

Detection of *Coxiella burnetii* DNA in Inhalable Airborne Dust Samples from Goat Farms after Mandatory Culling

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***Coxiella burnetii* is thought to infect humans primarily via airborne transmission. However, air measurements of *C. burnetii* are sparse. We detected *C. burnetii* DNA in inhalable and PM10 (particulate matter with an aerodynamic size of 10 μm or less) dust samples collected at three affected goat farms, demonstrating that low levels of *C. burnetii* DNA are present in inhalable size fractions.**

Q fever is a zoonotic disease caused by the Gram-negative intracellular bacterium *Coxiella burnetii* (1). Transmission of *C. burnetii* from animals to humans is considered to be primarily through air via inhalation of particles containing viable *C. burnetii* cells (1, 7, 8, 10). However, only a few published studies have actually demonstrated airborne *C. burnetii* (2, 3, 5, 12) and most did not take into account the size fraction of the dust particles collected (2, 3, 5). Given the respiratory route of infection, dust particles that can penetrate the respiratory organ should be considered. Examples of these are PM10 (particulate matter with an aerodynamic size of 10 μm or less) or inhalable dust particles (particulate matter with a 50% cutoff of 30 μm). One study detected *C. burnetii* in airborne inhalable dust samples in a sheep barn during shearing of an infected herd; however, the number of samples measured was very limited (12).

The motivation for this study was a human Q fever outbreak in the Netherlands. Infected dairy goats were identified as the primary source for the epidemic (8, 11), and to control the spread of *C. burnetii*, all pregnant goats on bulk tank milk (BTM)-positive farms were culled (6, 13, 14). The aim of the present study was to detect *C. burnetii* DNA in inhalable dust samples on a larger scale and to describe a method to detect *C. burnetii* DNA in inhalable dust and PM10 dust samples

Collection of samples. The presence of *C. burnetii* DNA was investigated in air samples collected on three BTM-positive goat farms. Mandatory culling of pregnant goats had taken place 32 to 76 days prior to sampling. Some does had already kidded prior to the culling date; therefore, those does and their kids were still present on the farm during sampling. In addition, does that were not pregnant (both lactating and nonlactating) as well as bucks were present. Sampling was conducted between 12 March and 6 April 2010. PM10 samples were captured on Teflon filters (2.0 μm pore size; Air Diagnostics and Engineering Inc., United States) with Personal Environmental Monitor collection heads (SKC Inc., United States), which were connected to a BGI pump (BGI USA, Waltham, MA) (airflow, 4 liters per min). Inhalable dust samples were captured on 37-mm-diameter glass fiber filters (GF/A; Whatman, USA) with GSP collection heads (JS Holdings, United Kingdom) connected to a Gilliair pump (Gillian, United Kingdom) (airflow, 3.5 liters per min). Sampling heads were installed at 1.50 m aboveground using a tripod. Both sampling sets (inhal-

able dust and PM10) were installed side by side. Several sets were placed at different sites around the farm, namely, in the milking parlor, milking doe pen, young stock pen, and buck pen, as well as inside the farmer's house and upwind and downwind of the farm (distance to farm between 20 and 70 m).

Sampling was performed during 4 h in a period of minimal activity. To investigate if routine farm activities would affect the outcome, sampling was also performed at one farm during two periods of activity: (i) during milking of the goats and (ii) when deep straw bedding was being removed from the stables. Due to logistical constraints, sampling during activity was not performed on all three farms.

In addition to the airborne dust samples, settled dust samples were collected with a clean (ethanol) plastic scraper from horizontal surfaces in the stables.

After sampling, filters and dust samples were stored at -20°C until processing began.

Sample processing and DNA extraction procedures. Filters were transferred to 50-ml tubes (Greiner Bio-one) containing 4 ml pyrogen-free water (Aqua B. Braun) with 0.05% Tween 20 (Calbiochem, United States). Tubes were agitated for 1 h on an end-over-end roller and centrifuged for 15 min at $1,000 \times g$. NucliSens lysis buffer (36 ml) (bioMérieux, France) was added to the tubes, followed by agitation on the end-over-end roller for 1 h. Samples were centrifuged for 15 min at $2,000 \times g$. The supernatant was transferred to a new tube. As an internal control for both DNA extraction and qPCR amplification, 7×10^4 spores of *Bacillus thuringiensis* (50 μl of a 1:10 diluted spore suspension; Raven Labs, Omaha, NE) was added to the supernatant, followed by DNA extraction with the NucliSens Magnetic Extraction Kit (bioMérieux, France) according to the manufacturer's protocol.

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TABLE 1 Presence of *C. burnetii* DNA in dust samples by sample type^a

Sample location	Result ^b for sample type		
	PM10	Inhalable dust	Settled dust
Kitchen			
Farm 1	–	–	n/a
Farm 2	–	–	n/a
Farm 3	+	+	n/a
Young stock			
Farm 1	+	–	++
Farm 2	–	–	++
Farm 3	–	++	++
Milking does			
Farm 1	–	–	++
Farm 1	–	–	–
Farm 2	++	–	++
Farm 3	–	+	++
Farm 3	–	–	++
Milking parlor			
Farm 1	–	–	++
Farm 2	–	–	++
Farm 3	–	–	++
Bucks			
Farm 1	–	+	++
Farm 2	–	+	++
Farm 3	–	Undetermined ^c	++
Downwind			
Farm 1	–	–	n/a
Farm 2	–	–	n/a
Farm 3	–	–	n/a
Upwind			
Farm 1	–	–	n/a
Farm 2	–	–	n/a
Farm 3	–	–	n/a

^a PM10, inhalable dust, and settled dust samples were collected during a period of minimal activity at various locations on three bulk tank milk-positive farms, 32 to 62 days after culling (35 days at farm 1, 62 days at farm 2, and 32 days at farm 3).
^b ++, *C. burnetii* IS1111 + *com1* positive; +, *C. burnetii* IS1111 positive; –, *C. burnetii* DNA negative; n/a, not available.
^c Undetermined sample showed PCR inhibition and no signal for internal control (*cry1b*).

Multiplex qPCR. *C. burnetii* DNA was determined by multiplex quantitative PCR (qPCR) as described previously by de Bruin et al. (4). Briefly, the qPCR detects one *B. thuringiensis* internal control target (*cry1b*) and two *C. burnetii* targets: a single-copy outer membrane coding gene, *com1*, and a multicopy insertion element, IS1111. The multiplex qPCR assays were carried out on a LightCycler 480 instrument (Roche Diagnostics Nederland B.V.), and analysis was performed with the instrument software (LightCycler 480 software release 1.5.0. SP3).

C. burnetii DNA-positive samples were categorized into two classes, with increasing *C. burnetii* DNA content: (i) IS1111 positive (+) and (ii) IS1111 + *com1* positive (++), as amplification of multicopy target IS1111 is expected to occur before amplification of single-copy target *com1* (9). Samples were scored as negative (–) when neither of the *C. burnetii* targets showed a positive signal

TABLE 2 Presence of *C. burnetii* DNA in dust samples by activity^a

Sample location	Result ^b for activity and sample type					
	Minimal activity, 62 days postculling		Milking activity, 75 days postculling		Bedding removal, 76 days postculling	
	PM10	Inhalable	PM10	Inhalable	PM10	Inhalable
Young stock	–	–	–	+	–	+
Milking does	++	–	–	–	+	+
Milking parlor	–	–	–	+	–	–
Downwind of farm	–	–	–	+	+	+
Upwind of farm	–	–	–	–	–	–
Downwind of manure heap	n/a	n/a	–	+	+	–
Upwind of manure heap	n/a	n/a	–	–	–	–

^a Presence of *C. burnetii* DNA in PM10 and inhalable dust samples collected during periods of minimal activity, during milking activity, and during deep straw bedding removal at bulk tank milk-positive farm 2 (62, 75, and 76 days after culling, respectively).

^b ++, *C. burnetii* IS1111 + *com1* positive; +, *C. burnetii* IS1111 positive; –, *C. burnetii* DNA negative; n/a, not available.

in the presence of a positive internal control (*cry1b*). Samples were scored undetermined when the internal control did not amplify.

Detection of *Coxiella burnetii* DNA in various samples collected at three farms. During a period of minimal activity, *C. burnetii* DNA was found in three out of a total of 23 PM10 samples (13.0%) and in five out of 23 inhalable dust samples (21.7%). In contrast, almost all settled dust samples were positive (Table 1).

***Coxiella burnetii* DNA in air samples during periods of activity.** The number of *C. burnetii* DNA-positive air samples increased when there was more activity on the farm (milking and cleaning), compared to a period of minimal activity. Additionally, even downwind air samples were positive during activity (Table 2).

In general, *C. burnetii* DNA was found more frequently in inhalable dust samples (12/37 [32.4%]) than in PM10 samples (6/37 [16.2%]). This suggests that *C. burnetii* binds or aggregates more frequently to larger dust particles. Moreover, the previous air sample studies in goat and sheep farms (2, 3, 5) detected *C. burnetii* DNA more often, which could be due to the larger particle size fractions collected in those studies. On the other hand, on farm 2 a single-copy gene of *C. burnetii* DNA was detected in a PM10 sample in the milking does area, but no *C. burnetii* DNA was found in the accompanying inhalable dust sample (Table 1). Future research is needed to look into the particle size distribution in relation to the presence of *C. burnetii* DNA.

Although this study is the largest study thus far investigating the presence of *C. burnetii* DNA in air samples that can enter the respiratory tract, the results should be interpreted with caution. The observed *C. burnetii* DNA levels were generally low, and most airborne samples were positive only for the multicopy gene. A previous study in the Netherlands also reported low *C. burnetii* DNA levels (3). It is not possible to determine whether low levels of airborne *C. burnetii* DNA are the result of minimal active shedding (high-risk goats had been culled) or minimal farm activity.

Interestingly, we collected more *C. burnetii* DNA-positive samples during periods with relatively higher activity, which suggests an increased formation of contaminated aerosols. These pos-

itive samples likely in part reflect resuspended dust. Whether this is of importance with regard to infectivity is unknown. In general, detection of *C. burnetii* DNA in air samples provides no information on the viability of the *C. burnetii* organism detected in the samples. Future research should look into the level of viable *C. burnetii* DNA in environmental samples to allow a proper human and animal infectiveness risk assessment from airborne exposures.

In conclusion, the results show that *C. burnetii* DNA can be detected in airborne dust samples of size fractions that can be inhaled by humans. This supports the general assertion that airborne transmission might indeed be a likely route of exposure, and it should be explored in more detail to understand the spread and transmission route of *C. burnetii* and the risk posed by *C. burnetii* for humans.

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REFERENCES

1. Angelakis E, Raoult D. 2010. Q fever. *Vet. Microbiol.* 140:297–309.
2. Astobiza I, et al. 2011. *Coxiella burnetii* shedding and environmental contamination at lambing in two highly naturally-infected dairy sheep flocks after vaccination. *Res. Vet. Sci.* 91:e58–e63. doi:10.1016/j.rvsc.2010.11.014.
3. de Bruin A. 2011. Q fever: the answer is blowing in the wind. Detection of *Coxiella burnetii* in aerosols. RIVM rapport 330291005. National Institute for Public Health and the Environment, Bilthoven, Netherlands. <http://www.rivm.nl/bibliotheek/rapporten/330291005.pdf>.
4. de Bruin A, et al. 2011. Detection of *Coxiella burnetii* in complex matrices by using multiplex qPCR during a major Q fever outbreak in the Netherlands. *Appl. Environ. Microbiol.* 77:6516–6523.
5. de Bruin A, et al. 2012. Detection of *Coxiella burnetii* DNA on small-ruminant farms during a Q fever outbreak in the Netherlands. *Appl. Environ. Microbiol.* 78:1652–1657.
6. Evaluatiecommissie Q-koorts. 2010. Van verwerping tot verheffing: Q-koortsbeleid in Nederland 2005–2010. Evaluatiecommissie Q-koorts, Den Haag, Netherlands. <http://www.rijksoverheid.nl/documenten-en-publicaties/rapporten/2010/11/22/van-verwerping-tot-verheffing.html>.
7. Hawker JJ, et al. 1998. A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? *Commun. Dis. Public Health* 1:180–187.
8. Karagiannis I, et al. 2007. Q fever outbreak in the Netherlands: a preliminary report. *Euro Surveill.* 12:E070809.2.
9. Klee SR, et al. 2006. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. *BMC Microbiol.* 6:2.
10. Maurin M, Raoult D. 1999. Q fever. *Clin. Microbiol. Rev.* 12:518–553.
11. Schimmer B, et al. 2010. The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infect. Dis.* 10:69. doi:10.1186/1471-2334-10-69.
12. Schulz J, Runge M, Schröder C, Ganter M, Hartung J. 2005. *Coxiella burnetii* isolated from the air of a sheep barn when sheared. *Dtsch. Tierärztl. Wochenschr.* 112:470–472. (In German.)
13. van den Brom R, et al. 2012. *Coxiella burnetii* in bulk tank milk samples from dairy goat and dairy sheep farms in The Netherlands in 2008. *Vet. Rec.* 170:310.
14. van der Hoek W, et al. 2010. Q fever in the Netherlands: an update on the epidemiology and control measures. *Euro Surveill.* 15:19520.