CHAPTER ELEVEN

General discussion

The central theme of this chapter is that essential, basic knowledge on the reservoir (paragraph one), transmission (paragraph two), epidemiology (paragraph three) and control measures (paragraph four) of Legionnaires’ disease (LD) are necessary ingredients for effective and efficient control. In paragraph five the application of the essential, basic knowledge is checked for the situation in the Netherlands after a large outbreak in 1999. Paragraph six gives suggestions for an evidence-based approach of LD control.

I. RESERVOIR

In this paragraph, the habitat of Legionella and the characteristics of the pathogen are discussed.

a. Habitat

Water and soil. Worldwide, Legionella is present in natural waters and soil where it lives as a parasite of protozoa. Legionella can also be found in wet soil. Little is known on the type of soil and combinations of species and soil types. Nor on the survival and capacity of Legionella to infect humans from the soil reservoir. Numerous reports have been published on transmission of Legionella longbeachae from potting soil. First in Australia, later in the United States and since 2006 [1] also in Europe (this thesis, chapter 5). It was assumed that in Europe potting soil was not a problem, as two studies demonstrated that European potting soil did not contain Legionella. After publication of the L. longbeachae cluster the Dutch Food and Consumer Product Safety Authority (vwa) ordered a potting soil survey for growth of Legionella. Also, in Switzerland a survey was performed that found 36% of potting soil samples culture-positive for Legionella species. Recently, a Legionella pneumophila serogroup 1 strain from soil was matched with an isolate from a patient suffering from Legionnaires’ disease who had been exposed to soil of a plant nursery. [2] This is in concordance with publications on an excavation site as reservoir of an outbreak involving 27 LD patients [3] and as an independent risk factor for LD. [4]

Despite the omnipresence of Legionella in lakes and rivers, direct transmission from these reservoirs is rare. There has been a case report on LD as a result from near drowning in freshwater. [5] Also, LD associated with natural spas has been reported. [6] The circumstances for transmission are favourable, since the concentration of Legionella in fresh waters seems sufficient to infect humans (10² to 10⁷ CFU/l), [7] aerosol formation occurs at the surface of such
waters, and floating biofilms have been identified which contain Legionella. [8] Explanations for the low attack rate may be that the selective pressure of other microorganisms in these environments is too low to enhance development of virulent traits in Legionella. Also, temperature and aerosol formation may not be optimal for successful transmission from freshwaters.

Legionella can also be cultured from groundwater, in concentrations between $10^2$ and $10^4$ CFU/l. [9] Both freshwater and ground water are raw materials for the production of drinking water. In the production process, physical and chemical substances as well as biological agents are removed and eliminated during extensive filtration and disinfection steps. In the Netherlands, testing for Legionella at critical points in the water mains is obligatory. Only very rarely contamination with Legionella is reported. Yet, indirect evidence has implicated the water mains as the source of infection [10] of the Bovenkarspel outbreak (this thesis, chapter two). Despite extensive sampling of the water distribution network of the outbreak premises, no Legionella could be cultured from water or biofilm swabs. However, a Legionella strain was cultured from three potential water sources two hundred meter apart that could not be distinguished from Legionella isolates originating from 28 LD patients. This shows that Legionella at undetectable concentrations can be introduced in water systems of end users.

A study comparing LD incidences at municipal level in the Netherlands (this thesis, chapter four) showed that high versus low price of water was a risk factor for high LD incidence in the 1987–2005 period. As higher drinking water prices are associated with the use of surface water as opposed to groundwater as raw material for drinking water production, it can be hypothesised that contamination of drinking water with Legionella in the Netherlands is related to the production process.

After delivery of drinking water by the water mains to the water distribution systems of end-users, Legionella has been shown to grow to detectable concentrations in household drinking water. Substantial differences in contamination of water with Legionella have been reported. These regional differences occur in private households [11] as well as in hospitals. [12] For example, in Chicago 30% of household water systems tested positive for L. pneumophila serogroup 1. [13] There is an association between contamination with Legionella and the size of a water distribution system. In a large survey 68% of hospital water systems were contaminated, some of them having over 30% of water samples tested positive for Legionella. [14]

Legionella is also cultured from water sampled from cooling towers. Surveys of cooling towers consistently report a high percentage of contamination. [15]

Amoebae and biofilm. As early as 1980, Rowbotham described that Legionella infects amoebae and he suggested that these protozoa play a role in the transmission of LD. [16] Later, he hypothesised that entire amoebae sizing 10–60 μm packed with 365–1483 bacteria could be the inoculum of LD. [17] Others confirmed that L pneumophila serogroup 1 can infect, multiply and cause lysis within Hartmanella, Acanthamoeba and Naegleria species. More than five genera have been described since. Cianciotto discovered a 24kDa surface protein (Mip = macrophage infectivity potentiator) required for optimal intracellular infection and involved in resistance to intracellular killing [18] both in the amoebae as in the human macrophages. [19] Legionella multiplies within amoebal cysts that are freed when the amoeba ruptures at the end of a parasite cycle. Amoebal cysts protect Legionella from harmful environments [20] and
possibly amoebal vesicles packed with *Legionella* act as vectors for the direct transmission of *L. pneumophila* to the human host. It has been calculated that these vesicles contain 20–200 *Legionella* bacteria \[21\]. Spread by amoebae and infection by amoebal vesicles is in accordance with findings on infectious dose and pathogenesis. It has been shown that amoebal passage of *Legionella*, enhances their pathogenic features by incorporating molecules of amoebal origin. \[22\] The amoebal vesicles seem quite robust and could protect *Legionella* from harmful environments. In addition, amoebae have been shown to resuscitate viable nonculturable *L. pneumophila* after disinfection by biocides, which may account for the re-emergence of *Legionella* in water systems after disinfection. \[23\]

In man-made environments, *Legionella* is not only protected by protozoa inclusion, but also by a micro layer of organic material, microorganisms and nutrients, called the biofilm. The biofilm protects against heat and disinfectants. \[24\]

### b. Characteristics of the pathogen

In the next section, the physical characteristics of *Legionella* in water and in air are discussed. Furthermore, LD pathogenesis and virulence traits of *Legionella* are elaborated on.

**Physical characteristics.** In water *Legionella* can survive 139 days in distilled water and 369 days in tap water. \[25\] Also, *Legionella* can survive low pH as well as temperatures up to 55 degrees Celsius thanks to protection by hosting protozoa and biofilm. \[24,26,27\] *Acanthamoeba* produce vesicles that protect *Legionella* against biocide exposure and freeze thawing. \[28\]

In vitro experiments studying airborne *Legionella* have indicated that the bacterium survives best in aerosols at a relative humidity of 65%. \[29\] Also in vitro, virulent strains of *Legionella* survived in aerosols for a longer time than avirulent strains. \[30\] The latter may be explained by assuming that the virulent strains originated from airborne amoebae or amoebal vesicles. Their higher resistance to evaporisation and capacity to containing hundreds of *Legionella* make them relevant infectious particles. Apart from that, there may be a role for humid weather in the transmission of LD \[31\], although other reports contradict seasonality in the presence of airborne *Legionella*. \[32\]

**Pathogenesis.** In the discovery of *Legionella*, guinea pigs played an essential role. Guinea pigs injected intra-peritoneally with LD patient material died of pneumonia. \[33\] As they also developed pneumonia when exposed to contaminated aerosols, airborne transmission was proved.

After being inhaled by humans, *Legionella* must reach the alveoli in order to find an appropriate host, which is the alveolar macrophage. Particles larger than five micrometer are retained in the nose. Only particles between one and five micrometer can reach the lung. The smallest of these can reach the alveoli (one micrometer). \[34\] This is the size of a few *Legionella*, which measure 0.3 to 0.9 in width and 2 to 20 micrometer in length. For amoebal vesicles to reach the lung alveoli, they will have to be ruptured in the bronchioli. Probably, this does not happen in the intact human host, whose mucocilliairy defence operating for particle sizes between one and five micrometer will clear such vesicles. However, in smokers’
airways with damaged mucociliary defence, amoebal vesicles may get stuck in the bronchioli and rupture while releasing hundreds of *Legionella* bacteria.

**Virulence traits.** As demonstrated with guinea pig models in the 1980s, some *L. pneumophila* serogroup 1 subtypes are more virulent than others. [35] At the same time, a role of protozoa was suggested. [36] Since then, the interaction between *Legionella* and its protozoan hosts has been studied extensively in vitro. Protozoan hosts are necessary for replication of *Legionella*. [37] It has been shown that temperature is one of the triggers for intracellular replication. [38] Intracellular *Legionella* in the growth phase was shown to be more virulent in a guinea pig model. [39] Tissue culture models confirmed that *L. pneumophila* is more invasive after growth within amoebae. [40] Generally, the *Legionella* virulence traits identified so far have been divided into motility, invasiveness and the capacity to replicate inside hosts and kill them. The difference in virulence between species is mostly due to replicating and apoptosis skills that are better developed in most *L. pneumophila* strains. [41]

Molecular epidemiology has also provided some clues on virulence. Genotyping efforts using monoclonal antibodies revealed that certain types are associated with community-acquired LD and others with nosocomial LD. Nosocomial infections are believed to result from infection with *Legionella* strains of low virulence. Furthermore, the different frequency distribution of genotypes in patients and in environmental sources hints that virulence differences with a genetic base do exist amongst *L. pneumophila* serogroup 1 strains. [42] In chapter six of this thesis, a study using systematically collected strains shows large distribution differences between the two. This finding leads to the hypothesis that yet unexplained selective environmental pressure at an environmental site can lead to *Legionella* strains at undetectable concentrations that are virulent for humans. These strains are at the same time dominated by genotypes that have a competitive advantage in this particular environmental niche. An alternative way to explain the distribution difference is by the existence of viable but nonculturable (vBNC) *Legionella*. [43] It remains to be demonstrated that vBNCs are truly infectious agents for humans.

2. TRANSMISSION

In this paragraph, waterborne transmission of LD is described briefly. The discussion is focussed on several aspects of airborne transmission of LD. First, because of its central role in transmission of LD the concept of aerosols and droplet nuclei is explained. Then, air sampling, and aerosol producing sources are discussed. At the end of this paragraph, susceptibility to LD is elaborated on.

a. Waterborne transmission

Although airborne transmission is the dominant infection route, drinking of contaminated water and subsequent aspiration has been described as transmission mode for LD. [44] These case reports mostly involve nosocomial LD patients with predisposing factors like head and neck surgery.
b. **Airborne transmission**

There has been extensive discussion on the strength of proof that certain implicated sources are indeed the cause of $\text{LD}$, be it in an outbreak of sporadic patient situation. Ideally, air-sampling results would be supportive in such cases. Some published results on this matter are presented in this section. First, a brief history of air as the source of human disease is given.

**Aerosols and droplet nuclei.** In the age of bacteriology the idea of airborne diseases was abandoned for quite some time. It came back in 1934, when Wells [45] proposed the idea of the droplet nuclei in airborne disease which he defined as a particle smaller than 10 micrometer in diameter resulting from evaporation of (bio)aerosols. In fact, droplet nuclei are dried residua of larger (respiratory) droplets. Later trials on experimental animals showed that small particles are capable of travelling considerable distances. This spurred research on the spread of bacteria [46] and virus (polio, influenza) [47] and showed that micro organisms stayed viable in the air for hours to days in the form of droplet nuclei of varying sizes. The most convincing studies [48,49] involved an experimental tuberculosis ward in Baltimore where guinea pigs were directly exposed to the air breathed out by hospitalised smear-positive tuberculosis patients. Later studies have shown that droplets between 60 and 100 micrometer can travel 1–6 meters before evaporating to droplet nuclei containing $\text{Mycobacterium tuberculosis}$ measuring 2 to 3 micrometer. [50] It was shown that for deep deposition in the human alveoli particles should measure between 0.5 and 2 micrometer. [51]

**Air sampling.** Several air sampling devices were used from the 1950s onwards, among them the Andersen sampler, which differentiates on size of droplet nuclei varying from 9.2 micrometer and larger to 1 micrometer and smaller. [53] These devices have been used to study several types of airborne transmission [52], but not for $\text{LD}$. It was guinea pigs that demonstrated airborne transmission of $\text{Legionella}$ initially. [54] In the Netherlands it was thus shown that nosocomial $\text{LD}$ was transmitted with tap water as the source. [55] Later, genotyping techniques, combined with epidemiology results replaced guinea pigs and air sampling as proof for $\text{LD}$ transmission. Only recently, a renewed interest in air sampling has occurred. [56,57] Several different techniques have been described, some commercially available [58,59], some by own design. [60–62] $\text{Legionella}$ have on a variety of occasions been demonstrated in air samples. In a comparative study, air samplers base on the liquid impingement method appeared superior. [63] Some findings indicate that repetitive sampling provides the best results. [64]

**Aerosol producing sources.** Initially, a correlation between the concentration of $\text{Legionella}$ in a water distribution system and the risk of $\text{LD}$ was suggested. [65] Later it became clear that there is no correlation between $\text{Legionella}$ in water and $\text{Legionella}$ in the air. [66] Thanks to air sampling, now slowly knowledge on the spread of airborne $\text{Legionella}$ is growing. Below, results of air sampling near the site of a proven or suspected source of $\text{LD}$ infection are described for showers, cooling towers, whirlpools, faucets, toilets and other aerosol producing devices.

On numerous occasions aerosols have been sampled and found contaminated with $\text{Legionella}$ within one-meter distance but not further from a showerhead. [67–71]
The longest distance away from a cooling tower that *Legionella* has been cultured by air sampling is 300 metres. The genotype was indistinguishable from that of a *Legionella* strain originating from the cooling tower that was suspected as the source of an outbreak of LD. However, this genotype was found in various other potential sources in the neighbourhood of the cooling tower, including private household water distribution systems. [72] Apart from this, *Legionella* has been cultured by air sampling at 30 centimetres from a cooling tower [73] and at the exhaust vent of an evaporative condenser [74] at which occasions the water was contaminated with *Legionella* in a concentration of $10^6$ CFU/l. Most evidence of airborne transmission is indirect and varies from having been at the tower for cleaning activities in the incubation period, [75] to being up to seven kilometres away from a cooling tower. [76–79] Most outbreak reports describe that the LD attack-rate is reciprocal to the distance from the cooling tower. [80] A large proportion of cooling towers is contaminated with *L. pneumophila* serogroup 1 at concentrations of $10^5$ or higher. [81,82] This concentration has been associated with solitary cases and small clusters of LD patients. [81] Concentrations of $10^6$ or higher are reported typical for cooling tower related outbreaks. [84] One study showed that amoebae in cooling towers are sixteen times more often infected with *Legionella* than amoebae in tap water. This may explain the strong association of LD with cooling towers and the relative high frequency of cooling tower related outbreaks as compared to other potential sources. [85]

Whirlpool spas on display have been implicated as the source of several outbreaks. [86–91] In this thesis, a large whirlpool associated outbreak is presented in chapter two. In this outbreak, air-sampling results were not available because the implicated source had been removed at the time of the outbreak investigation. Although two whirlpool spas contained a *L. pneumophila* serogroup 1 genotype that could not be distinguished from that isolated from sputum derived from 28 LD patients, two epidemiological studies implicated one of them as the source. Reports on LD associated with bathing in a whirlpool are rare, [92] possibly indicating that transmission is only noticed when the exposed population is substantial. No studies involving air sampling in the vicinity of a whirlpool have been published. Since most whirlpool spas are drained and displaced after an outbreak, no data are available on the concentration of *Legionella* in the water at the time of such outbreaks.

Faucets have been suggested as the source of sporadic LD [93] as well as outbreaks of LD. [94,95] In chapter ten of this thesis, one of the described LD clusters was genotypically linked to a faucet in a hospital. Rarely, air sampling has been used to substantiate the risk for LD transmission from water faucets. In one publication aerosols within one-meter distance from bathtub faucets were shown to contain *Legionella*. [96]

Toilets have not been implicated as a source of infection in LD. However, experiments using seeded water indicated that toilet flushing could spread bacteria and viruses, which can stay airborne for prolonged periods. [97]

Furthermore, numerous unique outbreaks have shown that LD transmission can involve any aerosol-producing device. Examples include a small decorative fountain in a restaurant, [98] a grocery mist machine [99], a footbath in a sauna [100] and a defect pump that unintentionally produced aerosols in a crawling space of a flooded bar. [101] In the latter example, air sampling and genotyping results revealed an indistinguishable *L. pneumophila* serogroup 1 genotype in
a sewage pump, in the air of the crawling space and of the bar as well as in sputum of LD patients. Apart from the above examples, many outbreaks have been published in which the source could not be identified. This includes an outbreak at a car parking. [102]

c. Susceptibility

In the whirlpool associated outbreak presented in chapter two of this thesis, the attack-rate increased linearly on a daily basis, from 0.011% to 0.56% six days later, probably due to larger amounts of airborne amoebae or vesicles corresponding to an increase in Legionella concentration in the whirlpool water. [103] This increasing attack-rate is a strong argument for general susceptibility of humans for Legionella infection. Furthermore, the whirlpool associated outbreak presented in chapter two and an outbreak of community-acquired LD due to a grocery mist machine [104] show similar host risk factor patterns. In the first, 29% had chronic underlying disease (smoking, alcohol intake, and immunosuppressive medication excluded), whereas in the other 62% had at least one current underlying disease (smoking, alcohol intake, and immunosuppressive medication included). Case-control studies for community-acquired LD that were matched for age and gender indicate that underlying disease is not an independent risk factor. [105] In chapter three of this thesis it is shown that probably travelling abroad is a confounder for this relation. [106]

Smoking is the only strong and independent risk factor for LD that is consistently reported from the original outbreak onwards. Furthermore, the risk was shown to increase significantly with the number of cigarettes smoked. [107] The prevalence of chronic bronchitis and emphysema is reported to be higher among LD patients than expected, based on US national morbidity data, [108] although not in case-control studies. Still, both findings strongly support the hypothesised pathogenesis of LD in which Legionella packed amoebae or vesicles are not removed by the mucociliary defence mechanism.

Numerous outbreaks of LD in hospitals show that health personnel are not affected, although they have been exposed to Legionella as shown by elevated titres of antibodies against Legionella in serum. [109] Especially transplant patients are vulnerable to infection with Legionella. [110] But also, other underlying diseases and immunosuppressive medication have been shown to increase the risk of nosocomial LD. Nosocomial infection is predominantly not due to Legionella serogroup 1, but to other serogroups [111] and other species [112] that are known to be less virulent.

Outbreaks of community-acquired LD are the best proof of general susceptibility. However, several publications have suggested that certain polymorphisms in the gene regulated innate host immune response may enhance susceptibility to LD as demonstrated in LD patients. [113,114]
3. EPIDEMIOLOGY

In this paragraph, the role of epidemiology in source identification and its role in understanding the transmission of LD are discussed. Different types of studies have implicated potential sources of infection with *Legionella*. Molecular epidemiology is becoming more prominent as shown by our recent discovery of a mismatch in *Legionella* genotype distribution (chapter six of this thesis). First, in this paragraph some typical LD transmission patterns are discussed: sporadic cases, serial cluster, clusters in time and space and outbreaks.

a. LD transmission patterns

Sporadic LD patients are single LD patients with no association to other LD patients in time and space. In the literature any LD patient who is not part of an outbreak or cluster is called sporadic. These LD patients are rarely linked to a source of infection. A study including 203 non-outbreak, non-travel community-acquired LD showed that many apparently sporadic cases are in fact serial clusters (LD patients spread over time, linked to one source) or clusters in time and place. [115] Sporadic LD patients may precede a cluster of cases (e.g. one patient six weeks before an outbreak of 16). [116] These findings made Bhopal in 1992 suggest that control of LD should include epidemiological and environmental investigation of sporadic LD patients, instead of waiting for two LD patients who could be linked to a common source. [117] One of the reasons that sources for sporadic LD patients are rarely identified, may be that risk factors for sporadic LD are not identical risk factors for outbreak related LD.

The finding of serial clusters of LD leads to the hypothesis that many apparently sporadic cases result from low intensity, intermittent exposure to a common source and are hence part of mini-outbreaks. [118] Examples of such undetected clusters include a group of 25 nosocomial LD patients in a period of 17 years in a university hospital, [119] and a cluster of six community acquired LD patients in a period of six years who visited the same sauna. [120]

The long period during which clusters are caused by the same environmental *Legionella* genotype in the same environmental niche has been used in public health intervention strategies. The European Working Group for *Legionella* Infections (EWGLI) implicates hotels which have been visited by two or more LD patients during their incubation period as a source of LD and stimulated participating governments to implement surveillance schemas. This strategy has lead to a successful decrease of hotel-related outbreaks. [121] The EWGLI strategy has been tried in the Netherlands on a national scale as described in chapter ten of this thesis, with positive and cost-effective results. [122] Based on a two-year evaluation, it was calculated that the programme would break even after six years. Despite the programme, small clusters may go undetected because of underdiagnosis and unawareness, or because detailed information on exposure in the incubation period is insufficient (e.g. postcodes).

There are several prerequisites for typical outbreaks of LD to occur: a large number of exposed individuals, among them male smokers of middle age, who inhale *Legionella* loaded (vesicles of) amoebae, which originate from an aerosol producing environmental niche. The *Legionella* are of the *L. pneumophila* species and serogroup 1 serotype, are MAb-3 positive as defined by monoclonal antibody typing, possess the Mip-gene, have been under selective
pressure from protozoa (most probably *Hartmanella vermiformis*) which potentiated their virulence, have grown at an optimal temperature of 37 degrees Celsius for a number of days to weeks, with sufficient nutrients available in the biofilm. The amount of aerosols produced, the ambient temperature and humidity as well as other climate conditions that favour spread and prevent dehydration of the airborne amoebae or vesicles further determine the size of the outbreak. The reason that outbreaks are rare events seems to be that the prerequisites are many and mostly do not all occur at the same time.

b. Epidemiological studies to implicate sources of LD

Ideally, the source of an outbreak is demonstrated by culture of the same *Legionella* genotype in LD patients’ sputum culture, air samples and in water from an aerosol-producing source. In most descriptions of LD outbreaks air sampling is missing. Transmission evidence in that case is further supported by epidemiological studies (cohort studies, case-control studies, sero-surveys, and molecular epidemiology).

In chapter two of this thesis a cohort study is described involving 742 healthy individuals who were exposed to *Legionella*. The cohort study was part of an investigation of an indoor community-acquired LD outbreak due to a whirlpool. [123] The geometric mean serum antibody titres against *Legionella* rose reciprocal to the distance to the source of the outbreak. [124] Using the same data, it was later demonstrated that sub-clinical infection with *Legionella* is very common (40% of workers within a 40 meter distance), [125,126] suggesting that a shorter distance to the source of infection was required for LD to occur.

A case-control study comparing 107 solitary LD patients to lung cancer patients revealed that the population living within 0.5 kilometre of a cooling tower had a three times higher risk of LD than people living more than 1 kilometre away. [127] A large outbreak involving 85 LD patients showed an association with living downwind of a cooling tower. [128] Visiting a retail shop next to an excavation and construction site was shown to be an independent risk factor in an outbreak of LD involving 27 patients. [129] Residing near an excavation site has been described as an independent risk factor as well as in a large case-control study. [130] In chapter three of this thesis a case-control study for risk factors of LD is described. The results of this study suggest that there are two distinct LD populations based on foreign travel. LD patients who travelled abroad were of better health then those who attracted their disease in the Netherlands.

Sero-surveys have been published suggesting the potential of certain sources to transmit LD. Among them several surveys aimed at quantifying the risk of dentist equipment. [131] However, numerous studies have failed to indicate this type of aerosol spread as a true source of LD. Results described in chapter three of this thesis confirm that visiting a dentist is not a risk factor for LD. In outbreaks of nosocomial LD, sero-surveys among hospital personnel showed elevated antibody titres and seroconversion in the absence of disease.

Different molecular biology techniques have been used to genotype *Legionella* isolates and environmental strains in order to implicate the source of an outbreak. Still, finding indistinguishable genotypes is no proof of transmission as they may be found in large geographical areas. [132] Essential in the interpretation of genotyping results is knowledge
on the background distribution of both clinical isolates as well as environmental *Legionella* strains, preferably based on prospective studies. Unfortunately, few such studies have been published, one of them is discussed below. [133]

c. Distribution mismatch

In a large prospective study in the Netherlands presented in chapter six of this thesis, patient isolates and environmental *Legionella* strains were collected and genotyped. The distribution of genotypes of the two groups appeared to differ substantially. Most prominent was the finding that the most common clinical isolate (EWG1 AFLP type 004 Lyon) was only found once in 6,500 environmental samples.

Several explanations are possible. The first is a laboratory contamination in the patient collection. This is not logic, since all 62 laboratories in the country have made clinical isolates in this collection available. The second possible explanation is that the environmental samples that were tested for *Legionella* had a low a priori chance to contain 004 Lyon. This is not the case, since only samples were taken that originated from potential sources to which LD patients had been exposed during their incubation period. Only types of sources with a documented association with LD had been included in the sampling procedures. The third possible explanation is that 004 Lyon is mostly found in cooling towers. As locations of cooling towers are generally not known in the Netherlands, they are rarely sampled. A fourth explanation is the choice of sampling sources that contain water per se. Instead, air as well as soil samples should have been taken in order to identify potential sources.

The most likely explanation is that strains of 004 Lyon were present at undetectable levels.

4. Intervention

Control of LD is possible at different levels. To describe these levels, in the section below the chosen direction is from the patients towards the environmental sources. First the role of diagnosis and treatment, notification, outbreak detection and source identification is discussed, followed by a summary of preventive measures. A differentiation is made between general measures and those aimed at the hospital situation.

a. Diagnosis and treatment

Without diagnosis, source identification and prevention are impossible. Since the use of urinary antigen testing, under-diagnosis of LD in the Netherlands has dramatically diminished, resulting in ever-rising incidences. Thanks to a more timely diagnosis and concurrent timely treatment, at the same time the case fatality rate has decreased from 15% to 8% in the Netherlands. [134] These data correspond to an even larger reduction of mortality in the USA, from 34% to 12%. [135]
b. Notification, outbreak detection and source identification

Since a large outbreak of LD in 1999, the notification process in the Netherlands has been speeded up by the introduction of a web-based notification system for all notifiable infectious diseases (osiris). As described in chapter ten of this thesis, an LD outbreak detection programme was installed in the Netherlands in 2002, aimed at identification of serial and geographical clusters as well as a short response time between notification and sampling of a potential source of LD. The response time has been significantly reduced as compared by the 1999 outbreak. This was evident in 2006, when an outbreak involving 30 LD patients was related by genotyping to a cooling tower in Amsterdam ten days after the first LD patient was notified. [136]

In the first two years, the outbreak detection programme identified seventeen clusters, twelve of which would not have been identified timely without its existence. Between 2002 and 2006, the programme was able to detect a potential source of infection for 25% of LD patients in the Netherlands, excluding patients who were part of the Amsterdam outbreak and those who had visited abroad in their incubation period. However, for most of the clusters in space and time, no specific source could be identified. A recent grant of the Netherlands Organisation for Health Research and Development (Zon-Mw) is being used to clarify these clusters.

c. Temporary disinfection

As soon as a source is identified, it should be controlled or eliminated. In most circumstances, checking of the water distribution system will reveal imperfections concerning stagnant water and inappropriate temperature, which can easily be corrected. If temperature levels cannot be adjusted, disinfection with heat or chemical substances may be needed. Unfortunately, these measures are not sufficient. As described in chapter ten of this thesis, 12 new LD patients occurred in a two-year period due to failing disinfection of identified LD patient-related potential sources. Even after a more rigorous second disinfection, two more LD patients occurred. Ultimately, removal and renewal of the entire water distribution system was required. [137] In case of a cooling tower as the source, mechanical cleaning and chemical disinfection are more appropriate. Chemical disinfection of cooling towers has shown to be difficult as well. [138]

d. Preventive measures

All of the above are interventions in the presence of LD patients. However, legislation on Legionella requires that preventive measures be taken in the absence of LD patients. In that case, installations that are notoriously contaminated with Legionella may become part of a Legionella control programme.

General preventive measures. The World Health Organisation (WHO) in 1990 stated in a memorandum from a WHO-meeting on epidemiology, prevention and control of legionellosis that general measures to control LD are ineffective. [139] In a 2007 update, [140] the WHO seems to have adapted its view since it now advises to apply water safety measures to potable water
and in-building distribution systems, cooling towers and evaporative condensers, health-care facilities, hotels and ships, natural spas, hot tubs and swimming pools.

The preventive measure most applied to control LD is temperature regulation. It has been shown that gas heaters are less often associated with growth of *Legionella* than electrical heaters. [141] Monitoring of the temperature in a water distribution system can be a sufficient control measure, provided that water in the entire system is held above 55 degrees Celsius. In practice, it is advised to keep the temperature of heaters above 60 degrees. Culture is the ultimate test for LD control of water distribution systems. Demonstration of *Legionella* by culture has a sensitivity of only 50% as measured by seeded tap water as golden standard. [142] Several conventional as well as real-time-PCR-based methods for detection of *Legionella* in water samples have been described. Joly recently reported that the type of water sample and inter-laboratory differences influences these assays. [143]

If temperature control cannot be achieved throughout a water distribution system, disinfection is warranted. Unfortunately, few controlled studies on *Legionella* disinfection have been published. Serial clusters can occur if temperature niches exist that create a stable microenvironment for growth of *Legionella*. Such niches are difficult to disinfect. Due to the protection of amoebae, *Legionella* is resistant to several disinfectants. [144] Also, amoebae can adapt to disinfectants. [145] In cooling towers, *Legionella* has shown to be highly resistant to disinfectants. [146]

*Preventive measures in hospitals.* All hospitals should be aware that immuno-compromised patients are at risk for LD caused by strains that are not pathogenic for healthy individuals. Furthermore, a virulent strain of *Legionella* can be introduced in the water distribution system at any time. Therefore, nosocomial pneumonia patients should be tested for LD.

Temperature monitoring and sanitation of the water distribution system are prerequisites for LD control in hospitals. Dead legs have been shown to contain *Legionella* concentrations up to $10^8$ CFU/l and should therefore be removed. [147] Removal of faucet aerators has been shown to reduce the risk of transmission. [148,149] Sampling and culturing of water two to four times per year will provide information on the effectiveness of LD control measures in place. *Legionella* is protected by amoebae [150] and biofilm [151] which makes disinfection problematic. Several strategies have been tried, the most common one being the so-called superheat-and-flush method. This method is based on a hospital outbreak in the early 1980s, involving 100 LD cases that was eventually stopped by heating of the water temperature above 60 degrees Celsius. [152] Later studies showed that the method gives only temporary results. [153,154] The use of copper-silver-ionisation, monochloramine, ultraviolet radiation and filters all has been described in uncontrolled studies. [155–157]

5. LD CONTROL IN THE NETHERLANDS

In this paragraph, LD control in the Netherlands after 1999 will be discussed. That is, as it took shape in the period following the large whirlpool-associated outbreak in Bovenkarspel in 1999 that was described in chapter two of this thesis.
a. Bovenkarspel and VROM

In 1999, the Ministry of Housing, Spatial planning and the Environment (VROM) in the Netherlands, responsible for the drinking water quality immediately took the initiative in Legionella control. VROM applied its own safety benchmark for environmental policies to the drinking water quality. The VROM safety benchmark allows for one death in a million exposed.

b. Drinking water legislation and public health actors

On October 15, 2000, a temporary legislation was issued aimed at reducing the overall level of Legionella in drinking water below 50 CFU/l. This general control strategy was consolidated two years later in the new Drinking Water Law, issued by VROM. Initially, all owners of a drinking water system to which third parties were exposed, were obliged to assess the risk of the system, create a control plan to contain growth of Legionella, and use a logbook to document control measures. If Legionella concentrations above 1000 CFU/l were cultured from water samples, water systems were closed and municipal health services consulted to assess the risk for LD transmission. In the new Drinking Water Law, the normative level was raised to 100 CFU/l and certain categories were excluded decreasing the impact from 600,000 to 10,000 owners of installations. The reduction was a direct consequence of intensive interaction of VROM with public health actors in the preceding three years. The public health actors consisted of the Municipal Health Services of the four biggest cities (Amsterdam, Rotterdam, Utrecht and The Hague), the Preparedness and Response Unit (LCI) and the National Centre for Hygiene and Safety (LCHV) of the National Institute of Health and the Environment (RIVM), the LD outbreak detection programme (BEL) and the National Society of Municipal Health Services in the Netherlands (GGD Nederland). The actors all pointed to the lack of evidence for the proposed Legionella control interventions to be effective and to the very high costs involved.

Since 2000, control of LD in hospitals falls under the jurisdiction of VROM. Control of LD related to cooling towers falls under the Ministry of Social Affairs and Employment.

c. A nine year evaluation

Nine years have passed since the 1999 outbreak. During this period VROM has actively stimulated LD control efforts and has actively pursued compliance to the Drinking Water Law. Below the impact of these efforts is discussed in terms of LD incidence and costs involved.

Incidence. The incidence of LD in the Netherlands was relatively stable at 2.7 per million in the 1987–1998 period. After a sharp increase in 1999 to 11 per million, the incidence has steadily continued to rise to 26 per million in 2006.

Costs. Most of the costs involved in control of Legionella in water distribution systems have not been made by the government, but by owners of collective water distribution systems divided over several sectors in society. They in their turn asked higher prices for their services.
to cover the investment in improved water distribution systems and installations. So, the
general public as the end-user of these services paid for the investments made by businesses
like hotels, saunas, camping sites, and sport facilities. The municipal health services of the
biggest Dutch cities calculated that in the 1999–2002 period a one-time investment of ten
billion Euros was spent, followed by a yearly investment of one billion Euros. The yearly costs
were reduced to 500 million Euros per year in the post 2002 period. [158]

In the Netherlands over a hundred hospitals exist all of which have complied with the
Drinking Water Law, making large investments in safe water distribution systems. These
investments were made using the normal budget for the health care sector. Given the financing
structure in the Netherlands, this means that every citizen contributed to these costs. There
are no data available on the height of the costs.

National and local government as owners of buildings like military training camps and
municipal indoor swimming pools have invested in Legionella control measures. The Associa-
tion of Netherlands Municipalities (vng) calculated that its members spent 48 million Euros
on general measures in the first years after 1999. The Ministry of Defence spent 16 million
Euros. [158]

There has not been a large investment in fundamental Legionella research. However, the
drinking water companies and vröm have invested in applied Legionella research by kiwa.
Also, 500,000 Euro was invested by a Ministry of Health Fund (Fonds Openbare Gezondheid)
in an outbreak detection programme. This implementation programme had some research
off-spin, which has been presented in parts two and four of this thesis.

The cost of Legionella prevention as expressed by quality of life adjusted life years (QALYs),
was estimated between Euro 100,000 and Euro 1,000,000 per QALY by the National Institute
of Public Health and the Environment. In comparison, in 2000 the minister for Health
considered that Euro 26,400 per QALY was not cost-effective enough to justify introduction of
hepatitis B vaccination in the National Vaccination Programme.

As QALYs are often used in calculating costs and benefits of public health interventions it
allows for comparisons to alternative interventions. Comparing this way, one QALY gained by
the Legionella prevention activities in the Netherlands was as expensive as 100 QALYs gained
by open-heart surgery. [159] When the Drinking Water Law was adjusted, limiting its range
to 10,000 water installations, the Legionella prevention was estimated to cost ten times lower.

6. An evidence based approach of LD control

As discussed in chapter five of this thesis, in the last nine years general LD control measures
in the Netherlands have not coincided with a decrease in LD incidence. In this paragraph a
more targeted approach is presented. The approach is based on the identification of several
circumscribed LD transmission situations for which different programmes can be developed:
outbreaks, hospitals, and cooling towers. Furthermore, two important contributions to pre-
vention are acknowledged for which a programme is suggested: a contamination database and
a cluster evaluation and disinfection expertise team. This paragraph finishes with recommend-
dations to practical research, results of which will enhance LD control measures.
a. Outbreak detection programme

Since 2002, an outbreak detection programme is operational in the Netherlands. Although it has been more successful in identification than in elimination of sources, its effectiveness could be enhanced by adding several tools: a geographic information system (GIS), air and soil sampling, and a culture procedure for amoebae.

Geographical information system. In outbreak situations as well as during surveillance efforts to identify clusters in time and space, GIS is a powerful tool as a visual aid to integrate complex data. In one view and at any desired level of geographical detail, LD patients as well as potential sources can be seen. Hypothesis can be generated and immediately tested for probability, as the GIS system can provide distances, sampling results, and seasonal information as desired. The GIS should contain detailed information on patients like their home address as a proxy for the site of infection, gender, date of birth, first day of illness, microbiology diagnosis, isolated genotype for culture-positive patients, smoking habit and presence of underlying diseases. The GIS should contain detailed information on cooling towers like the address and exact location on or at the building, the addresses and telephone numbers of the owner, the user and the maintenance company, the type of cooling tower and its capacity, a log with maintenance data, sampling results including the genotype(s) cultured from the water, the concentration in CFU/l and the disinfection regime. Also, address, type of use and maintenance details of potential sources of infection should be part of the GIS as identified during interviews with LD patients on activities during their incubation period. Available sampling results (genotype, concentration, disinfection) should be added. With informed consent of the owners, sampling results of contaminated water systems should also be part of the GIS. For certain types of installations, sampling is required by law. At present no central registration of contamination exists, whereas thousands of installations are sampled each year.

Air and soil sampling and culture for amoebae. Given that LD is an airborne disease and given the amount of controversy when implicating an installation as a potential source of infection, air sampling should be part of sampling procedures. For example, a cooling tower in Pas-de-Calais is considered to have caused LD patients at seven kilometres distance [160], whereas in a comparable outbreak in Amsterdam culture-positive patients have been within only 400 metres of the cooling tower implicated as the source. In both instances, positive air sampling results would have strengthened the microbiological and epidemiological findings. The same is true for the whirlpool-associated outbreak of chapter two of this thesis. In order to measure the spread of bacteria by aerosols, a study using an identical whirlpool was performed at the outbreak premises with Serratia marcescens as a marker, which was cultured on plates at varying heights and distances from the whirlpool. Unfortunately, air sampling was not part of this study.

Garden centres are among the premises most frequently visited by LD patients in their incubation period (this thesis, chapter five). Yet, rarely an environmental Legionella strain has been cultured from water samples during source identification efforts. It would be
worthwhile to take additional soil samples as soil has been implicated as a potential source of LD transmission.

Existing sampling procedures and culture techniques should be extended to enable demonstration of amoebae and other protozoa that play a crucial role in LD transmission.

b. Hospital control programme

Hospital associated LD comprises 15% of all LD patients who did not travel abroad in their incubation period. As these patients are clearly located, nosocomial LD is easier to prevent. Given that nosocomial LD is different from community-acquired LD with respect to the susceptible population and the virulence of the environmental Legionella strains involved, control should be more rigorous, especially for wards for transplantation patients. To date, no specific hospital control programme in the Netherlands exists. Recent experiences with a nosocomial outbreak involving nine LD patients in the Netherlands indicate that substantial progress can still be achieved here. A hospital control programme should be build around hospital microbiologists and hygienists who have experienced and successfully controlled nosocomial outbreaks.

c. Cooling tower control programme

In neighbouring countries up to 30% of all LD patients have been linked to cooling towers. After a cooling tower related outbreak in 2006 [161] these installations have received much attention. Presently in the Netherlands, municipalities are responsible for the prevention of cooling tower related outbreaks of LD. The have been advised by the Ministry of Housing, Spatial planning and the Environment (vrom) to explore their territory for the existence of cooling towers and urge owners to maintain them properly. For adequate outbreak detection, information on cooling towers is essential. A national register containing detailed information should fill this gap. Ideally, all known cooling towers should be sampled regularly. Sampling is an ultimate control tool and has the additional advantage that environmental strains can be genotyped, fluctuations in the concentration of Legionella can be monitored and disinfection efforts checked for effectiveness. Municipalities should be helped to identify cooling towers. Extensive experience in Rotterdam, Amsterdam and Haarlem has learned that these efforts consume substantial time and resources.

d. Cluster evaluation and disinfection expertise team

Outbreaks and clusters are rare events originating from rare environmental niches. Why of all saunas, hotels, camping sites, and shopping malls do patients get infected in this particular one? This is the central question that a cluster evaluation team should answer. During an outbreak or cluster this multidisciplinary team should be able to identify the most likely transmission route and find ways to control it. A lot can be learned from these unique situations. In the past, several clusters in the Netherlands have occurred that would have been controlled more rapidly had such a cluster evaluation team existed. Still, elimination
of the source of a cluster can take up to 59 days (this thesis, chapter ten). In the absence of an experienced team, precious time during outbreaks can be wasted due to lack of authority and coordination. Maybe the biggest problem in LD control is disinfection of contaminated water distribution systems and installations. In the Netherlands an authority in this domain is lacking, leading to a wide range of disinfection methods marketed and sold, without proper knowledge of its effect.

e. Contamination database

Water from thousands of installations in the Netherlands is being sampled and cultured for Legionella on a yearly basis. Workers in this branch indicate that up to five percent of cultures are positive for L. pneumophila serogroup 1. The sampling results are feed back to the owners of the installations and the positive cultures thrown away. If installation owners can be persuaded to hand over sampling results to a central database and if environmental Legionella strains were to be stored centrally instead of thrown away, useful information for Legionella control programmes would become available with little extra costs. Sampling results could be incorporated into the proposed GIS system (see paragraph 6a), speeding up response time in outbreaks and increasing the source identification percentage.

f. Research efforts

Essential information in LD control is still lacking as was described in the previous paragraphs. Research efforts should focus on transmission, virulence development, disinfection and amoebae.

A mismatch in distribution of patient-derived and environmental strains in the Netherlands has been described in chapter six of this thesis. Several studies could help identify the cause of the mismatch. After several years of data collection, the outbreak detection programme has identified 60 geographical clusters without a specific source. In cooperation with municipal health services air sampling at various spots within the boundaries of the identified clusters could pin down sources of LD infection. Apart from that, in the vicinity of different types of implicated sources of infection an air sampling study should give insight in the spreading pattern. Air sampling should include efforts to detect amoebae and other protozoa in the air.

The EWG1 AFLP genotype 004 Lyon is common in patients, but not in environmental samples. As molecular biology tools are theoretically more sensitive than culture, a PCR for 004 Lyon should be tested on water samples gathered during source identification efforts. Positive PCR results may indicate concentrations below the culture detection level or may confirm the existence of non-culturable but viable (NCBV) Legionella of this genotype.

If the mismatch finding is repeated in other countries, conclusions on virulence based on relative frequency can be drawn. Apart from specific PCRs, typing based on DNA micro-array and interaction with amoebae are promising study fields.

Preliminary typing results based on a DNA micro-array using a patient-derived and environmental Legionella strain collection indicate that both groups differ in genetic material. [162] This finding could form the basis of a virulence test, which would be useful in LD control
settings outside hospitals where decisions to disinfect or not may have substantial economic impact.

As amoebae and amoebal vesicles probably are the inoculum of LD, research effort should be directed to this field. Questions to be answered include: What amoebae are found in proven sources of LD transmission? Which types of protozoa render Legionella avirulent for human macrophages? Can disinfection strategies be build on host competition in water distribution systems? To which type of disinfectants are amoebae sensitive? Can amoebae be used as a marker for risk of transmission of LD?

Controlled studies in public health settings to evaluate the effect of disinfection are rare. However, there is a strong need for evidence-based disinfection practices in hospitals, hotels and other places where people gather.

It has been suggested that LD is a travellers disease paralleling E. coli infection, meaning that humans are exposed and naturally immunised to Legionella at home and only fall ill when exposed to Legionella in premises other than one’s own house. Such a relation is suggested in several studies involving workers in dental practices who are exposed on a daily basis to Legionella contaminated aerosols from dentist equipment inducing high antibody titres in the absence of LD. A study that would test the hypothesis would be a sero-survey in an exposed population using the environmental strain antigen to prepare a serum Legionella antibody test.

7. Conclusion

Fresh water and ground water are natural habitats for Legionella. As these raw materials are used for drinking water production, Legionella is introduced in end-users’ water distribution systems by the water mains. General measures aimed at reducing this contamination have been tried in the Netherlands in a period of nine year. During this period, the incidence of LD has risen from 11 to 26 per million inhabitants. Between 2002 and 2006, for 25% of LD patients a potential source of infection was identified by the national LD outbreak detection programme (BEL). Elimination of these patient-related sources has so far been moderately successful. To improve elimination and increase the percentage of identified potential sources, a geographical information system, additional sampling of air and soil, a cluster evaluation and disinfection expert team, and a contamination database will be supportive. Hospitals and cooling towers represent successful niches for LD transmission. These niches are well suited for specific LD control programmes, which must be rigorously executed to be effective. Implementing these measures will improve the efficiency of current LD control. However, LD will continue to be a public health problem as Legionella is ubiquitous in water, the exact mode of LD transmission is far from clarified and disinfection remains difficult to implement.
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