A query for Coxiella in veterinary and environmental matrices

Letter report 330291003/2009

A. de Bruin | B.J. van Rotterdam

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Arnout de Bruin (Researcher), RIVM
Bart van Rotterdam (Project Leader), RIVM

Contact:
Bart van Rotterdam
Laboratory for Zoonoses and Environmental Microbiology
bart.van.rotterdam@rivm.nl

This investigation has been performed by order and for the account of the Food and Consumer Product Safety Authority, within the framework of Deelproject 9.2.3.D Coxiella in kennisvraag livestock-borne zoonoses

Abstract

A query for Coxiella in veterinary and environmental matrices

Q fever, caused by *Coxiella burnetii*, is a zoonosis with a worldwide distribution that affects both humans and animals. In 2007, 2008, and 2009 large community outbreaks of Q fever were observed in the Netherlands. In 2008, several studies were started to investigate potential sources of *C. burnetii* infection and possible transmission routes. Temporal studies focussed on *C. burnetii* DNA content on farms, and their direct surroundings. *Coxiella burnetii* was found in veterinary and environmental samples obtained from a single farm, with an abortion wave among its goats in April 2007, during two successive years of Q fever outbreaks in 2007 and 2008. Within the Q fever outbreak of 2009, investigations at one location in Zuid-Limburg over a 16 week-interval demonstrated that the *C. burnetii* DNA content in both veterinary and environmental samples declined over time after the initial wave of abortions among goats. Although a decline of the *C. burnetii* DNA content was observed, environmental and veterinary samples were still found to be positive up to several months after the abortion wave at the farm.
Human outbreak linked source investigations focussed on veterinary and environmental matrices on farms, which in previous studies were found to contain the highest *C. burnetii* DNA content. These matrices included vaginal swabs from animals and surface area swabs from horizontal surfaces, to investigate the potential link between the putative Q fever-affected goat farms and (clusters of) human Q fever cases in the near vicinity of these farms. Screening results for vaginal swabs obtained from goats and/or sheep are consistent with results for surface area swabs taken on the same farm.

Keywords:
Coxiella burnetii, Q fever, qPCR

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1 Introduction

1.1 Q fever outbreaks in the Netherlands

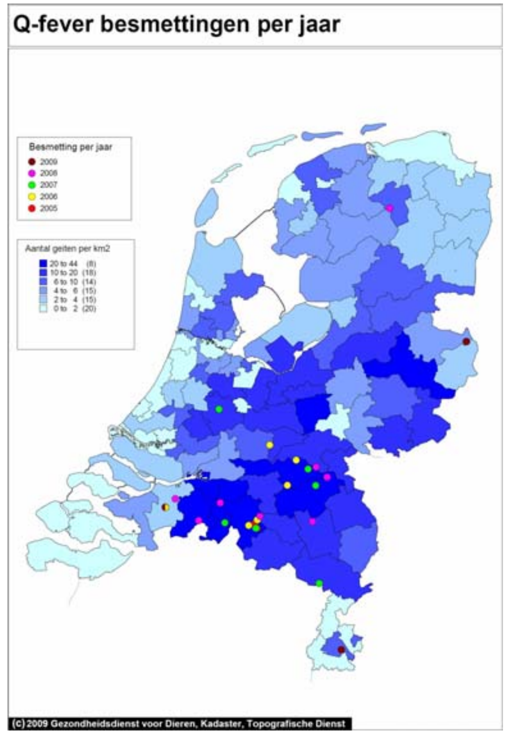
The impact of various transmission routes of Q fever is not well understood. Two years before the first documented outbreak in the Netherlands in 2007 (1), large abortion waves were reported on (primarily goat) farms in the same region as the Q fever outbreaks in humans in subsequent years (Figure 1). This implicated (goat) farms as potential sources for human Q fever infection, as later supported by another epidemiological study of a local outbreak (1,6).

Coxiella burnetii infection in humans can occur via close contact with infected animals, or contaminated animal products. In addition, *C. burnetii* can persist for long periods of time in the environment and transmission to animals and humans by inhalation of contaminated aerosols is thought to be the primary route (2,3). Infected animals, like goats, sheep, and cattle, often show no clinical signs of infection except for abortions or stillbirths that may occur due to infection of the placenta. When animals are infected, the main sources of *C. burnetii* shedding to the environment are manure, urine, milk, and most importantly birth materials like amnion fluid and placenta (4,5).

1.2 Report Outline

This report describes the current status of our investigations to investigate important environmental and veterinary sources of *Coxiella burnetii* that are relevant for source finding and risk assessment.

Temporal studies and source investigations were carried out during the Q fever outbreaks in 2007, 2008, and 2009, in which a large number of different environmental and veterinary matrices were screened for the presence of *C. burnetii* DNA by molecular detection via quantitative multiplex real time PCR. Samples were taken as part of source finding on request of municipal health services (GGD's), and for a project for the Ministry of Health, Welfare and Sport on persistence of *C. burnetii* DNA in contaminated farms and the environment over time.



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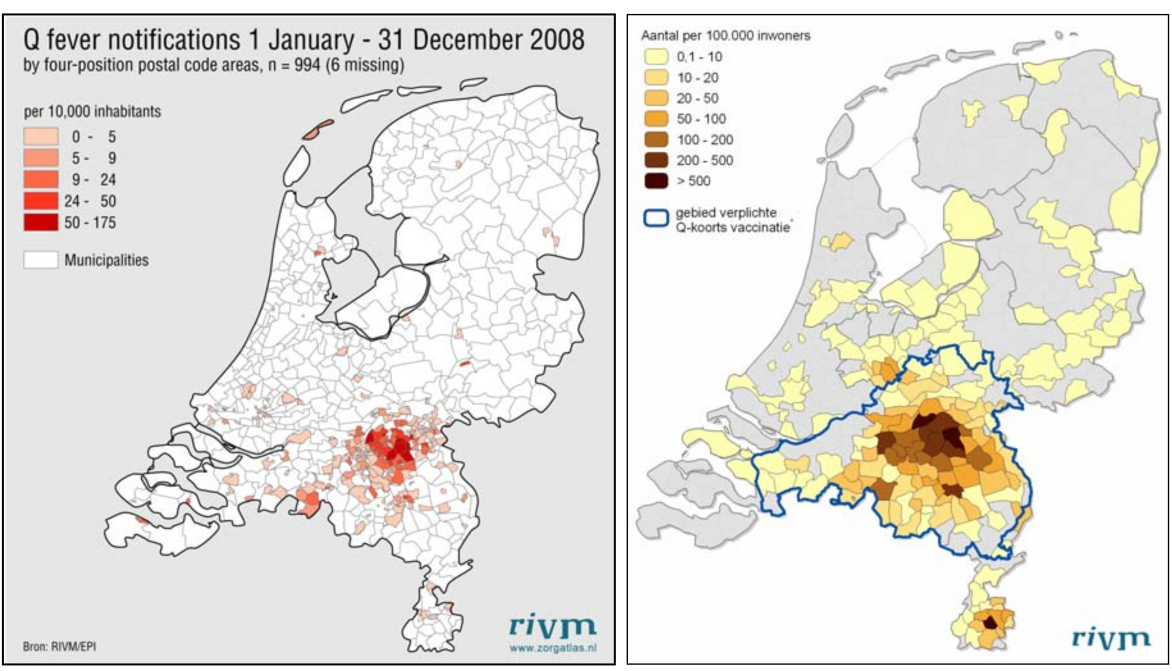

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Figure 1. Q fever on dairy goat farms in 2005-09 (A, Animal Health Service), and in humans in 2008 (B) and 2009 (C, RIVM Epidemiology & Surveillance).

2 Methods

2.1 Origin of samples screened for *C. burnetii* DNA presence

Environmental and veterinary samples were obtained from several locations in the Netherlands, during three successive years of Q fever outbreaks:

1. A farm involved in the first main human cluster area (Herpen) in two subsequent years (week 35 in 2007 & week 25 in 2008).
2. A farm, mandatory notified by the farmer, veterinarian, and the Animal Health Services, thought to be involved in a human Q fever cluster in Zuid-Limburg at three different weeks (14, 21 and 30) in 2009.
3. A farm mandatory notified by the farmer, veterinarian and the Animal Health Services in Overijssel at week 10 in 2009.
4. From several suspected Q fever affected locations, reported by the municipal health services based on human cases in 2008 (409 samples from 30 farms) and 2009 (1139 samples from 57 farms).

2.2 Sampling procedures for environmental and veterinary matrices

Goat farms, implicated as potential sources for Q fever in humans, were visited by employees of the Food and Consumer Product Safety Authority (VWA), or employees from the National Institute for Public Health and the Environment (RIVM) in 2007, 2008, and 2009. Potential sources of *C. burnetii* shedding to the environment are manure, urine, milk, and birth materials like amnion fluid and placenta. Therefore, veterinary matrices included samples from manure (droppings), milk (bulk or individuals), and (when available) placenta materials. In addition, vaginal swabs were obtained from a subset of the goat population on farms. Environmental matrices included samples from surface areas (swabs), water (drinking buckets), and aerosols (air samples).
Manure, water, and milk samples were collected in 50 ml Greiner tubes (Greiner Bio-one, the Netherlands). Surface area swabs and vaginal swabs of animals were taken using sterile cotton swabs (VWR International, the Netherlands). Placenta materials were obtained in frozen condition (-20ºC) from the Animal Health Service (GD). Aerosol samples were collected by using a Sartorius MD8 Airport. Aerosols were captured on nitrate-cellulose filters (pore size 8 µm), by sampling 500L of air using the pre-installed program of 50L per minute. After collection, all obtained environmental and veterinary samples were transported to the laboratory, stored at 4ºC, and processed within one week.

2.3 DNA extraction from environmental and veterinary matrices

DNA was extracted from environmental and animal samples using a single DNA extraction method, the Nuclisens Magnetic Extraction Kit (Biomerieux, France). Small modifications were made to the manufacturer's guidelines for DNA isolation from liquid samples, swabs, and manure samples. For the processing of liquid samples, 1 ml of liquid sample was added to 10 ml of NucliSens lysisbuffer. Surface area swabs and vaginal swabs were added to 10 ml of Nuclisens lysisbuffer. Processing manure samples was carried out by adding goat droppings to Phosphate-buffered Saline (PBS) in 50 ml Greiner tubes (Greiner Bio-one, the Netherlands), using a 1:1 ratio of manure and PBS.
This sample was homogenized for about 2 hours on a rotating tube holder at 10 rpm. Greiner tubes were centrifuged (Varifuge 3.2RS, Heraeus) at 2000 rpm for 10 minutes. The supernatant was transferred to a new Greiner tube, and 1 ml of

supernatant was added to 10 ml of NucliSens lysisbuffer. Cellulose Nitrate filters, used in aerosol sample collection, were placed in Petri dishes and submerged in 10 ml NucliSens lysisbuffer. Petri-dishes were then placed on a horizontal shaker for 2 hours at 50 rpm. To all samples, 1.2×10^5 spores of *Bacillus thuringiensis* were added as internal control for DNA extraction. In addition, 50 µl of magnetic beads were added to each sample and samples were placed at room temperature for one hour to complete lysis and hybridization of DNA to the magnetic beads. After lysis, samples were placed in a magnetic holder for 1 minute and the supernatant was removed. Further steps in DNA extraction were carried out according to the manufacturer's protocol. DNA from (positive) placenta materials was extracted under BSL-3 conditions using a QIAamp DNA Mini Kit according the manufacturer's protocol in the QIAamp DNA Blood Mini Kit Handbook (September 2001).

2.4 Detection of *C. burnetii* DNA by quantitative multiplex real-time PCR

To investigate possible routes of dispersion and transmission, serology cannot be applied. DNA based methods, like quantitative real time PCR (QPCR), detects DNA of the organism of interest directly and are more sensitive than serology based methods. In addition, QPCR can generate data not only on the presence of *C. burnetii* DNA, but also on the *C. burnetii* DNA content in various human, animal, and environmental matrices. For the current study we developed a quantitative multiplex real time PCR assay (QPCR) in which three genomic targets commonly used for the detection of *C. burnetii* DNA (*icd*, *com1* & *IS1111*) are combined into a single assay (Table 1). The development of this assay is extensively described in the 2008 report "Molecular detection and typing of *Coxiella burnetii* ". The three genomic targets selected are: the isocitrate dehydrogenase gene (*icd*), an outer membrane protein coding gene (*com1*) and a multi copy insertion element (*IS1111*). In addition, an internal control target (*B. thuringiensis* gene cry 1) was added to the assay to investigate possible inhibition on the QPCR assay, by the complex environmental and animal samples. PCR assays were carried out on a Roche LightCycler 480 PCR machine.

The results of qPCR assays on DNA extracts obtained from animal and veterinary matrices are categorised as negative or positive. In the following paragraphs the number and percentages of negative and positive samples are reported for the different studies.
The level of *C. burnetii* DNA in the various matrices is mentioned, however, the actual Cq values for the different *C. burnetii* targets are not reported. Including the actual data would make the tables and text very complex.

Primers & probe names		Primer and probe sequences (5'-> 3')	Positions	Product length
target *icd*				
forward primer	icdpri_f	GACCGACCCATTATTCCCT	1144122 - 1144138	
reverse primer	icdpri_r	CGGCGTAGATCTCCATCCA	1144001 - 1144019	139
probe (FAM)	Tqpro_icd	CGCCCGTCATGAAAAACGTGGTC	1144065 - 1144087	
target *com1*				
forward primer	compri_f	AAGCAATTAAAGAAAATGCAAAGAAATTAT	1829595 - 1829624	
reverse primer	compri_r	ACAGAATTCATGGCTTTGCAAT	1829706 - 1829727	133
probe (JOE)	Tqpro_com	CACATTGATAATCGAAAAATTCAACCAATG	1829673 - 1829702	
target *IS1111*				
forward primer	IS1pri_f	CGCAGCACGTCAAACCG	1715999 - 1716016	
reverse primer	IS1pri_r	TATCTTTAACAGCGCTTGAACGTC	1716122 - 1716145	146
probe (T-red)	Tqpro_IS1	ATGTCAAAAGTAACAAGAATGATCGTAAC	1716018 - 1716046	
target *Cry1*				
forward primer	Btpri_f	GCAACTATGAGTAGTGGGAGTAATTTAC		
reverse primer	Btpri_r	TTCATTGCCTGAATTGAAGACATGAG	n/a	132
probe (Cy5)	Tqpro_Bt	ACGTAAATACACTTGATCCATTTGAAAAG		

Table 1. Primers and probes for each target developed in Visual Omp 6 for the multiplex Q-PCR for *C. burnetii* DNA. Primer and probe sequences, the annealing positions on *C. burnetii* strain Nine Mile RSA phase I (RSA493) and product lengths obtained are given for *C. burnetii* targets *icd*, *com1*, and *IS1111*.

3 Results

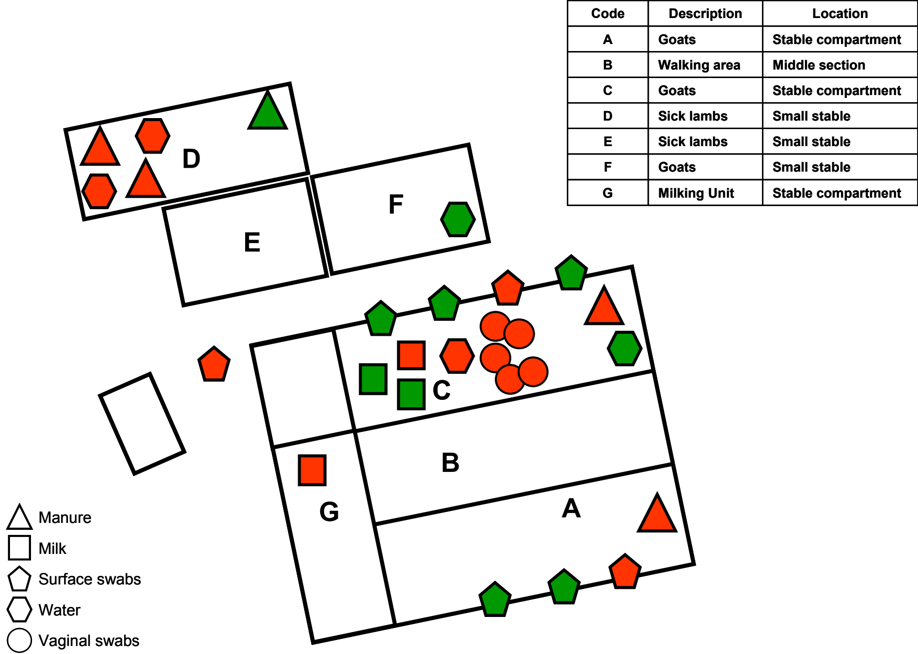
3.1 Temporal study I: monitoring a single farm in 2007 & 2008

In 2007, a single (goat)farm was implicated as one of the potential sources for the human Q fever outbreak in the vicinity of Herpen. On this farm a large abortion wave was reported among goats prior to the Q fever outbreak. In week 35 of 2007, samples of various environmental and animal matrices were obtained and screened to investigate if *C. burnetii* DNA was present. Environmental samples included surface area swabs, manure, and water. Animal samples were represented by milk and vaginal swabs of individual animals. A schematic representation of the farm can be found in Figure 2. In 2008, the same farm was visited again, this time in week 25 during the peak of the Q fever outbreak in the same rural area. Again, environmental and animal matrices were obtained from the same locations within the farm as in 2007.
A total of 68 animal and environmental samples were collected in the two successive years. The number of samples per category can be found in Table 2. Samples were screened using the multiplex quantitative real time PCR assay and were scored as positive when at least one of the *C. burnetii* DNA targets (*icd*, *com1*, or *IS1111*) showed a positive signal in combination with a positive signal in the internal control target (*cry1*). Samples were scored as negative when no *C. burnetii* targets showed positive signals in combination with a positive result for the internal control.
In 2007, 17 out of 27 samples were found to be positive for *C. burnetii* DNA. In 2008, the number of positive samples was 40, out of a total of 41 samples. All vaginal swabs, taken from goats, were found to be positive for *C. burnetii* DNA in the two successive years. Two out of the four milk samples were positive in 2007, while in 2008 all 10 milk samples were found to contain *C. burnetii* DNA. The number of manure samples showed comparable numbers of samples and positive samples between the two years. Water samples showed 3 out of 5 samples positive in 2007, while in 2008 all 6 water samples were found to be positive.

Category	Description	2007		2008	
		# Samples	# Positives	# Samples	# Positives
Animal	Vaginal Swabs (goats)	5	5	10	10
	Milk	4	2	10	10
	Manure	5	4	6	5
Environment	Surface Swabs	8	3	9	9
	Water	5	3	6	6

Table 2. Animal and environmental samples obtained from a commercial goat farm and screened with the multiplex real-time PCR assay.

Inhibition of the QPCR assay was occasionally observed. The strongest inhibition on the PCR assay was observed in environmental samples, like surface area swabs. In some PCR runs on these samples, the internal control (*B. thuringiensis* gene *cry1*) failed to produce a signal when undiluted DNA template was added to the PCR reaction mixture. Only after the samples were diluted 10 or 100 times, a positive signal could be observed for *cry 1*, indicating that these environmental matrices can inhibit our multiplex real time PCR assay quite strongly. The assay, however, was still able to detect *C. burnetii* DNA in these diluted samples.



Code	Description	Location
A	Goats	Stable compartment
B	Walking area	Middle section
C	Goats	Stable compartment
D	Sick lambs	Small stable
E	Sick lambs	Small stable
F	Goats	Small stable
G	Milking Unit	Stable compartment

Figure 2. Schematic representation of a dairy goat farm, implicated in the 2007 Q fever outbreak. Letters indicate different compartments within the farm where samples were taken. Symbols indicate the different sample categories. Symbols in red indicate positive samples, and symbols in green negative samples.

The monitoring of a single farm during two distinct Q fever outbreak seasons showed that a number of animals are at least carriers of the bacterium. These carriers may shed *C. burnetii* directly into the environment during lambing or regular excretion. In addition, *C. burnetii* DNA accumulates onto surface areas in relatively high concentrations compared to manure, milk, and water. This indicates that *C. burnetii* transmission from the environment (in stables) to animals and humans by contaminated aerosols is plausible.
Results from 2008 showed more positive samples than in 2007. The reason for this is not quite clear, since the number of abortions during the 2008 lambing season was below 5%, while this exceeded the 5% level substantially in 2007. The *C. burnetii* DNA content may vary over time and between matrices which may explain the results obtained from the two different outbreak seasons.

3.2 Temporal study II: a single farm in 2009, unrelated to human cases

A second temporal study was started in February 2009, when a large abortion wave among goats was observed on a farm in the province of Overijssel. Q fever among goats was diagnosed by the Animal Health Service (GD). This was the first reported Q fever positive farm in 2009 and located outside the vaccination area established in February 2009. No human cases were reported in the vicinity of this farm in the weeks following this abortion wave. Eventually, in 2009, 2 cases were reported in this area.
Environmental samples included surface area swabs and aerosol samples within the stables and in the courtyard of the farm. In addition, aerosol samples were also taken within 1 and 5 km radius of the farm in all directions (North, East, South & West).

Within the stable, surface area swabs contained the highest level of *C. burnetii* DNA in comparison to aerosol samples. In contrast, aerosol samples obtained from 8 locations (North, South, East, and West) within 1 and 5 km distance radius of the farm showed no *C. burnetii* DNA content. These findings are in concordance with the lack of human cases in this region. Despite the lack of human cases, the farmer was quite reluctant to cooperate when we announced to obtain aerosol samples in the direct surroundings of the farm.

3.3 Temporal study III: a single farm in 2009, related to human cases

We started a third temporal study in March 2009, when a large abortion wave among goats was observed on a farm in the province of Zuid-Limburg. Q fever among goats was diagnosed by the Animal Health Service. In addition, Q fever among humans on the farm was diagnosed by the Municipal Health Service (GGD) Zuid-Limburg. The farmer on this location was very cooperative and it was decided to follow the presence of *C. burnetii* DNA in environmental samples on this farm for at least 90 days as part of a newly started project for the Ministry of VWS. Ninety days is the time interval that manure excreted by the goats has to be confined and isolated on the farm itself and cannot be dispersed over meadows in fertilisation procedures.

In weeks 14, 21, and 30 of 2009, samples of various environmental and animal matrices were obtained and screened to investigate if *C. burnetii* DNA content changed during this time period. Environmental samples included surface area swabs, and aerosol samples within the stables and in the courtyard of the farm. In addition, aerosol samples were also taken within a 1 km radius of the farm in all directions (North, East, South & West). Animal samples were represented by manure droppings and mouth swabs of individual animals.

Category	Description	Week 14		Week 21		Week 30	
		# Samples	# Positives	# Samples	# Positives	# Samples	# Positives
Animal	Mouth Swab	1	1	-	-	4	4
	Manure	4	4	-	-	4	4*
Environment	Surface Swabs	5	5	-	-	4	4
	Aerosols (stables)	2	2	-	-	2	2
	Aerosols (courtyard)	2	2	-	-	2	2
	Aerosols (1 km North)	1	1	1	0	1	1
	Aerosols (1 km South)	1	1	1	1	1	1
	Aerosols (1 km East)	1	1	1	0	1	1
	Aerosols (1 km West)	1	1	1	1	1	1

Table 3. Animal and environmental samples obtained from a location in Zuid-Limburg in 2009.

Samples obtained in week 14 were all found to be positive, but differed in *C. burnetii* DNA content. *Coxiella burnetii* content in aerosol samples obtained in the courtyard was found to be lower compared to aerosol samples obtained in the stable. In addition, *C. burnetii* DNA content in aerosol samples, obtained from 4 locations (north, south, east, and west) within a 1 km distance radius of the farm was lower compared to aerosol samples obtained in the courtyard.
In week 21 we were able to take only aerosol samples from a 1 km distance radius in the 4 directions (north, west, east & south) from the farm. Two aerosol samples were found to be positive: the southern and western locations. The level of *C. burnetii* DNA content on these locations was lower than in week 14.
In week 30, Aerosol samples in stables again contained more *C. burnetii* DNA than aerosol samples obtained from the courtyard. Aerosol samples obtained from within a 1 km distance from the farm showed lower *C. burnetii* DNA

content than aerosol samples obtained from the courtyard. Surface area swabs again inhibited the PCR assay to some extent. In contrast, manure samples inhibited the PCR-assay quite severely, with one sample showing a negative result for the internal control even after a 100-fold dilution.
When all three sampling dates are compared, the number of positive samples is the same between weeks 14 and 30. However, *C. burnetii* DNA content detected within and around this farm is highest in week 14, just after the abortion wave among the goats and emerging human Q fever cases on the farm and in its near vicinity based. *C. burnetii* DNA content in all matrices sampled is quite consistent when samples from weeks 14 and 30 are compared. The highest level of *C. burnetii* DNA content, within sampling dates, is found in surface area swabs obtained in the stables and mouth swabs of goats, followed by aerosols sampled in the stables. The level of *C. burnetii* DNA in manure obtained from stables is lower and comparable to the content of *C. burnetii* DNA in aerosols sampled in the courtyard. The lowest *C. burnetii* DNA content is found in aerosols obtained from 1 km distance in all four wind directions.

3.4 Q fever source finding investigations in 2008 & 2009

In 2008, the Q fever outbreak started in the month May around week 19. One of the first reports of human Q fever started to emerge in the province of Gelderland, in the city of Nijmegen on a mental health care centre (7). In the subsequent weeks more human Q fever cases occurred all over the province. The Municipal Health Service (GGD) 'Hart voor Brabant' started an active source finding study by plotting human Q fever cases on the map of the province using 4-digit zip code addresses. This information was used, together with address information of (commercial) dairy goat farms, to pinpoint potential sources of *C. burnetii* infection.
The Municipal Health Service, in close collaboration with the Food and Consumer Product Safety Authority (VWA) made selections of the most likely sources of *C. burnetii* infection during the outbreak. Employees of the Food and Consumer Product Safety Authority sampled primarily animal matrices (vaginal swabs) on dairy goat farms, which were considered the most likely source for human Q fever cases. Samples were transported to the National Institute for Public Health and the Environment (RIVM), which conducted the actual screening for *C. burnetii* DNA by multiplex quantitative real time PCR (QPCR).

Between May and December of 2008, 409 samples divided over 30 farms were screened by the Laboratory for Zoonosis and Environmental Microbiology (LZO) of the RIVM. Samples screened were primarily animal matrices: vaginal swabs of goats, sheep & cattle, and to some extend blood, milk, and manure was screened. Environmental matrices were represented by surface area swabs. Results of this screening can be found in Table 4. Seven locations (23%) were unlikely sources for the Q fever human cases in their near vicinity, because none of the samples tested positive. On 23 locations (77%), at least one sample scored positive and in 16 locations (53%), 50% or more of the samples tested positive. The total number of positive samples was found to be 190 (46%). On most farms, positive results for vaginal swabs in goats and/or sheep were accompanied by positive results for the surface area swabs taken on the same farm, and vice versa, if animal matrices scored negative in the assay, surface area swabs on the same farm also scored negative. These results indicate that both animal matrices like vaginal swabs and environmental matrices like surface area swab are good indicators for the presence of *C. burnetii*.

In 2009, the Q fever outbreak started in April around week 14. Again, most cases started to emerge in the province of Brabant. The relevant Municipal Health Services (GGD's) started active source finding, again in close collaboration with the Food and Consumer Product Safety Authority (VWA) and RIVM. Between April and October of 2009, 1008 samples divided over 57 farms were screened by RIVM-LZO. The same matrices were sampled and screened as in 2008. Results of this screening can be found in Table 5.
The percentage of positive samples per location again showed a large variation. Sixteen locations (26%) were unlikely sources for the human cases in their near vicinity, because none of the samples tested positive. In 41 locations (72%), at least one sample scored positive and in 30 locations (53%), 50% or more of the samples tested positive. The total number of positive samples was found to be 488 (48%).

Again, on most farms, positive results for vaginal swabs in goats and/or sheep were accompanied by positive results for the surface area swabs taken on the same farm, and vice versa, if animal matrices scored negative in the qPCR assay, surface area swabs on the same farm also scored negative. Screening results were forwarded by the Food and Consumer Product Safety Authority (VWA) to the Municipal Health Service (GGD's).

Farm	Location	Sampling date	Source	Matrix	Positive	Negative	Total	% Positives
1	Afferden	6-8-2008	Goat	Vaginal swab	3	0	3	70
				Blood	0	3	3	
			Environment	Surface swab	4	0	4	
2	Bergharen	10-9-2008	Cow	Vaginal swab	0	19	19	0
3	Boven-Leeuwen		Environment	Surface swab	0	3	3	0
4	Echt	25-7-2008	Sheep & Goat	Vaginal swab	2	19	21	10
5	Goirle	23-6-2008	Goat	Vaginal swab	10	0	10	100
6	Groesbeek	15-5-2008	Sheep	Vaginal swab	5	0	5	83
				Milk	0	1	1	
7	Nijmegen		Sheep	Manure	4	2	6	67
8	Helmond	27-6-2008	Sheep & Goat	Vaginal swab	3	9	12	21
				Blood	2	10	12	
9	Herpen	19-5-2008	Goat	Vaginal swab	10	0	10	91
				Milk	10	0	10	
				Manure	4	2	6	
			Environment	Surface swab	5	1	6	
10	Horssen		Environment	Surface swab	5	0	5	100
11	Lith	11-12-2008	Goat	Vaginal swab	9	4	13	65
			Environment	Surface swab	4	3	7	
12	Maren-Kessel		Environment	Surface swab	5	0	5	100
13	Nuland	8-10-2008	Sheep & Goat	Vaginal swab	3	9	12	25
14	Odiliapeel	5-6-2008	Sheep	Vaginal swab	0	10	10	0
			Environment	Surface swab	0	5	5	
15	Odiliapeel	5-6-2008	Goat	Vaginal swab	0	4	4	0
				Surface swab	0	5	5	
16	Oijen	11-9-2008	Goat	Vaginal swab	10	9	19	53
17	Overasselt	19-5-2008	Sheep & Goat	Vaginal swab	2	10	12	14
				Milk	0	1	1	
				Manure	1	11	12	
				Surface swab	1	3	4	
18	Reek	5-6-2008	Goat	Vaginal swab	1	1	2	14
			Environment	Surface swab	0	5	5	
19	Reek	26-6-2008	Goat	Vaginal swab	1	2	3	33
20	Rosmalen	8-10-2008	Goat	Vaginal swab	17	3	20	85
21	Schaijk	26-6-2008	Goat	Vaginal swab	10	0	10	100
22	Schaijk	26-6-2008	Goat	Vaginal swab	10	0	10	100
23	Schijndel	5-9-2008	Sheep	Vaginal swab	0	16	16	0
24	St. Oedenrode	22-5-2008	Goat	Vaginal swab	10	0	10	100
			Environment	Surface swab	9	0	9	
25	Vinkel	22-5-2008	Cow	Vaginal swab	6	4	10	46
			Environment	Surface swab	0	3	3	
26	Wijk bij Duurstede	22-10-2008	Sheep & Goat	Vaginal swab	0	22	22	0
27	Zeeland	23-6-2008	Goat	Vaginal swab	0	4	4	0
28	Zeeland	23-6-2008	Sheep	Vaginal swab	2	7	9	22
29	Zeeland	16-6-2008	Sheep	Vaginal swab	8	4	12	67
30	Zeeland	19-6-2008	Cow & Sheep	Vaginal swab	14	5	19	74
Total number of positive and negative samples					190	219	409	
Percentage of positive and negative samples					46	54		

Table 4. Animal and environmental samples obtained from 30 locations within the province of Brabant in 2008. Indicated is the number of positive and negative samples, the total number of samples, and percentage of positive samples per farm.

Farm	Location	Sampling date	Source	Matrix	Positive	Negative	Total	% Positive samples
1	Aalst	17-7-2009	Sheep	Vaginal swab	11	2	13	88
			Environment	Surface area swab	4		4	
2	Aarle Rixel	13-5-2009	Sheep	Vaginal swab	8	26	34	24
3	Alem location 1	10-8-2009	Environment	Surface area swab	1		1	7
			Goat	Vaginal swab		14	14	
4	Alem location 2	10-8-2009	Goat	Vaginal swab		3	3	0
5	Appeltern	18-5-2009	Goat	Vaginal swab	2	12	14	11
			Environment	Surface area swab		4	4	
6	Bakel	13-5-2009	Goat	Vaginal swab	20		20	100
7	Belfeld	8-7-2009	Goat	Vaginal swab	20		20	100
8	Boven-Leeuwen	10-8-2009	Goat	Vaginal swab		22	22	0
9	Bunnik	15-7-2009	Goat	Vaginal swab	19		19	100
			Environment	Surface area swab	4		4	
10	Den Bosch	9-7-2009	Goat	Vaginal swab	6	14	20	30
11	Deventer	30-9-2009	Sheep & goat	Vaginal swab		20	20	0
12	Dreumel	7-8-2009	Goat	Vaginal swab	7	7	14	53
			Environment	Surface area swab	1		1	
13	Drunen	16-7-2009	Geit	Vaginal swab	10	10	20	50
14	Echt	13-7-2009	Sheep & goat	Vaginal swab	4	15	19	21
15	Haghorst	25-6-2009	Goat	Vaginal swab	20		20	100
16	Haren (NB)	11-8-2009	Goat	Vaginal swab	2	12	14	14
17	Heerhugowaard	9-7-2009	Goat	Vaginal swab	14	5	19	84
			Environment	Surface area swab	12		12	
18	Heerlen	2-7-2009	Sheep	Vaginal swab	9	11	20	45
19	Helvoirt location 1	16-7-2009	Geit	Vaginal swab		14	14	0
20	Helvoirt location 2	16-7-2009	Geit	Vaginal swab	10	9	19	53
21	Hilvarenbeek location 1	25-6-2009	Goat	Vaginal swab	16	3	19	84
22	Hilvarenbeek location 2	25-6-2009	Goat	Vaginal swab	19	1	20	95
23	Horssen	20-5-2009	Goat	Vaginal swab	19	1	20	95
24	Houten location 1	4-6-2009	Sheep & goat	Vaginal swab	1	13	14	7
25	Houten location 2	15-7-2009	Sheep	Vaginal swab		10	10	0
26	Houten location 3	15-7-2009	Goat	Vaginal swab	2	11	13	18
			Environment	Surface area swab	1	3	4	
27	Houten location 4	15-7-2009	Goat	Vaginal swab	13	7	20	68
			Environment	Surface area swab	2		2	
28	Leffen location 1	28-7-2009	Sheep	Vaginal swab	10	9	19	53
29	Leffen location 2	30-7-2009	Sheep	Vaginal swab	16	6	22	73
30	Lutten	26-8-2009	Goat	Vaginal swab		20	20	0
			Environment	Surface area swab		9	9	
31	Maren Kessel	11-8-2009	Goat	Vaginal swab	4	15	19	21
32	Megen	11-8-2009	Goat	Vaginal swab	14	6	20	70
33	Milsbeek	24-6-2009	Birds	Manure		8	8	0
			Environment	Surface area swab		4	4	
34	Nistelrode	9-9-2009	Sheep	Vaginal swab		20	20	0
35	Nuenen	15-5-2009	Sheep	Vaginal swab	18	1	19	95
36	Nuland	17-6-2009	Horse	Milk		3	3	0
				Vaginal swab		4	4	
37	Oerle-Veldhoven	25-6-2009	Goat	Vaginal swab	17	3	20	85
38	Oldenzaal	3-7-2009	Goat	Vaginal swab	13	7	20	65
39	Ommeren	21-8-2009	Sheep	Vaginal swab		20	20	0
40	Oss location 1	28-7-2009	Sheep	Vaginal swab	12	8	20	60
41	Oss location 2	28-7-2009	Goat	Vaginal swab	13	7	20	68
			Environment	Surface area swab	2		2	
42	Oss location 3	28-7-2009	Goat	Vaginal swab	4	1	5	67
			Environment	Surface area swab		1	1	
43	Rhenen	7-8-2009	Goat	Vaginal swab		5	5	0
			Environment	Surface area swab		1	1	
44	Schaijck lokation 1	8-7-2009	Goat	Vaginal swab	17	3	20	85
45	Schaijck lokation 2	8-7-2009	Goat	Vaginal swab	20		20	100
46	Schaijck location 3	3-9-2009	Cattle	Milk		5	5	0
			Environment	Filter		4	4	
47	Scherpenisse	17-7-2009	Sheep	Vaginal swab	3	2	5	67
			Environment	Surface area swab	1		1	
48	St-Michielsgestel	16-7-2009	Goat	Vaginal swab	1	19	20	5
49	Tilburg	27-7-2009	Goat	Vaginal swab	7	3	10	58
			Environment	Surface area swab		2	2	
50	Veghel	23-4-2009	Sheep	Vaginal swab		5	5	0
51	Venray	24-6-2009	Birds	Surface area swab	3	9	12	25
52	Voorschoten	18-9-2009	Sheep & goat	Vaginal swab		23	23	0
53	Vorstenbosch	9-9-2009	Sheep & goat	Vaginal swab		7	7	0
54	Vlijmen	17-6-2009	Goat	Vaginal swab	21		21	86
			Environment	Surface area swab	4	4	8	
55	Weerselo	19-6-2009	Goat	Vaginal swab	20		20	93
			Environment	Surface area swab	7	2	9	
56	Zeeland location 1	20-7-2009	Horse	Vaginal swab		20	20	0
				Milk		1	1	
			Environment	Surface area swab		1	1	
57	Zeeland location 2	8-7-2009	Red Deer	Manure		1	1	57
			Environment	Milk filter	2		2	
			Envrionment	Surface area swab	2	2	4	
Total number of positive and negative samples					488	520	1008	
Percentage of positive and negative samples					48	52		

Table 5. Animal and environmental samples obtained from 57 locations Within the province of Brabant in 2009. Indicated is the number of positive and negative samples, the total number of samples, and percentage of positive samples per farm.

4 Discussion

4.1 Temporal studies

In temporal study I, *Coxiella burnetii* was found in veterinary and environmental samples obtained from a single farm during two successive years of Q fever outbreaks (2007 & 2008). Abortions among goats were observed on a large scale only in 2007. In 2008 no abortions were observed on this particular farm. This indicates that *C. burnetii* can persist and be detected in large quantities on farms for prolonged periods of time, without causing abortions or stillbirths among goats or without being produced by these, as reported previously (8,9)
In temporal study II, *C. burnetii* DNA was found in all environmental samples examined and the Animal Health Service found *C. burnetii* in veterinary matrices. This particular farm differs from the farm in temporal study I in the fact that almost no human Q fever cases were observed in the near vicinity of this farm in 2009.
Temporal study III showed that within the Q fever outbreak of 2009, the *C. burnetii* DNA content in both veterinary and environmental samples declined after the initial wave of abortions among goats. It seems plausible, yet unproven, that the decline of the number of human Q fever cases can be related to the declining *C. burnetii* DNA concentrations found on farms and their direct surroundings in the months after the lambing season.
The level of *C. burnetii* DNA content in veterinary and environmental samples on farms in temporal study II & III is comparable. Both farms showed abortions among goats on a large scale in the lambing season of 2009. However, the farm in temporal study II can not be linked to a significant number of human Q fever cases in the near vicinity of the farm, while the farm in temporal study III is implicated to be involved in a human Q fever cluster in the same region. Both farms are located far outside the Q fever-affected area in the Netherlands, Noord-Brabant. The discrepancy between the farms in temporal studies I, II, and III in the involvement in human Q fever outbreaks is not entirely clear. Probably, differences in farming procedures, farm construction, or local climate differences (drought, rainfall, wind directions at the time of the abortion wave), and local population densities played a role in these differences.

4.2 Source investigations

The highest level of *C. burnetii* DNA content was found in placenta materials, obtained from aborting goats, during an abortion wave on dairy goat farms in 2007. These positive materials were provided by the Animal Health Service (GD) and were screened to investigate the level of *C. burnetii* DNA content, in comparison to other more easily obtainable animal and environmental matrices. Placenta materials are not easily obtained, because the moment of birth or abortion is unpredictable, and these materials are consumed by the goats or become untraceable due to dispersion in the stables.

Both temporal and source investigation studies showed that, after placenta materials, vaginal swabs obtained from goats contained the highest level of *C. burnetii* DNA in the veterinary matrix category. Milk samples, obtained from individual goats, also contained *C. burnetii* DNA, however, to a lesser extent than vaginal swabs obtained from the same animals. Manure droppings, obtained from within the stables, contained more *C. burnetii* DNA than milk samples, but less than in vaginal swabs. We have to take into account however that sample matrix and DNA extraction efficiency differs for these different

sample categories and hence the measured *C. burnetii* DNA content cannot be extrapolated to a number of *C. burnetii* bacteria present in the original sample material. Comparisons within one sample category are therefore justified. Comparisons between the different categories should be regarded as qualitative. They merely represent the overall efficiency for detecting *C. burnetii* DNA in relation to each other.

In the environmental category, surface area swabs contained the highest level of *C. burnetii* DNA and this level was comparable to the vaginal swabs of goats. However, surface area swabs can inhibit the PCR assay quite considerably, which underestimates the level of *C. burnetii* DNA content in this matrix. In most farms, positive results for vaginal swabs in goats and/or sheep were accompanied by positive results for the surface area swabs taken on the same farm, and vice versa, if animal matrices scored negative in the assay, surface area swabs on the same farm also scored negative. These results indicate that both animal matrices like vaginal swabs and environmental matrices like surface area swab are good indicators for the presence of *C. burnetii*.

Aerosols provide valuable information on potential transmission routes from the environment to animals and humans. The highest level of *C. burnetii* DNA content was found in aerosols sampled in the stables. The *C. burnetii* DNA content in aerosols sampled in the courtyard was lower than obtained from within the stables. The lowest *C. burnetii* DNA content was found in aerosols obtained from 1 km distance (in all four wind directions) of a Q fever affected farm. Although currently available data are limited, this seems a plausible outcome.

The relation between the *C. burnetii* DNA content in the matrices screened at one particular time point is quite consistent when followed over an extended period of time.

These findings are of importance, since shedding of *C. burnetii* directly into the environment via the vagina during lambing or regular excretion, and subsequent dispersion via contaminated aerosols is thought to be the primary route for human *C. burnetii* infection. Therefore, it is important to obtain at least (i) vaginal swabs from individual animals (goats or sheep), (ii) surface area swab from within the stables and (ii) aerosol samples from the stables and near surroundings (courtyard to 1-2 km distance) during sampling procedures in source finding investigations.

5 Conclusions

Coxiella burnetii was found in veterinary and environmental samples obtained from a single Q fever affected farm during two successive years of Q fever outbreaks (2007 & 2008). Therefore, the public health risk for farmers and individuals living in the vicinity of such a farm encompasses more than one lambing season.

C. burnetii DNA can be detected in aerosols on Q fever-affected goat farms, and their direct surroundings.

Within the Q fever outbreak of 2009, *C. burnetii* DNA could be detected for at least 16 weeks, although the *C. burnetii* DNA content in both veterinary and environmental samples declined over time after the initial wave of abortions among goats. Although the public health risk seems to decrease over time, consistent with the observed decrease in cases over the year, transmission from the environment to humans can take place up to several months following the lambing season.

Screening results for vaginal swabs obtained from goats and/or sheep are consistent with results for surface area swabs taken on the same farm.

During outbreak investigations it is important to obtain at least (i) vaginal swabs from individual animals (goats or sheep), (ii) surface area swabs from within the stables and (iii) aerosol samples from the stables and near surroundings courtyard to 1 to 2 km distance in source finding investigations.

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6 Literature

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