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Coxiella burnetii in sewage water at sewage water treatment plants in a Q fever epidemic area

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ABSTRACT

During 2007–2010, over 4000 persons in The Netherlands contracted Q-fever, a zoonosis caused by the bacterium *Coxiella burnetii*. Goats and sheep are the main reservoir of *C. burnetti* and infected animals shed the bacterium with their urine, faeces and birth products. Human infections may occur through direct contact with infected animals, or through inhalation of contaminated dust particles or aerosols. Discharge of waste water from Q fever contaminated goat farms may result in the presence of *C. burnetii* in sewage water and aerosols at sewage water treatment plants (SWTPs) which may pose a health risk for workers or neighbouring residents. The objectives of this study were to determine the presence of *C. burnetii* at SWTPs and to optimize available detection methods. In March–July 2011, sewage influent and aeration tank samples from four SWTPs receiving discharge from Q fever positive goat farms were examined by using a multiplex real-time PCR detecting *C. burnetii* DNA by targeting *IS1111* and *com1* genes. Influent (44%; $n = 16/36$) and active sludge (36%; $n = 13/36$) samples were positive with low *C. burnetii* DNA content. Percentage positive samples per SWTP were 28–61%. Positive samples were most frequent in March 2011 and least frequent in May 2011. The presence of *C. burnetii* DNA in sewage water samples suggests that SWTPs receiving waste water from Q fever contaminated goat farms may contribute to the spread of *C. burnetii* to the environment. The low levels of *C. burnetii* DNA in sewage water during the decline of the Q fever outbreak in The Netherlands in 2011 indicate a low health risk for SWTP workers and residents.

Introduction

From 2007 to 2010, The Netherlands faced a major outbreak of Q fever. In this period, over 4000 persons contracted the disease, mostly in the south-eastern part of the country where large, goat dairy farms are concentrated (Delsing et al., 2010; Schimmer et al., 2012). Schimmer et al. (2010) demonstrated a direct link between a 2008 cluster of Q fever patients and a dairy goat farm that experienced abortions as a result of Q fever. Persons that lived within a 2 km distance from the affected dairy goat farm had a much higher risk for Q fever (relative risk 31 (95% CI 16–59)) than persons that lived over 5 km away from that farm.

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii* (Maurin and Raoult, 1999; Raoult et al., 2005). Goats and sheep are the main reservoir, but cattle, pets, wildlife and birds may also be infected. In nature, the infection is maintained and transmitted by ticks as the main vector (Kazar, 2005). Infected animals shed the bacterium with their urine, faeces and birth products, and are generally asymptomatic except for abortions and stillbirths due to infection of the placenta (Maurin and Raoult, 1999). *C. burnetii* is well equipped to resist drought (Kazar, 2005), and when contaminated animal excreta dry and turn to dust, the bacterium spreads to the environment. Human infections may occur through inhalation of dust particles or aerosols (small droplets of water in the air) containing the bacterium, or through direct contact with (the dried excrements of) infected animals (Whelan et al., 2012). *C. burnetii* is extremely infectious; a low dose can cause an infection (Madariaga et al., 2003).

Most human infections are asymptomatic, or people may exhibit non-specific mild flu-like symptoms with fever. A limited number of the infected persons develop a chronic infection, months or years after the acute phase, of which endocarditis is the most common expression (Delsing et al., 2010; Raoult et al., 2005). In The Netherlands, approximately 52% of the Q fever patients still suffered from severe fatigue, one year after primary infection (Limonard et al., 2010).

Various actions were taken to control the Q fever epidemic in The Netherlands which included vaccination of dairy goat and sheep, a hygiene protocol for professional dairy goat and dairy sheep farms, manure handling measures, a transport ban of animals

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from Q fever positive farms and a visitor ban for such farms, culling of all pregnant goats and sheep on Q fever positive dairy farms, and a mandatory bulk tank milk monitoring for Q fever (Roest et al., 2011). Bulk tank milk monitoring on dairy goat and sheep farms and mandatory vaccination of all dairy goats and sheep were still ongoing in 2011.

The most likely route of dispersion of *C. burnetii* is through air, with dust particles and aerosols (Astobiza et al., 2011; Raoult et al., 2005). The formation of aerosols during active sludge aeration at sewage water treatment plants (SWTPs) is well known, and these aerosols may contain pathogenic micro-organisms originating from waste water (Brandi et al., 2000). Discharge of waste water from contaminated goat farms may result in the presence of *C. burnetii* in sewage water and aerosols at SWTPs which may pose a health risk for SWTP workers and people living in the vicinity of SWTPs.

We studied the presence of *C. burnetii* DNA in sewage water at four SWTPs in the Q fever epidemic area in The Netherlands during the decline of the epidemic in 2011. Available methods for detection of *C. burnetii* (De Bruin et al., 2011) were optimized for analysis of sewage water samples.

Materials and methods

Sampling sites and sampling

Sampling was done at four SWTPs in the south-eastern part of The Netherlands. The SWTPs received waste water from one or more goat farms that were bulk tank milk positive for *C. burnetii* at the onset of the study. The SWTPs treated waste water of a relatively small community (8.450–160.000 population equivalents of 136 g total oxygen demand per day) to decrease dilution of the goat farm(s) waste water with e.g. municipal waste water, and thus increase the probability of *C. burnetii* detection. SWTP A received the water used for washing the milk machine from one goat farm, as did SWTP B from another goat farm, SWTP C received all the waste water from a third goat farm and the water used for washing the milk machine from a fourth farm, whereas SWTP D received all the waste water from a fifth goat farm.

Sewage influent and aeration tank samples were taken in March–July 2011; each SWTP was sampled nine times. At each SWTP, automated systems continuously sampled influent rendering 24 h flow-proportional, homogeneous samples collected in mixing vessels. Influent samples were taken from these mixing vessels, whereas grab samples were taken from the aeration tanks with active sludge. Sample volumes were 500 ml; sampling was done according to ISO 19458 (International Organization for Standardization, 2006). Samples were cooled with ice packs and transported to the laboratory for analysis within 24 h from sampling.

Sample processing

Samples were mixed with lysis buffer (NucliSENS easyMAG, BioMerieux, no. 280134) in a series of three dilutions: (1) 5 ml influent or active sludge + 20 ml lysis buffer, (2) 1 ml influent or active sludge + 9 ml lysis buffer, and (3) 0.2 ml influent or active sludge + 4.5 ml lysis buffer. Samples were stored in lysis buffer overnight at 4 °C. Subsequently, 50 μl of a suspension of *Bacillus thuringiensis* spores (1.5×10^5 spores per 50 μl; Raven Labs, bioTrading Benelux B.V.; no. 29730) was added to each sample as an internal control for the DNA extraction, and establishment of the presence of inhibitory compounds in the sample. After mixing, the sample was left to settle at room temperature for 10 min, after which the supernatant was decanted in a clean tube and 50 μl of magnetic silica beads was added (NucliSENS, easyMAG, Magnetic Silica, BioMerieux, no. 280133). A standard NucliSens Magnetic DNA extraction (BioMerieux) was done according to the manufacturer's instructions, except that the fluid was transferred to a clean tube after the first washing with washing buffer 2. The extracted DNA was stored at −20 °C for later testing for *C. burnetii*.

The efficiency of the extraction of *C. burnetii* DNA from sewage water was tested by analyzing a duplicate dilution series of each sample to which 25 μl of cell culture medium infected with *C. burnetii* nine mile strain RSA 493 was added per dilution. Prior to use, the infected cell culture medium was heated (30 min, 100 °C) in a BSL-3 laboratory to inactivate *C. burnetii*.

Molecular detection of C. burnetii

The extracted DNA was examined for the presence of *C. burnetii* DNA by means of a multiplex quantitative real-time PCR assay, according to De Bruin et al. (2011), but with modifications. This PCR detects the two *C. burnetii* specific targets *IS1111* and *com1* and the *B. thuringiensis* specific target *cry1*. The PCR assays were run on a Roche Lightcycler 480 machine (Roche Diagnostics Netherlands B.V.) with amplification conditions: 5 min at 95 °C, 50 cycles of 5 s at 94 °C and 35 s at 60 °C, and 30 s at 50 °C. Each test contained 3 μl of the undiluted sample (or 3 μl of a ten times diluted sample in case of inhibition), 4 μl of 100 μmol l^{-1} of each of the six primers (Table 1), 4 μl (Tqpro_sBT (Cy5)) or 2 μl (Tqpro_sIS1 (FAM) and Tqpro_scom (JOE)) of 100 μmol l^{-1} of each of the probes (Table 1) and 168 μl Tris–EDTA buffer pH 8.0 (Sigma–Aldrich; no. 93283). All primers and probes were manufactured by Biolegio (Nijmegen, The Netherlands), except the *cry1* probe which was manufactured by Metabion (Martinsried, Germany).

Sample scoring

Samples positive for the *C. burnetii* target *IS1111* and the internal control *B. thuringiensis* target *cry1* were scored '*IS1111* positive', whereas samples that were additionally positive for the *C. burnetii* target *com 1* were scored '*IS1111+com1* positive'. *IS1111+com1* positive samples have a higher *C. burnetii* DNA content than *IS1111* positive samples. Samples for which the internal control *cry1* was negative were re-examined. Samples that did not produce a positive signal for the *C. burnetii* targets, but were positive for the internal control, were scored 'negative'.

Results and discussion

Detection of C. burnetii DNA

The optimized PCR assay for detection of *C. burnetii* DNA in sewage water samples facilitates a rapid onset of monitoring of sewage water at SWTPs in order to determine the health risk for SWTP workers and residents living nearby, in case the Q fever epidemic in The Netherlands revives or outbreaks of Q fever occur elsewhere, like recently in Serbia (Medić et al., 2012). Controls indicated that the high Cp-values in the PCR assays did not reflect inhibition, but demonstrated low concentrations of *C. burnetii* DNA in sewage water samples. Considerably higher concentrations of *C. burnetii* DNA were detected in vaginal swabs, faeces, goat and sheep milk, and dust on surfaces at contaminated farms, with Cp-values of 27–34 and both targets *IS1111* and *com1* positive (De Bruin et al., 2011, 2012). These samples were, however, taken during the peak of the Q fever outbreak, whereas the sewage water samples were taken during the decline of the outbreak in 2011 (Dijkstra et al., 2012).

The optimized PCR assay detects *C. burnetii* DNA that may originate from living as well as dead bacteria, and does not provide

Table 1
Primers and probes used to detect *Coxiella burnetii* DNA in waste water by multiplex real-time PCR.

Primer or probe		Sequence (5′–3′)	Product size (bp)
IS1111 forward primer	sIS1pri_f	CGGGTTAAGCGTGCTCAGTAT	75
IS1111 reverse primer	sISpri_r	TCCACACGCTTCCATCACCAC	
IS1111 probe (FAM)	Tqpro_sIS1	AGC CCA CCT TAA GAC TGG CTA CGG TGG AT – BHQ1	
com1 forward primer	scompri_f	AGCAGCCGCTAAACAAGGAAAAT	74
com1 reverse primer	scompri_r	GTTCTGATAATTGGCCGTCGACAC	
com1 probe (JOE)	Tqpro_scom	ATG CTT TCC ACG ACG CGC TGC TC – BHQ1	
cry1 forward primer	sBtpri_f	AGTTCGTGTCTGTCCGGGTC	85
cry1 reverse primer	sBtpri_r	CATGAATGGTTACGCAACCTTCT	
cry1 probe (Cy5)	Tqpro_sBT	ATC CCT CCT TGT ACG CTG TGA CAC GAA GGA – BHQ2	

information on infectivity. Whether or not bacteria are capable of causing an infection, determines their effect on public health. Therefore, valuable improvements to this and other current methods for the detection of *C. burnetii* would be the development and optimization of methods that allow distinguishing between dead and live bacteria, the latter being potentially infectious, such as cell culture infectivity assays and viability-PCR assays.

C. burnetii at SWTPs

Forty percent ($n = 29/72$) of the influent and active sludge samples from the studied SWTPs contained *C. burnetii* DNA. All, but one, were *IS1111* positive with relatively high Cp-values (33.2–40.2) in the PCR assay, indicating low concentrations of *C. burnetii* DNA. One influent sample was *IS1111+com1* positive albeit Cp-values were again relatively high (34.3–35.7). SWTP A had the highest frequency of positive samples ($n = 11/18$; 61%), followed by SWTP D ($n = 8/18$; 44%) and SWTPs B and C ($n = 5/18$; 28%) (Table 2). *C. burnetii* DNA was detected in 44% ($n = 16/36$) of the influent samples and in 36% ($n = 13/36$) of the active sludge samples.

C. burnetti DNA was most frequently detected in samples taken in March 2011 (Fig. 1), coinciding with the lambing season when infected goats shed large numbers of *C. burnetii* during partum. In April and May, the number of positive samples was low, which may be linked to the ending of the lambing season, but also with increasing level of vaccination. Vaccination does not eliminate the infection, but it largely decreases the prevalence and bacterial load in infected herds and thus reduces contamination of the environment e.g. through discharge of waste water (Hogerwerf et al., 2011). The goat farm that discharged onto SWTP B and one of the goat farms that discharged onto SWTP C became bulk tank milk negative during the study period. However, despite continued vaccination and the decrease in the total number of positive goat farms, new contaminated goat farms were reported in the study area during May and June 2011. These farms may be responsible for the slight increase in the number of positive samples in July 2011.

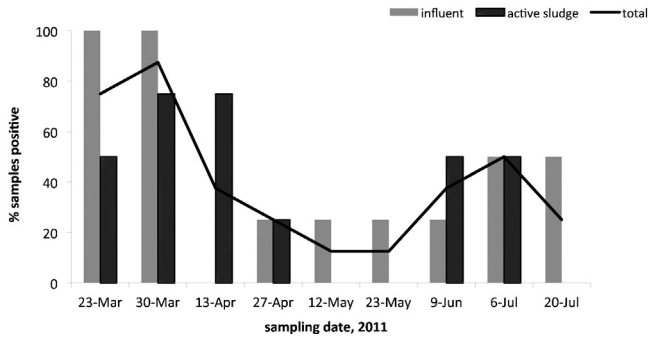

Fig. 1. Sewage water treatment plant (SWTP) influent and active sludge samples containing *C. burnetii* DNA, March–July 2011; four influent and four active sludge samples, taken at four different SWTPS, were tested per date.

This study aimed at examining sewage water samples from SWTPs in the Q fever epidemic area in the south-east of the country only; examination of samples taken outside this area, from SWTPs that did and did not receive wastewater from goat farms, was not included in the original assignment and was considered not opportune afterwards for various reasons, including the obtained results and the continued decline of the outbreak. Moreover, newly reported human cases and Q fever positive goat farms (far) outside the original outbreak area (Dijkstra et al., 2012) indicated that the disease had spread throughout most of the country thus largely limiting the possibility of sampling SWTPs in areas that were not or had not been affected by Q fever. Consequently, the detected low levels of *C. burnetii* DNA in sewage water may represent an elevation as a result of discharges from (newly reported) Q fever contaminated goat farms, but it cannot be ruled out that partially they reflect a background level of *C. burnetii* DNA in the environment. Transport of *C. burnetii* from contaminated goat farms to the environment may e.g. occur with soil, animal skin, wool or fur, non-pasteurized milk, and waste water. Since *C. burnetii* survives in the environment for months to years because of its resistance to heat, pressure and chemical stress (Kazar, 2005), and the most likely route of dispersion of the bacterium is through air with aerosols and dust particles (Astobiza et al., 2011; Raoult et al., 2005), the omniprevalence of *C. burnetii* DNA in the environment is plausible. Indications of a possible background level of *C. burnetii* DNA in the environment were obtained in 2010 when low concentrations of this DNA were regularly detected in samples of particulate matter taken in the Q fever epidemic area, but also at a reference site in Utrecht (Heederik and IJzermans, 2011). However, the elevated levels of *C. burnetii* DNA at SWTPs during the lambing season suggest a link with Q fever positive goat farms.

Exposure and health risks

The exposure of SWTP workers to aerosols depends on the frequency, duration and nature of their activities in the vicinity of aerosol producing processes (Medema et al., 2002; Sales-Ortells and Medema, 2012). On the basis of the obtained data, a quantitative assessment of the health risk for SWTP workers could not be performed due to the limited amount of data and the lack of information on *C. burnetii* infectivity. However, the detection of low levels of *C. burnetii* DNA in sewage water at SWTPs, led to the assumption that the health risk for SWTP workers exposed to aerosols at SWTPs was low since the dose often largely determines the risk outcome from exposure to pathogens in the environment. PCR detected DNA may originate from living as well as dead bacteria; the dead bacteria cannot cause infections, whereas the live and potentially infectious bacteria in influent or active sludge will not all end up in aerosols. At high epidemic times, the risk from exposure to *C. burnetii* in sewage water may be higher, but still, waste water from goat farms comprises only a fraction of the waste water that enters the SWTP and dilution will result in a lower

Table 2
Coxiella burnetii DNA in influent and active sludge at sewage water treatment plants (SWTPs) in The Netherlands.

SWTP	Number of samples							
	Influent				Active sludge			
	Total	*IS1111* positive[a] (%)	*IS1111+com1* positive[b] (%)	Negative[c] (%)	Total	*IS1111* positive[a] (%)	*IS1111+com1* positive[b] (%)	Negative[c] (%)
A	9	6 (67)	0	3 (33)	9	5 (56)	0	4 (44)
B	9	3 (33)	0	6 (67)	9	2 (22)	0	7 (78)
C	9	3 (33)	1 (11)	5 (56)	9	1 (11)	0	8 (89)
D	9	3 (33)	0	6 (67)	9	5 (56)	0	4 (44)
Total	36	15 (42)	1 (11)		36	13 (36)	0	

[a] Positive signal in PCR for *C. burnetii* target *IS1111* only.
[b] Positive signal in PCR for *C. burnetii* targets *IS1111* and *com1*.
[c] No *C. burnetii* DNA detected; no signal for *C. burnetii* targets *IS1111* and *com1*.

concentration of *C. burnetii* in influent and active sludge than in the crude waste water leaving a contaminated farm.

The presence of *C. burnetii* DNA in SWTP effluent was not studied, but given the low amount of this DNA in SWTP influent and active sludge, this was likely low during the study period. Conventional sewage water treatment does not specifically aim at removal of micro-organisms and generally removes approx. 1–3 logs (Ottoson et al., 2006a). High input of *C. burnetii* in conventional SWTPs may therefore result in further spread of the bacterium when SWTP effluent is discharged onto surface water or used for irrigation. Both in the water and soil environment, *C. burnetii* may be ingested by amoeba and survive within these organisms (La Scola and Raoult, 2001), thus creating potential environmental reservoirs. Advanced sewage water treatment by membrane bioreactors and/or disinfection of SWTP effluent by e.g. chlorination or UV radiation increase bacterial removal (Ottoson et al., 2006b) thus reducing the output of bacteria such as *C. burnetii* into the environment. The presence of *C. burnetii* DNA in sewage water samples suggests that conventional SWTPs receiving waste water from Q fever contaminated goat farms may contribute to the spread of *C. burnetii* to the environment.

A risk assessment framework based on scenarios using estimated concentrations during the peak of the epidemic, would be a useful tool to assess health risks from exposure to *C. burnetii* and other pathogens in sewage water, which spread through aerosols and thus pose a potential risk for SWTP workers and residents.

Limitations of the study

SWTPs were studied in 2011, which appeared to be during the decline of the Q fever outbreak in The Netherlands (Dijkstra et al., 2012). More valuable data could have been obtained when SWTPs were studied during the entire outbreak; however, research and funding priorities regarding the Q fever outbreak were set otherwise. Moreover, this pilot study aimed at studying sewage water samples from SWTPs receiving waste water from goat farms in the Q fever epidemic area only. The planned follow-up study, including sampling of SWTPs outside the epidemic area was not performed due to the continued decline of the outbreak and the spread of the disease throughout most of the country. Studying SWTPs that did or did not receive waste water from goat farms in Q fever free areas would have generated more information on the actual influence of goat farms on the presence of *C. burnetii* DNA in waste water at SWTPs and the background level of this DNA in Q fever free areas.

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References

Astobiza, I., Barandika, J.F., Ruiz-Fons, F., Hurtado, A., Povedano, I., Juste, R.A., García-Pérez, A.L., 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.
Brandi, G., Sisti, M., Amaglianiet, G., 2000. Evaluation of the environmental impact of microbial aerosols generated by wastewater treatment plants utilizing different aeration systems. J. Appl. Microbiol. 88, 845–852.
De Bruin, A., De Groot, A., De Heer, L., Bok, J., Wielinga, P.R., Hamans, M., Van Rotterdam, B.J., Janse, I., 2011. Detection of *Coxiella burnetii* in complex matrices by using multiplex quantitative PCR during a major Q fever outbreak in The Netherlands. Appl. Environ. Microbiol. 77, 6516–6523.
De Bruin, A., Van der Plaats, R.Q.J., De Heer, L., Paauwe, R., Schimmer, B., Vellema, P., Van Rotterdam, B.J., Van Duynhoven, Y.T.H.P., 2012. Detection of *Coxiella burnetii* on small ruminant farms during a Q fever outbreak in The Netherlands. Appl. Environ. Microbiol. 78, 1652–1657.
Delsing, C.E., Kullberg, B.J., Bleeker-Rovers, C.P., 2010. Q fever in The Netherlands from 2007 to 2010. Neth. J. Med. 68, 382–387.
Dijkstra, F., van der Hoek, W., Wijers, N., Schimmer, B., Rietveld, A., Wijkmans, C.J., Vellema, P., Schneeberger, P.M., 2012. The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol. Med. Microbiol. 64, 3–12.
Heederik, D.J.J., IJzermans, C.J. (Eds.), 2011. Mogelijke effecten van intensieve veehouderij op de gezondheid van omwonenden. IRAS Utrecht University, NIVEL, RIVM (in Dutch) http://www.nivel.nl/sites/default/files/bestanden/Rapport-Intensieve-Veehouderij.pdf
Hogerwerf, L., Van den Brom, R., Roest, H.I.J., Bouma, A., Vellema, P., Pieterse, M., Dercksen, D., Nielen, M., 2011. Reduction of *Coxiella burnetii* prevalence by vaccination of goats and sheep, The Netherlands. Emerg. Infect. Dis. 17, 379–386.
International Organization for Standardization, 2006. ISO 19458 Water Quality – Sampling for Microbiological Analysis. Geneva, Switzerland.
Kazar, J., 2005. Coxiella burnetii infection. Ann. N. Y. Acad. Sci. 1063, 105–114.
La Scola, B., Raoult, D., 2001. Survival of *Coxiella burnetii* within free-living amoeba *Acanthamoeba castellanii*. Clin. Microbiol. Infect. 7, 75–79.
Limonard, G.J., Peters, J.B., Nabuurs-Franssen, M.H., Weers-Pothoff, G., Besselink, R., Groot, C.A., Dekhuijzen, P.N., Vercoulen, J.H., 2010. Detailed analysis of health status of Q fever patients 1 year after the first Dutch outbreak: a case–control study. QJM 103, 953–958.
Madariaga, M.G., Rezai, K., Trenholme, G.M., Weinstein, R.A., 2003. Q fever: a biological weapon in your backyard. Lancet Infect. Dis. 3, 709–721.
Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518–553.
Medema, G.J., Koot, D., Brouwer, A., 2002. Risico van blootstelling aan Legionella op rwzi's. Stowa report 2002–16. Stowa, Utrecht, The Netherlands. http://www.stowa.nl/Upload/publicaties2/mID.4924.cID.3914.42349512.2002-16.blootstelling-Legionella.pdf (in Dutch with English summary).
Medić, S., Nitzan Kaluski, D., Šeguljev, Z., Obrenović, J., Rudan, P., Lazarević, M., Jandrić Kočić, J., Sajenković, D., Pušić, I., Bugarski, D., Vidanović, D., Šekler, M., 2012. Q fever outbreak in the village of Nočaj, Srem county, Vojvodina province, Serbia, January to February 2012. Euro Surveill. 17 (15), pII=20143.
Ottoson, J., Hansen, A., Westrell, T., Johansen, K., Norder, H., Stenström, T.A., 2006a. Removal of noro- and enteroviruses, Giardia cysts, *Cryptosporidium oocysts*, and fecal indicators at four secondary wastewater treatment plants in Sweden. Water Environ. Res. 78, 828–834.
Ottoson, J., Hansen, A., Bjölenius, B., Norder, H., Stenström, T.A., 2006b. Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. Water Res. 40, 1449–1457.
Raoult, D., Marrie, T.J., Mege, J.L., 2005. Natural history and pathophysiology of Q fever. Lancet Infect. Dis. 5, 219–226.
Roest, H.I.J., Tilburg, J.J.H.C., Van der Hoek, W., Vellema, P., Van Zijderveld, F.G., Klaassen, C.H.W., Raoult, D., 2011. The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol. Infect. 139, 1–12.

Sales-Ortells, H., Medema, G.J., 2012. Screening-level risk assessment of *Coxiella burnetii* (Q fever) transmission via aeration of drinking water. Environ. Sci. Technol., http://dx.doi.org/10.1021/es203744g.

Schimmer, B., Ter Schegget, R., Wegdam, M., Züchner, L., De Bruin, A., Schneeberger, P.M., Veenstra, T., Vellema, P., Van der Hoek, W., 2010. The use of a geographic information system to indentify a dairy goat farm as the most likely source of an urban Q fever outbreak. BMC Infect. Dis. 10 www.biomedcentral.com/1471-2334/10/69

Schimmer, B., Notermans, D., Harms, M.G., Reimerink, J.H.J., Bakker, J., Schneeberger, P., Mollema, L., Teunis, P., Van Pelt, W., Van Duynhoven, Y., 2012. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol. Infect. 140, 27–35.

Whelan, J., Schimmer, B., De Bruin, A., Van Beest Holle, M.R., Van der Hoek, W., Ter Schegget, R., 2012. Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009. Epidemiol. Infect. 140, 858–864.