Legionella pneumophila in Norwegian naval vessels

BACKGROUND Little is known about the occurrence of *Legionella pneumophila* in water supply systems on board ships. Our aim was to study the occurrence of *L. pneumophila* in the water supply system on board Norwegian naval vessels as the basis for framing preventive strategies against Legionella infection.

MATERIAL AND METHOD Water samples were collected from technical installations and from the water distribution network on board 41 vessels and from ten water filling (bunkering) stations, the sampling taking place in two rounds separated by a one-year interval. The samples were subjected to analysis, including serotyping and genotyping, with a view to identifying the presence of *L. pneumophila* and of free-living amoebae.

RESULTS *L. pneumophila* was found in 20 out of a total of 41 vessels in the first round of sampling, and live *L. pneumophila* serogroup 1 was isolated in seven of the 20 vessels. Free-living amoebae were found in the water supply system in most of the vessels, including all the vessels with *L. pneumophila*. The same genotype of *L. pneumophila* was identified in the water in bunkering stations and in the water on board the vessels.

INTERPRETATION *L. pneumophila* was not present in all the vessels, but all the vessels where the bacterium was found were also contaminated with free-living amoebae. We have demonstrated the probability of the fresh water from bunkering stations being the source of the contamination. In framing preventive strategies, importance should therefore be attached to identifying the source of contamination and the presence of free-living amoebae, as a premise for the establishment and growth of *L. pneumophila* in onboard water supply systems.

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MAIN POINTS

Legionella pneumophila was found in the water supply system of approximately 50 % of Norwegian naval vessels

One and the same genotype was found in three vessels

Genotypes found in two of the vessels were also found in the water filling station used by the vessels

Free-living amoebae appeared to be a premise for the growth of *L. pneumophila* in the water supply system

The Legionella bacterium is found in freshwater sources all over the world (1). The genus *Legionella* was not registered until 1979 – as a result of a major outbreak of Legionnaires' disease among members of the American Legion (war veterans) in 1976, where *Legionella pneumophila* was found to be the cause (2, 3). The first bacterium isolate which was subsequently thought to be *Legionella*, was isolated in 1943 in guinea pigs and appeared similar to the obligate intracellular bacterium *Rickettsia*. In 1954 a similar bacterium was described, which was found to infect free-living amoebae. This isolate was classified in 1995 as *Legionella* (4).

Today, we know *Legionella* as a small waterborne bacterium which can be found freely present in water. It is highly fastidious as regards the substrate it requires for growth, and therefore survives and multiplies in other organisms, especially in free-living freshwater amoebae (5, 6). *Legionella* can cause respiratory disease in humans if a person inhales aerosolised water containing the bacterium. This exposure may occur daily if showering using water from contaminated water systems, although without it necessarily resulting in illness.

Infection with *L. pneumophila* is called Legionellosis. The infection usually presents as two distinct clinical entities: Legionnaires' disease, a severe form of pneumonia with an approximately 30 % mortality rate; and Pontiac fever, an influenza-like illness of short duration (7). It has thus far not been demonstrated that these diseases can be transmitted from person to person (8).

To date, 53 different species of the Legionella bacterium have been identified (9), with some 20 of these being found in infections. The species *L. pneumophila* was found in more than 90 % of outbreaks and sporadic cases of Legionellosis, and more than 80 % of the *L. pneumophila* isolates belonged to serogroup 1 (10).

Legionella was registered in the 1980s and '90s in outbreaks of disease with fatalities in Europe, but it was not until 2001 that the first outbreak occurred in Norway. It happened in Stavanger, where there were seven deaths (11), followed by an outbreak in Fredrikstad/Sarpsborg in 2005, with ten deaths (12, 13). As a consequence of the latter outbreak the statutory microbiological control in Norway has been made much stricter, including a regulatory requirement designed to prevent the spread of Legionella from whirlpool spas and shower systems (14). New guidance has also been issued for the control and prevention of Legionella infection (8).

The Legionella regulations and guidance impose a high degree of responsibility on owners of devices and systems capable of

Table 1 Overview of sample material for analysis of *L pneumophila* during the study

Class of vessel	Number of vessels per class of vessel	Number of chosen sampling points
Minesweepers	6	9
Submarines	4	2
Coastal corvettes	2	6
Frigates	4	15
Defence logistics support & supply vessels	3	15
Port utility vessels	5	5
Coast guard vessels	13	15
Museum ships	1	8
Training ships	2	17
Specialised vessels	1	15
Total number	**41**	

spreading aerosolised water. In order to comply with the requirements, owners need to have a knowledge of which factors in the relevant water supply system may contribute to the growth and spread of the Legionella bacterium.

Where the Nordic fleet is concerned, the requirement is for all freshwater supplies on board ship, for both food and hygiene purposes, to be of drinking water quality. The freshwater supply is obtained either by filling fresh water in port (bunkering) or by freshwater production onboard ship via desalination of sea water using reverse osmosis (RO) or evaporation (EVA) (15). For ships, freshwater production from sea water will be the optimal choice, since *L. pneumophila* is a freshwater bacterium not naturally found in sea water.

The Royal Norwegian Navy has a large fleet of vessels which are subject to the Legionella regulations. A project was started in 2010 aimed at establishing the necessary knowledge for Legionella control on board naval vessels. It included surveys and risk assessments to identify the occurrence of *Legionella* in the water supply systems of all naval vessels, for the purpose of establishing a basis upon which the Navy could take the necessary measures to prevent the occurrence, growth and infection by the bacterium.

Material and method

The study comprised ten separate classes of vessel: minesweepers, submarines, coastal corvettes, frigates, defence logistics support and supply vessels, port utility vessels, coastguard vessels and others, making a total of 41 vessels.

Selection of water sampling points

The selection of water sampling points was based on an onboard inspection of one reference vessel from each class of vessel and on collected technical and procedural data. The following principles were applied:

- For vessels with fewer than ten sampling points, all the points were analysed
- For vessels with more than ten sampling points, up to 17 points were analysed

Out of a total of 3,807 possible sampling points, samples were taken from 439 (12 %). Samples were taken for analysis in two rounds separated by a one-year interval. In the second round of sampling, 36 of the 41 vessels were accessible for sampling.

Sample material

Table 1 contains an overview of water samples taken from vessels included in the study. In addition, samples were collected from 45 of the most used tapping points at the ten water filling (bunkering) stations; 43 of the tapping points were military and two civilian.

Water sampling

Water samples were collected in 1 L sterile flasks, dosed with sodium thiosulphate, from both the technical element of the water supply system (water tanks) and the distribution network (showers). The location of each sampling point was the same on every ship within the same class of vessel.

Microbiological analysis

Demonstration of presence of L. pneumophila. All the samples from the water supply systems were analysed with real-time PCR, as described previously (16). Positive samples were cultured on Legionella selective agar (buffered charcoal yeast extract, BCYE) plates to demonstrate live *L. pneumophila*, which is a prerequisite for infection. Where there was growth of *L. pneumophila* it was serotyped using MONOFLUO anti-Legionella Staining Reagent (Bio-Rad). All isolates of *L. pneumophila* serogroup 1 were genotyped using pulsed-field gel electrophoresis (PFGE) (17) in order to track possible collective routes of spread and contamination.

Demonstration of presence of free-living amoebae. As part of the effort to obtain systematic knowledge of the occurrence of free-living amoebae in water supply systems on board ships, parallel amoebic analyses and *L. pneumophila* analyses were carried out of all the samples from the water supply systems. The amoebic analyses were performed using cultivation and microscopic methods, in accordance with the methods described by Winiecka-Krusnell & Linder in 1999 (18).

Action upon findings of live L. pneumophila

Where *L. pneumophila* serogroup 1 was found, immediate action was taken to introduce disposable showerhead filters (0.2 μm) to prevent exposure to the Legionella bacterium from the shower water (using PALL water filters). Further follow-up was carried out in accordance with procedures described in the Legionella guidance issued by the Norwegian Institute of Public Health (8), that is to say mostly with respect to water heating systems.

Results

L. pneumophila

In the first round of sampling, analyses of 439 water samples from 41 naval vessels showed the presence of *L. pneumophila* DNA in 12 % of the samples. Positive samples were found in 20 out of 41 vessels. Live *L. pneumophila* serogroup 1 was isolated from seven of the vessels.

When the repeat survey was carried out one year later, the respective results of analysis were 13 positive vessels out of 36 surveyed and live *L. pneumophila* serogroup 1 in two of the vessels. These were vessels where no live *L. pneumophila* had been found in the first sampling round (Table 2).

Table 2 Occurrence of positive *Legionella* PCR, growth of *Legionella* and amoebae found on the first, second and both sampling rounds respectively

	Positive sample first sampling round (N = 41)	Positive sample second sampling round (n = 36)	Positive sample both sampling rounds (n = 36)
Legionella PCR	20	13	9
Culturing for Legionella	7	2	0
Amoebae found	34	30	28

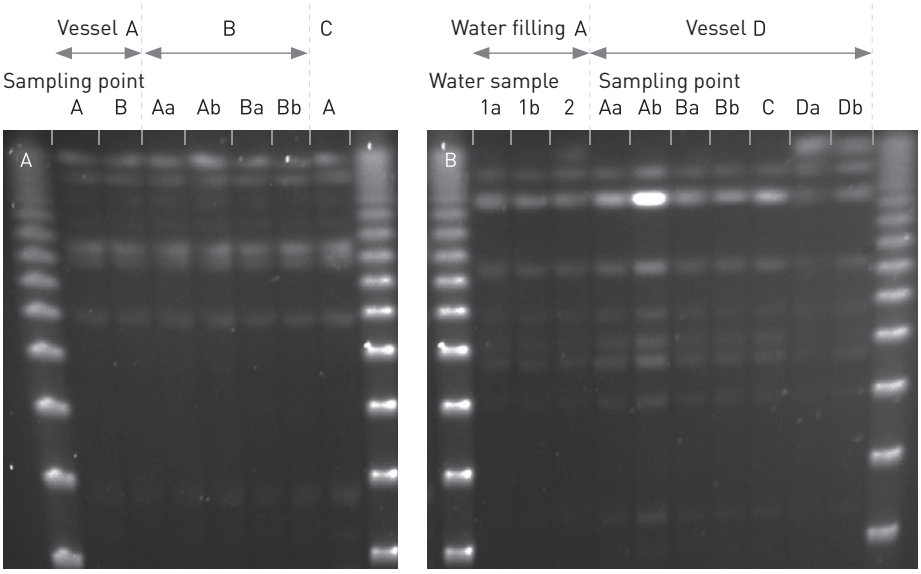

Figure 1 *a) Genotype pattern of* L. pneumophila *isolated from three different vessels in the same class. b) Genotype pattern of* L. pneumophila *from bunkering station and from the water supply system on one vessel (vessel D)*

Of the 41 vessels, 31 had pressure tanks (hydrophore tanks) installed in the water supply system. Eight of the samples with live *L. pneumophila* derived from vessels with hydrophore tanks (26 % of vessels). Of the ten vessels without hydrophore tanks, live *L. pneumophila* was found in one sample (10 %).

From the 45 bunkering points surveyed, *L. pneumophila* was found in 16 % in the first sampling round, of which live *L. pneumophila* serogroup 1 was isolated from two points.

Free-living amoebae

Free-living amoebae were found in 39 out of 41 vessels. *Acanthamoeba* and *Hartmannella* were the most commonly occurring genus. Free-living amoebae were present in all the vessels in which *L. pneumophila* serogroup 1 was demonstrated. In the first sampling round free-living amoebae were found in samples from ten out of the 45 bunkering points surveyed in the study.

Intracellular *L. pneumophila* was isolated from the amoebae and found to have the same genotype pattern as in the water samples from the vessel concerned (data not shown).

Genotyping of L. pneumophila

In genotyping the Legionella isolates, we found a very limited number of genotypes, and three vessels were even contaminated with the same genotype (Fig. 1a). In two of the vessels we isolated genotypes that were also found in the bunkering station that each vessel had used, as exemplified in Fig. 1b.

Discussion

L. pneumophila is, as demonstrated above, present in the freshwater supply systems on board Norwegian naval vessels. *L. pneumophila* DNA was found in 20 out of 41 vessels in the first sampling round. Live *L. pneumophila* serogroup 1 was isolated in seven of the vessels, and in these instances immediate action was taken to install disposable showerhead filters (0.2 µm) to prevent exposure to the Legionella bacterium from the shower water (using PALL water filters). Further follow-up was done in accordance with procedures described in the Legionella guidance issued by the Norwegian Institute of Public Health (8).

In the follow-up study a year later, no live bacteria were found on the seven vessels previously contaminated. However, infection was found on two other vessels – indicating that the actions taken after the first survey appear to have been inadequate. Free-living amoebae were found in the water supply system on most of the vessels, including all of the vessels with *L. pneumophila*. It was also possible to isolate *L. pneumophila* serogroup 1 from the amoebae, and these demonstrated the same genotype pattern otherwise found in the samples from the water supply system concerned. As it has been shown that the Legionella bacterium is able to survive and multiply in amoebae, this may explain its ability to survive for long periods in such water supply systems (19).

Samples were taken from 12 % of the relevant sampling points. The choice was based on inspections carried out on the reference vessel in each class and on whether the sampling point in question was representative of the exposure risk. *Legionella* can be difficult to detect, since the microbe colonises the walls in the water supply system and often only comes off in scale. However, on the basis of experience we considered an analysis of 12 % of possible sampling points as adequate for our purpose. The discovery of *L. pneumophila* with polymerase chain reaction (PCR) prior to culturing and genotyping is considered an appropriate strategy in view of the speed and sensitivity of the PCR method.

The fact that there is a tendency towards higher incidences of live *L. pneumophila* in vessels with hydrophore tanks, may indicate that these tanks play a certain role in terms of the growth of the bacterium. Hydrophore tanks are often sited in warm engine rooms, with resultant higher water temperatures than in the rest of the cold water system. In the light of recent knowledge about the intracellular growth of *L. pneumophila*, the water temperature may be decisive for the fate of the Legionella bacterium. Where temperatures are in excess of 20°C, the bacterium will be able to survive and multiply in the amoebae (20).

The same genotype of *L. pneumophila* was found in water from bunkering stations and in the water on board vessels that had filled water from the same bunkering station. This would appear to indicate that filling water from bunkering stations is one possible source of contamination.

Despite a number of outbreaks of Legionellosis being reported from passenger ships (21–23), few systematic studies have been made of Legionella colonisation in these ships' water distribution systems. Studies carried out on cruise ships and ferries show that Legionella bacteria of different species are widespread in onboard water systems (24). No studies have been made of the possible transfer of *L. pneumophila* from fresh water supply systems. The results of our study are assumed to have a transfer value to fresh water supply systems in general (25).

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