koppelgenoten of dieren uit andere koppels. Deze besmetting kan ook op een later tijdstip plaatsvinden omdat de kiem zeer lang persisteert in de omgeving. Het opsporen van uitscheidende dieren is moeilijk, onder andere omdat uitscheiding intermitterend is en dieren langdurig seropositief kunnen blijven terwijl ze de kiem niet meer uitscheiden.
Het opsporen en afvoeren van uitscheidende runderen heeft

een gunstig effect op de infectiegraad van de omgeving en lijkt effectief op bedrijfsniveau (36) en is dus aan te raden.

Algemene maatregelen

Besmetting met *C. burnetii* kan aërogeen (aërosolen van ingedroogde faeces en vruchtwater) of oraal optreden. Bronnen van besmetting zijn nageboorte, vruchtwater, vaginale uitvloeiing, wol of huiden, rauwe melk of kaas, gemaakt van niet gepasteuriseerde melk. Landbouwhuisdieren worden beschouwd als de belangrijkste bron van humane infecties (44). De kiemen kunnen met de wind over grotere afstanden worden overgebracht en daardoor humane infecties veroorzaken (60). Mogelijk kan ditzelfde optreden als oorzaak van infectie van dieren. Deze mogelijke wijze van overdracht betekent dat moet worden voorkomen dat dieren grote hoeveelheden kiemen uitscheiden, die vervolgens via direct contact of aërogeen overgebracht kunnen worden naar andere dieren of mensen.

Het is onduidelijk of de overdracht via andere transmissieroutes, zoals sperma (38), vlooien (39) of wilde, bruine ratten (63) een rol van betekenis speelt. Nader onderzoek is hiervoor nodig.

Naast de al genoemde maatregelen zijn op besmette bedrijven een aantal algemene maatregelen wenselijk (22, 36, 65) zoals:

- algemene hygiëne;
- het vernietigen van strooisel dat mogelijk besmet is met baarmoederinhoud (amnionvloeistof, nageboorte, etcetera) en dat is vrij gekomen tijdens en na de geboorte;
- het vernietigen van placenta's en verworpen vruchten. Dit kan gebeuren door verbranding of door het zo snel mogelijk laten ophalen door de kadaverophaaldienst;
- reinigen en desinfecteren van vloeren, voertuigen en gebruiksvoorwerpen. Daarbij moet wel in ogenschouw worden genomen dat de kiem bestand is tegen veel desinfectantia. Werkzame desinfecteermiddelen moeten gedurende 24 tot 48 uur worden toegepast (55). Werkzame desinfectantia zijn: ethanol, gasvormig formaldehyde, 5% peroxide, 0,05% hypochloriet (45). Mest kan worden behandeld met calciumcyanamide 0,6% gedurende één week (3);
- het afkalven/aflammeren in een aparte ruimte;
- geen dieren aankopen en zorgen voor een goede scheiding met dieren van naburige bedrijven;

Ook ter preventie van humane infecties is een optimale hygiëne bij het geboorteproces belangrijk. Zwangere vrouwen moeten contact met risicodieren/-materialen vermijden.

Bestrijdingsprogramma's

In Frankrijk is de benadering van deze ziekte de afgelopen jaren veranderd. In 1997 moest bij een klinische uitbraak de melk bij 85°C gedurende dertig seconden gepasteuriseerd worden en moest daarnaast het hele koppel geslacht worden. In 2004 is de regeling veranderd. De runderen hoeven niet meer geslacht te worden. Het pasteuriseren van de melk bleef gehandhaafd (72°C, vijftien seconden). Verwacht wordt dat in de komende jaren door ACERSA een nieuwe regeling wordt ingevoerd voor het diagnosticeren van Q fever en de aanpak van geïnfecteerde bedrijven.

In Duitsland is een werkgroep bezig een plan van aanpak op te stellen voor besmette bedrijven. In de deelstaat Hessen is er een plan van aanpak voor bedrijven waar rauwe melk of rauwmelkse kaas geproduceerd wordt (36). Deze bedrijven moeten jaarlijks een tankmelk PCR laten uitvoeren. Wanneer met PCR *C. burnetii* wordt aangetoond, worden nadere maatregelen genomen, bijvoorbeeld serologisch onderzoek, vernietiging van de rauwmelkse kaas en geen verkoop van rauwe melk. De effectiviteit van de afzonderlijke maatregelen is niet bekend.

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Ministerie van Landbouw, Natuur en Voedselkwaliteit

Directie Voedselkwaliteit en Diergezondheid

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uw brief van	uw kenmerk	ons kenmerk	datum
24 december 2007	1180653-60210028	VD 08.1057	12 juni 2008
onderwerp		doorkiesnummer	bijlagen
Definitieve bijdrage onderzoek q-fever (verpl. 2000974)		070-3784078	

Geachte mevrouw

Voor het onderzoek naar Q-fever in 2007 is met brief VD 07.2142/SW van 22 oktober 2007 een bijdrage in de kosten toegekend van maximaal € 27.306,- (inclusief BTW). Met uw bovenvermelde factuur informeert u mij over de realisatie. Gezien de door u verstrekte informatie, de kostenspecificatie, bepaal ik de definitieve bijdrage in de kosten op € 27.305,74.

Aan dit besluit zijn de volgende voorwaarden verbonden:

⇒ Aan de Auditdienst van het ministerie van Landbouw, Natuur en Voedselkwaliteit, alsmede aan andere door het departement aangewezen instanties dient op verzoek gelegenheid te worden geboden door boekenonderzoek of anderszins ter plaatse inlichtingen in te winnen die hun wenselijk voorkomen.

⇒ Indien blijkt dat dit besluit berust op onjuiste of onvolledige informatie, kan deze beschikking worden gewijzigd en wordt de GD verplicht reeds uitgekeerde bedragen geheel of gedeeltelijk te restitueren.

Er zijn geen voorschotten betaald, zodat u op korte termijn de betaling tegemoet kunt zien van € 27.305,74 door overmaking op uw bankrekeningnummer 10.88.07.045.

DE DIRECTEUR VOEDSELKWALITEIT EN DIERGEZONDHEID,

Ministerie van Landbouw,
Natuur en Voedselkwaliteit
Directie Voedselkwaliteit
en Diergezondheid
Cluster Financiën & Control
Bezuidenhoutseweg 73
Postadres: Postbus 20401
2500 EK Den Haag
Telefoon: 070-3785105
Fax: 070-3786141
Web: www.minlnv.nl

75

Aan: VWA), AID (nt), KNMvD (GD (
Betreft: Agenda overleg uitvoering Q-koortsmaatregelen
Van:
C.c.:

AGENDA

Zaal 6004

vrijdag 13 juni 2008

1. **welkom**
2. **stand van Zaken**
3. **procedure verdachtmelding**
4. **bezoek bedrijf en ~~bevestiging~~** monsternam e
5. **diagnose en bevestiging**
6. **toezicht op maatregelen**
7. **rondvraag**

Stukken:

- **Brief aan de Tweede Kamer d.d. 10 juni 2008**
- **Regelgeving meldplicht**
- **Regelgeving maatregelen**

Ministerie van Landbouw, Natuur en Voedselkwaliteit

Directie Voedselkwaliteit en Diergezondheid

75A

De Voorzitter van de Tweede Kamer
der Staten-Generaal
Postbus 20018
2500 EA 's-GRAVENHAGE

landbouw, natuur en voedselkwaliteit

uw brief van	uw kenmerk	ons kenmerk	datum
		VD. 2008/1191	10 juni 2008
onderwerp		doorkiesnummer	bijlagen
Maatregelen Q-koorts		3785447	

Geachte Voorzitter,

Inleiding
De afgelopen weken is opnieuw een aanzienlijke toename waargenomen van het aantal besmettingen bij de mens met Q-koorts in de noord-oostelijke regio van de provincie Noord-Brabant. Dit heeft geleid tot onrust bij de lokale bevolking.

Met deze brief brengen wij u op de hoogte van aanvullende maatregelen die wij uit voorzorg gaan treffen om de verspreiding van Q-koorts zoveel mogelijk te beperken.

Q-koorts
Q-koorts is een ziekte die wordt veroorzaakt door de bacterie Coxiella burnetii (hierna: bacterie). Q-koorts is een zoönose. Dit betekent dat verspreiding van dier naar de mens kan plaatsvinden. Q-koorts komt van oudsher over de hele wereld en bij veel diersoorten voor - niet alleen bij alle landbouwhuisdieren, maar ook bij soorten als vogels, honden, katten, ratten en in het wild levende dieren. Teken kunnen een vector zijn in de overdracht tussen dieren.

Met name kleine herkauwers worden beschouwd als een belangrijke besmettingsbron voor de mens. Na uitscheiding kan de bacterie lang overleven in de buitenlucht en soms over grote afstanden verspreid worden. Mensen kunnen besmet worden via een aantal wegen waaronder het inademen van besmette, fijne deeltjes. Bij de mens verloopt de ziekte vaak zonder of met alleen milde klachten. Er kan zich echter ook een ernstiger beloop voordoen.

Het belangrijkste klinische verschijnsel bij herkauwers is abortus (verhoogde verwerping) bij drachtige dieren, veroorzaakt door de bacterie. Tijdens en na de abortus scheidt een dier grote hoeveelheden bacteriën uit, die in de mest terechtkomen. Kleine herkauwers bestemd voor melkproductie worden voor het overgrote deel in zogenoemde potstallen gehouden.

Ministerie van Landbouw,
Natuur en Voedselkwaliteit
Directie Voedselkwaliteit
en Diergezondheid
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Een potstal is een stal waarbij de mest op gezette tijden wordt bedekt met een nieuwe laag stro. Als het mengsel van mest en stro een bepaalde hoogte heeft bereikt, wordt de stal geleegd. Vooral tijdens het uitmesten van de stal kunnen veel bacteriën in de lucht komen. Dit levert een risico op voor zowel de volks- als diergezondheid. Mogelijk is het uitrijden en onderwerken van de mest op het land ook een risicofactor, maar dat lijkt een minder grote rol te spelen dan het uitmesten van de stal. Veel van deze potstalmest wordt namelijk buiten de provincie Noord-Brabant afgezet en leidt daar voor zover bekend niet tot problemen bij de mens.

Reeds genomen initiatieven

Naar aanleiding van de uitbraak van Q-koorts in 2007 in Herpen, Noord-Brabant is al een aantal afspraken gemaakt tussen het ministerie van Volksgezondheid, Welzijn en Sport (VWS) en het ministerie van Landbouw, Natuur en Voedselkwaliteit (LNV) om beter zicht te krijgen op de problematiek rondom Q-koorts en om verspreiding van Q-koorts naar de mens zo veel mogelijk te voorkomen.

Zo zijn er aanvullende hygiëneadviezen voor bedrijven met kleine herkauwers opgesteld en heeft voorlichting hierover plaatsgevonden. Ook zijn de hygiënemaatregelen gepubliceerd op sites van het ministerie VWS en LNV en de Gezondheidsdienst voor Dieren (GD).

Voorts is onderzoek in gang gezet bij de GD bij zowel grote als kleine herkauwers om een beter inzicht te krijgen in de omvang van het probleem. Dit onderzoek wordt door zowel de sector als door de overheid gefinancierd.

Er vindt onderzoek plaats naar de risicofactoren voor de verspreiding van Q-koorts. De betrokken onderzoeksinstituten (het Rijksinstituut voor Volksgezondheid en Milieu (RIVM), het Centraal Veterinair Instituut (CVI) en de GD) zijn daarnaast bezig met het ontwikkelen en valideren van geschikte testmethodes om de bacterie te kunnen aantonen.

Als laatste initiatief geldt een onderzoek naar interventiestrategieën. Hierbij gaat de aandacht vooral uit naar een nieuw vaccin bij dieren dat op dit moment getest wordt in Denemarken en Frankrijk. Bekeken wordt of ook in Nederland dit vaccin experimenteel ingezet kan worden.

Aanwijzing Q-koorts als besmettelijke dierziekte en meldplicht

Om maatregelen op bedrijven te kunnen nemen, is het noodzakelijk Q-koorts als besmettelijke dierziekte aan te wijzen. De minister van LNV heeft deze aanwijzing inmiddels in gang gezet zodat spoedig maatregelen genomen kunnen worden. De aanwijzing is opgenomen in de Regeling preventie, bestrijding en monitoring van besmettelijke dierziekten, zoönosen en TSE's (Regeling preventie). Houders van kleine herkauwers, gehouden in potstallen, zijn verplicht verschijnselen van Q-koorts te melden. Deze meldplicht geldt ook voor de dierenarts.

Maatregelen ten aanzien van mest

Deskundigen zijn het erover eens dat mest waarschijnlijk een belangrijke rol speelt bij de verspreiding van Q-koorts in de provincie Noord-Brabant.

Als zinvolle, voorlopige maatregel op basis van het voorzorgsbeginsel zijn wij van plan een verbod voor de duur van drie maanden op te leggen voor het uitmesten en uitrijden van potstalmest van bedrijven waar een ernstige besmetting is vastgesteld. In deze periode vindt namelijk in de mest een aanzienlijke reductie plaats van de besmetting in de mest. Als uitmesten in deze periode onvermijdelijk is doordat de potstal vol is, zal onder nog nader uit te werken voorwaarden, de mest wel uit de stal verwijderd en eventueel afgevoerd kunnen worden.

Overige maatregelen en adviezen

Naast deze specifieke maatregel ten aanzien van mest op bedrijven met een besmetting zullen generieke adviezen gegeven worden om verspreiding van Q-koorts in de toekomst te voorkomen. Hierbij wordt gedacht aan het vervroegen van het moment van uitmesten tot voor het begin van het lammerseizoen. Hierdoor kan de mest tot zeker drie maanden na het lammerseizoen in de potstal blijven waardoor een grote reductie van een eventuele besmetting kan worden gerealiseerd.

Bedrijven met kleine herkauwers worden vaak bezocht door recreanten en andere geïnteresseerden. Contacten van burgers met besmette bedrijven zijn ook uit voorzorg onwenselijk. Tijdelijk bezoekers niet toelaten op zo'n bedrijf lijkt ons een zinvolle maatregel.

Er is ook een beperkt aantal bedrijven dat zelf kaas maakt. Dit gebeurt vaak met rauwe melk. Consumptie van rauwe producten afkomstig van besmette bedrijven wordt door het RIVM afgeraden. Het lijkt daarom in eerste instantie zinvol om pasteurisatie in bepaalde gevallen voor te schrijven. De minister van VWS gaat hierover in overleg met het RIVM.

Met bovenstaande maatregelen trachten wij de verspreiding van Q-koorts zo veel mogelijk te beperken. De genoemde maatregelen zijn erop gericht zo spoedig mogelijk actie te kunnen ondernemen om het risico van verspreiding te verkleinen. Wij werken ons beleid hierop verder uit. Indien nodig zullen wij u hier weer over informeren.

DE MINISTER VAN LANDBOUW, NATUUR EN VOEDSELKWALITEIT,

G. Verburg

DE MINISTER VAN VOLKSGEZONDHEID WELZIJN EN SPORT,

dr. A. Klink

75B

MINISTERIE VAN LANDBOUW,
NATUUR EN VOEDSELKWALITEIT

Regeling van de Minister van Landbouw, Natuur en
Voedselkwaliteit van 9 juni 2008, TRCJZ/2008/1622,
houdende aanwijzing van Q-koorts als besmettelijke dierziekte

DE MINISTER VAN LANDBOUW, NATUUR EN VOEDSELKWALITEIT,

Gelet op de artikelen 15, tweede lid, onderdeel a, 19, 100 en 107 van de Gezondheids- en welzijnswet voor dieren;

BESLUIT:

Artikel I

De Regeling preventie, bestrijding en monitoring van besmettelijke dierziekten, zoönosen en TSE's[1] wordt gewijzigd als volgt:

A

Onder vervanging van een punt door een puntkomma aan het einde van artikel 2, onderdeel ab, wordt aan artikel 2 een onderdeel toegevoegd, dat luidt:

ac. Q-koorts.

B

Na artikel 11 wordt een artikel toegevoegd, dat luidt:

Artikel 11a

De verplichting tot kennisgeving, bedoeld in de artikelen 19 en 100 van de wet, van verschijnselen van Q-koorts bij schapen en geiten die bestemd zijn voor de melkproductie, geldt in elk geval:

a. ten aanzien van bedrijven met 100 of meer volw

C

Na artikel 13 wordt een artikel toegevoegd, dat luidt:

Artikel 13a

1. Vrijstelling van de verplichting tot kennisgeving, bedoeld in artikel 19 van de wet, van de verschijnselen van Q-koorts, wordt verleend aan:
   a. de houder van een rund;
   b. de houder van een schaap, dat niet wordt gehouden voor de melkproductie;
   c. de houder van een geit, die niet wordt gehouden voor de melkproductie.
2. Het eerste lid is van overeenkomstige toepassing op de verplichting tot kennisgeving, bedoeld in artikel 100 van de wet, van verschijnselen van Q-koorts door de dierenarts.

Artikel II

Deze regeling treedt in werking met ingang van de tweede dag na publicatie in de Staatscourant.

Deze regeling zal met de toelichting in de Staatscourant worden geplaatst.

Den Haag, 9 juni 2008

DE MINISTER VAN LANDBOUW, NATUUR EN VOEDSELKWALITEIT,

G. Verburg

Toelichting

Inleiding

Met deze wijziging van de Regeling preventie, bestrijding en monitoring van besmettelijke dierziekten, zoönosen en TSE's (Regeling preventie), wordt de dierziekte Q-koorts aangewezen als besmettelijke dierziekte. Deze aanwijzing geschiedt op grond van artikel 15, tweede lid, onderdeel a, van de Gezondheids- en welzijnswet voor dieren (GWWD).

Q-koorts

Q-koorts is een ziekte die wordt veroorzaakt door de bacterie Coxiella burnetii (hierna: bacterie). Q-koorts is een zoönose. Dit betekent dat verspreiding naar de mens kan plaatsvinden door een bacterie die van dieren afkomstig is. Met name kleine herkauwers worden beschouwd als een belangrijke besmettingsbron voor de mens. Mensen kunnen besmet worden door het inademen van besmette fijne deeltjes.
Bij de mens verloopt de ziekte vaak zonder of met alleen milde klachten. Er kan zich echter ook een ernstiger beloop voordoen.

Het belangrijkste klinische verschijnsel bij herkauwers is abortus, veroorzaakt door de bacterie. Tijdens en na de abortus scheidt een dier grote hoeveelheden bacteriën uit via de placenta en andere vaginale excretie. Dit verschijnsel kan gepaard gaan met verminderde melkgift. Na uitscheiding kan de bacterie lang overleven in de buitenlucht en soms over grote afstanden verspreid worden.
Een infectie kan worden gediagnosticeerd door het aantonen van de bacterie of antistoffen tegen de bacterie. Het effect van het toedienen van antibiotica en vaccins voor therapeutische of preventieve doeleinden is nog onduidelijk. Tot op heden zijn er geen effectieve bestrijdingsprogramma's ontwikkeld.

Aanleiding

Aanleiding voor deze aanwijzing is de recente stijging van het aantal humane besmettingen van Q-koorts, die waarschijnlijk verband houdt met een uitbraak op schapen- en geitenbedrijven. Deze stijging komt met name voor in de provincie Noord-Brabant.

Gevolgen

Eén van de gevolgen van deze aanwijzing is dat de minister van Landbouw, Natuur en Voedselkwaliteit, de maatregelen bedoeld in hoofdstuk II, afdeling 3, van de GWWD kan toepassen. Deze maatregelen hebben betrekking op de preventie en bestrijding van besmettelijke dierziekten. Voorbeelden van dergelijke maatregelen zijn een verbod op het betreden van bedrijven door bezoekers en regels ten aanzien van het verwerken van mest.

Een ander gevolg van de aanwijzing van Q-koorts als besmettelijke dierziekte, is dat de meldplicht, bedoeld in artikel 100 van de GWWD, van toepassing is. Dit betekent dat een dierenarts, die weet of redelijkerwijs kan vermoeden, dat er Q-koorts is uitgebroken op een bedrijf, dit moet melden bij de Voedsel en Waren Autoriteit (VWA). Een dergelijke meldplicht geldt ook voor de houder (artikel 19 GWWD).
Aan houders van runderen en aan houders van schapen en geiten die niet zijn bestemd voor de melkproductie wordt vrijstelling verleend van de verplichting om de verschijnselen van Q-koorts bij deze dieren te melden aan de VWA (artikel 13a).
Dit betekent dat de meldplicht op basis van artikel 11a alleen geldt voor houders van schapen en geiten die bestemd zijn voor de melkproductie. Een enkele abortus bij dieren kan vele oorzaken hebben. Daarom is ervoor gekozen dat er in ieder geval gemeld moet worden als er meerdere abortussen in een beperkte periode plaatsvinden. Houders van 100 melkschapen of -geiten, of meer (grote bedrijven), moeten in ieder geval melden als er binnen een periode van 30 dagen bij meer dan 5% van de drachtige dieren abortus plaatsvindt. Houders van minder dan 100 melkschapen of -geiten (kleine bedrijven), moeten melden als er meer dan drie gevallen van abortus zijn binnen een periode van 30 dagen.

Reden voor het hierboven geschetste onderscheid tussen melkgeiten en melkschapen enerzijds en runderen en overige schapen of geiten anderzijds is vooral de manier van houden. Melkgeiten en melkschapen worden voor het overgrote deel in zogenoemde potstallen gehouden. Een potstal is een stal waarbij de mest op gezette tijden wordt bedekt met een nieuwe laag stro. Als het mengsel van mest en stro een bepaalde hoogte heeft bereikt, wordt de stal geleegd. De ondertussen goed aangestampte en gerijpte mest wordt verspreid en ondergewerkt over akkerbouwgronden. Omdat de schapen en geiten ook in de potstal lammeren, komt de bacterie bij een uitbraak ook in de mest terecht.

Administratieve lasten
De aanwijzing van Q-koorts als besmettelijke dierziekte brengt geen administratieve lasten met zich mee. De gevolgen van deze aanwijzing, namelijk dat de houder en de dierenarts verplicht zijn deze dierziekte te melden, brengt wel administratieve lasten met zich mee. Naar verwachting neemt het melden van een dierziekte circa 5 minuten in beslag. Voor de houder wordt uitgegaan van een tarief van € 30 per uur en voor de dierenarts van een tarief van € 60 per uur. In 2008 zijn er tot op heden vijf gevallen van een uitbraak op een schapen- of geitenbedrijf bekend. Verwachting is daarom dat het aantal meldingen beperkt zal blijven. Omdat de totale administratieve lasten, die voortvloeien uit de wijziging van deze regeling, naar verwachting onder de € 10.000 zullen blijven, is de regeling op voorhand niet voorgelegd aan het Adviescollege toetsing administratieve lasten (Actal).

DE MINISTER VAN LANDBOUW, NATUUR EN VOEDSELKWALITEIT,

G. Verburg

75C

LNV

Regeling houdende maatregelen ter preventie van Q-koorts

Regeling van de Minister van Landbouw, Natuur en Voedselkwaliteit van 12 juni 2008, nr. TRCJZ/2008/1645, houdende maatregelen ter preventie van Q-koorts

De Minister van Landbouw, Natuur en Voedselkwaliteit,
Gelet op de artikelen 17, 21, 24 en 31 van de Gezondheids- en welzijnswet voor dieren en de artikelen 2, onderdeel a, en 4 van het Besluit verdachte dieren;

Besluit:

Artikel I
De Regeling tijdelijke maatregelen dierziekten[1] wordt gewijzigd als volgt:

A

kend vanaf het tijdstip waarop de verdenking van besmetting met Q-koorts is ontstaan, verboden de stal waar deze dieren zijn of worden gehouden, te betreden.
2. Het eerste lid is niet van toepassing op personen voor wie het met het oog op de uitoefening van beroep of bedrijf, noodzakelijk is de stal te betreden.

Artikel 5.4
Indien de verdenking, bedoeld in artikel 2, onderdeel a, van het Besluit verdachte dieren, is beëindigd op grond van artikel 4, onderdeel b, van het Besluit verdachte dieren, zijn de artikelen 5.2 en 5.3 niet langer van toepassing.

Artikel II
Deze regeling wordt aan de media

Een ander gevolg van de aanwijzing van Q-koorts als besmettelijke dierziekte is dat de Minister van Landbouw, Natuur en Voedselkwaliteit maatregelen kan nemen ter preventie van Q-koorts. Met de onderhavige wijziging van de Regeling tijdelijke maatregelen dierziekten worden maatregelen ter preventie van Q-koorts getroffen. In het navolgende worden deze maatregelen toegelicht.

Maatregelen
Deskundigen achten het waarschijnlijk dat mest een belangrijke rol speelt bij de verspreiding van Q-koorts. Daarom is het ingevolge artikel 5.2 van de regeling verboden gedurende 90 dagen mest uit de stal te verwijderen. De termijn van 90 dagen begint te lopen vanaf het tijdstip waarop de verdenking is ontstaan

(76)

Van:

Verzonden: dinsdag 24 juni 2008 18:34

Aan:

CC:

Onderwerp: Werkafspraken uitvoering Q-koorts

Beste mensen,

Bijgaand de definitieve samenvatting van de afspraken, gemaakt op 13 juni jl. over de uitvoering van de maatregelen Q-koorts. Slechts een enkele reactie ontvangen. Er staat nog wel aantal vragen open (met name tussen VWA en CVI), waar ik nog wel in geïnteresseerd ben het vervolg te kennen. Ik neem daar nog wel contact over op. De juridische positie GD is al aan de orde geweest.

Groeten,

Ministerie van Landbouw, Natuur en Voedselkwaliteit
Directie Voedselkwaliteit en Diergezondheid (VD)
Postbus 20401. 2500 EK 's-Gravenhage
*Tel. **:* *141*
Email:

Aan:

- GD heeft 5 a 10 placenta's en vruchten nodig voor diagnostiek. Hierover wordt met boer afspraak gemaakt. Namelijk lastig verzamelen. Het is in zijn eigen belang zo snel mogelijk te verzamelen, want abortus kan ook andere oorzaak hebben waardoor de verdenking Q-koorts weer wordt ingetrokken.
- Gelijktijdig vaginale swabs bij geaborteerde (of geworpen) dieren nemen t.b.v. PCR-bepaling door CVI. CVI moet aangeven hoeveel monsters (5, 10 meer)
- Wie betaalt onderzoek GD en CVI? GD heeft één tarief voor dit sectiemateriaal onderzoek op basis waarvan differentiaal diagnose. Betekent dat er ook een aantal andere tests worden uitgevoerd. GD zoekt uit welke precies en wat de kosten hiervan zijn (Naar). Er wordt ervan uitgegaan dat dit niet substantieel is en dat de boer (zuivere benadering) hiervoor geen aparte rekening krijgt. Vormt zo klein voordeeltje voor boer. Rekening gaat naar VWA.

Diagnose en bevestiging

- GD voert (nu onder verantwoordelijkheid CVI) immunohistochemie uit. Als GD positief dan sprake van besmetting. Tevens bevestigingsonderzoek PCR door CVI. Als beide positief is besmetting definitief. Als alleen CVI positief en GD negatief dan is meer onderzoek nodig. VWA legt voor aan CVI voor advies met cc-tje naar VD.
- Al het placenta monstermateriaal gaat ook naar CVI voor validatieonderzoek.
- GD dient i.v.m. juridische bewijslast zeer veel zorgvuldigheid te betrachten met identificatie van monsters

toezicht op maatregelen en overig

- AID heeft al een concept handhavingsnotitie opgesteld in afstemming met VWA. Melding, toezicht mest en bezoekersregeling. Er worden ook uren begroot. Notitie gaat naar VD.
- Nathouden en stapelen afgedekt op eigen terrein lijkt de belangrijkste maatregel. Indien van bedrijf af is de vraag of het elders gestapeld moet worden of dat meteen uitrijen onderwerken de beste oplossing is. Bij voortijdig uitmesten van besmette mest wordt door VWA advies gevraagd aan CVI.
- Over bescherming privacy als gegevens door VWS/RIVM gebruikt worden, stuurt JZ een brief waarin gewezen wordt op eigen verantwoordelijkheid. Check door VD.
- Oude gevallen. Zeker één en waarschijnlijk twee bedrijven die momenteel aan de criteria voldoen om te melden. Deze bedrijven worden door GD gebeld. Houders zijn al op de hoogte.
- Arbo: Het advies is om zwangere vrouwen en vrouwen met een kinderwens niet in contact te laten komen met risicodieren. Geiten en schapen, omdat die relatief gemakkelijk aborteren

10 juni 2008

Stukken:
- Brief aan de Tweede Kamer d.d.
- Regelgeving meldplicht
- Regelgeving maatregelen

Ministerie van Landbouw, Natuur en Voedselkwaliteit

Directie Voedselkwaliteit en Diergezondheid

77

Gezondheidsdienst voor Dieren b.v.
[redacted]
Postbus 9
7400 AA DEVENTER

landbouw, natuur en voedselkwaliteit

uw brief van	uw kenmerk	ons kenmerk	datum
25 april 2008	200804-2566	VD.08.1235,	26 juni 2008
ondertekenaar		doorkiesnummer	bijlagen

[redacted]

Verplichtingennr. 2001053

Geachte heer

Ten aanzien van uw offerte met betrekking tot de uitvoering van de protocollaire benadering Q-feverbedrijven en controlebedrijven (projectnummer 2080017) deel ik u mee dat ik voor dit onderzoek een bijdrage toeken van 50% van de kosten tot een maximum van € 25.286,61 (dit is € 21.249,25 excl. BTW). Voor dit project geldt een maximale bijdrage van 50% vanwege een co-financiering door het Productschap Vee en Vlees.

8. Het project wordt - voor zover niet strijdig met het gestelde in deze brief - uitgevoerd conform het genoemde projectvoorstel.
9. De bijgevoegde Algemene Rijksvoorwaarden voor het verstrekken van opdrachten tot het verrichten van Diensten (ARVODI).
10. Mochten de door mij gestelde voorwaarden niet naar behoren worden nageleefd, dan behoud ik mij het recht voor de bijdrage geheel, of gedeeltelijk terug te vorderen.

U wordt verzocht om bij al uw correspondentie over dit project het bovengenoemde briefnummer en verplichtingennummer te vermelden.

De factuur dient onder vermelding van het relatie- en verplichtingennummer te worden verzonden naar:

Ministerie van Landbouw, Natuur en Voedselkwaliteit
Directie Financieel-Economische Zaken, afdeling Financieel Diensten Centrum
(klantteam 1, directie Voedselkwaliteit en Diergezondheid)
Postbus 20401
2500 EK DEN HAAG

Voor inhoudelijke zaken over het project verzoek ik u contact op te nemen met
an mijn directie.

DE DIRECTEUR VOEDSELKWALITEIT EN
DIERGEZONDHEID,

AZ

Vaststelling herziene Algemene Rijksvoorwaarden voor het verstrekken van opdrachten tot het verrichten van Diensten (ARVODI-2008)

Besluit van de Minister-President, Minister van Algemene Zaken, van 26 februari 2008, nr. 3313667, houdende vaststelling van de herziene Algemene Rijksvoorwaarden voor het verstrekken van opdrachten tot het verrichten van Diensten (ARVODI-2008)

De Minister-President, Minister van Algemene Zaken,
Handelende in overeenstemming met het gevoelen van de ministerraad;

Besluit :

Artikel 1
Vastgesteld worden de bij dit besluit gevoegde herziene Algemene Rijksvoorwaarden voor het verstrekken van opdrachten tot het verrichten van diensten, voortaan genaamd ARVODI-2008.

Artikel 2
Het besluit van de Minister-President, Minister van Algemene Zaken, van 5 maart 2004, nr. 04M464297, tot vaststelling van de Algemene Rijksvoorwaarden voor het verstrekken van opdrachten tot het verrichten van diensten, wordt ingetrokken.

Artikel 3
Dit besluit treedt in werking met ingang van de tweede dag na de dagtekening van de Staatscourant waarin het wordt geplaatst.

Dit besluit zal in de Staatscourant worden geplaatst.

De Minister-President, Minister van Algemene Zaken,
J.P. Balkenende.

Bijlage

I ALGEMEEN

1. Begrippen

In deze algemene voorwaarden worden de navolgende begrippen met een beginhoofdletter gebruikt. Onder deze begrippen wordt verstaan:

1.1 Beroepsfouten: tekortkomingen, zoals vergissingen, onachtzaamheden, nalatigheden, verzuimen, onjuiste adviezen, die een vakbekwame en zorgvuldige opdrachtnemer onder de gegeven omstandigheden met inachtneming van normale oplettendheid en bij een normale vakkennis en normale wijze van vakuitoefening, behoort te vermijden;

1.2 Bijlage: een aanhangsel bij de Overeenkomst dat na parafering door beide partijen deel uitmaakt van de Overeenkomst;

1.3 Diensten: de door Opdrachtnemer op basis van de Overeenkomst ten behoeve van Opdrachtgever te verrichten werkzaamheden;

1.4 Overeenkomst: de schriftelijke overeenkomst tussen Opdrachtgever en Opdrachtnemer, waarop de Voorwaarden van toepassing zijn verklaard;

1.5 Personeel van Opdrachtgever: het door Opdrachtgever op grond van de Overeenkomst ter beschikking te stellen personeel;

1.6 Personeel van Opdrachtnemer: de door Opdrachtnemer voor de uitvoering van de Overeenkomst in te schakelen personeelsleden of hulppersonen, die krachtens de Overeenkomst onder zijn verantwoordelijkheid zullen werken;

1.7 Voorwaarden: deze algemene voorwaarden die van toepassing zijn op en deel uitmaken van de Overeenkomst;

1.8 Werkdag: kalenderdagen, behoudens weekenden en algemeen erkende feestdagen in de zin van artikel 3, eerste lid, van de Algemene Termijnenwet, waarop de overeengekomen Diensten worden verricht.

2. Toepassing

2.1 Afwijkingen van de Voorwaarden zijn slechts bindend, voor zover zij uitdrukkelijk tussen partijen schriftelijk zijn overeengekomen.

2.2 In geval van strijdigheid tussen de Nederlandse tekst van deze Voorwaarden en vertalingen daarvan, prevaleert steeds de Nederlandse tekst.

II UITVOERING VAN DE OVEREENKOMST

3. Garanties van de Opdrachtnemer

3.1 Opdrachtnemer garandeert dat de door of namens hem te verrichten Diensten voldoen aan de in de Overeenkomst vastgelegde eisen.

3.2 Opdrachtnemer garandeert dat de door of namens hem te verrichten Diensten op vakbekwame wijze worden uitgevoerd.

4. Acceptatie en toetsing

4.1 Indien Opdrachtgever de resultaten van de dienstverlening als onvoldoende beoordeelt, worden de resultaten van de Diensten niet geaccepteerd. In dat geval is hoofdstuk VI van toepassing.

4.2 Opdrachtgever kan de resultaten van de geleverde Diensten laten toetsen. Hij wijst daartoe een of meer functionarissen aan die bevoegd zijn voor hem de toetsing uit te voeren.

5. Vervanging personen die belast zijn met de uitvoering van de Diensten

5.1 Vervanging van personen die zijn belast met de uitvoering van de Diensten, kan door Opdrachtnemer slechts bij uitzondering plaatsvinden.

5.2 Opdrachtnemer kan personen die zijn belast met de uitvoering van de Diensten niet zonder voorafgaande toestemming van Opdrachtgever tijdelijk of definitief vervangen. Opdrachtgever weigert zijn toestemming niet op onredelijke gronden en kan aan deze toestemming voorwaarden verbinden. De voor de oorspronkelijke personen geldende tarieven kunnen bij vervanging niet worden verhoogd.

5.3 Indien Opdrachtgever vervanging verlangt van personen die zijn belast met de uitvoering van de Diensten, omdat hij meent dat dit in het belang van een goede uitvoering van de Overeenkomst nodig of wenselijk is, geeft Opdrachtnemer hieraan gevolg. Daarbij wordt een tarief in rekening gebracht dat niet hoger is dan het tarief dat voor de persoon die wordt vervangen in de Overeenkomst is vastgelegd.

5.4 Bij een vervanging van personen die belast zijn met de uitvoering van de Overeenkomst, stelt Opdrachtnemer personen beschikbaar die qua deskundigheid, opleiding en ervaring ten minste gelijkwaardig zijn aan de te vervangen personen.

6. Gebruik van zaken van Opdrachtgever en diensten van derden

6.1 Bij het verrichten van de Diensten kan Opdrachtnemer gebruik maken van zaken die eigendom zijn van Opdrachtgever, en die voor dat doel aan Opdrachtnemer in bruikleen worden gegeven. Aan deze bruikleen kunnen voorwaarden worden verbonden.

6.2. Bij het uitvoeren van de Overeenkomst maakt Opdrachtnemer slechts na toestemming van Opdrachtgever gebruik van de diensten van derden. Opdrachtgever onthoudt deze toestemming niet op onredelijke gronden. Aan de toestemming kan hij voorwaarden verbinden. De door Opdrachtgever verleende toestemming laat onverlet de verantwoordelijkheid en aansprakelijkheid van Opdrachtnemer voor de nakoming van de krachtens de Overeenkomst op hem rustende verplichtingen en de krachtens de

belasting- en socialeverzekeringswetgeving op hem als werkgever rustende verplichtingen.

III VERHOUDING TUSSEN PARTIJEN EN BEGELEIDING

7. Voortgangsrapportage

Opdrachtnemer rapporteert over de voortgang van de werkzaamheden aan Opdrachtgever zo vaak en op de wijze als in de Overeenkomst is bepaald dan wel Opdrachtgever nodig acht.

8. Contactpersonen

8.1 Beide partijen wijzen een contactpersoon aan, die de contacten over de uitvoering van de Overeenkomst onderhoudt. Partijen informeren elkaar schriftelijk over degene die zij als contactpersoon hebben aangewezen.

8.2 Contactpersonen kunnen hun partij vertegenwoordigen en binden tenzij bij de Overeenkomst anders is bepaald.

9. Begeleidingscommissie/stuurgroep

De Overeenkomst kan voorzien in de instelling van een begeleidingscommissie of stuurgroep. De taken en bevoegdheden, alsmede de samenstelling van de begeleidingscommissie of stuurgroep kunnen in de Overeenkomst nader worden bepaald.

10. Wijze van kennis geven

10.1 Kennisgevingen van partijen op grond van de Overeenkomst worden schriftelijk gedaan.

10.2 Mondelinge mededelingen, toezeggingen of afspraken hebben geen rechtskracht, tenzij deze schriftelijk zijn bevestigd.

11. Geheimhouding

11.1 Opdrachtnemer maakt hetgeen hem bij de uitvoering van de Overeenkomst ter kennis komt en waarvan hij het vertrouwelijke karakter kent of redelijkerwijs kan vermoeden op geen enkele wijze verder bekend, behalve voorzover enig wettelijk voorschrift of een uitspraak van de rechter hem tot bekendmaking daarvan verplicht.

11.2 Opdrachtnemer verplicht zijn Personeel deze geheimhoudingsverplichting na te leven.

11.3 Opdrachtnemer is er verantwoordelijk voor dat Personeel van Opdrachtnemer dat betrokken is bij de uitvoering van werkzaamheden in het kader van de uitvoering van de Overeenkomst, voor zover deze bij Opdrachtgever worden verricht, de door Opdrachtgever aangegeven privacyregels in acht neemt.

11.4 Beide partijen geven geen persberichten uit en doen geen andere openbare mededelingen met betrekking tot de onderhavige opdracht dan na voorafgaande toestemming van de andere partij. Toestemming is niet nodig, indien de verstrekking van informatie berust op een wettelijke verplichting.

11.5 Indien Opdrachtgever besluit over de resultaten van de geleverde Diensten en de daarmee gemoeide kosten de Tweede Kamer te informeren is de toestemming, bedoeld in het vierde lid, evenmin nodig.

11.6 Opdrachtnemer stelt alle gegevens (schriftelijke stukken, computerbestanden, etc.) die hij in het kader van de uitvoering van de Overeenkomst onder zich heeft, binnen 10 Werkdagen na beëindiging van de desbetreffende werkzaamheden aan Opdrachtgever ter beschikking.

11.7 Opdrachtgever kan bij de Overeenkomst een boete stellen op het schenden van de geheimhoudingsverplichtingen. Betaling van de boete, die onmiddellijk opeisbaar is, laat de gehoudenheid van Opdrachtnemer de schade die het gevolg is van de schending te vergoeden, onverlet.

12. Beveiliging

12.1 Opdrachtnemer draagt zijn Personeel dat betrokken is bij de uitvoering van de werkzaamheden voor zover die bij Opdrachtgever worden verricht, op de door Opdrachtgever aangegeven beveiligingsprocedures en huisregels in acht te nemen. Opdrachtgever informeert Opdrachtnemer tijdig over deze procedures en regels.

12.2 Opdrachtgever kan vorderen dat van Personeel van Opdrachtnemer minimaal drie Werkdagen voor aanvang van de werkzaamheden bij Opdrachtgever verklaringen omtrent het gedrag worden overgelegd.

12.3 Opdrachtgever kan Personeel van Opdrachtnemer onderwerpen aan een veiligheidsonderzoek, overeenkomstig de bij Opdrachtgever gebruikelijke regels. Opdrachtnemer verleent aan dit onderzoek zijn volledige medewerking. Opdrachtgever kan op grond van de uitkomsten van een dergelijk veiligheidsonderzoek de inzet van het betrokken personeelslid bij de uitvoering van de Overeenkomst zonder opgave van redenen weigeren.

IV VERGOEDING, MEERWERK EN MINDERWERK

13.1 Opdrachtgever zal aan Opdrachtnemer de werkelijk door hem gemaakte kosten en uren vergoeden, tenzij in de Overeenkomst een vaste prijs is overeengekomen.

13.2 Indien door aanvullende wensen of gewijzigde inzichten van Opdrachtgever of door wijziging van de voor de te verrichten prestaties van belang zijnde wettelijke voorschriften, de prestaties die Opdrachtnemer op grond van de Overeenkomst moet verrichten, aantoonbaar worden verzwaard dan wel uitgebreid, is sprake van meerwerk, dat voor vergoeding in aanmerking komt. Tot meerwerk worden niet gerekend aanvullende werkzaamheden of gewijzigde inzichten die Opdrachtnemer bij het sluiten van de Overeenkomst had behoren te voorzien. Indien een partij meent dat van

meerwerk sprake is, zal zij daarvan zo spoedig mogelijk mededeling doen aan de andere partij.

13.3 Opdrachtnemer vangt niet aan met meerwerk alvorens hij daartoe schriftelijke opdracht van Opdrachtgever heeft gekregen.
Opdrachtnemer brengt ter verkrijging van een opdracht een schriftelijke offerte uit met betrekking tot de omvang van het verwachte meerwerk en de daaraan verbonden tijdsduur en kosten. Ter zake van het door Opdrachtnemer te verrichten meerwerk gelden de bepalingen van de Overeenkomst, waaronder de tarieven en eventuele kortingen, voorzover deze door de nadere schriftelijke opdracht niet worden gewijzigd. Opdrachtnemer kan bij het uitbrengen van een offerte geen nadere dan wel zwaardere voorwaarden stellen, dan die waarmee Opdrachtgever instemt.

13.4 Opdrachtnemer zal een opdracht tot meerwerk tot een maximum van 15% van de oorspronkelijke opdracht aanvaarden en uitvoeren. Een dergelijke opdracht tot meerwerk wordt uitgevoerd onder de bepalingen van de Overeenkomst.

13.5 Indien door gewijzigde inzichten van Opdrachtgever of door wijziging van de voor de te verrichten prestaties van belang zijnde wettelijke voorschriften de prestaties die Opdrachtnemer op grond van de Overeenkomst moet verrichten, aantoonbaar worden verlicht dan wel verminderd, is sprake van minderwerk, dat voor verrekening in aanmerking komt. Indien een partij meent dat van minderwerk sprake is, doet zij daarvan zo spoedig mogelijk schriftelijk mededeling aan de andere partij. Indien een vaste prijs is overeengekomen, bepalen partijen in onderling overleg het bedrag van het minderwerk, dat met de te betalen prijs zal worden verrekend.

V FINANCIËLE BEPALINGEN

14. Facturering

14.1 Het recht op betaling ontstaat na acceptatie door Opdrachtgever van de resultaten van de verrichte Diensten. Indien geen acceptatie is verzonden binnen 30 dagen na de uitvoering van de Diensten, worden deze geacht geaccepteerd te zijn. Opdrachtnemer factureert binnen 30 dagen na acceptatie.

14.2 Opdrachtnemer zendt de factuur toe aan Opdrachtgever onder vermelding van datum en nummer van de Overeenkomst, van het BTW-bedrag en, indien van toepassing op gerond van artikel 14.1, onder overlegging van een afschrift van de kennisgeving van acceptatie, alsmede andere door Opdrachtgever verlangde gegevens.

14.3 Indien is overeengekomen dat betaling volgens nacalculatie plaatsvindt, zal Opdrachtnemer de factuur specificeren en in door Opdrachtgever eventueel nader aangegeven vorm factureren. In de factuur doet Opdrachtnemer opgave van het aantal en de data van de werkelijk en noodzakelijk bestede dagen of uren, waarbij Opdrachtnemer een korte omschrijving van de verrichte werkzaamheden geeft, alsmede een omschrijving van de eventuele reis- en verblijfkosten, indien deze niet zijn inbegrepen in de dag- of uurtarieven.

14.4 Meerwerk wordt door Opdrachtnemer na voltooiing van de meerwerkzaamheden, en acceptatie daarvan door Opdrachtgever, apart gefactureerd. De aard en de omvang van de verrichte meerwerkzaamheden worden in de facturen uitdrukkelijk vermeld en, aan de hand van authentieke documenten, gespecificeerd.

15. Betaling en controle

15.1 Opdrachtgever betaalt de door hem op basis van de Overeenkomst verschuldigde bedragen uiterlijk binnen 45 dagen na ontvangst en goedkeuring van de desbetreffende factuur aan Opdrachtnemer.

15.2 Indien Opdrachtgever een factuur zonder geldige reden niet binnen het verstrijken van de in het vorige lid genoemde termijn heeft voldaan, is hij van rechtswege de wettelijke rente over het openstaande bedrag verschuldigd. Op de rentevergoeding kan Opdrachtnemer geen aanspraak maken, indien de desbetreffende factuur niet aan het gestelde in artikel 14, tweede tot en met vierde lid, voldoet.

15.3 Opdrachtgever kan de door Opdrachtnemer verzonden factuur door een door Opdrachtgever aan te wijzen accountant als bedoeld in artikel 393, eerste lid, van Boek 2 van het Burgerlijk Wetboek op inhoudelijke juistheid laten controleren. Opdrachtnemer verleent de betrokken accountant inzage in boeken en bescheiden en verstrekt hem alle gegevens en informatie die deze verlangt. De controle is vertrouwelijk en strekt zich niet verder uit dan voor het verifiëren van de facturen is vereist. De accountant brengt zijn rapportage zo spoedig mogelijk aan partijen uit. De kosten van het accountantsonderzoek komen voor rekening van Opdrachtgever, tenzij uit het onderzoek van de accountant blijkt dat de factuur niet juist dan wel onvolledig is, in welk geval bedoelde kosten voor rekening van Opdrachtnemer komen.

15.4 Opdrachtgever kan de betaling van een factuur of een deel daarvan, waarover tussen partijen geen overeenstemming bestaat, opschorten gedurende de periode van het accountantsonderzoek. Van deze bevoegdheid maakt Opdrachtgever uitsluitend gebruik, indien bij hem redelijke twijfel bestaat omtrent de juistheid van de desbetreffende factuur.

15.5 Overschrijding van een betalingstermijn door Opdrachtgever of niet-betaling van een factuur op grond van vermoedelijke inhoudelijke onjuistheid daarvan of ingeval van ondeugdelijkheid van de gefactureerde Diensten geeft Opdrachtnemer niet het recht zijn werkzaamheden op te schorten dan wel te beëindigen.

16. Voorschot

16.1 Indien Opdrachtgever ter uitvoering van de Overeenkomst (een) betaling(en) verricht voor Diensten die nog niet zijn geleverd, kan hij verlangen dat door Opdrachtnemer voorafgaande aan die betaling(en) een kredietinstellingsgarantie "op afroep" aan Opdrachtgever wordt afgegeven ter waarde van het (de) betaalde bedrag(en). Aan de garantie zijn voor Opdrachtgever geen kosten verbonden. Worden vanwege enige tekortkoming aan de zijde van Opdrachtnemer Diensten niet binnen de overeengekomen termijn geaccepteerd, dan is Opdrachtnemer de wettelijke rente over het voorschot verschuldigd voor de tijd dat de tekortkoming voortduurt.

16.2 De kredietinstellingsgarantie "op afroep" wordt afgegeven door een door Opdrachtgever aanvaarde kredietinstelling, overeenkomstig het bij deze Voorwaarden gevoegde model (bijlage 1).

VI TEKORTSCHIETEN IN DE NAKOMING, ONTBINDING EN OPZEGGING

17. Dreigende vertraging

17.1 Indien de voortgang van de werkzaamheden vertraging dreigt te ondervinden, bericht Opdrachtnemer dat onmiddellijk aan Opdrachtgever met vermelding van oorzaak en consequenties daarvan. Tevens stelt Opdrachtnemer maatregelen voor om verdere vertraging te voorkomen.

17.2 Binnen 14 dagen na ontvangst van de in het vorige lid bedoelde melding, bericht Opdrachtgever of hij al dan niet instemt met de voorgestelde maatregelen en de genoemde consequenties. Instemming houdt niet in dat Opdrachtgever de oorzaak van de dreigende vertraging erkent en laat alle andere rechten of vorderingen die Opdrachtgever op grond van de Overeenkomst toekomen, onverlet.

18. Boete

18.1 Indien de volledige Diensten niet binnen de overeengekomen dan wel verlengde termijn zijn verricht op een wijze die aan de Overeenkomst beantwoordt, is Opdrachtnemer aan Opdrachtgever een onmiddellijk opeisbare boete verschuldigd van 0,1 % van de totale dan wel maximale prijs die met de Overeenkomst is gemoeid voor elke dag dat de tekortkoming voortduurt tot een maximum van 10 % daarvan. Indien nakoming anders dan door overmacht blijvend onmogelijk is geworden, is de boete onmiddellijk in haar geheel verschuldigd.

18.2 De boete komt Opdrachtgever toe, onverminderd alle andere rechten of vorderingen, daaronder mede begrepen:
a. zijn vordering tot nakoming van de overeengekomen verplichting tot het verrichten van de Diensten;
b. zijn recht op schadevergoeding.

18.3 De boete wordt verrekend met de door Opdrachtgever verschuldigde betalingen, ongeacht of de vordering tot betaling daarvan op een derde is overgegaan.

19. Aansprakelijkheid

19.1 Indien één der partijen tekortschiet in de nakoming van haar verplichtingen uit de Overeenkomst, kan de andere partij haar in gebreke stellen. De nalatige partij is echter onmiddellijk in verzuim als nakoming van de desbetreffende verplichtingen anders dan door overmacht binnen de overeengekomen termijn reeds blijvend onmogelijk is. De ingebrekestelling geschiedt schriftelijk, waarbij aan de nalatige partij een redelijke termijn wordt gegund om alsnog haar verplichtingen na te komen. Deze termijn is een fatale termijn. Indien nakoming binnen deze termijn uitblijft, is de nalatige partij in verzuim.

19.2 De in het vorige lid genoemde ingebrekestelling is niet vereist indien de termijn waarbinnen de overeengekomen Diensten verricht hadden moeten zijn, voor de afloop daarvan is verlengd. Indien de in het vorige lid bedoelde nakoming ook niet heeft plaatsgevonden voor het eind van de verlengde termijn, is de nalatige partij vanaf dat moment direct in verzuim.

19.3 De partij die toerekenbaar tekortschiet in de nakoming van haar verplichtingen, is tegenover de andere partij aansprakelijk voor de door de andere partij geleden dan wel te lijden schade.

19.4 Opdrachtnemer vrijwaart Opdrachtgever tegen eventuele aanspraken van derden op vergoeding van schade als gevolg van het tekortschieten als bedoeld in het derde lid.

19.5 Indien Opdrachtnemer voor het verrichten van de Diensten gebruik maakt van zaken als bedoeld in artikel 6, eerste lid, die eigendom zijn van Opdrachtgever, is Opdrachtnemer aansprakelijk voor de schade die aan deze zaken wordt toegebracht. Indien als gevolg van de aanwezigheid van zaken van Opdrachtgever bij Opdrachtnemer ter uitvoering van de Overeenkomst schade aan Opdrachtnemer of aan derden wordt toegebracht, op welke wijze dan ook, is deze schade geheel voor rekening en risico van Opdrachtnemer. In voorkomende gevallen zal Opdrachtnemer Opdrachtgever vrijwaren tegen aanspraken van derden.

19.6 Alle verplichtingen met betrekking tot het Personeel van Opdrachtnemer, ook die krachtens de belasting- en socialeverzekeringswetgeving, komen ten laste van Opdrachtnemer. Opdrachtnemer vrijwaart Opdrachtgever tegen elke aansprakelijkheid in dit verband.

20. Overmacht

Onder overmacht wordt in ieder geval niet verstaan: gebrek aan personeel, stakingen, ziekte van personeel, verlate aanlevering of ongeschiktheid van voor de uitvoering van de werkzaamheden benodigde goederen, liquiditeits- of solvabiliteitsproblemen aan de zijde van Opdrachtnemer of tekortschieten van door hem ingeschakelde derden.

21. Ontbinding en opzegging

21.1 Onverminderd hetgeen overigens in de Overeenkomst is vastgelegd, kan elk van de partijen de Overeenkomst door middel van een aangetekend schrijven buiten rechte geheel of gedeeltelijk ontbinden, indien de andere partij in verzuim is dan wel nakoming blijvend of tijdelijk onmogelijk is.

21.2 Indien één der partijen gedurende een bij de Overeenkomst te bepalen periode ten gevolge van overmacht haar verplichtingen op grond van de Overeenkomst niet kan nakomen, heeft de andere partij het recht de Overeenkomst door middel van een aangetekend schrijven met onmiddellijke ingang buiten rechte geheel of gedeeltelijk te ontbinden, zonder dat daardoor enig recht op schadevergoeding zal ontstaan.

21.3 In geval van overmacht gaan partijen niet eerder tot ontbinding over dan na het verstrijken van een termijn van 15 Werkdagen, tenzij partijen een andere termijn overeenkomen.

21.4 Opdrachtgever kan, zonder enige aanmaning of ingebrekestelling, met onmiddellijke ingang buiten rechte de Overeenkomst door middel van een aangetekend schrijven ontbinden, indien Opdrachtnemer (voorlopige) surseance van betaling aanvraagt of hem (voorlopige) surseance van betaling wordt verleend, Opdrachtnemer zijn faillissement aanvraagt of in staat van faillissement wordt verklaard, de onderneming van Opdrachtnemer wordt geliquideerd, Opdrachtnemer zijn onderneming staakt, op een aanmerkelijk deel van het vermogen van Opdrachtnemer beslag wordt gelegd, Opdrachtnemer een fusie of splitsing aangaat of wordt ontbonden, dan wel Opdrachtnemer anderszins niet langer in staat moet worden geacht de verplichtingen uit de Overeenkomst na te kunnen komen.

21.5 Opdrachtgever kan voorts de Overeenkomst door middel van een aangetekend schrijven te allen tijde opzeggen. Tussen Opdrachtgever en Opdrachtnemer zal alsdan afrekening plaatsvinden op basis van de door de Opdrachtnemer ter zake van de uitvoering van de onderhavige opdracht verrichte Diensten en in redelijkheid gemaakte kosten en van de voor de uitvoering van de opdracht in redelijkheid voor de toekomst reeds aangegane verplichtingen. Opdrachtgever hoeft Opdrachtnemer op generlei wijze anderszins schadeloos te stellen voor de gevolgen van de opzegging van de Overeenkomst.

22. Behoud recht nakoming te vorderen

Het nalaten door één van de partijen om binnen een in de Overeenkomst genoemde termijn nakoming van enige bepaling te verlangen, tast het recht om alsnog nakoming te verlangen niet aan tenzij de desbetreffende partij uitdrukkelijk en schriftelijk met de niet-nakoming heeft ingestemd.

VII DIVERSEN

23. Intellectuele eigendomsrechten

23.1 Alle intellectuele eigendomsrechten die kunnen of zullen kunnen worden uitgeoefend - waar en wanneer dan ook - ten aanzien van de resultaten van de verrichte Diensten die Opdrachtnemer voor Opdrachtgever verricht, berusten bij Opdrachtgever.
Deze rechten worden op grond van de Overeenkomst door Opdrachtnemer op het moment van het ontstaan daarvan aan Opdrachtgever overgedragen, welke overdracht door Opdrachtgever reeds nu voor alsdan wordt aanvaard.

23.2 Voor zover de resultaten, bedoeld in het eerste lid, tot stand komen met gebruikmaking van reeds bestaande, niet aan Opdrachtgever toekomende Intellectuele eigendomsrechten, verleent Opdrachtnemer aan Opdrachtgever een niet-exclusief gebruiksrecht van onbepaalde duur. Opdrachtnemer garandeert in dat geval gerechtigd te zijn tot het verlenen van vorenbedoeld gebruiksrecht.

23.3 Voor zover voor de overdracht van de rechten, bedoeld in het eerste lid, een nadere akte zou zijn vereist, machtigt Opdrachtnemer Opdrachtgever reeds nu voor alsdan onherroepelijk om zodanige akte op te maken en namens Opdrachtnemer te ondertekenen, onverminderd de verplichting van Opdrachtnemer om op eerste verzoek

van Opdrachtgever aan de overdracht van deze rechten medewerking te verlenen, zonder daarbij voorwaarden te kunnen stellen. Opdrachtnemer machtigt voor zover nodig Opdrachtgever hierdoor onherroepelijk om de overdracht van deze intellectuele eigendomsrechten in de desbetreffende registers te doen in- of overschrijven.

23.4 Indien tussen partijen verschil van mening bestaat over intellectuele eigendomsrechten ten aanzien van de resultaten van de verrichte Diensten wordt er, behoudens tegenbewijs, van uitgegaan dat die rechten bij Opdrachtgever berusten. In alle gevallen mag Opdrachtgever het bij de Overeenkomst beoogde gebruik van de uitkomst van de resultaten maken.

23.5 Opdrachtnemer doet hierbij afstand jegens Opdrachtgever van alle eventueel aan hem, Opdrachtnemer, toekomende zogenaamde persoonlijkheidsrechten als bedoeld in de Auteurswet 1912, in de mate als de toepasselijke regelgeving zodanige afstand toelaat. Opdrachtnemer doet, hiertoe gevolmachtigd, ook namens het aan zijn zijde betrokken Personeel, afstand jegens Opdrachtgever van alle eventueel aan deze personeelsleden toekomende persoonlijkheidsrechten, in de mate als de toepasselijke regelgeving zodanige afstand toelaat.

23.6 Opdrachtnemer mag de resultaten van de verrichte Diensten in generlei vorm aan derden beschikbaar stellen, noch hierover aan derden enige inlichting te verschaffen, tenzij Opdrachtgever uitdrukkelijk toestemming hiervoor heeft verleend. Opdrachtgever kan aan deze toestemming voorwaarden verbinden.

23.7 Opdrachtnemer vrijwaart Opdrachtgever tegen aanspraken van derden ter zake van (eventuele) inbreuk op intellectuele eigendomsrechten van die derden, vergelijkbare aanspraken met betrekking tot kennis, ongeoorloofde mededinging en dergelijke daaronder begrepen. Opdrachtnemer verplicht zich tot het op zijn kosten treffen van alle maatregelen die kunnen bijdragen tot voorkoming van stagnatie en tot beperking van de te maken extra kosten en/of te lijden schade als gevolg van bedoelde inbreuken.

23.8 Onverminderd het hiervoor bepaalde kan Opdrachtgever, indien derden Opdrachtgever ter zake van schending van intellectuele eigendomsrechten aansprakelijk stellen, de Overeenkomst schriftelijk, buiten rechte, geheel of gedeeltelijk ontbinden. Van zijn recht tot ontbinding van de Overeenkomst zal Opdrachtgever geen gebruik maken dan na voorafgaand overleg met Opdrachtnemer.

24. Overdracht rechten en verplichtingen uit de Overeenkomst

Partijen mogen de uit de Overeenkomst voortvloeiende rechten en verplichtingen niet zonder toestemming van de andere partij aan een derde overdragen. Toestemming wordt niet zonder redelijke grond geweigerd. Partijen kunnen daaraan voorwaarden verbinden.

25. Verzekering

25.1 Opdrachtnemer heeft zich adequaat verzekerd en zal zich adequaat verzekerd houden voor de navolgende risico's:
a. beroepsaansprakelijkheid (risico's die voortvloeien uit Beroepsfouten);

b. bedrijfsaansprakelijkheid (waaronder aansprakelijkheid voor schade toegebracht aan personen of zaken die eigendom zijn van Opdrachtgever);
c. verlies van en schade aan bedrijfsinventaris (waaronder door brand en diefstal), inclusief de zaken die eigendom zijn van Opdrachtgever.

25.2 Opdrachtnemer legt op verzoek van Opdrachtgever onverwijld (een gewaarmerkt afschrift van) de polissen en de bewijzen van premiebetaling ter zake van de in het eerste lid bedoelde verzekeringen dan wel een verklaring van de verzekeraar betreffende het bestaan van deze verzekeringen en het betaald zijn van de premie over. Opdrachtnemer beëindigt niet zonder voorafgaande schriftelijke toestemming van Opdrachtgever de verzekeringsovereenkomsten dan wel de condities waaronder deze zijn aangegaan. Evenmin wijzigt Opdrachtnemer het verzekerde bedrag ten nadele van Opdrachtgever zonder bedoelde toestemming. De door Opdrachtnemer verschuldigde verzekeringspremies worden geacht in de overeengekomen prijzen en tarieven te zijn begrepen.

25.3 Opdrachtnemer cedeert bij voorbaat aan Opdrachtgever alle aanspraken op uitkeringen van verzekeringspenningen, als bedoeld in het eerste lid en voor zover betrekking hebbende op schade, waarvoor Opdrachtnemer op grond van de Overeenkomst jegens Opdrachtgever aansprakelijk is. Opdrachtnemer verplicht zich om deze cessie schriftelijk ter kennis van zijn verzekeraar te brengen en hiervan een afschrift aan Opdrachtgever te zenden, onverminderd de bevoegdheid van Opdrachtgever om hiervan aan deze verzekeraar melding te doen.

25.4 Verzekeringspenningen die door verzekeringsmaatschappijen rechtstreeks aan Opdrachtgever worden uitbetaald, worden in mindering gebracht op de door Opdrachtnemer voor het verzekerde voorval aan Opdrachtgever te betalen schadevergoeding.

26. Overname van Personeel, omkoping, belangenverstrengeling

26.1 Partijen zullen zonder toestemming van de wederpartij, tijdens de uitvoering van de Overeenkomst en binnen één jaar na beëindiging daarvan, geen personeel van de wederpartij in dienst nemen of met dat personeel over indiensttreding onderhandelen. Deze toestemming wordt niet zonder redelijke grond onthouden.

26.2 Partijen zullen aan elkaar noch aan derden aanbieden, noch van elkaar of derden vragen, accepteren of toegezegd krijgen, voor henzelf of enige andere partij, enige schenking, beloning, compensatie of profijt van welke aard dan ook die uitgelegd kan worden als een onwettige praktijk. Een dergelijke praktijk kan reden zijn voor gehele of gedeeltelijke ontbinding van de Overeenkomst.

26.3 Indien blijkt dat een lid van het Personeel van Opdrachtgever een al dan niet betaalde nevenfunctie vervult bij Opdrachtnemer of ten tijde van de onderhandelingen over de totstandkoming van de Overeenkomst heeft vervuld, zonder dat Opdrachtgever daarover vóór het sluiten van de Overeenkomst is ingelicht, kan Opdrachtgever de Overeenkomst zonder ingebrekestelling met onmiddellijke ingang ontbinden zonder tot enige schadevergoeding te zijn gehouden.

26.4 Opdrachtnemer betrekt geen personen, anders dan met toestemming van Opdrachtgever, bij de uitvoering van de Overeenkomst die in een periode van twee jaar voorafgaand aan de werkzaamheden bij Opdrachtgever in dienst zijn geweest.

27. Nietige en vernietigde bepalingen

Indien één of meer bepalingen van de Voorwaarden of de Overeenkomst nietig blijken te zijn of door de rechter vernietigd worden, behouden de overige bepalingen van de Voorwaarden of de Overeenkomst hun rechtskracht. Partijen zullen over de nietige of vernietigde bepalingen overleg voeren teneinde een vervangende regeling te treffen. De vervangende regeling zal de strekking van de Voorwaarden of de Overeenkomst niet aantasten.

28. Vervolgopdracht

Opdrachtnemer kan aan de Overeenkomst geen enkel recht ontlenen voor de verkrijging van een vervolgopdracht.

29. Melding in publicaties of reclame-uitingen

Opdrachtnemer maakt in publicaties of reclame-uitingen geen melding van de opdrachtverlening en gebruikt de naam van Opdrachtgever niet als referentie dan na toestemming van Opdrachtgever.

30. Voortdurende verplichtingen

Verplichtingen die naar hun aard bestemd zijn om ook na afloop van de Overeenkomst voort te duren, behouden nadien hun werking. Tot deze verplichtingen behoren onder meer: vrijwaring voor schending van intellectuele eigendomsrechten, geheimhouding, cessie van verzekeringspenningen, geschillenbeslechting, domiciliekeuze en toepasselijk recht.

31. Geschillen en toepasselijk recht

31.1 Ieder geschil tussen partijen ter zake van de Overeenkomst zal bij uitsluiting worden voorgelegd aan de daartoe bevoegde rechter in het arrondissement Den Haag, tenzij partijen alsnog een andere vorm van geschillenbeslechting zullen overeenkomen.

31.2 Op de Overeenkomst is Nederlands recht van toepassing.

-+-+-+-+-+-

Van:
Verzonden: donderdag 24 juli 2008 9:04
Aan:

Onderwerp: Q fever

Bijlagen: Round table conference on Q fever 22.07.2008.doc

Hierbij mijn verslag van de Round table conference on Q fever van afgelopen week. Wij proberen de informatie over de vaccinatie van geiten boven water te krijgen en daarna wil ik graag een keer overleggen. Als jullie nog vragen hebben hoor ik dat graag.

Met vriendelijke groet,

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78 A

Round table conference on Q fever

datum: 22 juli 2008
Plaats: Hotel Grand Karel V, Utrecht
Programma: zie bijlage
aantal deelnemers: 32
De bijeenkomst was met de nodige pressie georganiseerd door de Gezondheidsraad (De Gezondheidsraad is een onafhankelijk adviesorgaan dat als taak heeft ministers en parlement te adviseren over de stand van wetenschap op het gebied van de volksgezondheid) en het RIVM.

Kernvragen die in de discussie aan de orde kwamen waren:

1. is het noodzakelijk om een screening uit te voeren van zwangere vrouwen in het gebied waar nu Q-fever speelt?
2. is het noodzakelijk om maatregelen te nemen voor bloeddonoren in het betreffende gebied.

De incidentie in Herpen in 2007 was 1500 Q-fever gevallen per 100.000 inwoners. In 2008 gaat het om 43 gevallen per 100.000 inwoners maar op de dag van de bijeenkomst was het aantal humane gevallen inmiddels gestegen tot ongeveer 650. In Herpen wordt gesproken over een uitbraak waarschijnlijk vanuit één bron (hoewel dit niet is bewezen); niet veel informatie over risico's (contact met hooi, stro en mest als belangrijke risicofactor genoemd), roken is risicofactor. Bij de veel grotere aantallen patiënten tot nu toe in 2008 wordt niet gesproken over een uitbraak en er zou sprake zijn van meerdere bronnen.

In de discussie wordt uitgebreid stil gestaan bij:

1. de noodzaak om de huidige situatie in Brabant met spoed beter in beeld te brengen. RIVM neemt nog deze week initiatief; daarna situatie eventueel opnieuw bespreken in Outbreak Management Team; benodigd onderzoek: environmental contamination, behandeling, strain isolatie, uitscheiding bij geiten (volgens de Canadees excretie het grootste en het langst bij geiten, dan bij schapen en runderen in laatste plaats);
2. bij het belang om meer informatie te krijgen van de veterinaire risico's;
3. de noodzaak om zwangeren te screenen: er wordt besloten om niet tot een screening over te gaan maar wel de verzamelde informatie en bloedmonsters retrospectief te onderzoeken om achteraf de risico's beter in beeld te hebben om bij een eventuele volgende uitbraak met meer kennis van zaken een beslissing te kunnen nemen; mocht uit de analyse onder 1. naar voren komen dat er locaal sprake is van duidelijke clustering dan zou ter plaatse een ander besluit kunnen worden genomen; balans tussen maatregelen en risico's;
4. de beschikbare kennis om besmette mensen te behandelen;
5. de noodzaak om bloed donoren uit te sluiten of andere maatregelen om risico's te beperken: er wordt besloten om geen maatregelen te nemen maar ook hier geldt dat bij eventuele locale clustering locaal tot andere maatregelen kan worden besloten; balans tussen maatregelen en risico's;
6. het nauwkeurig volgen wat nu gebeurt; hoewel het de bedoeling was om tijdens dit overleg de risico-analyse te scheiden van het maken van voorstellen voor vervolgonderzoek, lukte dat in veel gevallen niet helemaal;
7. laboratoriumonderzoek: gebruik wordt gemaakt van ELISA, IgG en IgM onderscheidende testen, CBR, PCR. Uitslagen regelmatig aanleiding tot verwarring.

Op de site van de Gezondheidsraad stond de dag na de conferentie de bijeenkomst als volgt samengevat:

Conferentie over Q-koorts

23 juli 2008 - Op 22 juli organiseerden het RIVM en de Gezondheidsraad een conferentie over Q-koorts in Nederland. Aan de bijeenkomst, die zeer waardevol bleek, namen experts deel uit Canada, Frankrijk, Duitsland, Denemarken, Zweden en Nederland. Door het uitwisselen van ervaringen kwam aan het licht dat de situatie in Nederland uniek is in de wereld. Ook in andere landen zijn incidentele uitbraken van Q-koorts geweest, maar niet in zo'n uitgebreid en dichtbevolkt gebied als nu in Nederland. Maatregelen bij uitbraken in andere landen zijn dan ook niet zomaar in Nederland toepasbaar.

23.07.2008,

rivm

Draft Programme
Round table conference on Q-fever, 22nd of July
Location: Hotel Grand Karel V, Utrecht, the Netherlands
Chair:

AIM: To review the scientific evidence around the public health risk management principles for screening of pregnant women and blood donors with possible exposure to *Coxiella burnetii* in outbreak and endemic situations

Welcome and opening address

Introduction of the participants

Session one: The state of the art in Q-fever

- clinical picture of Q-fever ( ): veel chronische vermoeidheid (20% van de gevallen; duurt soms jaren), vaak neurologische klachten, aantal uitbraken kat en konijn gerelateerd, dd legionella, tularaemie; "In Novoscotia Q fever disappeared, just because nobody was interested."
- diagnosis of Q-fever ( ): small cell and large cell variant; fase I (virulent) en fase II antigeen; na toedienen antibiotica bij mens: PCR negatief; soms erg late seroconversies; chronische infecties: hele langzame vermeerdering van Coxiella, heel lang behandelen; ELISA: 60% sensitiviteit en hoge specificiteit.
- Q-fever and blood donations (Sanquin: wil graag contact met GD om te praten over beschikbaarheid commerciële testen en eigenschappen daarvan. Insteek Sanquin: risk assessment and risk reduction
- Q-fever in pregnancy ( ), Marseille; goed verhaal; ook vanuit referentie-laboratorium in Marseille worden soms adviezen gegeven terwijl niet in alle gevallen voldoende feitelijke kennis beschikbaar is. Bij Q-fever besmette vrouw zou borstvoeding gecontraïndiceerd zijn. Vrouwen zijn iets minder gevoelig voor Q-fever als mannen van dezelfde leeftijd, kinderen zijn nog ongevoeliger en zwangere vrouwen zijn heel erg ongevoelig maar als ze worden besmet kan dat leiden tot placentitis en verlies van de vrucht, niet alleen bij de zwangerschap tijdens de besmetting maar in een deel van de gevallen ook bij volgende zwangerschappen.

Coffee

Round table – Summary of significant issues

Session two: Managing Q-fever during outbreaks and in endemic areas, emphasis on pregnancy and blood donors

- Dutch outbreaks 2007-2008

- the human outbreak): Herpen 2007: 72,7% seronegatief; 8,6% oude infectie, 28,7% nieuwe infectie; 2008: nu al ongeveer 650 gevallen;
- Q fever outbreak in small ruminants : schets van 2005 tot nu; de aanwezigheid van besmette geitenbedrijven aan de ene kant en humane gevallen aan de andere kant leidt te gemakkelijk tot de conclusie dat de problemen direct zijn gelinkt, zonder dat naar andere bronnen is gekeken;
- modeling the epidemic): beschrijving hoe wordt geprobeerd te komen tot modellering van de uitbraak;
- Q fever outbreaks in Germany): beschrijving van twee uitbraken in Duitsland, in Soest in mei 2003 (0,1-0,5 gevallen per 100.000 inwoners: na geboorte lam op een markt met veel bezoekers); en in juni 2005 in Jena/Winzerla: 331 gevallen vanuit een koppel schapen dat aan de rand van de stad twee weken had gegraasd; 46% van de schapen seropositief.

- recent experiences in Denmark : verslag van onderzoek rundvee in Denemarken: ongeveer 50% van de tankmelkmonsters sero- (en PCR-?)positief; ondanks gerichte humane screening sinds enkele jaren van 1200 personen per jaar, aantal besmettingen bij de mens in Denemarken heel erg laag.

Lunch

Session three: round table

Which criteria could be applied to decide whether screening of pregnant women living in the endemic area is justifiable?

Coffee

Are restrictive measures needed with respect to donation of blood and blood products in the endemic area?

Closing session: conclusions

79

Van:
Verzonden: donderdag 24 juli 2008 9:09
Aan.

Onderwerp: AD Q fever

'Nederlandse Q-koortsuitbraak is uniek'

23 jul 2008 16:46

Maatregelen die in andere landen zijn getroffen tegen Q-koorts zijn niet zomaar in Nederland toepasbaar.
Dat is de conclusie van een rondetafelgesprek dat het Centrum Infectieziektebestrijding van het RIVM in samenwerking met de Gezondheidsraad heeft gehouden. Experts uit Canada, Frankrijk, Duitsland, Denemarken, Zweden en Nederland bespraken mogelijkheden om verspreiding van Q-koorts tegen te gaan.

Ook in andere landen zijn incidentele uitbraken van Q-koorts geweest, maar niet in een uitgebreid en dichtbevolkt gebied als nu in Noord-Brabant. Wetenschappers concluderen dat de situatie in Nederland uniek is in de wereld.

Afgesproken is dat het RIVM met veterinaire deskundigen gaat kijken hoe de overdracht van Q-koorts van dier naar mens gebeurt en of verdere maatregelen mogelijk of nodig zijn om dat te voorkomen.

Bloedtransfusie lijkt een verwaarloosbaar risico te zijn voor de overdracht van Q-koorts. Dit leidt ertoe dat het beleid voor bloeddonoren voorlopig niet aangepast zal worden.

Tijdens de conferentie bleek ook dat een verantwoorde screening van zwangeren in de regio niet mogelijk is omdat er onvoldoende bekend is over de kans op zwangerschapscomplicaties bij een Q-koortsinfectie. Ook zijn de beschikbare laboratoriumtesten om Q-koorts vast te stellen nog niet betrouwbaar genoeg voor screening van grote groepen.

Tevens is niet bekend wat de effectiviteit en mogelijke nadelen zijn van langdurige behandeling van Q-koorts met antibiotica tijdens zwangerschap. Het RIVM gaat nader onderzoek doen om meer inzicht te krijgen in de risico's van Q-koorts tijdens de zwangerschap.

Met vriendelijke groet,

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80

5 FW RE Vaccine trial Q-fever

Van: [redacted]mineleni.nl]
Verzonden: woensdag 6 augustus 2008 16:42
Aan:
dr.
CC:
Onderwerp: FW: RE Vaccine trial Q-fever

Ter info. Het contact met Ceva is gelegd. CEVA bekijkt nu of het mogelijk is om op korte termijn een bijeenkomst te organiseren.

1 expert zit in Budapest, 1 expert in Frankrijk en [redacted] in Brussel, dus ik hoop dat het mogelijk is om snel een afspraak te maken.

Beleidsmedewerker
Directie Voedselkwaliteit en Diergezondheid

Van:
Verzonden: woensdag 6 augustus 2008 16:33
Aan:
Onderwerp: RE Vaccine trial Q-fever

Dear

I got your email, I will revert to you as soon as I can get in touch my my colleagues in charge of Coxevac.

Best regards,

Ceva Animal Health Benelux

06/08/2008 16:00

A [redacted]@ceva.com>

CC

Objet
Vaccine trial Q-fever

5 FW RE Vaccine trial Q-fever

Dear

As discussed over the telephone, the Netherlands is interested in organising a vaccine trial for Q-fever on very short notice.
The situation in the Netherlands is very serious. At this moment there are 700 reported human cases of Q-fever in the eastern part of Noord Brabant and adjoining part of Gelderland. Although it is not clear how people have become infected, experts say that the infection most likely come from goats and sheep. The pressure to do something to prevent this from happening next year is very high.
Therefore we are looking into the possibility to set up a vaccination trial to see if vaccinating sheep and goats can diminish the amount of bacteria that reach people. We are thinking of vaccinating lactating goats and sheep in the infected and surrounding area (approximately 50.000 – 150.000 animals, depending on the size of the area).

If we want to see an effect of this vaccination trial next year, we have to move fast. Vaccination should than take place before mating, this means within the next few months. We would like to discuss the possibilities with your organisation. I hope we can organise a meeting on short notice. The meeting can take place anywhere most convenient for you.

Best regards,

Beleidsmedewerker
Directie Voedselkwaliteit en Diergezondheid

01

Van:
Verzonden: woensdag 20 augustus 2008 7:10
Aan:
Onderwerp: Q-fever

Bijlagen: Coxevac vaccin.pdf

Hierbij het artikel dat ik gisteren noemde: gaat over geiten, ELISA en PCR.

Met vriendelijke groet,

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ONLY PHASE I Q FEVER VACCINE PROTECTS PREGNANT GOATS AGAINST CHALLENGE WITH COXIELLA BURNETII

Nathalie Arricau-Bouvery, Armel Souriau, Christelle Bodier and Annie Rodolakis
Pathologie Infectieuse et Immunologie, INRA, Tours-Nouzilly, F-37380 Nouzilly, France

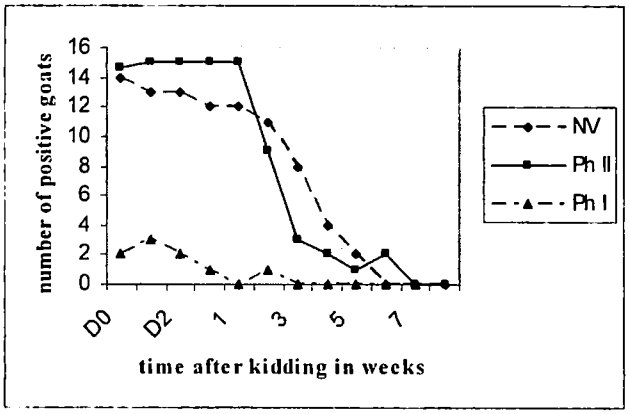
Introduction

Q fever, a zoonosis caused by the obligate intracellular bacteria *Coxiella burnetii* is endemic throughout the world and infects arthropods, birds, pets, domestic and wild mammals and humans. The disease is known since the 1930th and has been reported worldwide except in Antarctic region and perhaps in New Zealand where its presence is not really confirmed (5). In livestock, *C. burnetii* is associated with reproductive disorders: abortion, stillbirth, and delivery of weak and unviable newborns, placentitis, endometritis and infertility (7). Such reproductive failures are accompanied with shedding of great number of *Coxiella* into birth products, urine, faeces and milk of infected animals. In human, the acute disease currently appears like a flu-like, usually self-limiting illness accompanied by myalgia and severe headache. Complications may occur such as pneumonia infected one, with an efficient vaccine preventing abortion and shedding of the bacteria. Several vaccines have been developed for this purpose. However *C. burnetii* presents phase variation, which is similar to smooth-rough variation in the lipopolysaccharide (LPS) of enterobacteria (2). Phase I that corresponds to smooth LPS is infectious for animals and humans contrary to phase II that is obtained after several passages in chicken embryos or cells culture.

Phase I vaccines are difficult and hazardous to obtain but are described as the only efficient vaccines (9). So in this study the efficacy of 2 commercial vaccines compounded of inactivated *C. burnetii* reference strain Nine Mile, one phase I vaccine (Coxevac, CEVA Santé Animale France) and one phase II vaccine (Chlamyvax-FQ, Merial France) were assessed in goats by comparing the 2 vaccinated groups with a control one for the kidding performances

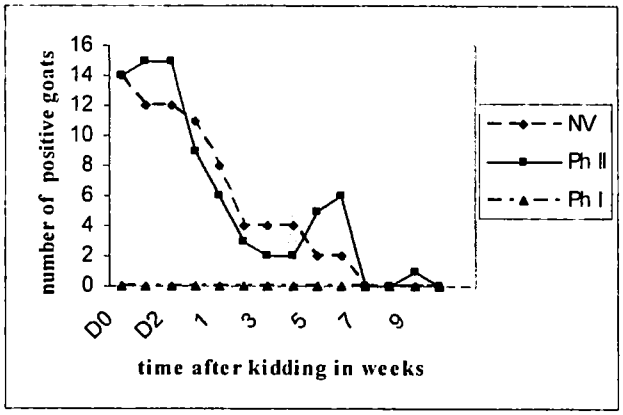
(153±3) but was too short (134±15 and 141±8 respectively) for the groups Ph II and NV. The kidding performances of the group Ph I, 22/26 (85%) live kids, were similar to the one observed in does of original flock when only 7/23 (30%) kids survived in group NV and 9/18 (33%) in group Ph II.

Fig1 Shedding of C burnetti in vaginal mucus



C burnetii was detected in vaginal mucus (Fig 1), faeces or milk samples (Fig 2) of all the goats of group Ph II and NV while none of the milk samples was positive in group Ph I, only 7/17 goats had a transient bacterial shedding in vaginal mucus (1.5 days in average in comparison to 16 days and 22 for groups Ph II and NV respectively) and 12/17 in faeces (10 days in average in comparison to 28 days and 27 for groups Ph II and NV respectively).

Fig2 Shedding of C burnetti in milk



Antibodies after challenge increased following almost the same pattern in the phase II vaccinated and the unvaccinated animals whereas, their increasing was quickly stabilized in the phase I vaccinated goats.

Discussion

The efficacy of vaccines against Q fever has never been tested in experimentally infected goats. The used dose of *C burnetii* CbC1 strain has been established in a previous experimental infection (1). It induced the abortion of about 80 % of the non immune pregnant goats, which is sometimes but extremely hardly ever observed in field conditions. Indeed, often in ruminants' herds, few females abort while the others are asymptomatic but shed the bacteria during several months (4). However in some caprine flocks more than 30% and even 90% of the pregnant female abort the reason of this difference of gravity of the disease is always unknown, nevertheless the phase I vaccine is able to protect the pregnant goats even against a very high challenge.

Conclusion

In our experimental conditions, which were very severe, only Coxevac vaccine was efficient and dramatically reduced abortion and excretion of bacteria in the milk, vaginal mucus and faeces, reducing environmental contamination and thus the risk of transmission to humans. In contrast, Chlamyvax FQ did not modify the course of the disease. So phase 1 vaccine must be used to control the disease. The large use of such a vaccine in cattle in Slovakia in the 70-ties and 80-ties has significantly reduced the occurrence of Q fever in this country (6, 11).

Acknowledgements

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