Limited presence of *Waddlia chondrophila* in drinking water systems in the Netherlands

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Abstract

*Waddlia chondrophila* is an emerging pathogen belonging to the order of Chlamydiales. This obligate intracellular bacterium was initially isolated from an aborted bovine fetus and is associated with adverse pregnancy outcomes in women. The ability of *W. chondrophila* to reside and replicate within a range of free-living amoebae implies a possible widespread environmental presence. Potential hosts of *W. chondrophila* are present in Dutch drinking water. This study therefore investigated the presence of *W. chondrophila* DNA in drinking water by analysing 59 samples from ten drinking water systems throughout the Netherlands. Samples were taken at three distances from the treatment plant, during both summer and winter. Twelve of the samples were positive, originating from two of the treatment plants, of which three samples were quantifiable.

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Introduction

*Waddlia chondrophila* is an emerging pathogen belonging to the order of Chlamydiales. It is an obligate intracellular bacterium that was initially isolated from an aborted bovine fetus [1]. Later, a second case of *W. chondrophila* was found in a septic stillborn calf [2]. *Waddlia chondrophila* is a zoonotic bacterium and has been associated with bovine abortion [3], as well as adverse pregnancy outcomes and infertility problems in women, such as tubal factor infertility [4–6]. Furthermore, *W. chondrophila* has been detected in samples from children with respiratory infections and in individuals with community-acquired pneumonia [7,8]. The ability of *W. chondrophila* to induce respiratory infections was demonstrated in an experimental animal model [9].

Unlike the well-known *Chlamydia trachomatis*, which mainly spreads through sexual contact, the transmission routes of *W. chondrophila* have not yet been fully elucidated. Potential routes of infection include the consumption of milk and uncooked meat, as well as contact with animals [10]. Sexual transmission of *W. chondrophila* is unlikely given the low numbers of patients being positive for both *C. trachomatis* (a typical agent of sexually transmitted infections) and *W. chondrophila* [5]. Its ability to reside and replicate within a range of free-living amoebae (FLA) implies a possible widespread environmental occurrence of *W. chondrophila* [11,12].

The ability of amoeba-resistant microbes (ARM) to infect FLA provides them with the advantage of transportation within the environment. By forming persistent cysts, FLA provide protection for ARM against water disinfectants such as chlorine, and other stresses [13,14]. The ubiquitous presence of FLA in...
soil, air, animals, plants and water facilitates transport into drinking water systems. FLAs have been reported to break through the treatment barrier and enter water distribution systems, where they can colonize and regrow [15,16]. The colonization of pathogenic ARM in drinking water systems might pose a clinical risk, as has been observed in the case of Legionella pneumophila [17].

Waddlia chondrophila can infect, among others, Acanthamoeba spp. and Vermamoeba vermiformis (formerly Hartmannella vermiformis) [11], both of which have been identified in drinking water distribution systems and in treated drinking water in many countries worldwide [15]. Moreover, W. chondrophila DNA has been identified in drinking water sources in various European countries, such as France, Spain and Switzerland [18–20]. Although no Acanthamoeba spp. were detected in Dutch drinking water systems [21], the presence of V. vermiformis has been confirmed in distributed drinking water [16]. As a possible protozoan host for W. chondrophila is present in Dutch drinking water, this study was performed to investigate the presence of W. chondrophila DNA in drinking water systems in the Netherlands.

Materials and methods

Sample selection
In total, 59 drinking water samples were measured, obtained from the distribution systems of ten treatment plants throughout the Netherlands (plants A–J). Treatment plants A–E use surface water, which is treated with a multiple barrier approach, involving pre-treatment (e.g. rapid sand filtration, coagulation/sedimentation), disinfection process (e.g. dune infiltration, ozonation, UV, or UV/H2O2) and post-treatment (e.g. active carbon filtration, slow sand filtration). Plants F–J use groundwater, which is treated with aeration followed by rapid media filtration. From each treatment plant, samples were taken during summer and winter at three distances from the treatment plants (proximal, central and distal location). This provided six samples per treatment plant, except for plant F, for which no sample was available from the central location in summer. The kitchen water tap was flushed for 4 minutes before sampling, to make sure that microorganisms present in the premise’s plumbing system were flushed out and the results displayed microorganisms from the distribution systems. Table 1 shows more details of the different treatment plants, including water temperature, total organic carbon and adenosine triphosphate levels.

DNA extraction and analysis
DNA extraction was performed by the KWR Water Research Institute [21]. In short, 1 L of each drinking water sample was filtered through a 25-mm polycarbonate filter (0.22-μm pore size). DNA extraction was performed following the protocol of the PowerBio™ DNA Isolation kit (MoBio, Carlsbad, CA, USA).

Quantitative PCR analysis for W. chondrophila, Acanthamoeba spp. and V. vermiformis
DNA was analysed for the presence of W. chondrophila-specific DNA using quantitative PCR as previously developed by Goy et al. [7]. A calibration curve was used as a positive control and for quantification, consisting of the W. chondrophila-specific 16S rRNA gene plasmid containing a 100-bp fragment. Gene copy numbers were calculated by comparing the threshold cycle (CT) values of the samples with those of the calibration curve.

TABLE 1. Detailed information on treatment plants and drinking water samples

| Treatment plant | Region in the Netherlands | Water source | Water source | TOC (mg C/L) | Season | Date samples taken | Temperature (°C) | ATP (ng ATP/L) |
|-----------------|---------------------------|--------------|--------------|-------------|--------|-------------------|-----------------|----------------|
| A               | West                      | SW           | 1.9          | Summer      | 10-09-2012 | 19.4 ± 1.0        | 4.8 ± 4.3       |
| B               | West                      | SW           | 2.1          | Winter      | 13-02-2013 | 6.0 ± 0.6         | 1.3 ± 0.3       |
| C               | West                      | SW           | 2.1          | Winter      | 27-08-2012 | 20.5 ± 0.8        | 4.7 ± 0.7       |
| D               | West                      | SW           | 3.4          | Summer      | 09-01-2013 | 8.9 ± 0.9         | 2.1 ± 0.1       |
| E               | West                      | SW           | 2.2          | Summer      | 29-08-2012 | 18.6 ± 0.8        | 5.4 ± 1.8       |
| F               | West                      | GW           | 8.0          | Winter      | 14-01-2013 | 9.0 ± 0.6         | 1.0 ± 0.0       |
| G               | East                      | GW           | 0.3          | Summer      | 03-09-2012 | 20.0 ± 0.6        | 3.9 ± 0.3       |
| H               | South                     | GW           | 3.4          | Winter      | 18-09-2012 | 7.2 ± 0.1         | 3.1 ± 0.5       |
| I               | North                     | GW           | 4.3          | Summer      | 04-09-2012 | 18.4 ± 1.3        | 1.9 ± 0.9       |
| J               | North                     | GW           | 2.0          | Winter      | 28-01-2013 | 7.5 ± 0.5         | 1.5 ± 0.2       |

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Sensitivity of the quantitative PCR was ten gene copies, based on the lowest detected dilution of the positive plasmid control (quantification limit).

DNA isolated from the water samples of plants A to D was analysed for the presence of Acanthamoeba spp. and V. vermiformis-specific DNA using the quantitative PCR analyses as previously described [21,22]. Quantifications were based on comparison of the sample CT value with the CT values of a calibration curve based on known copy numbers of the respective gene from Acanthamoeba or V. vermiformis.

Results

The analysis of the 59 samples taken from ten water treatment plants throughout the Netherlands showed a low number of positive samples for W. chondrophila DNA in drinking water derived from treatment plants A and C (Table 2). Three of these samples, all from treatment plant C and taken during winter, were quantifiable. The sample that was taken at a proximal location from the treatment plant showed the highest copy number of W. chondrophila DNA. All other samples from treatment plants A and C, with copy numbers of <10 per litre (Table 2), showed a W. chondrophila-specific amplification curve below the quantification limit. In contrast to the samples from treatment plants A and C, no W. chondrophila DNA was detected in the samples from the remaining eight treatment plants. In these samples no W. chondrophila-specific amplification curve was observed, indicating that these 47 samples were all negative for the organism.

The presence of DNA from Acanthamoeba spp. and V. vermiformis was also examined on the samples from plants A to D. Acanthamoeba spp. could not be detected in any of these samples. V. vermiformis DNA was detected in 11 of the 12 samples taken from the distribution systems of plants A and C, with one sample having levels above the quantification limit (Table 2). In addition, V. vermiformis DNA was detected in all samples taken from plants B and D with levels above and below the quantification limits (data not shown).

Discussion

The current study shows the presence of W. chondrophila DNA in Dutch drinking water. This is in concordance with three other European studies that investigated its occurrence in (drinking) water systems. In Spain, Codony et al. detected W. chondrophila DNA in 10 of the 40 analysed well water sources, but all 30 drinking water samples were negative [19]. In France, Agusti et al. detected low W. chondrophila DNA levels in 12 of the 59 investigated samples from non-domestic hot water systems [18]. Three of the twelve positive samples could be quantified, nine samples showed a qualitative positive detection but were below the quantification limit. In addition, they observed that more water systems were positive for W. chondrophila than for Legionella spp., respectively nine versus four. In contrast to our study, they sampled hot water (average temperature 57.3°C, ranging from 28.0°C to 65.3°C), whereas we sampled cold water (ranging from 5.4°C to 20.5°C). In Switzerland, W. chondrophila DNA was detected in one of the 48 domestic drinking water samples and one biofilm sample [20]. Although biofilms were not investigated in our study, the study by Lienard et al. indicates that biofilms could form a possible niche for W. chondrophila, as well as for various other Chlamydiales [20].

In order to determine whether drinking water provides a possible transfer route of W. chondrophila, it is important to know which infected FLA hosts carry W. chondrophila in the water distribution system. Acanthamoeba spp. and V. vermiformis were found to be the most suitable hosts, but also Vahlkampfia ovis and Dicyostelium discoideum could be infected with W. chondrophila [11]. We could not detect Acanthamoeba spp. in the samples from the two plants that were positive for W. chondrophila DNA, but V. vermiformis was detected at low levels in most of the samples from the two plants that were positive for W. chondrophila DNA. Therefore, this amoeba might serve as a host for W. chondrophila. However, samples from two W. chondrophila-negative plants (plants B and D), were also positive for V. vermiformis DNA. This indicates that the presence of V. vermiformis is not a reliable indicator for the presence of W. chondrophila. The two other reported host protozoans (Vahlkampfia ovis and D. discoideum) were not
detected in an extensive 18S RNA gene analysis of drinking water sampled from two groundwater treatment plants in the Netherlands [16], but it remains uncertain if these two hosts were also absent in drinking water from plants A and C.

In contrast to the Netherlands, where Acanthamoeba spp. were not detected, samples from Spanish, French and Swiss water sources were positive for Acanthamoeba and/or V. vermiformis [21,23–25]. However, the higher prevalence of Acanthamoeba spp. in water sources in Spain, France and Switzerland does not seem to influence the presence of W. chondrophila, as the current study showed its presence in Dutch drinking water despite the absence of Acanthamoeba spp. In the Swiss study, Lienard et al. also detected V. vermiformis in some of the drinking water and biofilm samples. However, the samples that were positive for W. chondrophila were negative for V. vermiformis [20]. It is possible that W. chondrophila uses V. vermiformis as a host in drinking water, but that its main source is another FLA host that is as yet unknown.

Our results showed a higher presence of W. chondrophila DNA in samples taken during winter, than in those taken during summer at the same treatment plant. This is in contrast to most findings, where higher numbers of various microbes are found in the summer season due to higher water temperatures [16,21]. To our knowledge, we are the first to investigate seasonal associations with W. chondrophila specifically. It can be hypothesized that W. chondrophila or its hosts have lower optimum temperatures, leading to the higher numbers during winter. Alternatively, the higher temperature might be favourable to some bacterial species that overgrow and are detrimental to W. chondrophila or its eukaryotic hosts.

As mentioned before, higher numbers of W. chondrophila DNA were detected in the drinking water samples proximal to the treatment plant than in the more distal parts of the distribution system. The highest numbers of V. vermiformis in the summer were also observed at the proximal location of treatment plant A (Table 2). At treatment plants B and D, the highest V. vermiformis numbers were again observed at the proximal site, either in the winter (plant B) or summer and winter (plant D). The concurrent occurrence of W. chondrophila and V. vermiformis at the proximal sites of the distribution system suggests that W. chondrophila might use V. vermiformis as a protozoan host.

A question that remains unanswered in this study is whether there is a relation between drinking W. chondrophila-containing water and human infection. The clinical impact and risk of the presence of W. chondrophila in drinking water has yet to be examined. First, it remains unknown whether the presence of W. chondrophila DNA in the samples indicates the presence of live W. chondrophila that is capable of infecting another host. As W. chondrophila is an obligate intracellular bacterium, it cannot be cultured on an agar plate and live bacteria cannot be easily quantified. A possible direction for their study might be a co-culture of the drinking water samples with amoebae. Second, it is unknown what transmission route would be used by W. chondrophila to infect hosts via drinking water. The association between drinking W. chondrophila-containing water and infection has never been made. It is however known that the cysts of FLA, possibly containing ARM, can travel into the human respiratory tract via aerosols [15]. Increased exposure to aerosols through, for example, air conditioning systems could therefore possibly lead to an increased infection rate [20], as it does for the intracellular bacterium Legionella pneumophila [17]. The effects of exposure to W. chondrophila-containing drinking water or aerosols may be more likely to occur in the respiratory tract than in the reproductive system.

In summary, this study showed that in eight of the ten analysed Dutch treatment plants, W. chondrophila could not be detected in the distributed drinking water. Nonetheless, drinking water from two treatment plants showed positive samples for W. chondrophila. As only DNA was detected and gene copy numbers were low, it remains unlikely that these two drinking water types are an important source for infection with W. chondrophila. In conclusion, drinking water from the Netherlands does not seem to be a likely infection route for W. chondrophila. However, future studies are needed to investigate whether low concentrations of W. chondrophila could lead to infection, and whether this might result in infected tissues and related clinical implications. As W. chondrophila is a zoonotic pathogen, it would furthermore be interesting to investigate its occurrence in water sources around farms, to obtain more knowledge of the environmental presence of W. chondrophila and its implications from a One Health approach.

Conflicts of interest

None declared.

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