Screening-level assessment of the risk of *Coxiella burnetii* (Q fever) related to aeration of drinking water.

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KWR Watercycle Research Institute

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Colofon

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Samenvatting

Achtergrond
In 2007-2009 hebben zich drie epidemieën van Q-koorts voorgedaan in Nederland. De omvang van de epidemieën, de tijd en plaats en de analyse van de *case-history* van de ziektegevallen suggereerde verspreiding van de verwekker van Q-koorts , *Coxiela burnetii,* via de lucht vanuit besmette geiten- en schapenstallen. In het gebied waar de Q-koorts vooral heerste wordt grondwater gebruikt voor de productie van drinkwater. Grondwater wordt belucht om zuurstof in het water te brengen en ongewenste vluchtige stoffen (zoals sulfide en methaan) te strippen. Bij diverse typen beluchting wordt omgevingslucht van het grondwaterpompstation actief aangezogen en vindt intensief contact plaats tussen de lucht en het water. Filtratie van omgevingslucht vindt niet altijd plaats. Als de omgevingslucht is besmet met *Coxiella burnetii* vanuit een nabijgelegen besmette geitenstal zou de *Coxiella* overgebracht kunnen worden in het drinkwater en via deze route naar de drinkwatergebruikers worden getransporteerd. Bij het drinken van dit water of douchen met dit water zou de bacterie op de drinkwatergebruiker kunnen worden overgebracht.

Doel
Doel van deze studie was om te bepalen hoe groot het risico is dat drinkwaterconsumenten Q-koorts oplopen via drinkwater in een gebied waar besmette geitenstallen en winning en beluchting van (anaeroob) grondwater zich in elkaars nabijheid bevinden.

Aanpak
Er is een *screening-level* risicoanalyse uitgevoerd. Er is een risicoscenario ontwikkeld waar een grondwaterpompstation met geforceerde beluchting zonder luchtfiltratie zich op 1 km afstand van een besmette geitenstal bevond. Er zijn gegevens verzameld uit de literatuur over de verschillende stappen van de transmissie van *Coxiella* van de geitenstal via de lucht, afhankelijk van de windrichting, en beluchting naar het grondwater, en vervolgens de verwijdering in de nazuivering, verspreiding via het net en blootstelling van de drinkwatergebruiker via inademen van aerosolen tijdens het douchen of inslikken bij het drinken van water. Met de dosis-responsrelatie van (inhalatie van) *Coxiella burnetii* was het risico dat de drinkwatergebruiker via deze route Q-koorts oploopt in te schatten.
Omdat veel gegevens uit de literatuur moesten worden geëxtrapoleerd, moesten aannames worden gedaan. Waar meerdere gegevens beschikbaar waren is gekozen voor de gegevens die het meest conservatief waren, tenzij duidelijk was dat deze gegevens niet representatief waren voor het risicoscenario. Middels een gevoeligheidsanalyse is gekeken voor welke aannames deze risicoanalyse wel en niet gevoelig is.

Resultaat
De risicoanalyse gaf aan dat het risico date en drinkwaterconsument via dit risicoscenario Q-koorts oploopt 1.1×10^{-6} per jaar is.
De gevoeligheidsanalyse laat zien dat het berekende risico gevoelig is voor het gebruikte model voor de volgende parameters:

- transport/overleving van bioaerosolen in de lucht,
- het gebruik van luchtfiltratie door het waterbedrijf (HEPA filters verlagen het risico met 99,95%)
- de mate van bioaerosol vorming tijdens het douchen. Een studie gaf een veel hogere bioaerosol vorming dan een reeks van studies. Nadere analyse van deze studie gaf aan dat deze gegevens onwaarschijnlijk hoog zijn.

Een dosis responsrelatie is alleen beschikbaar voor inhalatie. Dit lijkt ook verreweg de belangrijkste route van overdracht van Q-koorts. Blootstelling door inslikken via drinkwater is daarom niet mee gerekend.
De brongegevens over *Coxiella* concentraties in de lucht van besmette geitenstallen was beperkt en de omstandigheden in de twee studies die hierover zijn gerapporteerd zouden mogelijk tot lagere concentraties *Coxiella* kunnen leiden dan de omstandigheden in Nederland. Het RIVM onderzoekt de lucht in en rondom besmette geitenstallen, maar had geen kwantitatieve gegevens beschikbaar die in deze studie konden worden gebruikt.

De concentratie *Coxiella* in het water is direct gekoppeld aan die in de lucht en die is sterk afhankelijk van de afstand tussen stal en pompstation. 41 pompstations bevonden zich in de 5 km radius van een besmette stal, 4 in de 1-km radius. Bij een afstand van 360 m (de kortst gemeten afstand tussen besmette stal en pompstation) is het risico 6x hoger. Bij een afstand van 85m zou het risico boven de 10^{-4} (de eis uit het Waterleidingbesluit) komen, waarbij opgemerkt wordt dat hier een ziekterisico is berekend, terwijl het Waterleidingbesluit spreekt over een infectierisico (infectie is (nog) geen ziekte).

Ter vergelijking van het risico via drinkwater is ook berekend wat het risico is op het oplopen van Q-koorts voor de gemiddelde Nederlander in 2009 (via alle transmissieroutes samen): 1.4×10^{-4} per jaar (2357 Q-koorts gevallen op een bevolking van 16.5 miljoen). Voor mensen die in de 5 km radius van een besmette geitenstal wonen was het risico op het oplopen van Q-koorts in 2009 7×10^{-4} per jaar.

Conclusie
De bijdrage van de overdracht via drinkwater aan Q-koorts in Nederland is verwaarloosbaar. Dat is gebaseerd op:

- het lage jaarrisico dat is berekend via de water route in de *screening-level* risicoanalyse (1.1×10^{-6});
- het relatief hoge jaarrisico via andere routes ($1.4 – 7 \times 10^{-4}$);
- de conservatieve aanpak in de *screening-level* risicoanalyse;
- de afstand tussen besmette geitenstallen en grondwaterpompstations (>1 km voor op 4 na alle pompstations).

Aanbevelingen
Deze studie geeft aan dat er geen specifieke maatregelen nodig lijken te zijn, omdat het risico op overdracht van Q-koorts via deze water route verwaarloosbaar is, zelfs bij de kleinst gemeten afstand tussen besmette geitenstal en pompstation. Desalniettemin wordt aanbevolen om het luchtfiltratie beleid bij intensieve beluchting te evalueren in het licht van het feit dat stallen kunnen fungeren als bron voor ziekteverwekkers voor mens en dier.

Als zich een besmette boerderij in de nabijheid van een drinkwaterpompstation bevindt wordt aanbevolen dat waterbedrijven de gezondheidsinstanties (GGD) consulteren om vast te stellen of er maatregelen nodig zijn voor de bescherming van het personeel dat werkzaam is op het pompstation. In deze situatie kunnen werkzaamheden aan de luchtfiltratie beter plaatsvinden op dagen dat de wind niet vanuit de besmette boerderij naar het pompstation waait.

Abstract

A screening level risk assessment of Q fever through drinking water produced from groundwater and exposed to aeration in the Netherlands was performed. Quantitative data from scientific literature was used and a Quantitative Microbial Risk Assessment (QMRA) approach was followed. An exposure model was developed to calculate the dose to which consumers of aerated ground water are exposed through drinking water from the tap and inhaling aerosols during showering. Starting from the concentration of the bacteria in the air of infected barnyards, the concentration of the bacteria was calculated at each step: at the air inlet of the water treatment plant, in the air after filtration, in raw water after aeration, in treated water, in tap water and in shower aerosols, in order to calculate the ingestion and inhalation dose. A dose-response relationship was only available for inhalation. The exposure assessment and hazard characterization were integrated in a screening level risk characterization of the QMRA to determine the risk of Q fever through tap water. The data contained uncertainties, fore some factors assumptions were needed and variable factors were found. Hence, a nominal range sensitivity analysis was performed. For each uncertainty, assumption and variable value, an alternative value was selected and its effect on the risk was determined. A risk lower than 10^{-4} was calculated, concluding that the risk of transmission through drinking water is very low. In the sensitivity analysis can be seen that the most uncertain parameters are the emission of *C. burnetii* from the barnyard, the air transport and the aerosolization in the shower. Compared to direct airborne exposure, the risk of exposure through drinking water is negligible.

Content

1 Background

Q fever is a worldwide zoonosis caused by the bacteria *Coxiella burnetii*. Infected domestic animals, especially goats, sheep and cows, secrete it in high concentrations in placentas, birthing/abortion fluids, feces or urine and it is also present in the ticks that these animals carry. Once in the environment, *Coxiella* can travel long distances in the air (20 km if the meteorological and geographic conditions are favorable)[1].

The Netherlands is a crowded country. Therefore, barnyards are located in close proximity to urban areas, facilitating the spread of a disease like Q fever (figure 1). During the last decade, the raising of goats especially for milk production increased in The Netherlands. Goats have been found the primary source of Q fever infection in this country. Also two sheep farms have been declared infected to date. The bacteria have been found in the air 5km and even 10km away from infected farms [2].

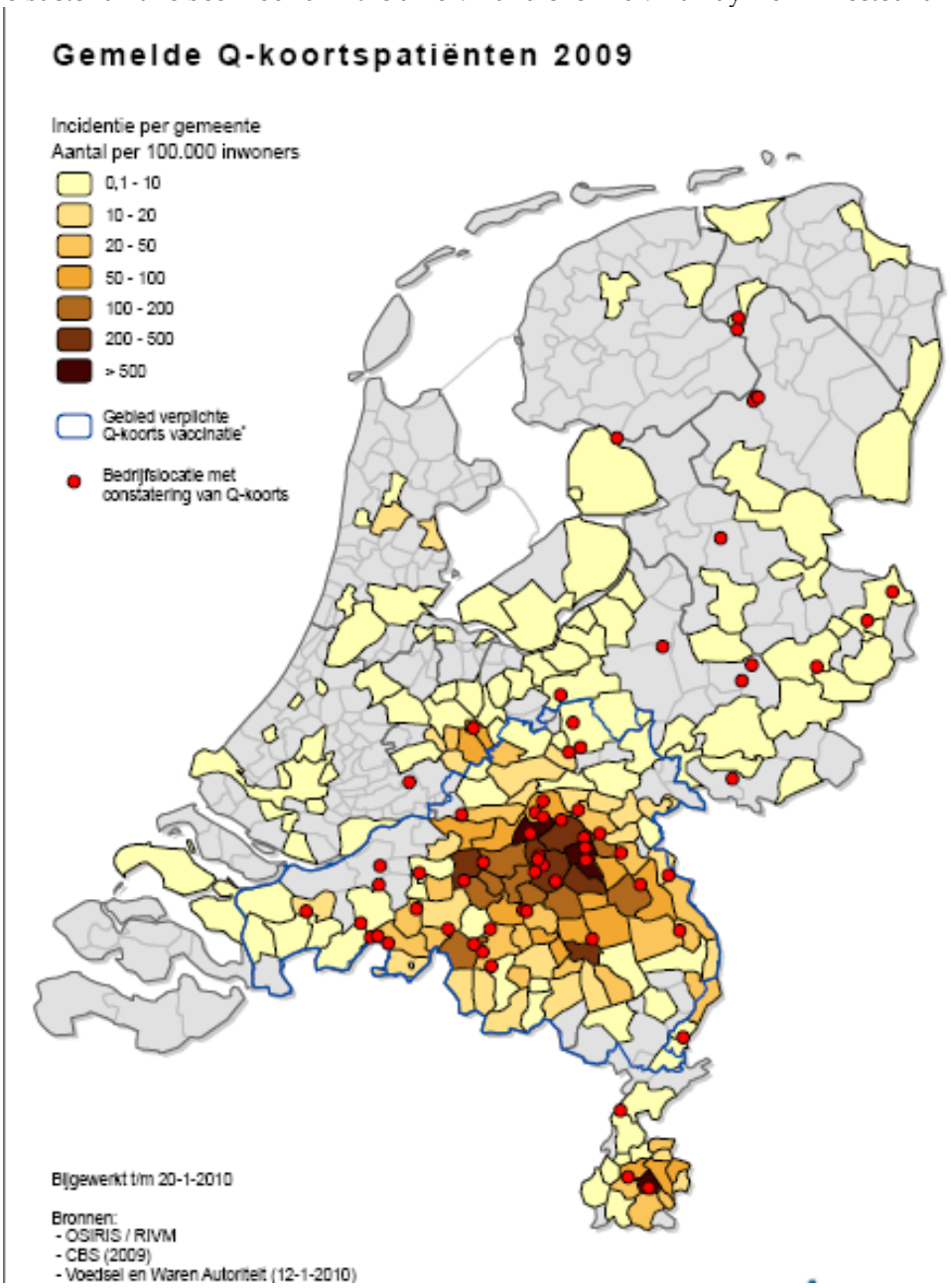

Figure 1. Amount of human cases of Q fever per 100.000 inhabitants and farms declared contaminated through milk tests in The Netherlands, 2009[3].

In the last 3 years, 3 outbreaks of Q fever in humans took place in The Netherlands right after the kidding season, period when goats give birth. The extent of the outbreaks, the case history and the spread of the cases suggest that a wide-scale environmental contamination or multiple point-source contamination sites are more probable as transmission sources than direct contact with animals, consumption of contaminated unpasteurized milk or contact with parturient pet animal [4]. So, the occurrence of Q fever in the air is considered as a probable route of transmission.

Groundwater is commonly used in Noord-Brabant in particular and in the Netherlands in general as a source for drinking water production. The addresses of infected barnyards have been published, allowing us to locate them together with the ground water treatment plants in a map (figure 2). The map shows that a high number of groundwater treatment plants are located in the vicinity of infected barnyards, 41 inside the 5km radius zone and 4 of them less than 1km from an infected barnyard[5].

An aeration step is necessary for many ground waters to increase the oxygen concentration and stop undesirable volatile compounds present in anaerobic ground water [6]. During forced aeration close contact takes place between air and water. If the air is contaminated with *C. burnetii*, the bacteria can be transmitted to the water, survive during the treatment and distribution and reach the consumers' taps, who may ingest it or inhale it during showering.

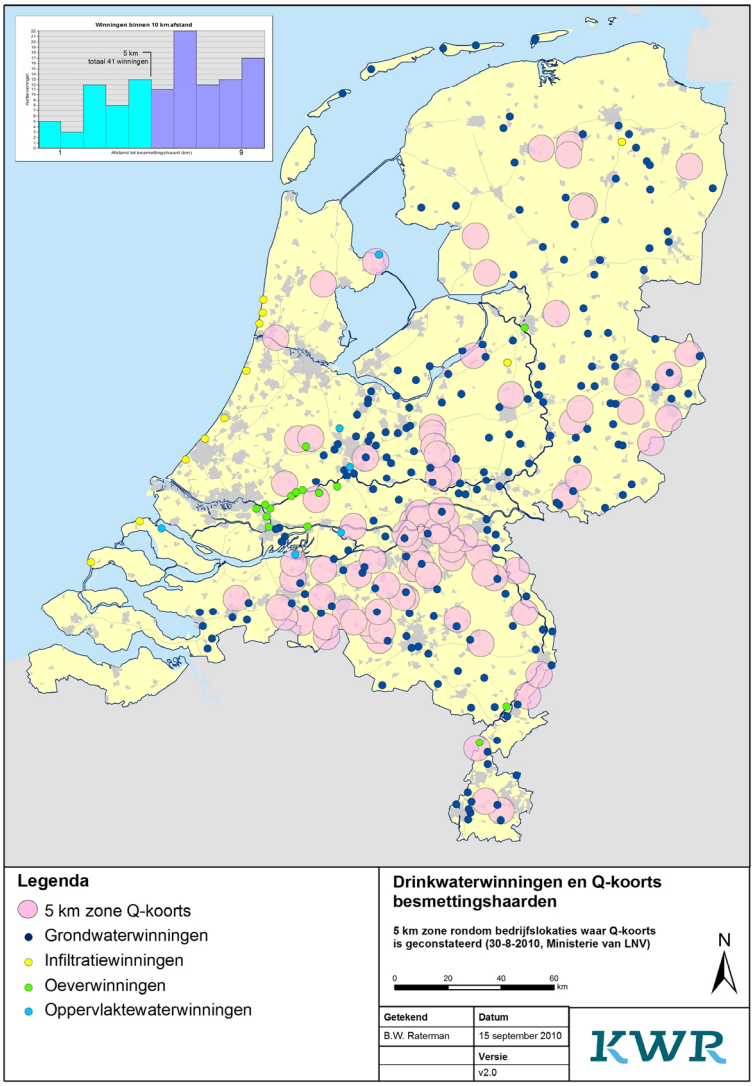

Figure 2. 5 km zone around infected goat farms (pink circles) and the location of drinking water stations (ground water treatment plants in blue dots)[5].

2 Scope and objectives

This is a screening-level risk assessment to evaluate the magnitude of the risk of infection with *Coxiella burnetii*, the causative agent of Q fever, through municipal water supply. The risk assessment targets the general population in areas where: 1. goat farms are infected with *Coxiella burnetii* and 2. anaerobic groundwater is used for drinking water production and the groundwater is aerated to introduce oxygen and strip methane, hydrogen sulphide and other unwanted volatile compounds. The exposure scenarios are through ingestion of tap water and inhalation of tap waterborne aerosols while taking a shower. The exposure scenarios also consider the possibility that the air that is used for aeration of anaerobic groundwater is previously passed through air filters with different filtration efficiencies that can remove aerosols/particles that contain *Coxiella.*
The objective of this study is to determine the probability of infection of the general population with *Coxiella burnetii* in areas with concurrent presence of goat farms with Q fever and municipal water supply that uses aerated anaerobic groundwater with or without air filtration.
The study was performed following a deterministic and conservative approach. In each step the literature value that gave a higher probability of clinical illness was selected. When the highest value was considered far from reality or not representative of the Dutch population, a lower value was used. A sensitivity analysis was performed with alternative data for each step.

The risk assessment guidelines for microbial contaminants in drinking water in the Netherlands states that the probability of infection should be $<10^{-4}$ per person per year [7]. This risk guideline is derived from fecal contamination of drinking water with enteric pathogens. However, it is not exclusive and this study uses this risk level to compare to the estimated probability of infection with *Coxiella burnetii*.

3 Hazard identification

3.1 Q fever

Q fever is a zoonotic disease caused by *Coxiella burnetii,* a species of bacteria that is distributed globally. Q fever is a notifiable disease in the Netherlands. 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 high rates of abortion in goats and sheep have 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 (sheep placenta can contain 10^{10} infectious doses of *C.burnetii* per gram of tissue[1]). 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 or meat, 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 [8].

3.2 Signs and symptoms in humans

Most acute cases of Q fever begin with sudden onset of one or more of the following: high fevers, 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. Only about 40% of the people infected with *C. burnetii* show signs of clinical illness. Twenty percent of the infected people develop flu-like symptoms. Additionally, a majority of patients have abnormal results on liver function tests. The other twenty percent of patients with a symptomatic infection will develop acute disease with pneumonia and/or hepatitis. Some 25% of the cases are hospitalized. In general, most patients will recover to good health within several months without any treatment. 1%-3% 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 (developing in 1-5% of the acute Q fever cases), 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[2, 8]. Another manifestation (but rare) of chronic Q fever is Hepatitis[9].

International literature suggests that a Q fever infection during pregnancy in humans may lead to adverse pregnancy outcome in a large percentage of cases. However, in a recently completed retrospective study in the high incidence area in the Netherlands, the presence of antibodies against *C.burnetii* in early pregnancy was not significantly associated with preterm delivery, low birth weight or perinatal mortality[10]. A literature review on Q fever concluded that, though there are indications of severe disease and progress towards chronic infection or chronic disease in pregnant women, the comparison with non-pregnant women and risk groups like people with damaged heart valve or heart valve prosthesis cannot be quantified. Transplacental transmission is possible but no association has been found with adverse obstetrical outcomes (e.g. fetal death, premature delivery, infection of the

neonate). The same report recommended that pregnant women should not visit infected farms, but not enough evidence was found to warn them not to travel to affected areas [11].

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 3-4 weeks after exposure. Those who recover fully from infection may possess lifelong immunity against re-infection.

3.3 Cases in the Netherlands

Q fever was rare in the Netherlands before 2007, with only around 15 cases reported annually. Since 2007, the number of cases has increased, starting with an outbreak in Noord-Brabant in 2007 with 168 cases. In 2008, 1000 cases were reported in Noord-Brabant and the southern part of Gelderland and in 2009 2355 cases were found in the Netherlands, with 6 fatalities. In 2010, only 407 cases have been declared until the 3rd of June, 2010; this decrease is probably due to the measures undertaken by the Dutch government (vaccination of goats or culling of the pregnant goats in infected barnyards before the kidding season) preventing the emission of high amounts of bacteria (Figure 3) [2].

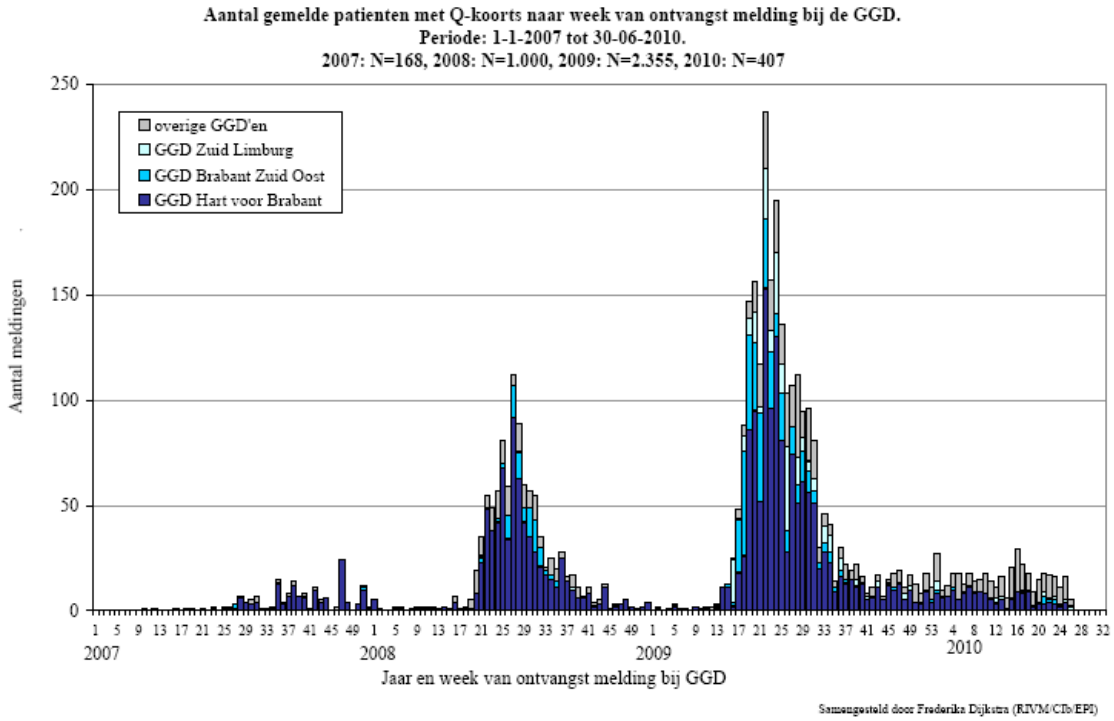

Figure 3. Number of human cases with Q fever in The Netherlands in 2007, 2008, 2009 and 2010[12].

There is an association between the concentration of goats, the occurrence of abortions in goats and human cases of Q fever. For most cases, there is no indication for direct contact or close proximity to infected barnyards. Presence in the risk area is a common factor for all cases. In 2009, 59% of the notified human Q fever cases lived within a radius of 5 km of an infected dairy goat or dairy sheep farm, while only 12% of the Dutch population live within these zones. The incidence of Q fever in 2009 was 69 per 100,000 population within, and six per 100,000 outside the 5 km-areas. Q fever is found in 7.8% of the goats and 18% of the goat farms are contaminated. Q fever is found in 427 of the 450 milk samples from goat and sheep farms[2]. In 2008, the average number of goats per farm, in farms that showed abortion outbreaks, was 900 and the abortion rate ranged from 10-60%, with an average of 20% [13].The types of Q fever bacteria in goats and humans show a large degree of similarity.

3.4 Coxiella burnetii

Coxiella burnetii is a bacterium that lives and reproduces inside the cells of suitable hosts. Livestock (goats, sheep, cattle) and pets are major reservoirs of *C. burnetii.* The bacteria are found inside the host

cells in two forms: an inactive spore-like small cell variant (0.2-0.4 µm) and a large cell variant (up to 2 µm). The small cell variant is very resistant to drying, UV irradiation, acid or alkaline pH, disinfectants and other chemicals. At 4 °C, viability of the small cell variant is retained for 1 year in unchlorinated tap water. This small cell variant, also known as endospore, appears to be a resistant stage adapted to survive in the environment and be transmitted to new hosts.
16S rRNA typing showed that *Coxiella* belongs to the Gammaproteobacteria and has *Legionella* as close relative. Like *Legionella pneumophila, Coxiella* is an intracellular parasite but while for the former this characteristic is facultative, *Coxiella* is an obligate intracellular bacterium.

3.5 Transmission pathway through drinking water?

Airborne bacteria in dust or aerosols from goat and sheep farms is reported as transmission pathway of Q fever bacteria to humans. Q fever bacteria may travel considerable distances through the air. Preliminary results of a study that is currently conducted in the Netherlands demonstrate that Q fever bacteria may be detected in the air 500 and 1000m from a contaminated goat farm [2].
Drinking water plants are abstracting groundwater for the production of drinking water. Aeration is usually one of the processes in groundwater treatment to increase the oxygen content of the water and remove carbon dioxide, methane, hydrogen sulfide and other volatile compounds that may be present in groundwater. This is achieved by intimate contact between air and water by spraying, cascades or aerators. Bacteria that are present in the air may enter the water during this intensive contact.
Once in the water, they may be removed by subsequent water treatment steps. If these are not present or not effective, the bacteria may enter the distribution mains and are transported to the homes of the consumers. The consumer may either ingest the bacteria with the water or inhale the bacteria via aerosols generated, for instance in the shower. Both inhalation (of Q fever bacteria in barnyard dust) and ingestion (of Q fever bacteria in raw milk) have been reported to transmit the disease although according to some authors, ingestion of moderate doses of *Coxiella* would be unlikely to produce clinical symptoms and drinking milk contaminated with *C.burnetii* has caused seroconversion in human volunteers, without clinical disease. However, nothing is known about the use of massive doses of *Coxiella* in the case of an intentional contamination of food products or water[14].

4 Problem formulation

Is it possible to compute the risk of clinical disease with Q fever through drinking water produced from groundwater that is aerated with or without air treatment based on exposure assessment scenario and dose-response relationship using real world data?

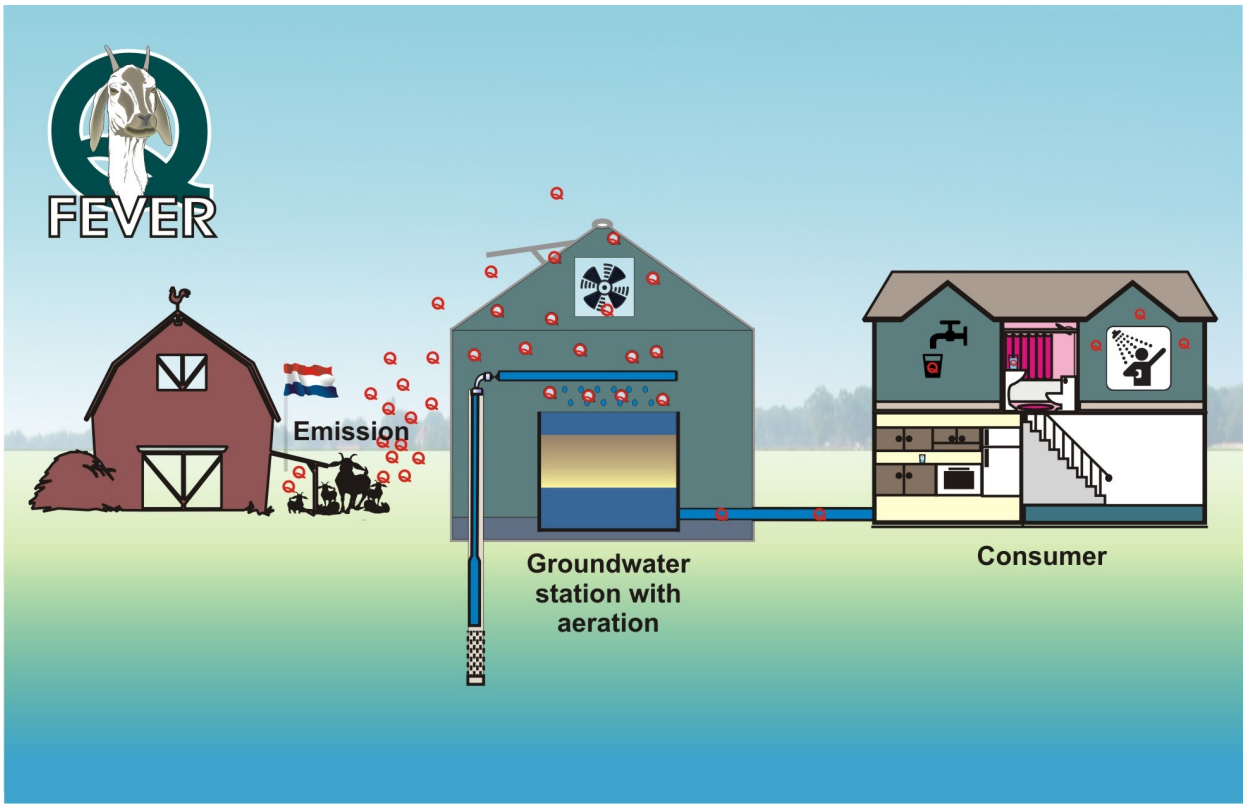

Figure 4: Q fever pathway from the infected barnyard to the drinking water consumer.

5 Exposure assessment

The pathway from infected goat litter to the drinking water consumer is long and has many steps (Figures 4 and 5).

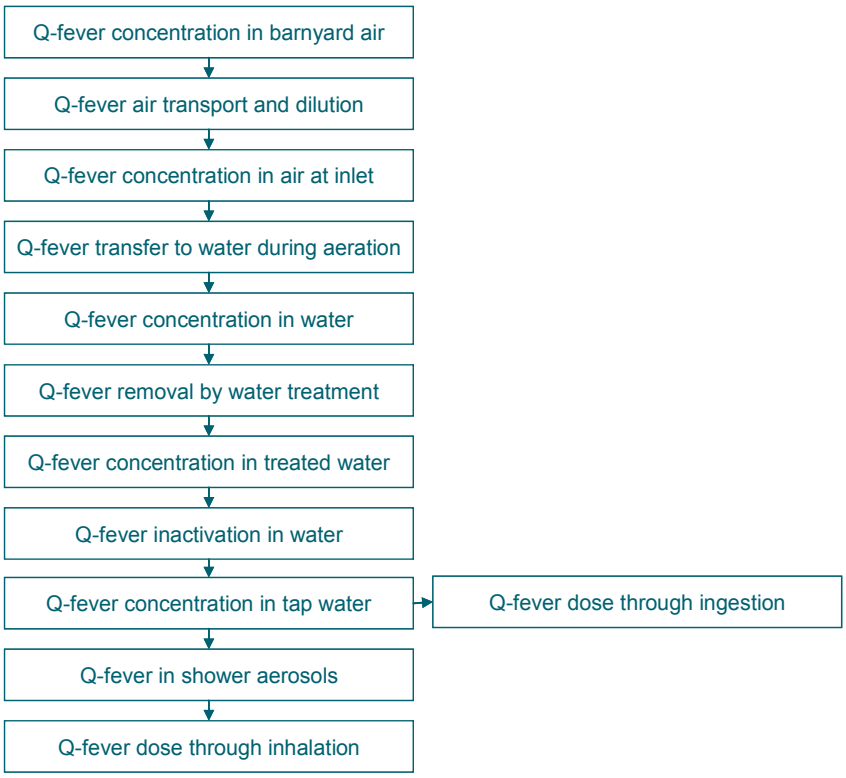

Figure 5. Exposure assessment steps

5.1 Q fever concentration in barnyard air

There is surprisingly little information published about the concentration of Q fever bacteria in the air in barns or on and around infected farms. *Coxiella burnetii* is excreted by infected animals during partiturion in the placenta and birth fluids, and afterwards in the feces [15].
A study of Delay et al. (1950) sampled dust from approximately 800 liters of air in a barn on a sheep farm. The barn had been inhabited by sheep 30 days prior to the sampling. The floor was covered with loose, dry fine soil and manure that was disturbed during sampling to mimic the activities of sheep in the barn. The air was bubbled through 35 mL of beef extract broth. After 45-90 min of sampling, 10-25 mL of broth remained. This was used to inoculate guinea pigs to determine the presence of *Coxiella burnetii*. Guinea pigs that were inoculated with 2.5 mL of broth developed an immune response to *Coxiella burnetii*, and *Coxiella burnetii* was isolated from guinea pigs that were inoculated with 5 mL of broth. Similar results were obtained with samples taken from the yard outside this barn[16].
Direct calculation of the concentration of *Coxiella burnetii* in the air of this barn is not possible. The transfer rate of airborne *Coxiella* to the broth during sampling is not reported, neither is the sensitivity of the guinea pig model. If we assume the transfer rate to be 1 and 1 *Coxiella* bacterium to be enough to cause the response in guinea pigs, the concentration of *Coxiella* in the barn air can be calculated from:

1 *Coxiella* in inoculum volume (2.5-3 mL) / remaining broth volume (10-25 mL) x volume of air sample (800 L) is 4.2 - 12.5 *Coxiella* per m^3 air.

Schulz et al. (2005) isolated and detected *C. burnetii* in the air of an enclosed sheep barn during shearing of a herd which had tested positive for *C. burnetii*. The samples were taken from the air of the barn, with an area of approximately 500 m^2 during 270 minutes. During the sampling, the windows were opened and there were around 500 sheep in the barn. Two sheep were sheared at a time and samples were taken in two sites, both at 3 meters from the shearing place at a height of 1.5 m. For sampling, glass filters and polycarbonate filters of 0.8 µm of diameter were used. The air was picked up with a vacuum pump with a 2.5 L/min flow. The glass fiber filters were used to determine the dust concentration while the polycarbonate filters were treated for PCR analysis of a specific DNA section of *C. burnetii*. The method used was based on the one developed by Willems et al. (1994) [17]. The results were positive for *C. burnetii* for samples taken in both sites, with a sequence homology close to 100%, while negative for the control sample. The concentration of the bacteria was calculated indirectly, assuming that at least 120 microorganisms have to be present in a sample of 135 L to have a positive response with PCR. Their conclusion was that 600 microorganisms were contained in a 675L sample[18], which indicates a concentration of 880 *Coxiella*/m^3 in the barnyard air.

Based on this information, for the risk assessment it was decided to use the concentration found during the shearing season (880 cells/m^3) by Schulz as the point estimate for the barnyard air as it is the most conservative value of those found in the literature. We divided the year in two periods: the kidding season and the rest of the year. The highest concentration of Q fever is emitted during the kidding season, while during the rest of the year the emission is lower. Therefore, we used the value of 880 *Coxiella*/m^3 for the kidding season and 8.3 *Coxiella*/m^3 for the rest of the year.

The uncertain and variable factors found in this part of the study are shown in table 1. The sensitivity table shows the sources of uncertainty and variability found during the QMRA process and indicates the effect that each of those factors has on the risk of developing Q fever: increases (+) or decreases (-). When none or very low effect is produced, it is indicated with a 0, while +/- means that it can either increase or decrease the risk. Although no quantification is given, the magnitude of the effect is showed by adding more signs. The same criterion has been used along the whole QMRA process.

Table 1. Sensitivity table Coxiella concentration in barnyard air

Source of uncertainty/variability	**Direction & magnitude**
Delay study	
Sheep were not in barn for 30 days. During occupation the concentration in the air may be higher.	+
Recovery efficiency of the method is not 100% but lower.	+
Guinea pig model is less sensitive. Dose (and concentration in the air) is higher.	+
Coxiella may be present in the dust in aggregates and aerosolized as such. Concentration and guinea pig dose is higher.	+/-
Schulz study	
Data from sheering season used for the kidding season	+
PCR may detect nonviable cells. Concentration of infectious *Coxiella* bacteria would be lower.	-
To calculate the concentration of Q fever, the lower limit of PCR detection is being used (120 microorganisms in 135L of sample)	+
The data belong to sheep studies, while in The Netherlands most infected farms are goat farms.	0

5.2 Transport, inactivation and dilution in air

The locations of infected farms and of the drinking water stations are known. Figure 2 depicts the 5km zone around infected goat farms and the location of drinking water stations. Of all 242 drinking water stations 88 were within a 10 kilometer radius of an infected goat farm, 32 of these were within a 5 km radius, 13 within a 3 km radius and 3 within a 1 km radius. The closest any drinking water station was to an infected goat farm was 360m. Given the dynamic nature of Q fever in farms, this is a snapshot in time and closer vicinities cannot be excluded.

The airborne particles released from its substrate or environment in different ways are transported up in the atmosphere due to turbulence and air currents. The concentration of particles in a volume of air above the ground depends on the amount of particles released from the source per unit time, on the meteorological conditions in the air mass and also on the characteristics of the particles like mass and shape [19].

It is not known whether *Coxiella burnetii* is transported through the air individually, in clusters or attached to aerosols; however, Hunink et al. (2010) state that the aerial infection of Q fever occurs through inhalation of contaminated dust particles[4]. The way a microorganism is transported in air determines the distance it can travel due to its weight, and also its concentration and distribution. According to Cambra-López et al. (2010), particulate matter in livestock houses can enter the environment in high concentrations and can absorb micro-organisms. Particulate matter levels are influenced by the type of housing and feeding, animal type and environmental factors[20].

Several models describe the transport, dilution and inactivation of bacteria through the air. Lighthart and Frisch (1976) estimated the downwind concentration of viable microbes using a modified Pasquill inert particle dispersion model, which is an empirical model based on observations of the dispersion of tracers in the atmosphere. To use this model, it is necessary to know the emission rate of the target microbe, the death rate of the microorganism and the meteorological conditions[21].

To calculate the emission rate, the concentration of the microorganisms in the air at the source and the ventilation rate are needed. A study by Seedorf et al (1998) determined the ventilation rates in cattle, pigs and poultry barnyards in several countries in Northern Europe, including The Netherlands. To estimate the ventilation rate, they used the CO_2 balance method. The CO_2 concentrations were measured with an infra-red analyzer controlled by a computer. They substituted CO_2 with artificial tracer gases to avoid the effect of CO_2 emitted from other sources in mechanically ventilated animal houses. The sampling point taken as the indoor reference was situated close to an air outlet and the external reference point was situated outside the barnyard. Measurements were made each hour during the measurement period (24 hours). The ventilation rates differed among countries, seasons (winter and summer), type of housing (litter, cubicles, slats…) and livestock. Expressing the ventilation rate in $m^3/h/LU$ (Livestock Unit, equivalent to 500 kg) to compare the results between animals with different weights and herd size, they observed a lack of equivalence for animals differing in body weight and, therefore their results cannot be extrapolated to other animals[22]. However, due to the lack of data for goats and sheep, in the present study the values of ventilation rate obtained by Seedorf et al. for dairy cows in cubicles in The Netherlands has been used. When observing the results of Seedorf et al., several factors can be noticed contributing to the difference in ventilation rate: weight (difference between beef and calves), activity (difference between dairy cows and beef) and kind of housing (difference between litter, cubicles and slats). Based on these facts and assuming that in The Netherlands the goats are mainly used to obtain dairy products and using the most conservative approach the maximum value of ventilation rate obtained for dairy cows in cubicles in The Netherlands in winter was selected (938 $m^3/h/LU$). One LU corresponds to 500kg; if we assume a mean weight for goats of 100kg, we can calculate the LUs present in an infected farm housing 900 goats (average number of goats per farm in the Netherlands in the period 2006-2008 [13]). The uncertain and variable factors referred to the emission rate are shown in table 2. Figure 6 shows a goat's barnyard in The Netherlands.

Figure 6. Goat's barnyard in The Netherlands.

Table 2. Sensitivity table Emission rate.

Source of uncertainty/variability	**Direction & magnitude**
Extrapolation from cows/calves is being used.	0
Change in ventilation rate depending on kind of bedding (cubicles, litters…), season, using average instead of maximum…	-
A difference between outdoor and indoor concentrations of at least 250ppm CO_2 was necessary for accurate calculation of the ventilation rate.	+
In naturally ventilated barnyards (which do not use a mechanical system), the ventilation rates measurements are more inaccurate and the ventilation rate will be lower.	-
The concentration of Q fever might vary through the day and season due to animal activity, stocking density, state of the litter or manure, frequency of manure removal…	+/-
The number of goats per barn used is an average (900 goats per farm). Some dairy goat farms are very large (>5000goats) [23] but there are also smaller barnyards.	+/-
Goats' weight	0
The emission is not continuous but in peaks, depending on the abortions and breeding.	+/-
Other practices that might contribute to emission: transportation of animals through populated areas has caused outbreaks previously; burning fresh straw bedding and birth products outside may facilitate windborne spread of *C.burnetii* by releasing incompletely burnt contaminated material into the air [24-25]	+

During its transport through the air, microorganisms can suffer inactivation due to several factors like UV radiation, high temperatures or low relative humidity. The small cell variant of *Coxiella burnetii*, can survive for long periods in the environment. It resists high temperatures, drying, and UV light[26], although it suffers inactivation when exposed to 600μW/cm^2 during 15 sec in suspension (10^8 organisms/ml) or within guinea pig peritoneal macrophages[27]. It can survive for 7-10 months on walls at 15-20ºC, for more than one month on meat in cold storage and for more than 40 months in skimmed milk at room temperature [9].
Due to its ability to survive for long periods in the environment, and following a conservative criterion, it is assumed that no inactivation of the bacteria is taking place during its transport through the air.

The Pasquill's equation adapted by Lighthart and Frisch is written as:

$$\frac{\chi}{Q}\cdot\frac{\overline{U}}{Exp\left(-\lambda x/\overline{U}\right)}=\frac{1}{2\pi\sigma_y\sigma_z}\cdot EXP\left[-\left(\frac{H^2}{2\sigma_z^2}\right)\right]\equiv g \qquad \textit{Equation 1}$$

where χ is the number of particles per cubic meter in the air inlet at the water treatment plant (C_I), Q is the number of particles emitted from the source per second, $\overline{U}$ is the mean air speed in meters per second, λ is the microbial inactivation rate, x the downwind distance from the source, σ_y and σ_z are the diffusion factors in the y and z directions in meters (crosswind and vertical direction respectively) and are functions of meteorological conditions (stability class and wind speed) and downwind distance from the source, and H is the source height in meters. The source is located at (x, y, z) = (0, 0, 10). The second part of the equation can be expressed as g and extrapolated from the graphs in figure 7 [21].

If 10-m-height point source (H) is assumed for *Coxiella* coming from animals calving in a mechanical ventilated barnyard, where 4.1x10^4 particles/s are emitted, situated 1km upwind from a drinking water plant where aeration is used for groundwater treatment, with a C atmospheric class (average atmospheric class during the kidding season), a mean wind speed of 4.2m/s (average wind speed during the kidding season)[28], extrapolating from the graphs in figure 7 we find out that g = 1,5x10^{-4}.

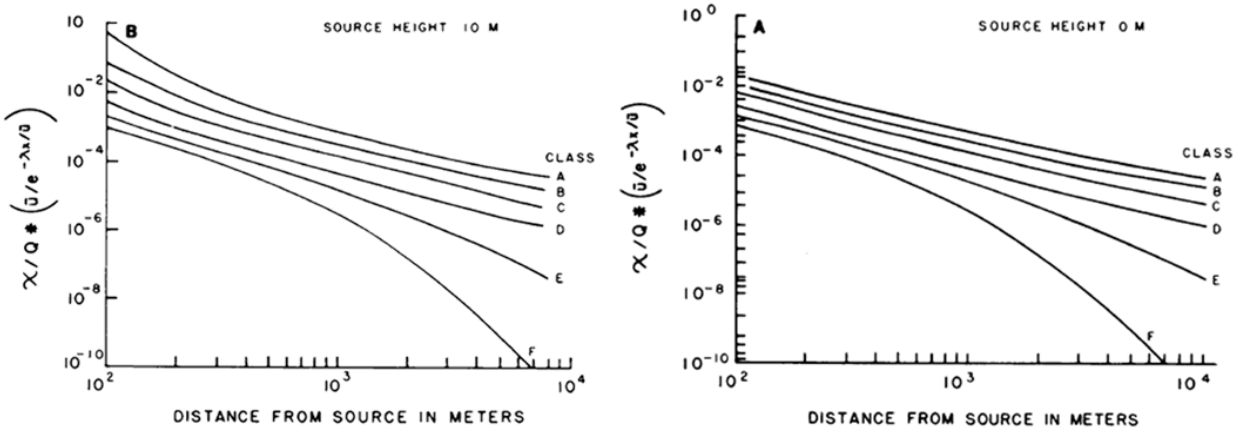

Figure 7. Function of $X/Q*\left(\overline{U}/e^{-\lambda x/\overline{U}}\right)$ versus distance from the source in meters for the indicated stability classes and 2 source heights: 0m and 10m [21].

As it is assumed that there is no inactivation of *Coxiella* during air transport (λ = 0), we can simplify equation 1 and calculate the value of χ :

$$\frac{\chi}{Q}\cdot\overline{U}=g \qquad \textit{Equation 2}$$

Though *x* cannot be seen in the equation anymore, *X* still depends on the distance of the groundwater treatment plant from the source as *g* is dependent on it (figure 7).
From equation 2, it is possible to calculate the concentration of *Coxiella* in the air at the water treatment plant inlet.

When plotting the concentration of Q fever in the air versus the distance downwind from the source, it can be seen that the concentration of *C.burnetii* decreases following a power pattern (figure 8).

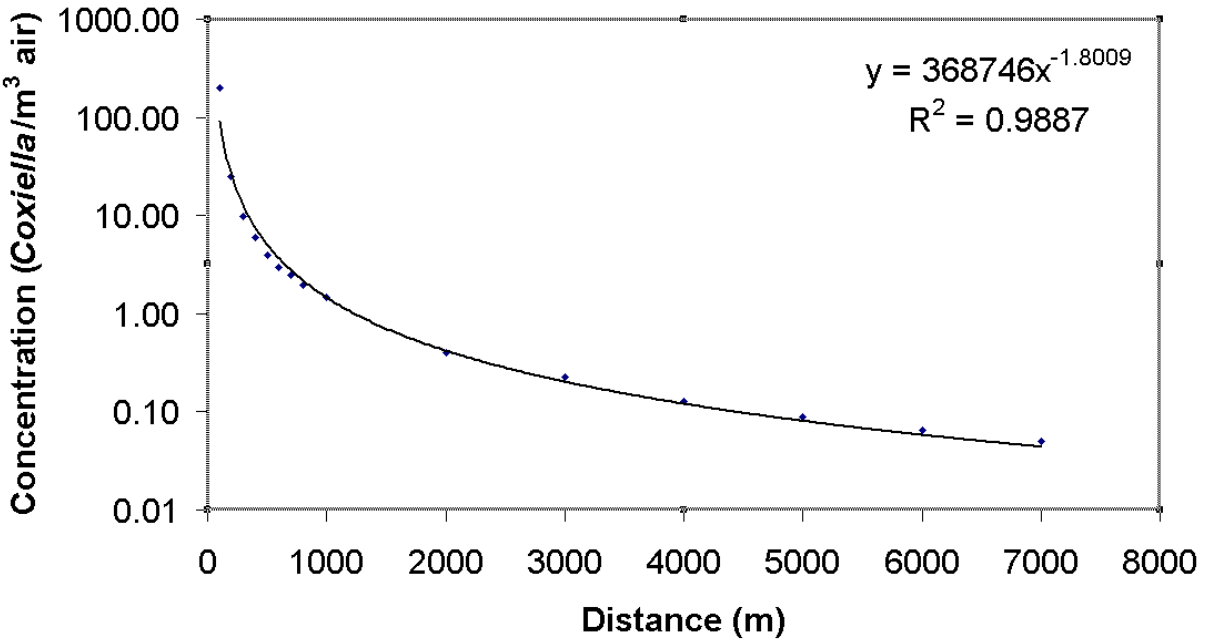

Figure 8. Dilution of Q fever concentration with the distance downwind from the source using Lighthart's model, windspeed 4.19m/s, emission 2.9x10⁴ Coxiella/m³ air, source height 10m and atmospheric class C.

Only water treatment plants located directly in the downwind direction from the emission source can be evaluated with this model. This is appropriate in the present study because is the worse case scenario. When a plant is located some kilometers cross-wind, it cannot be evaluated with this model. Instead, a more general equation that includes y and $z \neq 0$ can be used and the values of σ_y and σ_z can be estimated as a function of distance using other graphs published elsewhere [29].

This model does not include the effect of (wet)deposition which is, in other words, the deposition of the dust particles suspended on air, for example when it rains [19], and the resuspension of these particles when insolation of the soil originates unstable conditions on the air at ground level. However, it assumes that all the particles that hit the floor are reflected to the air.

An alternative approach is to use data from experimental studies on the downwind dispersion of micro-organisms. Paez-Rubio & Peccia (2006) studied the biological, chemical and physical characteristics of source and downwind aerosols emitted during disk incorporation where biosolids had been applied. Biological and particulate matter (PM_{10}) samplers were situated downwind from the plume source at a distance of 0, 10 and 170m. Sterile liquid impingers were used to collect aerosol samples of total coliforms, sulfite reducing *Clostridia*, heterogenic plate-count (HPC) and total bacteria. PM_{10} was measured using real-time PM_{10} monitors. Culture-based assays were run to quantify the microorganisms at the different points. The results for downwind concentrations of PM_{10}, sulfite reducing *Clostridia* and HPC are shown in graphs[30]. In the present study, data was extracted from those graphs (figure 9) and a regression line was fitted and adapted to the Q fever data available and the log reduction of *Coxiella* cells after 1Km was calculated. The same method was used with a graph published by Seedorf (2007) showing the decreasing of *Staphylococci* concentration in the air downwind from a broiler barn[31]. The log reduction obtained was 4 from sulfite reducing Clostridia and Staphylococci data, 5 from HPC and 7 from PM_{10}.

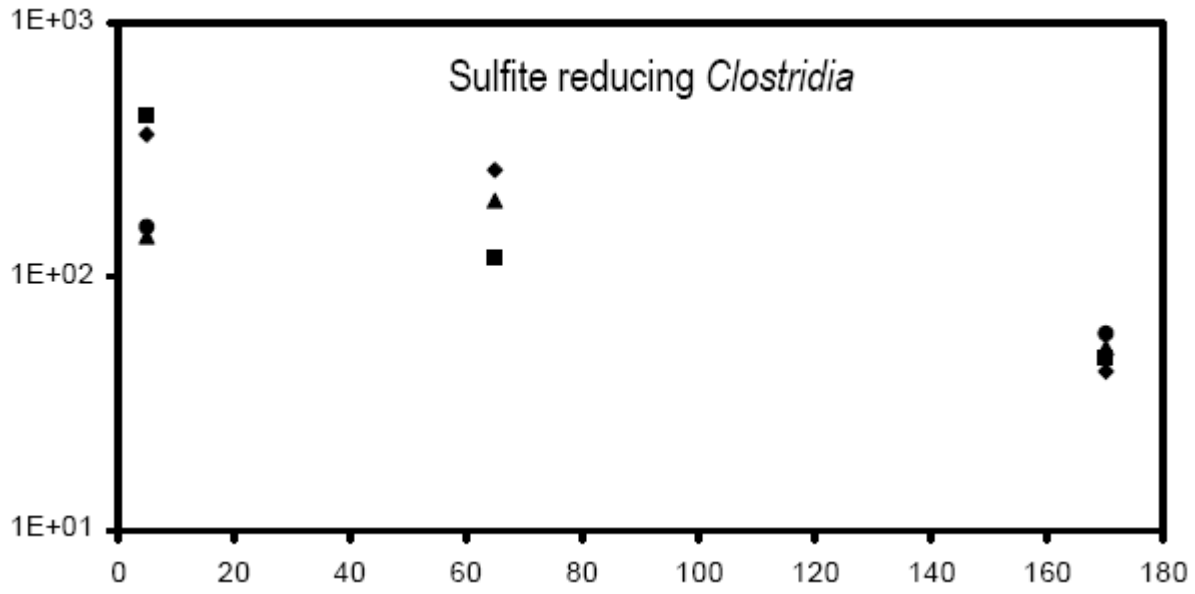

Figure 9: Downwind concentration of Sulfite reducing Clostridia at 0, 70 and 170 m from the plume source [30].

Figure 10 shows an exponential decreasing of the air concentration of Q fever with the distance from the source when using adapted data from Paez-Rubio, in comparison with the power decreasing of the concentration when using Lighthart's model. It can also be seen that, for the scale used, little difference is found in the concentration decay for different height sources or atmospheric conditions.

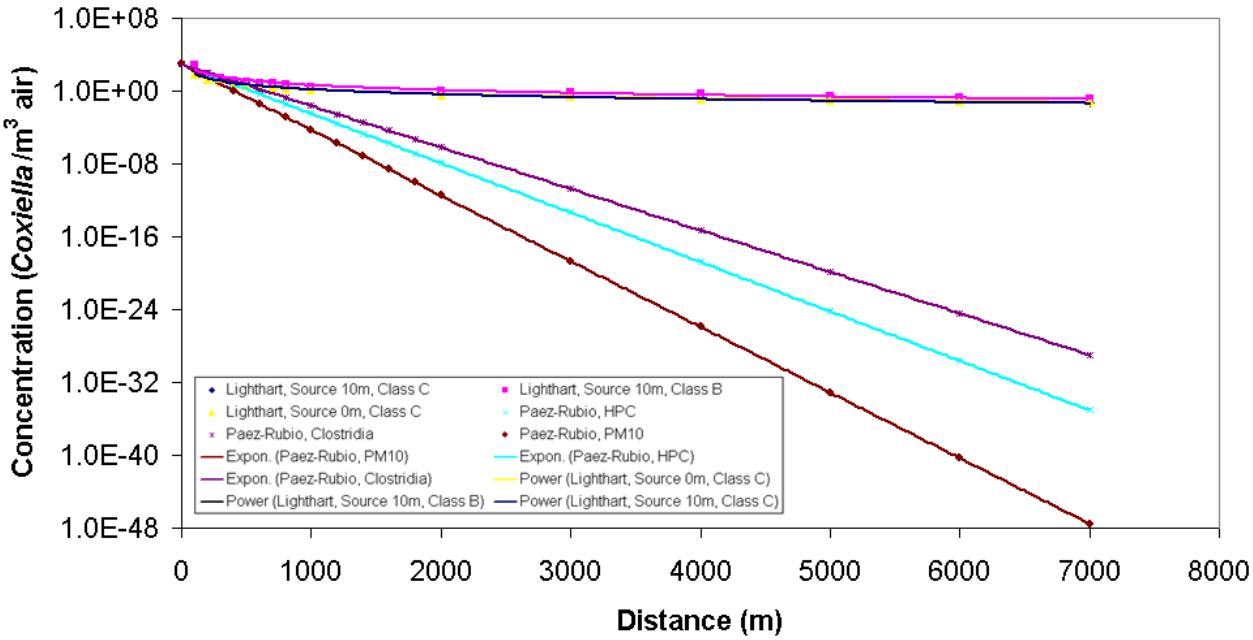

Figure 10 Decay of the Q fever concentration in the air with the distance downwind from the source. Comparison of Lighthart's model and Paez-Rubio's adapted data.

Lighthart's model has been used in this risk assessment to calculate the transport and dilution of Q fever in the air, while for the sensitivity analysis, a 4 log reduction in the air concentration from the barnyard air to a water treatment plant situated 1 km downwind from the farm has been used based on the work by Paez-Rubio & Peccia and Seedorf.
Uncertainties about the air transport and dilution are shown in table 3.

Table 3. Sensitivity table Air transport and dilution.

Source of uncertainty/variability	Direction & magnitude
Emission and transport of *Coxiella*: attached to dust? In clusters? Inside the placenta cells?	0
When the water treatment plant is close to several emission sources (contaminated barnyards) then the concentration at the inlet is higher	++
Figure 2 is based on contaminated milk. Barnyards can be infected without shedding microorganisms in the milk; therefore, the number of contaminated farms may be higher	+
Lighthart model	
Wind speed and meteorological conditions are variable from day to day.	+/-
Source height for mechanical ventilated barnyards, the ventilation outlet can be at some height point, but we don't know where; for natural ventilated barnyards, the emission source height is considered 0m	-
The model considers complete reflection of the particles that hit the floor during the air transport	-
Wet deposition due to the rain is not included in the model	-
Transport of contaminated manure to other regions, far from contaminated barnyards	+
The model only allow to calculate particle concentration at ground level	-
The air inlet is sucking air from the environment	+
It doesn't take into account the effect of barriers (e.g. trees and buildings)	-
Paez-Rubio	
The aerosols are emitted during land disking, so their source height is lower than ours	+
Our extrapolation from the graph is made from a point out of the data given (after 170m)	+/-
We are using the log reduction corresponding to Clostridium spores and Staphylococcus. Using data for HPC, the log reduction would be higher	-

5.3 Concentration at the inlet of a groundwater treatment plant

Groundwater treatment plants are situated, in the Netherlands, relatively close to demonstrated Q fever outbreaks [5]. The closest one is 360 m from a source. Several studies have stated the ability of bioaerosols of travelling long distances. Christensen et al. (2005) demonstrated epidemiologically the travelling of the foot-and-mouth disease (FMD) over a distance of 70 km, based on molecular and meteorological data [32].

Using Lighthart's model for a distance between the water treatment plant and the contaminated barnyard of 1 km, the calculated Q fever concentration at the air inlet of the water treatment plant (C_I) is 1.5 *Coxiella*/m^3 of air. For a water treatment plant situated 360m from a farm, the C_I is 9.2 *Coxiella*/m^3 and 0.08 *Coxiella*/m^3 at 5km. The value obtained from the uncertainty analysis (using data from Paez-Rubio & Peccia) is $1.5x10^{-2}$ *Coxiella*/m^3 of air at 1 km downwind from the source, 20.1 *Coxiella*/m^3 after 360m and it is negligible at 5km.

Table 4 shows the point estimate concentration of *Coxiella* in the air at the water treatment plant inlet (C_I) derived from the calculations using Lighthart model and the value used for the uncertainty analysis obtained from the data from Paez-Rubio & Peccia for Sulfite reducing *Clostridium*.

Table 4. Concentration of Coxiella in the air at the water treatment plant inlet.

Distance (m)	C_I Point estimate (*Coxiella*/m³ air)	C_I Uncertainty Analysis (*Coxiella*/m³ air)
360	9.19	20.1
1000	1.46	$2.42x10^{-2}$
5000	0.08	$1.39x10^{-20}$

5.4 Air filtration

Data from a questionnaire from Brabant Water, WML and Evides showed that several different types of air filtration exist. In some systems, the air inside the drinking water station building is filtered only through a screen (vliegengaas), in some systems the air passes fine dust filters (EN13779 filter class F7) and in some systems HEPA filters (EN1822 filter class F13).
Removal of particles is very different between the different air filter types. Screens do not remove small particles. Table 5 shows test grading and the BS EN1822 standard based on measuring the most penetrating particle size (MPPS) to establish the performance for high efficiency filters. Air quality at point of introduction into the drinking water station is the result of air filter efficiency and filter framework integrity.

Table 5. Air filter efficiency

General Filter Type	Filter Test Reference and Classification		Filter Test Type and Application for the Food Industry
Primary filters to collect coarse dust	BS EN779 *Arrestance %*		Average value for collection of large particles using synthetic test dust. Filters used to prevent mechanical system fouling and as pre-filters to secondary and semi-HEPA range.
	G1	<65	
	G2	65<80	
	G3	80<90	
	G4	>90	
Secondary filters to collect and retain small particle dust	BS EN779 *Efficiency %*		Average percentage value test using atmospheric air. Filters installed to keep general food processing areas clean and free from airborne pollution. Some high care use with risk assessment.
	F5	40<60	
	F6	60<80	
	F7	80<90	
	F8	90<95	
	F9	>95	
Small particulate air filters of the semi-HEPA and HEPA type for specific particulate control	BS EN1822 *Minimum MPPS %*		Filters installed for high care/high risk food process applications. Was Eurovent 4/4 Sodium chloride test Now BS EN1822 aerosol MPPS
	H10	85	
	H11	95	
	H12	99.5	
	H13	99.95	
	H14	99.995	
Highly efficient air filters ULPA type	BS EN1822 *Minimum MPPS %*		Filters used for Laboratory work, in cabinets and some mini environments. BS EN1822 aerosol MPPS
	U15	99.9995	
	U16	99.99995	
	U17	99.999995	

MPPS - Most penetrating particle size
HEPA - High Efficiency Particulate Air (filters)
ULPA - Ultra Low Penetration Air (filters)

BS6540 / BS EN779 arrestance and efficiency test references are based on average percentage values. Primary and secondary filters are at their lowest efficiency when they are new, and at their most efficient at the end of their useful life. BS EN1822 tested filters offer a guarantee of minimum performance when newly installed.

Based on the questionnaire data, there was no correlation between filter type and type of aeration (like more intense aeration, more efficient air filtration). Some of the groundwater treatment plants do not use air filtration. Hence, we selected no air filtration as the setting for the risk assessment, as it results in a higher amount of bacteria being transmitted to water. In the sensitivity analysis, we placed fine dust filters of class F7 (80% removal) or HEPA filters (99.95% removal). When no filtration is applied, the concentration of bacteria in raw water (C_R) is 30 *Coxiella*/m^3 (see next section for values on air to water transfer), while when using F7 and HEPA filters the C_R results in 5.9 and 0.01 *Coxiella*/m^3 , respectively.

5.5 Groundwater aeration

Data from a questionnaire from Brabant Water, WML and Evides showed that several different types of aeration exist, with forced and non-forced airflow. Non-forced systems worked with natural ventilation. In forced air systems the RQ (air flow to water flow ratio) depends on the type of aeration. For increase of the oxygen concentration, a RQ of 0.5 is usually sufficient, while an RQ of 5 or higher is needed for removal of methane, carbon dioxide and hydrogen sulfide [6]. Data from the water utilities showed that a RQ of 20 is not uncommon.

The transfer of *Coxiella* bacteria from air to water in these situations is unknown. Although the conditions are not identical, it is assumed that the transfer rate in this aeration is comparable to the transfer rate of *Coxiella* bacteria from barn air to the liquid broth during air sampling at the barn[16]. Data from detection of *Legionella* in water and shower aerosols is available. Aerosols were sampled from the air using liquid impingement, impaction onto agar and filtration. To determine the recovery rate of the methods used to measure the concentration of *L.pneumophila* in the air, a recovery rate was calculated as the ratio between its concentration in aerosols (measured with an optical particle counter) and its culturable fraction from sampled aerosols. The recovery rate for the liquid impingement method was found to be 0.1, which means that 1 CFU was transferred from the air to the liquid out of 10 particles in aerosols[33]. As the liquid impingement method simulates the phenomenon that takes place during the water aeration process, this data can be used to calculate the transfer of bacteria from air to water at the groundwater treatment plant.

Table 6. Sensitivity table Aeration

Source of uncertainty/variability	Direction & magnitude
Theoretically, lower RQ ratios (5 and 0.5 L air per L of water) are applied.	-
Deloge-Abarkan et al. (2006)	
Recovery rate for liquid impingement method = 10%	-
Experiments performed under laboratory conditions	+/-
Experiments performed with *L.pneumophila*	0
The recovery rate was calculated between the *L.pneumophila* concentration in the aerosol and their culturable fraction; therefore, viable but non-culturable cells were not taken into account	+

Following the conservative approach, the RQ value of 20L of air per L of water is selected and a transfer rate of 1 (all the bacteria present in the air are transferred to the water) is chosen due to the high air flow to water ratio. This results in an amount of 30 *Coxiella*/m^3 of C_R. For the sensitivity analysis, the theoretical RQ values (5 and 0.5) have been used, giving a C_R of 7.4 and 0.7 *Coxiella*/m^3 respectively. The 10% transfer of bacteria from air to water was used in the sensitivity analysis resulting in 3 *Coxiella*/m^3.

5.6 Groundwater treatment

Groundwater that is aerated is usually also treated by rapid sand filtration. No data are available for removal of *Coxiella burnetii* bacteria through rapid sand filters. They are small Gram-negative rod shaped bacteria, and also exist as spores. We assume that the removal by sand filtration is similar to the removal

of *E. coli* and *Clostridium* spores. Hijnen et al. (2004) reviewed the literature. Removal of *E.coli* through rapid sand filtration was 0.5 +/- 0.3 log and *Clostridium* spores were removed by 0.7 +/- 0.5 log[34].

Gale et al (2001) reported that drinking water treatment, consisting of ferric coagulation, blanket clarification and rapid sand filtration, induced spatial heterogeneity of indigenous raw water aerobic spores and *Bacillus subtilis* var *niger* spores, although did not affect spatial distribution of vegetative bacteria (total coliform and heterotrophic bacteria) [35]. The effect of water treatment on the spatial heterogeneity of spores is not included in the present assessment but is discussed later.

Table 7. Sensitivity table Groundwater treatment

Source of uncertainty/variability	Direction & magnitude
The removal used is for *E. coli* (no data for *Coxiella burnetii*) and equivalence for *C.burnetii* is unknown	+/-
The removal for *Clostridium* spores is higher (lower *Coxiella* concentration at the end)	-
Only rapid sand filtration is considered (each water treatment plant must be slightly different, maybe other treatment steps affect the microbial concentration?)	-
Spatial heterogeneity due to water treatment	+/-

Applying the 0.5 log removal, the concentration of *Coxiella* in treated water (C_T) becomes 9.4 *Coxiella*/m^3. If instead of 0.5, a 0.7 log removal is used, the C_T becomes 5.9 *Coxiella*/m^3. These values are used as point estimate and for the sensitivity analysis, respectively.

5.7 Inactivation in water

Coxiella burnetii is able to survive in the environment for long periods. Survival times of 20 – 30 days in soil or barn litter and 30 months in tap water are reported [26]. Given the short residence time of treated water in the distribution network (hours – days), no significant inactivation is assumed to occur during the transport from the treatment plant to the consumers' home.

5.8 Concentration in tap water

As no inactivation during the permanence of the bacteria in the water distribution network is assumed, the concentration in tap water (C_W) is equivalent to C_T.

5.9 Drinking water consumption

Consumption of (unheated) tap water in The Netherlands is well described. The average consumption of cold tap water in the Netherlands is 0.6 L pppd [36]. The health authorities recommend an ingestion of 2 L of water per day. Mons et al. (2007) reviewed water consumption studies and fitted statistical data from the Netherlands, Great Britain, Germany and Australia finding a mean daily consumption variation from 0.10 to 1.55L[37]. Following the conservative approach and considering that the average of 0.6 Lpppd is not covering the whole population in The Netherlands, 2 L is the value for drinking water consumption (I) used as a point estimate. The value of 0.6Lpppd is used in the sensitivity analysis.

Table 8. Sensitivity table Drinking water consumption

Source of uncertainty/variability	Direction & magnitude
Foekema et al., 2007: 0.6 Lpppd (mean amount of water drank per person per day in The Netherlands in 2007)	-

5.10 Dose through ingestion

Considering the data described above, the dose of *C.burnetii* through ingestion (D) is 1.9×10^{-2} *Coxiella* pppd. Using 0.6L instead of 2L of drinking water consumption, D is equal to 5.6×10^{-3} *Coxiella* pppd.

5.11 Transfer to air during showering

There is no data available about the transfer of *Coxiella* from water to air during showering. However, some studies have been done with *Legionella pneumophila*, which is similar to *Coxiella.* Both are cocobacillus resistant to high temperatures and they cause infection by the inhalation route, although this is the only route for *Legionella*, while there is evidence that *Coxiella* can infect humans through ingestion as well. *Legionella* is a non-spore forming bacteria and is bigger than *Coxiella*. Despite these differences between both microorganisms and due to the lack of data available for *C.burnetii*, information relative to *Legionella* has been used for some steps of this part of the risk assessment.

When showering, aerosols are generated from the shower heads. Bacteria, as well as other pollutants, are transferred from water to aerosols. The bacteria air/water ratio can be calculated from the data from several authors' studies. Bollin et al. (1985) studied the concentration of *Legionella* in shower aerosols and the concentration of the microorganism in water sampled from the same shower heads. They found 0.006 CFU per liter in the air culture, while more than 200000 CFU/liter in the water samples. This makes an air to water ratio of 3×10^{-8}. They also found that approximately 90% of the *L. pneumophila* recovered were trapped in aerosol particles between 1 and 5µm in diameter, hence, inhalable [38]. Perkins et al. (2009) found an average of total bacterial counts of 2.2×10^{7} cells/liter in shower water and 3.4×10^{1} cells/liter in shower aerosols, which means a 1.55×10^{-6} air/water ratio [39]. Another study run by Dennis et al. (1984) reported 1000 *Legionella* CFU per liter in water and 0.00033 CFU/L in the air surrounding the showers where the water samples were taken (air samples taken before the showers were started gave negative results). This means a 3.3×10^{-7} ratio[40]. More recently, the feasibility of the detection of airborne *Legionella* while showering in two health care centers in northern France has been studied, sampling the hot water from the shower and the air after the water faucet was closed. Three different bioaerosol sampling methods were tested: solid impaction, liquid impingement and filtration, to evaluate their influence on the culturability of the aerosolized bacteria, and the in situ hybridization method (FISH) to detect airborne *Legionella*. The recovery rate from 18 samples was calculated as the ratio of the concentration of *Legionella* in aerosols, measured using an optical particle counter, by its culturable fraction after sampling, and it was observed a higher recovery rate when the impingement method was used (1 CFU for every 10 airborne particles, while 1:40 and $1:7x10^{3}$ for impaction onto agar and filtration, respectively). With the FISH method, it was obtained a 74% detection of aerosolized *L.pneumophila*, while only 12% of the bacteria were detected with the culture analysis. An average of 4.65×10^{8} *Legionella*/m^3 in water was found using the FISH method and 2.71×10^{6} *Legionella*/m^3 in air, using the liquid impingement sampling technique and the FISH method[33]. This gives a ratio of *Legionella* from water to air of 5.83×10^{-2} cells. Although this data give the most conservative value in our risk assessment, it suggests that all the bacteria present in the water leaving the showerhead are aerosolized. This is unlikely, as some of the bacteria will stay in the water and leave the shower through the floor drain [41].Therefore, we use data from the culturable *Legionella spp* obtained in the same study. Culturable *Legionella* spp detected in water was 10 fold lower than when the FISH method was used ($4.03x10^{7}$ CFU/m^3). Only 2 CFU/m^3 air where detected from aerosol samples collected from the air with agar impaction; considering the recovery rate for agar impaction, this gives a ratio of $1.99x10^{-6}$ *Legionella* from water to air, this generates a C_A of $1.9x10^{-5}$.

Table 9. Sensitivity table transfer of Coxiella from water to air.

Source of uncertainty/variability	Direction & magnitude
Deloge-Abarkan (2006)	
Legionella (extrapolation between species)	0
Laboratory conditions	-
Liquid impingement instead of filtration	+++
Higher ratio with FISH	+
Other studies	
Ratio from Bollin et al. (1985)	-
Ratio from Perkins et al.(2009)	0
Ratio from Dennis et al. (1984)	-
No recovery rate (in the other studies)	++

5.12 Q fever dose through inhalation

The dose of bacteria that every person inhales during showering depends on several factors: the concentration of bacteria in aerosols, the volume of air inhaled, and therefore, the breathing rate and the shower duration, the size of aerosols, that will determine the quantity of aerosols and, hence, bacteria that will reach the alveolar region and cause infection, etc.

The average shower duration among the Dutch population during 2007 was between 7.7 and 7.9 minutes and every person took, in average, 0.8 showers per day. People between 18-44 years old shower an average of 0.91-0.93 times/day; those living in the 3 big cities shower more often: 1.11 times per day, suggesting that some people shower more than once per day. Immigrants shower 1.37 times, while Dutch people 0.74 [36].
Information about the average showering duration in The Netherlands is also available. During the week days, people spend an average of 8.1 min in the shower. During the weekends the time spent is lower (7.3 minutes). In the present study, it has been considered a shower frequency of 1 for the whole population and shower duration of 8.1 min. For the sensitivity analysis, the values of 0.8 showers per week and 7.7min per shower have been chosen.

The breathing rate of an average healthy person during rest is around 12 breaths/min[40, 42]. Benchetrit et al. reported values of 31breaths/min in a study made by Hutchinson in 1850, in which breathing frequency values in 1714 adult subjects during rest were obtained by observation[43]. The average breathing or tidal volume, which is the air inspired or expired during a normal respiration, is 500 mL [42]. Values of 1549mL have been reported by Benchetrit [43]. From this data it is possible to calculate the respiratory minute volume:

$$V_E = V_T \cdot f_R \quad \textit{Equation 3 [42]}$$

During exercise, values of 32.2 L/min for light exercise, 50 L/min for moderate exercise and 80.4 L/min for heavy exercise were obtained in a study made to nine healthy male subjects between 19-35 years old [44]. Ventilation rates exceeding 200 L/min in large individuals during acute exercise have also been reported [45]. Zhou et al (2007) assumed that showering was a light exercise and, based on the ICRP deposition model (1994), used the value of 20 breaths/min and a tidal volume of 1.25 L, which makes a breathing rate of 25 L/min[46]. After exercise, the breathing rate does not decrease immediately to the rest values but gradually. It starts decreasing fast, followed by a slow stage which duration depends on the intensity and duration of the physical activity [47]. Complete recovery can take many hours [48]. The values of 500mL for the breathing volume and 12 breathings/minute for the breathing rate have been used as point estimates because they are representative of the average population. Therefore, the respiratory minute volume is, according to equation 3, 6 L/min, and the total air inhaled during a shower 0.05m^3. Assuming not only that showering is considered a light exercise, but also that a percentage of the population is having a shower right after developing a moderate or heavy exercise, without giving enough time to their respiratory system to recover, it has been used the value of 31 breaths/min and 1549mL of tidal volume, obtaining a respiratory minute volume of 48.02 L/min, in the sensitivity analysis. This means an amount of air inhaled during a shower of 0.39m^3.

A study performed by Zhou et al. (2007) calculated the particle deposition fraction during showering with mouth and nose breathing. A bathroom with a shower stall of 2 m^3 and a mannequin simulating the human body were used. Two sampling positions were situated, one inside the shower stall and the other one outside, at 1.5m from the shower floor. The total mass concentration of particles was determined using a Data RAM real-time particle monitor, which measures particles in the concentration range of 0.1µg/m^3-400mg/m^3 for particles less than 10µm in diameter. An Aerodynamic Particle Sizer measured particle number and mass size distributions, which completed 1 measurement every minute, so 10 replicate measurements were made during each 10-min showering period. The ICRP deposition model was used to calculate the particle deposition fractions in the extrathoracic, bronchial, bronchiolar and alveolar regions. Three different flow rates (5.1, 6.6, and 9.0 L/min) were used. The temperature of the hot water was controlled at 43-44^{o}C. The particle concentration in the shower stall ranged from 300µg/m^3 to 14000µg/m^3 and the mass median diameter (MMD) of the droplets were between 6.3 and

7.5 µm. The deposition fraction differed for hot and cold water, for different shower flow rates and for mouth and nose breathing [46]. For hot water, the total inhaled water particles was 75 times higher than for cold water. Considering the conservative approach taken in the present study and assuming that the majority of people in The Netherlands use warm water during showering, only the values for hot water from Zhou's study have been used in the point estimate study, while the data from cold water has been used in the sensitivity analysis. For mouth breathing, the average fraction of bronchiolar (bb) deposition for the three shower flow rates was 4.6% while during nose breathing it was only 0.57%. In the alveolar (Al) region, the deposition rate during mouth breathing was 8.13%, and during nose breathing 1.17% of the particles inhaled. For mixed breathing, the values should be within these parameters [46]. Due to the lack of information about mixed breathing, we use the rate referred to mouth breathing for being the most conservative one (12.7% of the inhaled aerosols are deposited in the bb and Al region, approximately). We assume that the alveolar and bronchiolar regions are the paths through which the microorganism can cause infection.

Bollin et al. (1985) found out that 90% of *L.pneumophila* cells that were aerosolized from shower faucets were trapped in aerosol particles between 1 and 5 µm in diameter. They state that this range correspond to inhalable aerosols [38]. However, other authors claim that inhalable aerosols measure between 0.5 and 12 µm, and aerosols in the range of 1 to 5 µm are the ones that reach the alveolar region, where can be deposited, while those with a diameter higher than 10 µm (large particles) deposit in the upper respiratory tract[49]. Following the conservative approach, we take the second statement as valid and consider that particles between 1 and 5 µm can reach the alveolar region. However, we use the data from Zhou et al. (1997), for being better described, to estimate the bacteria deposition in the alveoli, and the data from Bollin et al. (1985), though being more conservative, in the sensitivity analysis.

The amount of *Coxiella* inhaled during a shower is calculated as the amount of Q fever in shower aerosols times the volume of air inhaled during a shower. This gives an amount of 9.1×10^{-7} Coxiella inhaled per person during a shower (C_S) and a total deposition of *C.burnetii* in the bronchiolar and alveolar region during a shower or, in other words, the dose of bacteria through inhalation (d), of 1.15×10^{-7} *Coxiella* pppd.

However, exposure to aerosolized *C.burnetii* doesn't limit to shower aerosols. Although not daily, people are exposed to other aerosols generated by urban water devices (e.g. fountains), cooling towers, whirlpools, etc. (whirlpool workers, for example, would be exposed daily). It is difficult to determine the magnitude of this exposure, and the variability among Dutch population is probably very high.

Table 10. Sensitivity table Q fever dose through inhalation

Source of uncertainty/variability	**Direction & magnitude**
Population variability in shower duration and shower frequency	0
Population variability in breathing rate and tidal volume	+
The deposition calculated by Zhou et al. and used in our study is for mouth breathing. For nose breathing it is lower.	-
Zhou et al. do not consider suction effect of breathing during inhalation.	+
After showering, bioaerosols generated during showering are still in the bathroom, so the time spent in the bathroom after showering and before opening the bathroom door should be considered	+
We are considering only hot water, for cold water the deposition pattern and the inhalation is different	+
We assume that deposition of *Coxiella* in alveolar but also in bronchiolar region can cause infection	-

Table 11 shows a summary of all the exposure assumptions of the Q fever risk assessment through drinking water.

Table 11. Exposure assessment assumptions

Assumptions	Value	Units	Reference
Q fever concentration at the barnyard air	880/8.35	Cells/m^3	[18], [16]
Air Model			[21]
Ventilation rate	938	m^3/h/LU	[22]
Number of goats in the farm	900	n	[13]
Goat's weight	100	Kg	Assumption
Inactivation of *C.burnetii* in the air	0	-	Assumption[26]
Distance between barnyard and groundwater treatment plant	1000	m	Assumption
Source height	10	m	Assumption
Wind speed	4.17/3.92	m/s	[28]
Insolation	Slight	-	[28]
Air filtration efficiency	0		Questionnaire
RQ ratio	20	L.air/L.water	Questionnaire
Transfer rate of *C.burnetii*	1	-	Assumption
Removal by water treatment	0.5	log	[34]
Inactivation of *C.burnetii* in water	0	-	Assumption[9, 26]
Water ingestion	2	L	Assumption
Ratio Cair/Cwater	$2x10^{-6}$	-	[33]
Shower frequency	1	pppd	Assumption[33, 36]
Shower duration	8.1	min	[36]
Breathing rate	12	Breaths/min	[42]
Breathing volume	500	mL	[42]
Deposition in the lower respiratory tract	12.7	%	[46]

6 Hazard Characterization

Recently, a dose-response model for *C. burnetii* has been established [50]. In that study, different models (exponential, beta-Poisson and log-probit) were fit to data using the method of maximum likelihood estimation (MLE). The data from the literature corresponded to 3 studies performed with mice (SCID mice, C57BL/6J mice and C57BL/10ScN mice) exposed intraperitoneally and one with humans (young adult male men) exposed to aerosolized *C.burnetii*. The best fit dose response model was the Beta-Poisson for the human aerosol exposure data, the SCID mice and the C57BL/10ScN mice, while the exponential showed a best fit for the C57BL/6J mice (table 12). The Beta-Poisson model with human data gave a N_{50} of 6.6, which means that around 6 microorganisms are sufficient to cause clinical symptoms in 50% of the population exposed to *C.burnetii*. A single bacterium is estimated to cause illness in 19% of the exposed population (Figure 11).

Table 12. Best fit model for different Q fever studies [50]

Subject	Route of Exposure	Response	Best fit model	Model Parameters
Mice (SCID)	Intraperitoneal	Death	Beta-Poisson	α=0.492 N_{50}=6.77x10^{-5}
Mice (C57BL/6J)	Intraperitoneal	Death	Exponential	r=5.7x10^{-11}
Mice (C57BL/10ScN)	Intraperitoneal	Death	Beta-Poisson	α=0.356 N_{50}=4.925x10^{8}
Adult male men	Inhalation	Clinical disease	Beta-Poisson	α=0.414 N_{50}=6.623

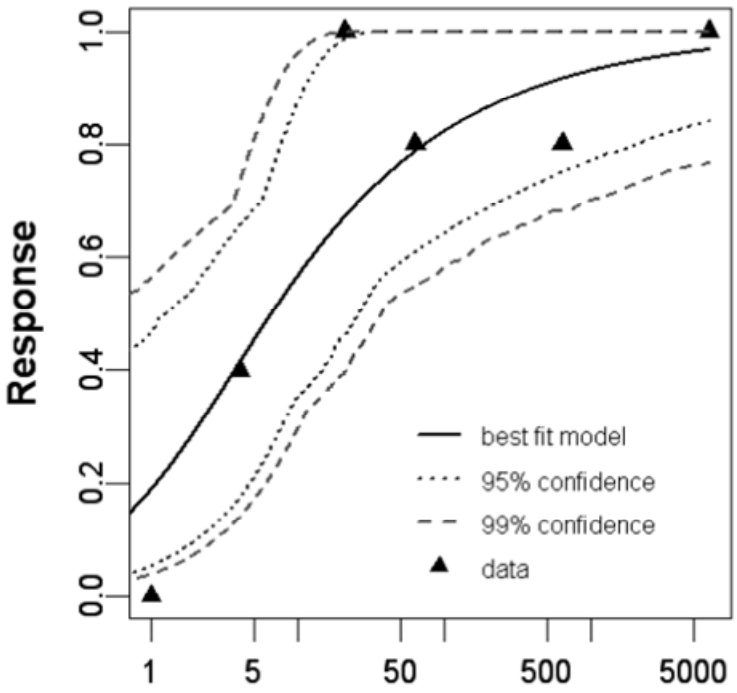

Figure 11. Dose response Data and Beta-Poisson Model Fit for Clinical Disease in Aerosol Exposed Human to C. burnetii [50].

In the present study, the Beta-Poisson model (equation 5) was used:

$$P(d) = 1 - \left[1 + \left(\frac{d}{N_{50}}\right) \cdot \left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \qquad \textit{Equation 4}$$

where N_{50} is 6.623 and α is a slope parameter and equals to 0.414. In the sensitivity analysis, an exponential model was used instead.

Although it is known that *C.burnetii* can cause infection through the ingestion of contaminated milk or meat, no studies have been found that determine the dose-response relationship through the ingestion route. According to Madariaga et al. (2003), ingestion of moderate doses of *Coxiella* would be unlikely to produce clinical symptoms [14]. In the literature, only relations between ingestion of contaminated milk and seroconversion have been found. A study performed by Fishbein and Raoult (1992) showed the same incidence of Q fever seropositivity among farm workers and persons who had consumed unpasteurized milk coming from the same farm (75 % in both cases) [51]. In another study, 120 men were given unpasteurized milk contaminated with *Coxiella burnetii*, while 2 control groups, with 112 and 99 men each, were given only pasteurized milk. From the men exposed, 35% were serologically positive and 28.6% had positive conversions (were serologically negative originally or developed fourfold or greater increases in Q fever titers during the test period). In the control groups, positive titers were found in 8.9% and 4% of the men, but none of them had positive conversions. No relation was found between febrile or respiratory illnesses and seropositivity or seronegativity for *C.burnetii* [52].

As stated above, the dose-response model used in the risk assessment has only been fit for clinical symptoms developed after inhalation exposure to *C.burnetii* and no dose-response data has been found for the ingestion route. We assume that the model is not valid for the ingestion route as literature suggest that the bacterial dose necessary to cause clinical symptoms in humans through ingestion should be higher than for inhalation. Therefore, the data concerning probability of developing clinical illness due to ingestion of drinking water is not taken into account.

The dose-response model for the susceptible population is also not known. The data fit by Tamrakar et al. from a study made on SCID mice correspond to a combination of immunodeficient and immunocompetent mice. The best fit model was the Beta-Poisson model but with different parameters values, being N_{50} equal to 6.77×10^{-5} cells and α 0.492. However, the data correspond to the intraperitoneal route of exposure and, moreover, the study was performed in mice. Hence, this data was not considered suitable for the present study.

The uncertainties corresponding to this part of the risk assessment are showed in table 13.

Table 13. Sensitivity table Dose Response Model

Source of uncertainty/variability	**Direction & magnitude**
Extrapolation of a study made on young healthy volunteers to a heterogenic population	+
Study made on subjects from US (not known whether a difference of susceptibility between US and Dutch population exists)	?
Dose-response relation in susceptible population is unknown	+
Dose-response through ingestion route is unknown	+/-

Although the exponential model results in a higher risk of Q fever disease, the Beta-Poisson model has been selected to calculate the point estimate risk, while the exponential model has been used to obtain an alternative value.

7 Risk Characterization

7.1 Results

In order to calculate the probability of infection of *C.burnetii* through drinking water, a hypothetic scenario consisting of a ground water treatment plant situated 1km North-East from a barnyard with infected goats was studied for the year period 2009. Three situations were established: A, days corresponding to the kidding season when the wind was blowing from the South-West (so that the water treatment plant was situated downwind from the barnyard); B, days of the rest of the year (out of the kidding season) when the wind was blowing from the South-West; and C, days when the wind was not blowing from the South-West but from other directions, so the water treatment plant was not located downwind from the source (including kidding season and rest of the year). For the first situation, the particles emitted from the source were assumed to be 880 *Coxiella*/m^3; for the second situation 8.35 *Coxiella*/m^3 and for the third situation it was assumed to be 0 (as the particles were emitted in another direction).
It was difficult to determine the kidding season months. Hatchette et al. (2001) studied an outbreak of Q fever associated to goats that occurred in the spring of 1999 in Newfoundland, USA. They observed that, for that year and in the eight farms within a 170km^2 area in Newfoundland, the kidding season began in January 6 and ended in April 24, 1999, while the first abortion occurred in December of the previous year, but the rest took place between January 14 and April 24, 1999. Serum samples were analyzed for *C.burnetii* in 147 of the 174 goats in the eight farms as well as from livestock from other farms throughout Newfoundland to determine the background seroprevalence of Q fever. *Coxiella* was identified in placental samples examined by using electron microscopy and light microscopy, and *C.burnetii* DNA was demonstrated in all three placental samples with PCR. A total of 30 abortions were recorded at six of the eight farms, with abortion rates of 16-22%. No relationship was found between seropositivity in goats and frequency of abortion. It was also pointed that *Coxiella* is shed in high numbers in birth products and aerosolization of the microorganism can persist for days after partiturion, despite immediate removal of the highly infectious placenta [53]. Sanford et al. (1994) studied the relation between abortions and *C.burnetii* infection in 5 herds of goats from Ontario and New Brunswick that had been exposed to the bacteria at the 1991 Royal Winter Fair in Toronto. A total of 107 goats were tested serologically, giving a positive result in 50%-85% of the animals. Abortions occurred in 20-47% of the pregnant goats. The first abortion was observed on December 16, 1991, but the rest reproductive failures (including abortion, stillbirth, premature kidding and weak newborn kids) occurred between January 1 and March 7, 1992 [54].

Based on the information above the kidding and abortion season should occur between January 1 and April 30, 2009. However, this period is in discordance with the data found about the disease incidence in the Netherlands for 2009 (Figure 12) and the information about the incubation period of the bacteria in humans. In figure 12 can be seen that the peak of the illness occurred between week 14th and week 23rd of the year (beginning of April to beginning of June). Knowing the incubation period of Q fever in humans, we can back-calculate when the bacteria was emitted from the barnyard.

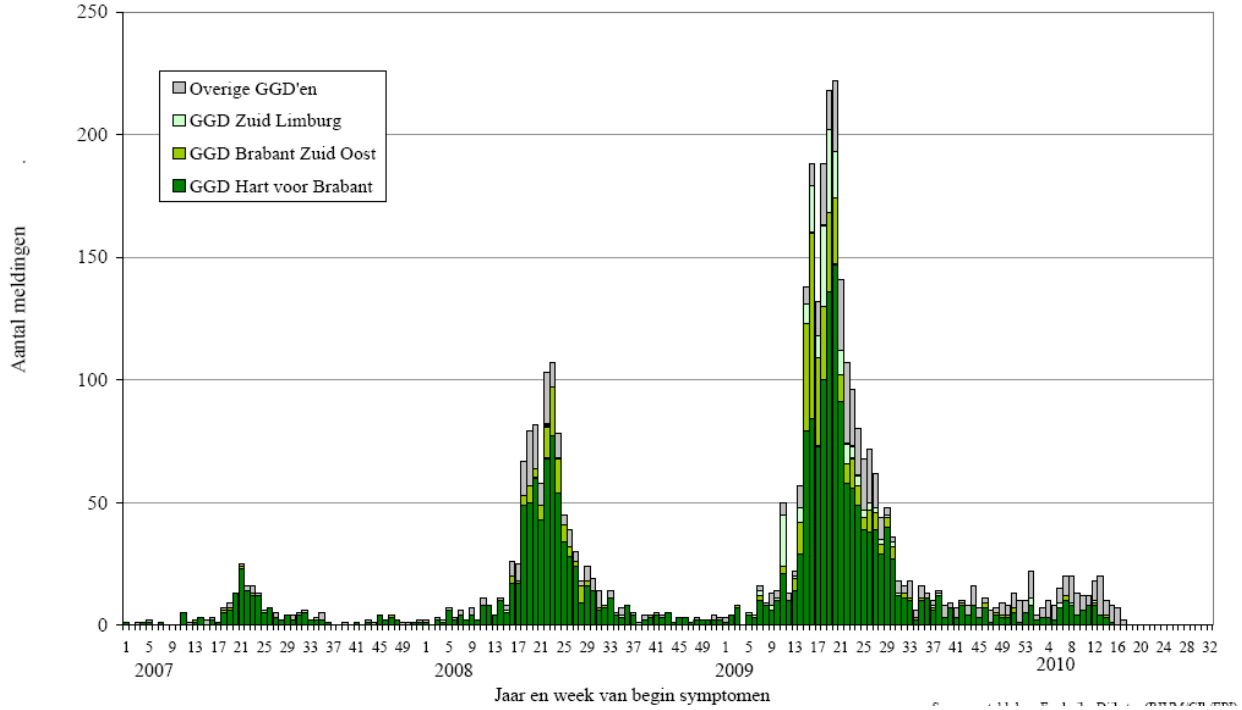

Figure 12. Number of Q fever cases in The Netherlands per year and week of the onset of symptoms.

Baca and Paretsky, 1983, claim that the incubation period for Q fever is 15 days or less, varying with the route of exposure, dosage of bacteria and age of victim [55]. However, Marrie et al. published a study in 1989 about a Q fever outbreak in a truck repair plant due to aerosols from clothing contaminated by contact with newborn kittens and found that the clinical symptoms appeared from 12 to 21 days after exposure [56]. In a previous study, they found an incubation period from 4 to 30 days, but most cases appeared 2 weeks after exposure [57]. Also Marrie et al., in 1996, observed that human volunteers who inhaled one infectious dose of Q fever had an incubation period of 16 days, while those who were exposed to 1500 infectious doses had an incubation period of 10 days [58]. Miller et al. (1996) observed an incubation period from 1 to 3 weeks [59]. In order to follow the conservative approach and based on the literature data, in the present study it was decided to consider an incubation period from 1 to 3 weeks, covering a wide range.

Considering the onset of the outbreak in 2009 and the incubation period of Q fever, the bacteria would have been released from the barnyard during the period between the 8th of March and the 30th of May. This period does not coincide with the kidding and abortion period found in the literature. A reason can be that, although kidding and abortions start in January, most of them occur at the end of the season (March-April) and if the birthing fluids and placentas are left without cleaning, the bacteria have more time to be emitted to the environment. A time lap exists between the birthing and the releasing of the bacteria from the placenta that may contribute to this difference between the kidding season and human exposure. Moreover, during April and May there are more hours of sun, favoring the releasing of the bacteria from the fluids and placentas on the ground to the environment. In a study about a Q fever outbreak in The Netherlands in 2008, it was estimated that the likely period of infection was from mid-April to mid-June. The estimation was also based on the incubation period and onset of symptoms in the affected population [23].

To determine the wind speed and direction and the atmospheric conditions, data from the KNMI website corresponding to 2009 has been used. The atmospheric conditions have been determined only for the daylight hours considering the relative insolation and the wind speed. Slight insolation was assumed to be between 0-33%, moderate between 34-66% and strong between 67-100%. Only the days when the wind was blowing from the South-West (considering between 180° and 269°) were used for the study, which made a total of 34 days for the Q fever emission period in 2009. This wind direction was selected for the present risk assessment for being the predominant direction in the Netherlands (45% of the days during 2009, meteorological station Eindhoven[28]). Slow wind speed (less than 2m/s) and

strong daytime insolation corresponds to atmospheric stability class A, which favors high concentration of aerosols downwind from the source, while fast wind speed (more than 6m/s) and slight insolation corresponds to class D, which is the least favorable. In between these extremes, several combinations are possible. Between the 8th of March and 30th of May only one day was A stability class, 9 were B, 12 C and 12 D, being the average C. The average mean wind speed for the 34 days was 4.2m/s, while for the days with atmospheric class C it was 4.1 m/s.
For situation B (from January to the 7th of March and June to December), the average wind speed was found to be 3.9 and the insolation was light. Therefore, the average stability class was also C.
Table 14 shows the risk characterization for Q fever through aerated water for situations A, B and C.

Table 14. Risk characterization of the different situations

	Situation A	**Situation B**	**Situation C**
Months	March-May	June-February	January-December
Wind direction	180-269º (S-W)	180-269º (S-W)	270-179º
Number of days	34	137	194
Q fever concentration at the barnyard air (cells/m^3)	880	8.35	880/8.35
Q fever concentration at the air inlet of the water utility (cells/m^3)	1.5	0.02	0
Dose through ingestion (cells pppd)	$5.6x10^{-3}$	$1.9x10^{-4}$	0
Dose through inhalation (cells pppd)	1.210^{-7}	$1.2x10^{-9}$	0
P disease through inhalation (n/y)	$1.1x10^{-6}$	$4.32x10^{-8}$	0

The probability of Q fever disease is higher during the kidding months than during the rest of the year, when the wind is blowing from a contaminated farm to a ground water treatment plant. Indeed, the total risk depends on the probability of exposure during the kidding period.

7.2 Sensitivity analysis

In order to know in which magnitude and direction each model input influences the risk of developing Q fever disease, a nominal range sensitivity analysis was performed and the results are shown in table 15 and figure 13. Some uncertainties are not included because of their negligible effect on the output, e.g. the goats' weight; others because no information about their magnitude is available, e.g. aspiration of infected aerosols during drinking of tap water. It was found that the steps that affect disease risk most are the concentration in the barnyard air, the distance from the barn, the air model, the RQ value in the aeration process, the efficiency of the air filters, and the water to air ratio during shower aerosolization. The later and the air filtration efficiency were the factors that produced a higher effect on the risk, due to the magnitude of the difference between the point estimate value and the alternative value. Most of the alternative values produce a decrease in the probability. This is what we expected, as we have been following a conservative approach selecting the literature values for the point estimates that gave a higher probability of disease, therefore the alternative values necessarily generate a lower output. As stated before, when the most conservative value isn't representative of the population or is considered far from reality, another value has been selected and, in this case, the alterative value generates a higher probability of developing Q fever. This is the case of the water to air ratio, the number of goats in the farm, the distance between the groundwater treatment plant and the barnyard, the respiratory minute volume, the deposition pattern of the bacteria in the respiratory ducts, the use of cold water for showering and the use of the exponential model.

Four of the exposure steps show an inverse relation with the model output (an increase in the magnitude of the assumption generates a decrease on the probability of Q fever and vice versa). These steps are the

distance between the water treatment plant and the barnyard, the stability class and wind speed, the efficiency of the air filtration and the efficiency of the water treatment process (log reduction of bacteria). It is important to indicate that due to the lack of data available for *C.burnetii*, lots of assumptions had to be made based on data available for other microorganisms like *L. pneumophila* or *E.coli*. This is the case of the removal efficiency of water sand filtration or the ratio of bacterial transference from air to water. Also an interspecies extrapolation was made to estimate the concentration of Q fever bacteria in the barnyard air (data from sheep was used due to the non-availability of goats' data) and the emission (data from cows was used for the ventilation rate).
In table 15, only the influence of the distance on the risk for 360m and 5000m are shown. The first have been chosen because is the closest that a water treatment plant has been found from an infected barnyard to the date. The second because is the perimeter around infected barnyards where high concentrations of the bacteria have been found. More information about the effect of the distance between a groundwater treatment plant and the infected barn is shown in figure 14. The risk shows potency decay with the distance. A groundwater treatment plant located 85m downwind from an infected barnyard would pose a risk of $1.0x10^{-4}$ per year through inhalation to the population using drinking water from that plant. It is known that the closest water plant to an infected barnyard is situated 360m from it. However, more barnyards can be declared infected in the future and new farms can be built within the 85 m radius from water treatment plants.

Table 15. Sensitivity analysis

				Probability (n/y)		
Step	**Units**	**P.E. value**	**U/V value**	**P_A**	**P_B**	**P_{TOTAL}**
Point estimate value				**$1.1x10^{-06}$**	**$4.3x10^{-08}$**	**$1.1x10^{-06}$**
Concentration barnyard air[3]	cells/m^3	880/8.35	8.35	$1.0x10^{-08}$	$4.3x10^{-08}$	$5.3x10^{-08}$
Ventilation rate[3]	cells/s	938	268	$3.0x10^{-07}$	$1.2x10^{-08}$	$3.2x10^{-07}$
Number of Goats[2]	n	900	5000	$5.9x10^{-06}$	$2.4x10^{-07}$	$6.1x10^{-06}$
Distance from the barn[2]	m	1000	360	$6.6x10^{-06}$	$2.7x10^{-07}$	$6.8x10^{-06}$
Distance from the barn[2]	m	1000	5000	$5.8x10^{-08}$	$2.3x10^{-09}$	$6.0x10^{-08}$
Stability class and wind speed[3]	m/s	C, 4.2/3.9	D, 5.7	$2.3x10^{-07}$	$8.9x10^{-09}$	$2.4x10^{-07}$
Source height[1]	m	10	0	$8.5x10^{-07}$	$3.5x10^{-08}$	$8.8x10^{-07}$
Air model[1]	-	Lighthart	4log reduction	$1.7x10^{-08}$	$6.6x10^{-10}$	$1.8x10^{-08}$
Aeration (RQ)[1]	Lair/Lwater	20	0.5	$2.7x10^{-08}$	$1.1x10^{-09}$	$2.8x10^{-08}$
Transfer rate *Coxiella*[1]	ratio	1	0.1	$1.1x10^{-07}$	$4.3x10^{-09}$	$1.1x10^{-07}$
Air filtration efficiency[3]	%	0	99.95	$5.3x10^{-10}$	$2.2x10^{-11}$	$5.5x10^{-10}$
Air filtration efficiency[3]	%	0	80	$2.1x10^{-07}$	$8.6x10^{-09}$	$2.2x10^{-07}$
Water treatment[1]	log	0.5	0.7	$6.7x10^{-07}$	$2.7x10^{-08}$	$7.0x10^{-07}$
Higher Ratio Cair/Cwater[3]	ratio	$2x10^{-06}$	$5.8x10^{-02}$	$3.1x10^{-02}$	$1.3x10^{-03}$	$3.2x10^{-02}$
Lower Ratio Cair/Cwater[3]	ratio	$2x10^{-06}$	$3x10^{-08}$	$1.6x10^{-08}$	$6.5x10^{-10}$	$1.7x10^{-08}$
Shower frequency and duration[2]	pppd; min	1; 8.1	0.8; 7.7	$8.1x10^{-07}$	$4.1x10^{-08}$	$8.5x10^{-07}$
Respiratory minute volume[2]	L/min	6	48	$8.5x10^{-06}$	$3.5x10^{-07}$	$8.8x10^{-06}$
***Coxiella* deposition pattern[3]**	%	12	90	$7.5x10^{-06}$	$3.1x10^{-07}$	$7.8x10^{-06}$
Cold water[2]	%	12	27	$1.6x10^{-06}$	$6.7x10^{-08}$	$1.7x10^{-06}$
Nose breathing[3]	Cells pppd	$1.2x10^{-07}$	$1.6x10^{-08}$	$1.5x10^{-07}$	$5.9x10^{-09}$	$1.5x10^{-07}$
Only alveolar deposition[1]	Cells pppd	$1.2x10^{-07}$	$7.4x10^{-08}$	$6.8x10^{-07}$	$2.8x10^{-08}$	$7.1x10^{-07}$
Scenario (wind direction)[1]	-	S-W	SSW-WSW	$5.6x10^{-07}$	$2.9x10^{-08}$	$5.9x10^{-07}$
Dose response model[1]	-	Beta-Poisson	Exp	$3.9x10^{-06}$	$1.6x10^{-07}$	$4.1x10^{-06}$

[1] Uncertainty,[2] Variability, [3] Uncertainty and variability

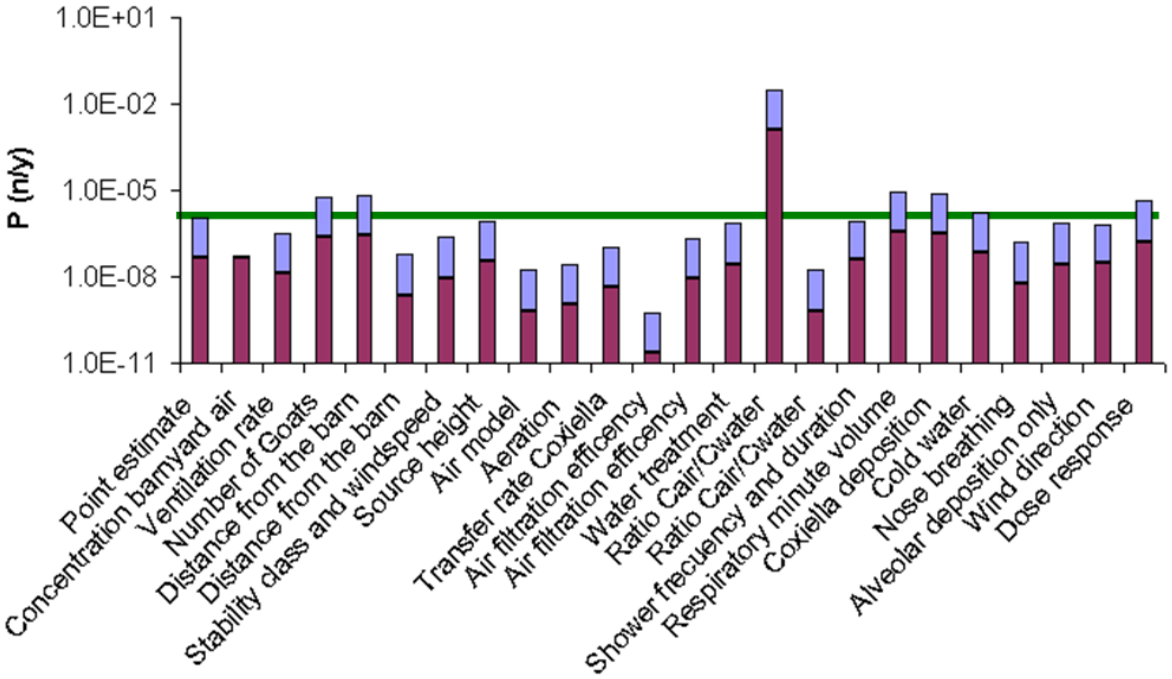

Figure 13. Effect of each alternative value on the risk of Q fever disease. Situation A in blue and situation B in purple. Green line represents the point estimate risk for situation A.

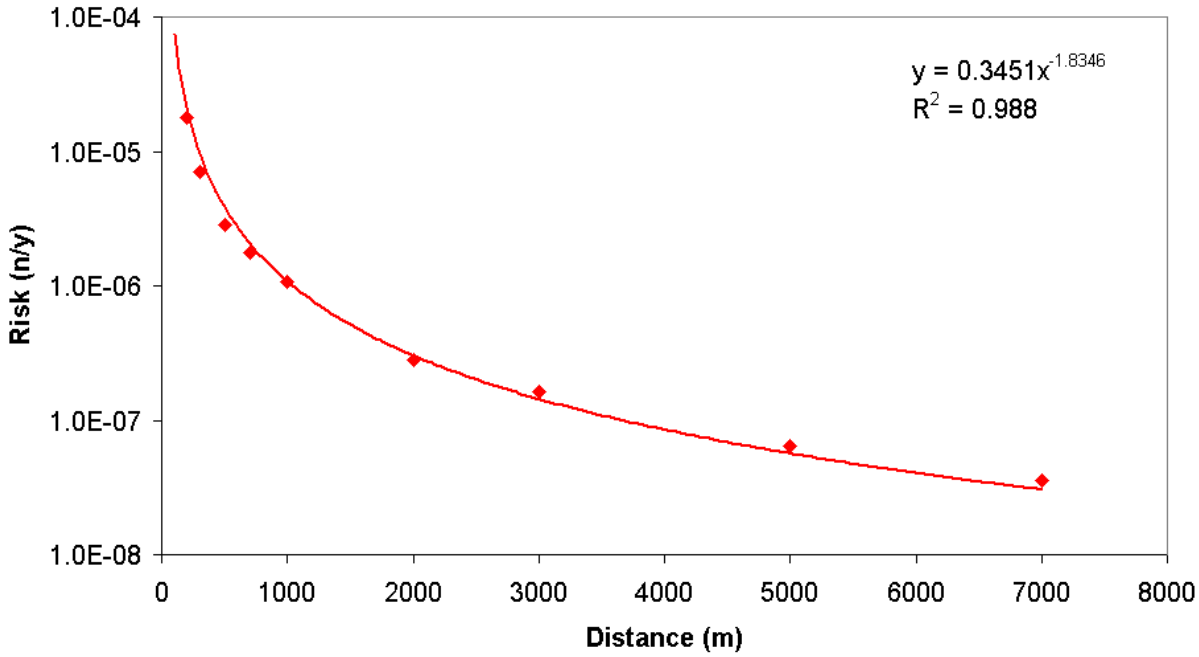

Figure 14. Risk of developing Q fever versus downwind distance of the groundwater treatment plant from the infected barn, situation A only.

Regarding the shower water temperature, it can be seen that the probability of developing Q fever increases slightly when using cold water instead of warm water. This is due to the fact that the aerosols generated with cold water are smaller and, therefore, a higher amount can reach the bronchiolar and alveolar regions compared to the aerosols generated with warm water, which are bigger. However, warm water generates more aerosols than cold water, but this factor is not included in the model. This is addressed later on in the discussion.

Earlier in this report, the specific scenario to which the present risk assessment study is applied has been described. The wind blows from the SW and carries the *C.burnetii* particles emitted from a contaminated barnyard to the air inlet of a groundwater treatment plant located 1km downwind. In the term SW we have included 180 to 270 degrees, wind blowing from between the south and the west. In the sensitivity analysis we are using a more specific wind direction: from between the south-southwest (SSW) and the west-southwest (WSW) (202.5-247.5 degrees). This assumption reduces the number of days when the wind carries the infective cells from the barn to the water treatment plant to 17 days in the situation A and 80 days in the situation B [28] and, therefore, the risk of illness is also reduced. The average wind speed is also affected by this change, being 3.95m/s in situation A and 4.04m/s in situation B. The mean stability class is still C, but the mode is now D.

Regarding the dose-response model, the sensitivity analysis shows an increase on the risk of developing the disease when the exponential model is used (equation 5). However, the Beta-Poisson model has been demonstrated as the best dose-response relationship model for Q fever in humans through infection.

$$P(d) = 1 - e^{-rd} \qquad \textit{Equation 5}$$

Moreover, the r applied in the model is 1, which means that the bacteria have a 100% probability of surviving in the host organism to cause an infection, which is a quite conservative assumption. Despite of these arguments, the exponential model has been included in the sensitivity analysis because it is still not known which dose-response model fits better the risk of Q fever in humans through the ingestion route.

7.3 Risk of Q fever through the air

In order to compare the water borne risk of developing Q fever with the direct air transmission risk, the risk for a person standing 1km downwind from an infected barnyard was determined. The same air model and assumptions were applied for the exposure assessment steps: a person standing every day 1 km north-west from an infected barnyard for 8.1 minutes, with a breathing rate of 12 breaths/min, a tidal volume of 500 mL and assuming that 12.7% of the particles inhaled reach the lower respiratory tract. The dose to which people are exposed in this case is 9.2×10^{-3} and the risk of Q fever disease is 8.4×10^{-2} n/y. Figure 15 represents the decreasing of the risk with the distance.

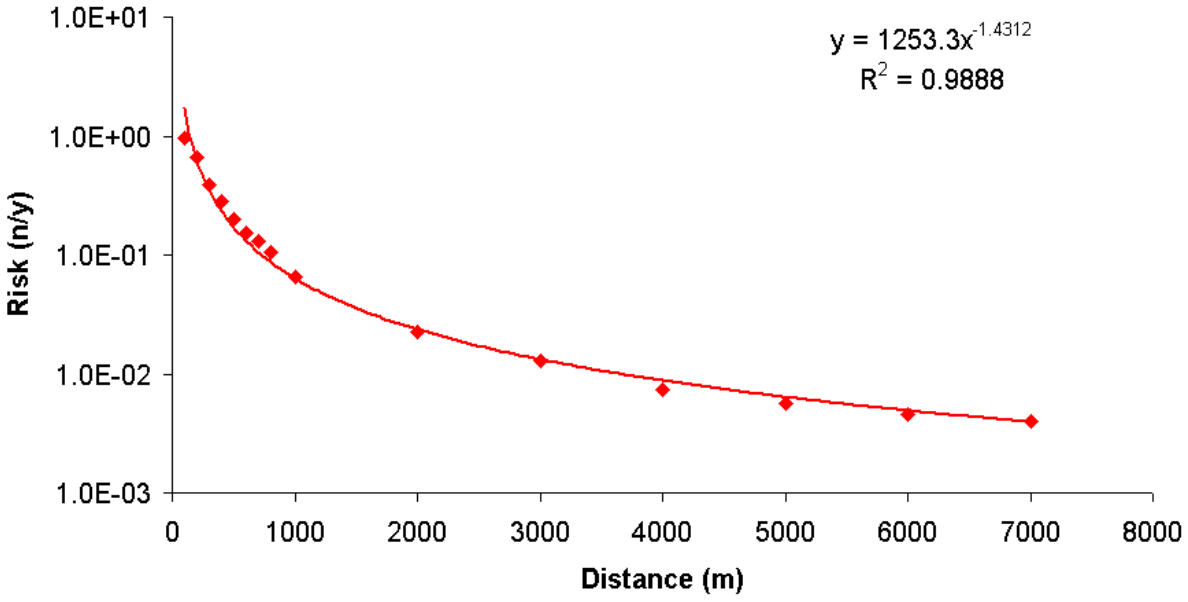

Figure 15: Risk versus distance for people standing downwind from an infected barnyard.

As expected, the risk is higher than for showering, as the dilution process due to the water treatment is skipped in this model. Schimmer et al. (2010) studied a Q fever outbreak that took place in Noord-Brabant in May 2008 to relate it with a suspected animal source. They combined epidemiological data on notified cases, veterinary and meteorological data in a generic geographic information system to analyze the cluster. They found an attack rate of 3.8×10^{-3} in residents in the 0-2 km area[23]. Our calculated risk is 1 log higher, but it is calculated for 1 km and with data from the year 2009 (more Q fever cases were reported in 2009 than in 2008, so the 2009 attack rate is also higher).

8 Discussion

We have performed a screening level risk assessment of Q fever through drinking water in the Netherlands following a conservative approach. Our results indicate that the use of air contaminated with *C.burnetii* to aerate groundwater pose a very low risk of Q fever disease to the population through inhalation of aerosols during showering. The sensitivity analysis showed a high risk when using the most conservative water to air ratio. The risk of infection through drinking fresh tap water could not be evaluated due to the lack of a dose-response model for ingestion.

No data is available about the concentration of *C.burnetii* at the barnyards air in The Netherlands. To estimate the concentration of *C. burnetii* in the air of a barnyard, it is necessary to know the number of goats in that specific barnyard, the prevalence of Q fever among them, the number of parturient/aborted goats, the concentration of bacteria in the placenta, birthing fluids, feces and urine, the rate of transmission from the infected materials to the air, the rate of decomposition of the placentas, etc. and consider as well the volume of the barnyard and the rate of air exchange with the atmosphere. As some of these data is not available and this calculation would generate numerous uncertainties, literature data about the concentration of the bacteria in the air of a barnyard has been used in the present study. As stated before, no data about concentration of Q fever bacteria in the air of goat barns was found. Only 2 studies were found that showed data which allowed the calculation of the bacteria in the air. However, both studies studied sheep barns. Moreover, the highest concentration was found when sampling the air during sheep shearing and we are using that data for the kidding period emission, when the secretion of the bacteria to the environment is higher. Despite the uncertainties generated and due to the lack of data available, we considered this the best and simplest way to estimate the concentration of bacteria in air but future research can contribute with new data that help to improve the model.

To determine the emission of bacteria from the barnyard, data of the concentration of bacteria in the air of contaminated barnyards and data of ventilation rates measured in different barns have been used. It has been suggested that Q fever bacteria is emitted to the environment attached to dust particles[4]. If this is true, the emission of dust necessarily affects the emission of bacterial cells and it should be included in the model, as well as factors that affect dust emission. Hunink et al. (2010) observed that farms with low vegetation in the surroundings have a higher probability of transmitting Q fever to humans and related this phenomenon to the effect of vegetation in reducing the amount of dust available for dispersion of the bacteria. They also found out that the depth of the groundwater around the farm has an influence on the dust emission, as shallow water generates high moisture soil, decreasing the dust production and its emission [4]. Further definition of the model would include local environmental conditions of the area where the barnyard is located and its influence in the dust and bacteria emission.

A simple plume model has been used to estimate the transport and dilution of the bacteria in the air. It only allows calculating the concentration of particles in the air at the ground level, while the air inlet of the groundwater treatment plants is located some meters above the ground. It doesn't include the effect of buildings or trees that can be located between the source and the plant and that act as a barrier reducing the amount of particles that reach the air inlet. But to include this factor in the model, it would be necessary to study a real case and estimate the barriers found between the barn and the water treatment plant. The model should be modified to estimate the particles concentration at different levels in the air during the transport, to be able to calculate the reduction due to those barriers, as particles travelling higher than the building and trees are not affected by this phenomenon. A verification of the concentration of Q fever in the air at the inlet of the ground water treatment plant would be useful. Air samples should be taken with a good air sampling method (i.e. with a high recovery rate), e.g. liquid impingement[33].
The method used to determine the parameter g in the model is not accurate. The extrapolation from the graph is imprecise and can be subjective, generating errors in the result. Another way to solve the model has been tried, consisting in finding the values of σ_z and σ_y, but this implies also the use of graphs, which pose the same problem of imprecision.

The model assumes 100% reflection of the particles that hit the floor. This is quite unrealistic and can be modified changing the last member of the equation:

$$Exp\left(-\frac{H^2}{2\sigma_z^2}\right)$$

However, as the amount of reflected particles is not known, this would pose estimating a reflection fraction, which wouldn't reduce the uncertainty. Other studies found in the literature assume 100% reflection as well [21, 60].
When comparing the reduction of the concentration of *C.burnetii* in the air with the distance (figure 10) it can be observed that the difference in concentration between the plume model and the extrapolation form Páez-Rubio after 5km is bigger than after 1km. The Páez-Rubio model shows an exponential decay of the concentration with the distance, while the plume model shows a power pattern decay. The RIVM has sampled air at several distances from infected barnyards. Occasionally, they found the bacteria incidentally at 5km from the source with PCR. Assuming a sample size of 50 L and a sensitivity of the PCR method of 100%, this would mean a concentration of (at least) 1 Coxiella (DNA)/50 L at 5 km from the source. This value is closer to the one obtained with the plume model (0.1 cells/m^3 at 5 km downwind) than with the Páez-Rubio extrapolation ($1.4x10^{-20}$).

During the air transport, rain can drag particles from the air and deposit them on the ground. When the rain stops, the soil is wet so it can take some days till the aerosols can be suspended again on the air, and this generates late delivery of cells into the water treatment plant. This is not included in the model.

The water treatment process has been observed to modify the spatial heterogeneity of micro-organisms in the water[35]. This statement would implicate a change in our model. If the bacteria are not homogeneously distributed in the water after its treatment but some regions have higher concentrations of bacteria than others, then the dose would be either 0 (or very low) or higher than the dose calculated. The individual probability of developing Q fever disease from inhalation of drinking water aerosols would be random, either 0 or a value higher than our estimated value.

In the scope and objective of the present study, we stated that the exposure scenario considered was the drinking water ingestion and inhalation of aerosols during showering. It was observed that the risk through ingestion was higher than the risk through inhalation. However, due to the lack of an existing dose-response model through the ingestion route for *C.burnetii*, we decided to reject this exposure scenario. A study has been done to find out the best fit dose-response model of Q fever based on literature data. For the inhalation route, the best fit model was the Beta-Poison model. No studies have been found that relate dose of ingested *C.burnetii* with clinical symptoms in humans. An attempt was carried out to find an ingestion dose-response relationship in humans, but the only response found was seroconversion[52]. The dose-response modeling in mice exposed intraperitoneally to *C.burnetii* suggested that the Beta-Poisson model gave the best fit (it gave the best fit for two of the studies, while the exponential model gave the best fit for one of them). Nonetheless, we can not extrapolate the results from mice to humans and we cannot assume that the intraperitoneal exposure route equals the ingestion exposure, as through ingestion the microorganism encounters several host barriers (e.g. oral antibodies, stomach acid, intestinal wall) that reduce its probability of surviving and causing an illness, while these barriers are not present in the intraperitoneal route. More research should be done about the clinical disease produced through ingestion of *C.burnetii* and the dose-response relation.

On the other hand, the risk of aspiration of infected particles has not been taken into account. When swallowing, a little fraction of water is aspirated through the respiratory system. No information has been found about the proportion of liquid that is aspirated during liquid ingestion and, hence, it is not possible to quantify the amount of water and, therefore, infectious cells, that are aspirated. Presumably, this fraction is not very high in healthy people, but it can be in elderly [61], dysphagic patients with neurological disorders, Parkinson, alcoholism, children with dysphagia [62] or hospitalized patients (e.g. subjected to nasogastric tube feeding), as has been observed for *L.pneumophila* [63]. Fifty percent of

the healthy adult population aspire small amount of fluids during sleep [64], but no information has been found about the prevalence of aspiration during eating/drinking.

During showering with warm water (38-42 ^{o}C), the air in the shower stall can reach RH of 80% [33] or even nearly 100% [41] and under these conditions, the aerosol formation is higher than under lower water temperatures. In this study , the aerosolization of bacteria has been estimated using a ratio calculated from data of experiments performed at 20^{o}C (+/-1^{o}C) and RH between 30-35% [33]. Furthermore, when using warm water the concentration of aerosols might be higher in the upper half of the shower stall, including the breathing area, due to the chimney-like convection flow originated by the hot water heating the air [46]. Hence, we are probably underestimating the aerosol formation and the concentration of *C.burnetii* in the air of the shower stall during warm water showering.

Other issues not taken into account in our case study are the emission of Q fever particles from other barnyards located close to the groundwater treatment plant but in other directions. When the wind is not blowing from the SW but from the direction where the water plant is located downwind from other barnyards, the plant will still receive the bacteria. This scenario is quite probable in Noord-Brabant, where many farms were declared contaminated and plenty groundwater treatment plants are located between them (figure 2).

Transport of manure from barnyards to far locations is a common practice in the Netherlands. The manure can be kept for months in piles until it is transported to other regions in opened trucks. In the way, particles are released and can be transported to water treatment plants. This practice can produce an increase in the risk of Q fever transmission out of the kidding season. A Q fever urban outbreak in residents along a road over which farm vehicles travelled transporting contaminated straw and manure has been reported elsewhere [65].

The present study has been based in a specific groundwater treatment plant during the year 2009. The model allows for changes in the characteristics of the water treatment plant and in the time period. For example, a plant located 2km North-West (NW) from an infected barnyard can be studied changing the distance from the source and analyzing the meteorological data to find the days when the wind was blowing from the South-East (SE), its speed and the stability conditions. It can also be studied another year or location in the Netherlands with the appropriate meteorological data that can be found in the KNMI website [28].

Only the risk of Q fever in the healthy population has been assessed in the present study. Children, elderly, immunosupressed patients, people with heart valve problems, dysphagia and pregnant women should be addressed differently. Higher prevalence of *C.burnetii* seropositivity in HIV positive patients compared to healthy blood donors as well as high ratio of immunocompromised patients (20%) among Q fever patients have been reported [66]. However, no Q fever dose-response relationship has been established for immunocompromised humans. In the Netherlands, 15% of the population is 65 years old or older (old population). 1.8% of the population and 6.8% of the old population suffered from severe heart disease in 2009. 1.4% of the population and 4.5% of the old population had cancer in 2009, hence, were immunosupressed [67]. The median age of the Q fever patients in The Netherlands in 2009 was 49 years. Six deaths were reported the same year, all in patients with other underlying diseases [10].

9 Conclusions and Recommendations

This study was a screening-level risk assessment to determine the potential health risk of contamination of groundwater used for drinking water during the aeration process with *Coxiella burnetii*. A scenario was developed to perform the screening level risk assessment: a groundwater plant in the close vicinity (1 km) of an infected goat farm that used forced aeration (air/water ratio of 20) that does not filter the air it takes in for the aeration. The screening-levelrisk assessment was deterministic and conservative. The risk assessment indicated that for this risk scenario, the annual risk that a resident in the area contracted Q fever through this pathway was 1.1×10^{-6}.
In comparison: the average annual risk in 2009 in the Netherlands of contracting Q fever by any exposure route was 1.4×10^{-4} (2357 cases in a population of 16.5 million). For people living in a 5 km radius around infected goat farms, the annual risk in 2009 was 7×10^{-4}.

The sensitivity analysis showed that the calculated risk strongly depended on the air transport model, the use of air filtration and the aerosolization of bacteria during showering.

At a distance below 1 km, the risk rapidly increases. Most of the groundwater stations are >5 km from an infected goat farm. 41 groundwater stations are within the 5 km radius and 4 within a 1 km radius. At 360 m (the closest distance observed between an infected goat barn and groundwater station) the annual risk is 6.6×10^{-6}. At 85 m the estimated annual risk would become higher than 10^{-4}, the risk of infection level that is considered acceptable for enteric pathogens in drinking water, with the notion that this study estimates a risk of illness, not risk of infection.

The use of air filtration with HEPA filters reduced the risk with 99,95%.

The contribution of the pathway through drinking water to the occurrence of Q fever in the Dutch population is considered negligible. This is based on:

- the low annual risk of the water pathway that is estimated in this screening level risk assessment (1.1×10^{-6});
- the relatively high annual risk of other pathways that has resulted in the Q fever cases in the Netherlands ($1.4 – 7 \times 10^{-4}$);
- the conservative approach taken in this screening level risk assessment;
- the distance between infected goat farms and ground water stations that is more than 1 km in all but 3 groundwater stations.

Three factors could seriously increase the estimated risk of Q fever transmission through water.

1. The aerosolization of bacteria during showering. The study of Deloge-Abarkan on *Legionella* aerosolization based on the FISH-assay suggested a much higher aerosolization than the culture assay in this study and all assays in other studies. The high aerosolization rate was considered an outlier and hence not used in the risk assessment.
2. The dose response of ingestion of *Coxiella burnetti* is comparable to the dose response for inhalation. In that case, the estimated risk of waterborne transmission would be considerably higher. As indicated, no dose response data for ingestion are available.
3. The real concentration of bacteria in the air, which couldn't be quantified due to the lack of data available.

Recommendation for water utilities:
This study suggests that there is no specific action needed, since the risk of waterborne transmission is negligible, even at the closest distance observed between an infected farm and groundwater station.

Water utilities are suggested to evaluate their air filtration policy, taking into account the vicinity of farms that could serve as a source of pathogens and the intensity of their aeration process.

When infected farms are located nearby groundwater treatment plants, water utilities are recommended to consult health authorities in order to protect plants' workers. It is also suggested to program the maintenance of the aeration facilities for the periods when the wind is not blowing in the direction from infected barnyards, when possible.

10 Literature

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