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The presence and growth of *Legionella* species in thermostatic shower mixer taps: an exploratory field study

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Abstract

Legislation in the Netherlands requires routine analysis of drinking water samples for cultivable *Legionella* species from high-priority installations. A field study was conducted to investigate the presence of *Legionella* species in thermostatic shower mixer taps. Water samples and the interior of ten thermostatic shower mixer taps were investigated for cultivable *Legionella* species. In seven cases, *Legionella* species was found in at least one of the samples. In four cases, *Legionella* species was detected in the biofilm on the thermostatic shower mixer taps interior, with the highest values on rubber parts, and in five cases in the cold supply water. These results show that thermostatic shower mixer taps can play a role in exceeding the threshold limit for cultivable *Legionella* species, but the cold supply water can also be responsible.

Practical implications: This study showed that contamination of thermostatic shower mixer taps (TSMTs) with *Legionella* spp. was frequently observed in combination with contamination of the water system. Consequently, a combined focus is necessary to prevent the proliferation of cultivable *Legionella* spp. in TSMTs. In addition, the results also demonstrated that biofilms on rubbers inside the TSMT had high numbers of *Legionella* spp., probably because rubber contains relatively high concentrations of biodegradable substrates. Therefore, improvement of the rubber materials is necessary to reduce the proliferation of cultivable *Legionella* spp. in TSMTs.

Keywords

Legionella, shower tap, rubber, premise plumbing system, microbial water quality

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Introduction

Legionnaires' disease or legionellosis, Pontiac fever and acute pneumonia are caused by *Legionella (pneumophila)*. In 2009 and 2010, a total of 5518 and 6296 cases of Legionnaires' disease were reported in Europe.[1,2] In more than 95% of these culture-confirmed cases, *Legionella pneumophila* was isolated from the patient, indicating that *L. pneumophila* is the most virulent *Legionella* species (spp.). *Legionella* spp. can be present in drinking water systems in buildings.[3] The inhalation of small airborne water droplets that are contaminated with pathogenic *Legionella* might result in legionellosis. Uncultivable *Legionella* spp. are always present in the drinking water in the Netherlands, but cultivable *Legionella* spp. are not always observed in drinking water.[4] Most of these species have not been related to disease or are only mildly virulent. However, the presence of *L. pneumophila* in premise plumbing systems is a serious threat to public health.

Legislation in the Netherlands calls for water systems in so-called high-priority installations like hotels, prisons, hospitals and saunas, to comply with special regulations for the prevention of growth of cultivable *Legionella* spp. in the premise plumbing system. Especially in healthcare settings, where residents may have a compromised immune system, aspects of drinking water safety and security are important design criteria. Despite all efforts to comply with legislation in the Netherlands, the occurrence of *Legionella* spp. in premise plumbing systems remains a challenge. There can be several places in premise plumbing systems where *Legionella* spp. might proliferate, but the exact locations remain unknown. Thermostatic shower mixer taps (TSMTs) are locations where *Legionella* spp. might proliferate, because the cold and hot water meet each other in such mixer taps and the mixed water temperature in the tap can be around the optimal growth temperature for pathogenic *Legionella* strains. Therefore, our study focuses on TSMTs in healthcare facilities and hotels as a potential source of excess *Legionella* spp. levels in water systems. The objectives of our study are to determine (1) whether shower mixer taps contribute to excess levels of cultivable *Legionella* spp. in tap water and (2) to what extent are cultivable *Legionella* bacteria present inside TSMTs and the supplying drinking water pipes.

Methodology

Field study and locations

A total of 10 thermostatic shower mixer taps (TSMT) coming from five locations (three healthcare facilities and two hotels) with centralized boiler types were investigated between September 2012 and May 2013. These TSMT locations had a history of *Legionella* spp. contamination, i.e. a number of positive test results during the last 2 years. Informed consent was obtained from the healthcare facility or hotel for their participation in the study, the analysis of the water and TSMT, and the publication of the results in an anonymized manner. The pH of the water supplied to the five locations varied between 7.8 and 8.0.

The field study consisted of sampling the water from the TSMT, demounting of the TSMT, the closure of the incoming cold and warm water supplies in combination with sampling water from both supplies. Additional checklists were filled out in order to gain data on the use of the TSMT of the past two years and other factors that may have an impact on the growth of *Legionella* spp. The water samples and demounted TSMTs were transported to the laboratory at 4°C and processed within 24 hours. At the laboratory, demounted TSMTs were opened and visually screened for biofilm in a so-called autopsy. Swab samples were then taken from suspected areas in the interior of the TSMT for further analyses.

Sampling procedure in the field

The sampling procedure is as follows:

- Take a photograph of the tap;

- Disconnect the shower hose;
- The tap was set for cold water;
- Water sampling of the TSMT;
  - Sample 1: The first 10 mL of water from the TSMT was analyzed for adenosine-5'-triphosphate (ATP), which is a measure for active biomass.
  - Sample 2: Subsequent 250 mL of water was taken from the TSMT/premise plumbing system and analyzed for *Legionella* spp. (S_{tap})
- Both cold and warm water supplies were closed;
- The TSMT was demounted from the wall;
- A new TSMT was installed;
- The new TSMT was set to warm water, and the tap was run for 3 seconds (in order to flush away any lose particulate matter present in the TSMT), and a 250 mL of warm water sample (S_{warm}) was taken from the new TSMT;
- Subsequently, the TSMT was set to cold water, and run for 3 seconds (same reason as above) and 250 mL of cold water sample (S_{cold}) was taken from the new TSMT;
- Determination of temperature gradient of the cold water during 2 minutes for every 10-second interval;
- Determination of temperature gradient of the warm water during 2 minutes for every 10-second interval. The S_{warm} sample was only analyzed if the subsequent temperature measurement did not exceed 55°C[5];
- All demounted TSMTs were stored at 4°C until further analyses.

Checklists

The checklist was used to collect data about the TSMT use and operational conditions of the premise plumbing system. The checklist was based on ISSO Publication 55.1.[6] It contained a description of the room where the TSMT was installed, the type, age and frequency of (non)use of TSMT and the material of the premise plumbing system pipes. The checklist also contained questions about the type of water used (warm, cold, mixed temperature water), whether the temperature of the water was limited to a certain temperature, if a flushing protocol was being applied[7] and about the maintenance of the TSMT and premise plumbing system, including questions on the history of *Legionella* spp. and sampling procedures.

Analysis of water and swab samples

The demounted taps were quite similar in terms of the number of compartments; the design of the interior chambers was somewhat different. In this study, two types of TSMTs were distinguished: types A and B (Figures 1 and 2). Five of the TSMTs were analyzed for the presence of biofilm at six separate swab positions, whereas the interior of five other TSMT were collectively swabbed (Figures 3 and 4). The swab samples that were collected during the autopsy were investigated in a following manner. The cotton bud swabs were placed, cotton side down, in a sterile test tube (190/25, called the collective test tube), which was filled with 10 mL of sterile water. The swab was then mixed with a vortexer, for 2 minutes in an ultrasonic water bath, so that the biofilm got separated from the swab. The liquid from the mixed test tubes was transferred into a sterile test tube and placed on ice. Another 10 mL of sterile water was added to the sample, again mixed with a vortexer for 2 minutes. This liquid was added to the collective test tube. This procedure was repeated twice. Subsequently, cultivable *Legionella* spp. were determined in the pooled sample (30 mL).

Legionella spp. was determined in water and swab samples using the buffered charcoal yeast extract agar (BCYE) according to NEN 6265.[8] Obtained colonies on the BCYE agar medium were subsequently confirmed as *Legionella* spp. using the polymerase chain reaction (PCR) as previously described.[9,10] In addition, PCR was also used to determine whether *Legionella* spp. positive colonies belonged to the pathogenic species *L. pneumophila*. ATP was determined according to the method described by van der Wielen and van der Kooij.[11]

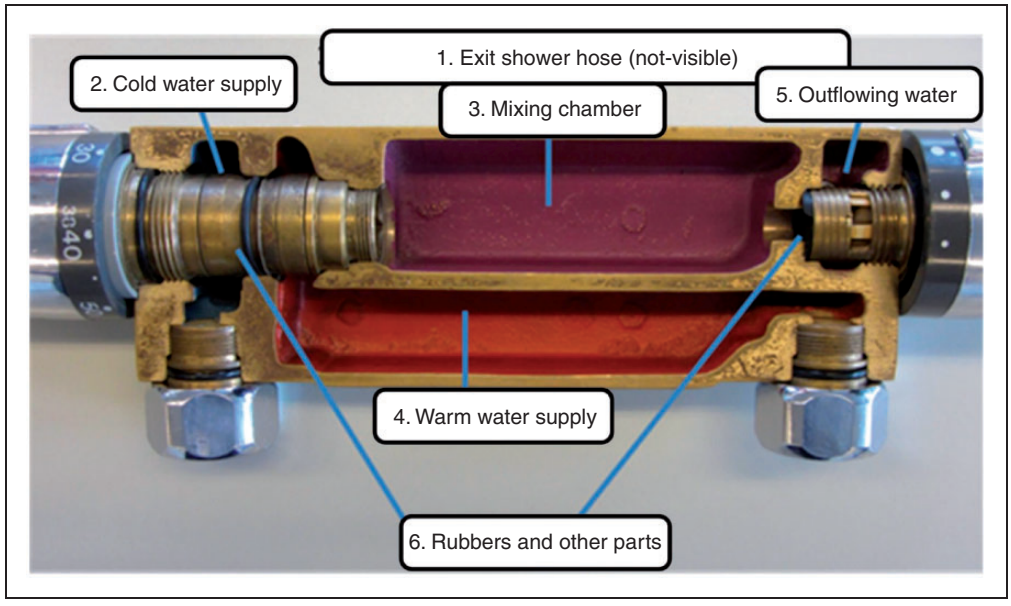

Figure 1. Cross section of a thermostatic shower mixer tap type A showing the six investigated compartments.

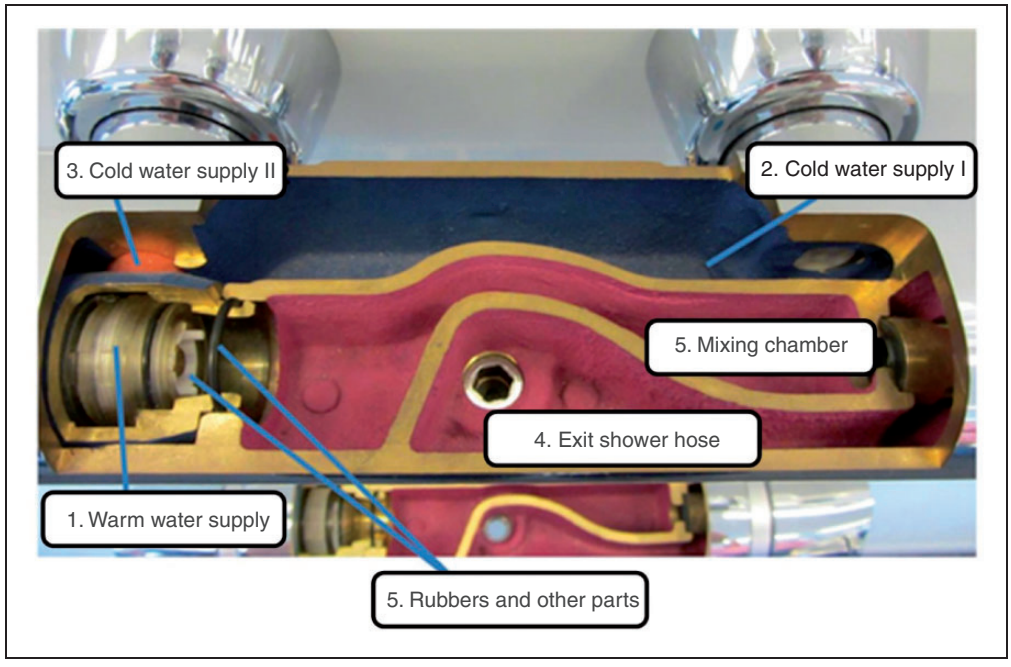

Figure 2. Cross section of a thermostatic shower mixer tap type B showing the six investigated compartments.

Results

Overview of TSMTs and results of check list

An overview of locations and the history of *Legionella* spp. as well as the properties of the taps and their maintenance are given in Table 1. All TSMTs were used for tapping mixed warm-cold water. A total of nine out of ten TSMTs had a history of testing positive for *Legionella* spp. TSMT 3 did not test positive in the last 2 years and is used as a reference tap in this investigation.

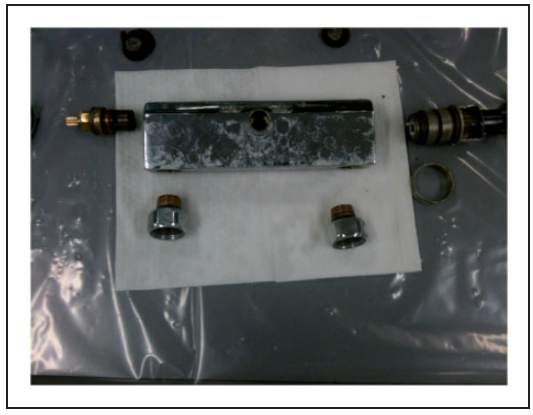

Figure 3. Demounting of a TSMT prior to taking swab samples.

Water samples and autopsy

Cultivable *Legionella* spp. numbers and ATP concentrations in the water and swab samples are presented in Table 2. In 50% of TSMTs, the warm water temperature of the warm water did not reach the obligatory 55°C. All 10 TSMTs were investigated via swabs to see whether *Legionella* spp. was present in the encountered biofilm.

In three out of ten taps, *Legionella* spp. could not be detected in the water and swab samples. Two of them, TSMTs 4 and 5, have tested positive before. There can be different explanations for this apparent discrepancy. It may be that the

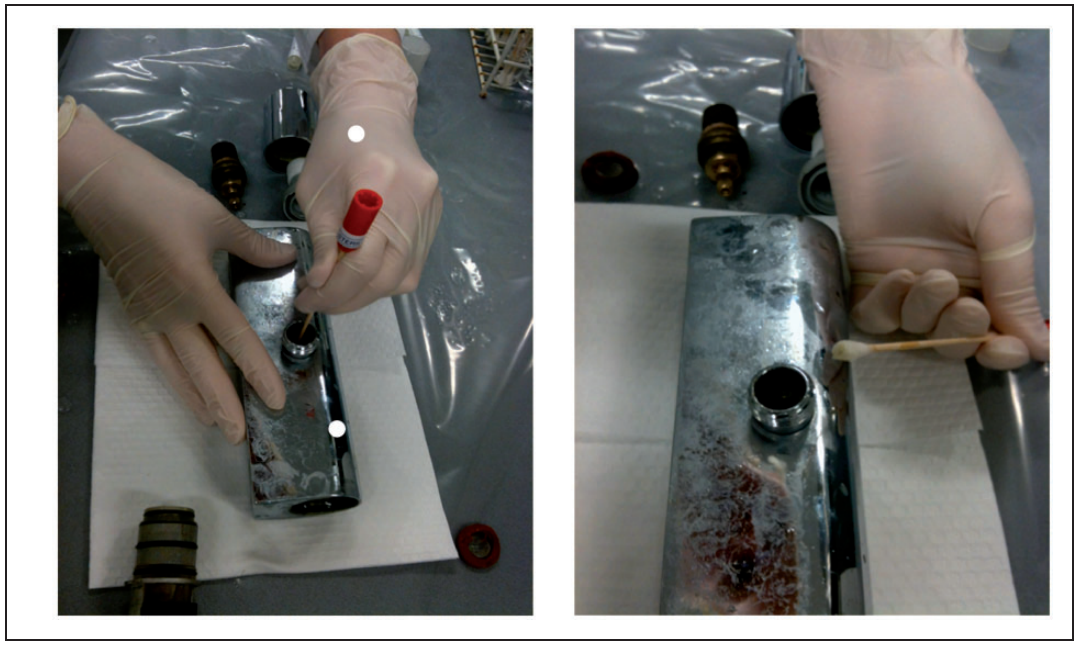

Figure 4. Autopsy of the interior compartments of a TSMT using swabs.

Table 1. Overview of locations and history of test results for cultivable *Legionella* spp. during the last 2 years, and the self-reported results of the checklist.

TSMT	Location	History of positive testing for *Legionella* spp.	Frequency of sampling in past 2 years	Number which tested positive for *Legionella* spp.	Pipe material	TSMT age (years)	Frequency of use (per week)	Time not in use	Water temperature limit (°C)	Variations in water temperature during use	Ambient temperature (°C)	Flushing protocol at 60°C	Maintenance
1	1, hotel	Yes	2	1	Copper	0.5–1	1 or more	2–7 days	38	Between cold and warm	<25	After 5 days of vacancy	January 2012, water meter disinfected. Summer 2012 main tap replaced.
2	1, hotel	Yes	3	3	Copper	0.5–1	1 or more	2–7 days	38	Between cold and warm	<25	After 5 days of vacancy	January 2012, water meter disinfected. Summer 2012 main tap replaced
3	2, hotel	No[a]	0	0	Copper	2–3	1 or more	0–2 days	No limit	Between cold and warm	<25	After contamination	No
4	3, healthcare	Yes	3	3	Copper	5–10	1 or more	0–2 days	40	Between cold and the limit	<25, higher in winter	After contamination	No
5	3, healthcare	Yes	3	3	Copper	5–10	1 or more	0–2 days	40	Between cold and the limit	<25, higher in winter	After contamination	No
6	4, healthcare	Yes	3	3	Plastic[b]	1–2	1 or more	In use	38	Between cold and the limit	<25	Technologically possible, not done yet	No
7	4, healthcare	Yes	3	3	Plastic[b]	1–2	1 or more	In use	38	Between cold and the limit	<25	Technologically possible, not done yet	No

(continued)

Table 1. Continued.

TSMT	Location	History of positive testing for *Legionella* spp.	Frequency of sampling in past 2 years	Number which tested positive for *Legionella* spp.	Pipe material	TSMT age (years)	Frequency of use (per week)	Time not in use	Water temperature limit (°C)	Variations in water temperature during use	Ambient temperature (°C)	Flushing protocol at 60°C	Maintenance
8	4, healthcare	Yes	3	3	Plastic[b]	1–2	1 or more	In use	38	Between cold and the limit	<25	Technologically possible, not done yet	No
9[c]	5, healthcare	Yes	2	2	Copper	5–10	Less than once	Unoccupied	38	Always at 38°C	<25	When needed	No
10[c]	5, healthcare	Yes	2	2	Copper	5–10	Less than once	Unoccupied	38	Always at 38°C	<25	When needed	No

PEX: cross-linked polyethylene.
[a]Reference tap.
[b]Multilayer PEX-Al-PEX (PEX/aluminum sandwich, consisting of aluminum pipe sandwiched between layers of PEX). These pipes are connected with modified brass compression fittings.
[c]At this location, the rooms have not been used for a number of months. The boiler has been turned off.

Table 2. *Legionella* spp. and adenosine-5'-triphosphate (ATP) concentrations in the water and swab samples taken directly from the tap (S_{tap}) and in water from the cold (S_{cold}) and warm (S_{warm}) tap, based on a 250-mL sample.

TSMT	S_{tap} (cfu L^{-1})	S_{cold} (cfu L^{-1})	S_{warm} (cfu L^{-1})	ATP (ng L^{-1})	Swab (Separate vs Collective)	Number of positive swabs
1	<100	11,000	[a]	10	S	0
2	1400	<100	[a]	14	S	2
3	<100	<100	[a]	38	S	0
4	<100	<100	<100	21	S	0
5	<100	<100	<100	15	C	0
6	<100	330	[a]	10	S	6
7	1800	<100	[a]	8	C	1 (collective sample)
8	<100	6600	2600	3	C	1 (collective sample)
9	<100	100	<100	9	C	0
10	100	900	<100	8	C	0

[a]Water temperature exceeded 55°C.

Legionella spp. numbers reduced during the first positive testing and current sampling. Another option is that *Legionella* spp. came from the shower hose or shower head and not from water from the TSMT. Water from the shower hose was not included in this study, but routine sampling for *Legionella* spp. collects the sample from the shower hose. In the other seven TSMTs, one or more samples tested positive for *Legionella* spp. In five out of these seven TSMTs, *Legionella* spp. was found in the cold water supply with numbers varying between 100 and 11,000 cfu L^{-1}. In one case *Legionella* spp. was observed in the warm water supply (2600 cfu L^{-1}). In the latter case, both the warm and cold water supplies contained *Legionella* spp. In three cases, *Legionella* spp. was found in the water coming from the TSMT with numbers varying between 100 and 1800 cfu L^{-1}. In TSMTs 2 and 7, *Legionella* spp. was found only in the TSMT, and not in the water from the cold or warm water supply. The temperature of the warm water supply reached 60.3°C in TSMT2, and 59.6°C in TSMT7. At these temperatures *Legionella* spp. is unable to survive,[12] and it is, therefore, assumed that no *Legionella* spp. is present in the warm water supply. The swab samples taken after autopsy of these two TSMTs showed that these two taps contained a biofilm with *Legionella* spp. In these two cases, the water samples might have become positive by the *Legionella* spp. in the biofilm of the TSMT itself. Apart from TSMT1, all cold water had an eventual temperature of lower than 20°C.

The interior of ten TSMTs has been investigated for cultivable *Legionella* spp. using swabs. In four TSMTs, *Legionella* spp. was detected after swabbing the biofilm on the internal compartments (Table 3).

In TSMTs 2 and 7, *Legionella* spp. was found in the tap but not in the supply water. It can, therefore, be concluded that the water in the tap was contaminated with *Legionella* spp. bacteria that were present in the tap. In TSMTs 6 and 8, *Legionella* spp. was found in the cold water supply (TSMT6), the warm water supply (TSMT8) and in the swabs taken from the biofilm on the interior parts. *Legionella* spp., however, was not found in the water that came directly from the TSMT (S_{tap}). In case of TSMT6, the number of colony forming units of *Legionella* spp. in the swabs is high, but the water from S_{tap} is not contaminated with detectable levels. Although the TSMTs 6, 7 and 8 are all of type B, the presence of *Legionella* spp.

Table 3. Results from the swabs of the biofilms on the internal compartments in the TSMTs. The *Legionella* spp. numbers are shown in cfu L^{-1} (based on a 30-mL water sample, there is no absolute relationship between the swabbed surface and cfu).

		Swab						
TSMT	Type	1	2	3	4	5	6	Collective
2[a]	A	<13	25	<13	<13	<13	36	
6[b]	B	750	900	4500	900	1800	18,000	
7	B							11,000
8	B							150

[a]Swab 1 = exit chamber of shower hose, swab 2 = incoming cold water, swab 3 = mixing chamber, swab 4 = incoming warm water, swab 5 = exit, swab 6 = rubbers and other parts.
[b]Swab 1 = incoming warm water, swab 2 = incoming cold water 1, swab 3 = incoming cold water 2, swab 4 = exit chamber of shower, swab 5 = mixing chamber, swab 6 = rubbers and other parts.

cannot be allocated to just the type of tap, as the three taps were taken from the same institution. Further investigation showed that the water supply pipelines were made of plastic, and that there were contaminations with *Legionella* spp. throughout the water system. There are, thus, multiple factors that can have an effect on the presence of *Legionella* spp. in the samples.

The results of the swab tests showed the presence of *Legionella* spp. in TSMTs 2 and 6. In TSMT2, two samples tested positive: the compartment for incoming cold water and the rubbers that are present in the tap. In TSMT6, all compartments, rubbers and other parts tested positive. In both taps, the rubber materials showed the highest number of *Legionella* spp. The rubber material in the taps may, thus, be the most vulnerable place for biofilm formation. In all positive cases (water and swab samples), the cultivable *Legionella* spp. were non-pneumophila species.

The ATP-content of the water is a measure for the amount of active biomass. A normal value of ATP for drinking water is between 1 and 10 ng L^{-1}.[11] The ATP-values observed in our study show a large range (2.9 to 38 ng L^{-1}). In addition, there seems to be no correlation between the TSMTs that tested positive for *Legionella* and the ATP concentration in the water. It might be possible that the ATP-content of the water is not a good predictor for the biofilm amount on pipework and taps.

Relation between checklist data and the occurence of Legionella spp

The TSMTs were investigated at five locations. Based on the results of the checklists and the number of investigated taps it is not possible to draw statistically significant conclusions. There are, however, a number of remarkable observations. There is one location with plastic pipework (multilayer polyethylene). Three sample sets were taken from this location, and the swab tests of these three samples were positive. In addition, there was no flushing protocol available at this location, and the taps were of type B. Multiple parameters differed from the other locations. The age of the taps does not seem to be a main factor in the occurrence of *Legionella* spp. Apart from TSMTs 9 and 10, most taps were in regular or permanent use. Location number 5 has a copper-silver ionization[13–15] system put in place. Nevertheless, this facility tested positive for *Legionella* spp. Only at location 1 (TSMTs 1 and 2), maintenance work has been carried out on the water system and during maintenance the water meter was disinfected after *Legionella* spp. was found in the water. Despite this effort, *Legionella* spp.

was still detected in the cold water supply of TSMT 1.

Discussion

Legionella spp. in supply water

In this study, nine TSMTs from which the water was tested positive for *Legionella* spp. in the last 2 years and one TSMT from which the water did not test positive were investigated for the number of cultivable *Legionella* spp. in the tap water, the compartments of the tap and both cold and warm supply water. At 50% of the cases, *Legionella* spp. was found in the cold supply water. Under normal conditions, cultivable *Legionella* spp. are not detected in cold water. Consequently, the detection of cultivable *Legionella* spp. suggests an inadequate design of the premise plumbing system. Moreover, the temperature of the warm supply water did not exceed 55°C in 50% of the cases. In two of these cases, the boiler was turned off because of temporary vacancy. However, the boiler was in operation at the other three cases, indicating that the boiler system was not sufficient to provide water temperatures of 55°C at every tap. In TSMT8, *Legionella* spp. was actually found in the warm water supply. Although this study only investigated a small amount of TSMTs, it can still be concluded that the supply water was remarkably often positive for cultivable *Legionella* spp. Consequently, the control of cultivable *Legionella* spp. in premise plumbing systems can be improved when a correct premise plumbing system design is made and a correct operation of the system and boiler is performed.

Can Legionella spp. maintain or multiply themselves in TSMTs?

In a number of investigated TSMTs, *Legionella* spp. were detected by swabbing the biofilm from internal parts of the TSMTs. *Legionell*a spp. are known to multiply inside protozoa that graze on these biofilms.[16] As a result, the analysis of biofilms provides information on the presence of *Legionella* spp. In this study, *Legionella* spp. were detected in four of the ten TSMTs. In these taps, *Legionella* spp. settled and presumably multiplied inside protozoa that graze on the biofilm in the various compartments of the TSMTs. In three other TSMTs, *Legionella* spp. were detected in the water but not in the biofilm on internal parts of the TSMT. Thus, there was no physical evidence of *Legionella* spp. being present in the biofilm of these three TSMTs. It is, therefore, concluded that *Legionella* spp. can be present in TSMTs, but that *Legionella* spp. can also colonize other parts of the premise plumbing systems then the TSMTs.

Sampling and materials

In other *Legionella* spp. investigations, it was possible to sample defined surface area for *Legionella* spp., which made it possible to report *Legionella* spp. numbers per cm squared. Unfortunately, it was not possible in our study to give an exact estimation of the surface area, because not all compartments were easily accessible by swab and surfaces were irregular in shape and size. However, we observed that the surface area of the rubber parts of the TSMTs was relatively small compared to the metal surfaces. Still, the highest cultivable *Legionella* spp. were observed in the biofilm that has developed on the rubber material. Previous research has demonstrated that rubber enhance the growth of bacteria 100 to 200 times more than metal materials.[17] A higher biofilm concentration results probably in higher numbers of protozoa and *Legionella* spp. Niedeveld et al.[18] studied the proliferation of *L. pneumophila* serogroups 1, 9 and 10 in naturally contaminated hot potable water after the addition of various rubbers and their constituents. In the experiment, rubbers produced a 10- to 100,000-fold increase in the number of *L. pneumophila* organisms. Niedeveld et al.[18] concluded that thiuram-containing rubbers should be used in water systems. Consequently, rubber materials in TSMTs might provide a higher risk for growth of *Legionella* spp. There is also the issue of

cross-contamination of the biofilm or contaminated water after demounting and during transportation and in the laboratory, for instance, via water in the remaining gaps between the rubber parts, which may have influenced the *Legionella* spp. counts. Additional research should establish whether a different composition of rubber can reduce biofilm formation and growth of *Legionella* spp. In addition, other studies have demonstrated that PEX pipe materials (cross-linked polyethylene) were found to contain higher median values of biofilm concentration with higher median values of *Legionella* spp. concentrations in water compared to copper and stainless steel pipes in the initial phase of the experiment.[19,20] Therefore, material use in premise plumbing system might influence biofilm formation and growth of *Legionella* spp.

This study did not specifically consider the pH levels of the water. According to Dutch legislation concerning the production and distribution of potable water, the pH levels of the potable water need to range from 7.0 to 9.5. The water delivered to the five locations had a pH varying from 7.8 to 8.0. Wadowsky et al.[21] found that naturally occurring *L. pneumophila* multiplied at a temperature between 25 and 37°C, at pH levels of 5.5 to 9.2. Katz and Hammel[22] showed *L. pneumophila* showed a two-log drop in viable cells after being held for 1 month in tap water varying in pH from 4 to 7. At pH 8, there was a six-log drop in viability for *L. pneumophila*. One of the healthcare facilities in this study, number 5, installed a copper-silver ionization system. Research by Lin et al.[23] suggested that the pH level may be an important factor in the efficacy of copper-silver ionization in controlling *Legionella* spp. in water systems, with higher levels reducing its biocidal capacity. Therefore, pH level of the water should be considered in future studies.

The internal parts of TSMTs 6 and 8 both contained cultivable *Legionella* spp., but *Legionella* spp. were not detected in the water coming from the tap. Apparently, *Legionella* spp. cells do not always release in sufficient amounts from the biofilm in the water so that they will be detected. This result shows that finding *Legionella* spp. negative water samples during regular water sampling (maintenance) does not automatically mean that cultivable *Legionella* spp. are not present in the biofilm of the premise plumbing system and/or TSMTs. In addition, this result can also explain why water samples from some premise plumbing systems are sometimes positive and sometimes negative for cultivable *Legionella* spp. In future studies, background flora should also be tested when there are no detectable numbers of *Legionella* spp. found. The new TSMTs replacing the old ones should be disinfected before installing, as these taps may contain other types of microorganisms. Furthermore, water is often taken from the shower head itself during routine sampling after optional filters are removed. During such sampling actions, the contamination with *Legionella* spp. can be present only inside the shower hose or shower head, because these parts of a shower are often made from materials that enhance biofilm formation and *Legionella* spp. growth. Therefore, one should consider taking water samples from the shower hoses and heads, as well, in order to account for this potential source of contamination.

In four TSMTs, *Legionella* spp. was found inside the tap. These four TSMTs were present at locations 1 and 4. We observed that *Legionella* spp. was present in the premise plumbing systems of these two locations. It is likely that at these two locations, *Legionella* spp. bacteria from the premise plumbing system have also settled in the TSMTs. It cannot be concluded from this study whether *Legionella* spp. can settle in a TSMT if there are no detectable levels of *Legionella* spp. in the supply water, because there were no positive results from the autopsy with negative results from the supplies.

Conclusions

This research shows that *Legionella* spp. can settle inside a TSMT, and that these TSMTs

can play a role in exceeding the threshold limit for cultivable *Legionella* spp. It was observed that both the contaminations found in the supply water and material type used in the taps (for instance, rubber) can play a role in *Legionella* spp. growth. At the same time, cases were observed in which TSMTs did not play a role in the occurrence of *Legionella* spp. in the water, even when the water sample tested positive for *Legionella* spp.; that water samples that tested negative for *Legionella* spp. can be false negatives, as was observed in two cases, and that *Legionella* spp. was found inside the TSMTs but not in the sampled water coming out of the tap. This study does not allow differentiation of the conclusions based on the type of TSMT or the type of building (healthcare versus hotel). Finally, we conclude that replacement of taps, when the water system itself is positive for *Legionella* spp., is pointless.

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Conflict of interest

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