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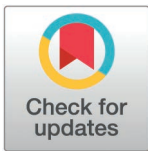Please check the document version above.

RESEARCH ARTICLE

Quantification of *Legionella pneumophila* in building potable water systems: A meta-analysis comparing qPCR and culture-based detection methods

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Abstract

Quantitative polymerase chain reaction (qPCR) offers a rapid, automated, and potentially on-site method for quantifying *L. pneumophila* in building potable water systems, complementing and potentially replacing traditional culture-based techniques. However, its application in assessing human health risks is complicated by a tendency to overestimate risks due to the detection of genomic copies unassociated with viable, infectious bacteria. This study examines the relationship between *L. pneumophila* measurements via qPCR and culture-based methods, aiming to establish qPCR-to-culture concentration ratios needed to inform associated health risks. Eligible studies collected quantitative data on *L. pneumophila* concentrations using molecular and culture-based methods within paired water samples. We developed a Poisson lognormal ratio model and a random-effects meta-analysis model to analyze variations in qPCR-to-culture ratios within and across sites. Of the 17 studies in the systematic review, seven, including 23 site-specific data sets, were used for meta-analysis. Our findings indicate these ratios typically vary from 1:1 to 100:1, with ratios close to 1:1 predicted at all sites. Consequently, adopting a default 1:1 conversion factor appears necessary as a cautious approach to convert qPCR concentrations to culturable concentrations for use in health risk models, such as quantitative microbial risk assessment (QMRA). Where this approach may be too conservative, viability-qPCR could improve the accuracy of qPCR-based QMRA. Standardizing qPCR and culture-based methods and reporting site-specific environmental factors affecting *L. pneumophila* culturability would improve understanding of the relationship between the two methods. The ratio model introduced here advances beyond simple correlation analyses, facilitating investigations of temporal and spatial heterogeneities in the relationship. This analysis is a step forward in the integration of QMRA and molecular biology, and the framework demonstrated for *L. pneumophila* is applicable to other pathogens monitored in the environment.

List of symbols: c, *Legionella pneumophila concentration*; k, Individual study; n, Sample size; Q, Chi-square statistic; $\bar{r}$, Arithmetic mean qPCR-to-culture ratio on the raw scale; V, Sample volume; x, *Legionella pneumophila count*; μ, Mean parameter of the lognormal distribution; $\mu_{Culture}$, Mean parameter of the lognormal distribution of culture concentrations; μ_{PCR}, Mean parameter of the lognormal distribution of qPCR concentrations; μ_{ratio}, Mean parameter of the lognormal distribution of qPCR-to-culture ratios; θ_{ratio}, True mean log qPCR-to-culture ratio; σ, Standard deviation parameter of the lognormal distribution; $\sigma_{Culture}$, Standard deviation parameter of the lognormal distribution of culture concentrations; σ_{PCR}, Standard deviation parameter of the lognormal distribution of qPCR concentrations; σ_{Ratio}, Standard deviation parameter of the lognormal distribution of qPCR-to-culture ratios; $\sigma_{PCR, Culture}$, Covariance between qPCR and culture results on the log scale; $\sigma_{\mu ratio}$, Standard error of μ_{ratio}; $\sigma_{\theta ratio}$, Standard error of θ_{ratio}; τ^2, Between-study variance of θ_{ratio}; $\text{e.s.e}\left(\ln\left(\bar{r}\right)\right)$, Estimator of the standard error (e.s.e.) of $\ln\left(\bar{r}\right)$; $\text{e.s.e}\left(\widehat{\log_{10}\left(\bar{r}\right)}\right)$, Estimator of the standard error (e.s.e.) of $\bar{r}$ on the log10 scale

1 Introduction

Legionella pneumophila is widely regarded as the primary causative agent of Legionnaires' disease [1]. Therefore, accurately quantifying *L. pneumophila* concentrations in water is central to prevention strategies. Culture-based methods, defined by standards like ISO 11731:2017, NF T90-431:2017, and ASTM D8429-21, are commonly used to quantify *L. pneumophila* concentrations in colony-forming units (CFU) or most probable number (MPN). These cultivation-derived data serve as inputs for quantitative microbial risk assessment (QMRA), enabling predictions of probabilities of infection upon exposure [2,3]. However, the widespread application of culture-based methods is hindered by several limitations [4], including their time-consuming nature (8–14 days to obtain results), low processing throughput, the need for specialized cultivation expertise, and concerns about so-called viable but not culturable (VBNC) bacteria. Culture-based methods can also produce false positives due to the growth of non-target organisms on selective media [5].

In some circumstances, molecular techniques like quantitative polymerase chain reaction (qPCR) present a solution to some limitations. With their capacity for high-throughput, rapid, and specific *L. pneumophila* genome copies quantification, as well as benefits such as automation and on-site implementation, qPCR methods offer a compelling tool to use in conjunction with cultivation [6,7]. While standardized qPCR is well established for *L. pneumophila* detection [8,9], cultivation will most likely remain the dominant reference method in the foreseeable future. Hence, a comprehensive understanding of the relationship between cultivation and qPCR data is crucial for advancing the use of qPCR for monitoring and risk assessment.

Studies comparing *L. pneumophila* concentrations obtained through qPCR and culture-based methods often report correlations [10,11]. However, the relationship between qPCR and culture is not expected to be conserved due to various factors, such as environmental conditions affecting the viability and cultivability of the organism (e.g., disinfectant concentration, water temperature, age of pipes/biofilms, water flow conditions) [12–14], and method-specific performances related to analytical recovery and detection/quantification limits [15]. In some instances, qPCR might overestimate human health risks by detecting DNA from intact cells (culturable, VBNC, and dead), as well as extracellular DNA, which can exist as free, particle-bound, or encapsulated forms.

Emerging techniques, which target DNA within intact cells, often referred to as viability-qPCR, may better align concentration estimates with associated health risks [16] but are still not widely adopted in qPCR-based applications. Additionally, bacterial aggregation in environmental samples [17,18] and parasitization by free-living amoebae may result in qPCR yielding higher concentrations than cultivation. Thus, employing a ratio as a model could help identify situations with notable discrepancies between the two quantification methods [15], improving our understanding of their interplay.

This study presents a systematic review and meta-analysis of the qPCR-to-culture concentration ratio for *L. pneumophila* in building potable water systems. Our objectives were to (i) develop statistical models to describe the variability in the qPCR-to-culture relationship within and across various studies to generate a comprehensive understanding of literature data (ii) apply these models in interpreting qPCR results within a QMRA framework, and (iii) evaluate and interpret factors influencing the qPCR-to-culture ratio.

2 Systematic review

The studies included in this work were identified, screened, and selected following the guidelines established in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework [19]. A systematic review protocol was initially developed and amended

during the study (S1 Text). The PRISMA checklist is provided in S1 PRISMA Checklist. The review protocol has not been registered.

2.1 Eligibility criteria

This review focused on the analysis of water samples collected in full-scale building potable water systems. Eligible studies collected quantitative data on the concentrations of *L. pneumophila* in water using both molecular methods (qPCR, viability-qPCR) and culture-based methods (ISO 11731, Legiolert) within paired water samples. Both most probable number (MPN) and CFU were included for the quantification of culturable concentrations because both approaches aim to assess viable *L. pneumophila* populations. This inclusion allows for a more comprehensive analysis across a broader range of studies. Studies using alternative molecular methods, such as digital droplet PCR (ddPCR) and reverse-transcription PCR (RT-qPCR), were excluded. The search was restricted to peer-reviewed journals and governmental reports published in English, French, or German languages.

2.2 Search strategy

Backward and forward searches were conducted by the first and second reviewers (WR and ES), starting with a narrative review paper on methods comparison for *Legionella* enumeration [20] and the National Academies of Science, Engineering, and Medicine (NASEM) report Management of *Legionella* in Water Systems [4]. Based on the information gathered from these sources, an electronic search was conducted in PubMed, Scopus, and Web of Science for studies published between January 2000 and July 2024 using the following search string:

"(Legio* AND qPCR AND culture) OR (Legio* AND viability PCR AND culture) OR (Legio* AND EMA AND culture) OR (Legio* AND PMA AND culture) OR (Legio* AND Methods AND Compare)."

WR conducted title and abstract screenings, with ES verifying excluded articles. WR and ES duplicated the full-text screening, and discrepancies were resolved through discussion. Reasons for excluding studies were recorded. A collaborative spreadsheet software (Google Sheets, Alphabet Inc) was used to retrieve citations, screen citations, and record reasons for study exclusion.

2.3 Data extraction

Water system information, sample processing details, and measurement information, as shown in Table 1, were extracted from the selected studies by WR, ES, and a research intern. Extracted data were verified by a second reviewer (WR or ES) for all studies to ensure accuracy. For 10 studies where concentrations or counts were not provided or reported in tables, WebPlotDigitizer, a data extraction program with nearly perfect accuracy [21], was used to manually extract approximated concentrations from relevant figures by magnifying them at 500%. All data extracted from studies were stored in the collaborative spreadsheet software, facilitating collaborative document editing and ensuring consistency.

2.4 Quality assessment and risk of bias

Only studies including data sets with at least 20% quantifiable samples were selected to ensure reliable statistical assessments. Studies reporting building-specific data and pooled data from multiple buildings were included in the review, but only building-specific data were used for meta-analysis. A risk of bias tool was developed to systematically evaluate the quality control

Table 1. Reporting elements necessary for evaluating the relationship between concentrations of Legionella pneumophila measured by qPCR and culture-based methods in building potable water systems.

Category	Parameter
Water system information	Building type Water system (e.g., hot/cold) Outlet Draw (e.g., first draw, flushed)
Water treatment	Preventive control measure(s) Curative control measure(s)
Water quality parameter	Total chlorine Free chlorine Monochloramine Water temperature
Sample processing information	Sampling location and date Sample type Sample volume for qPCR Sample volume for cultivation Sample dilution/concentration factor for qPCR Sample dilution/concentration factor for cultivation Volume processed for qPCR Volume processed for cultivation
Measurement information[a]	qPCR method Gene target for qPCR Number of gene copies within the genome Specificity of the qPCR assay Culture-based method (e.g., CFU, MPN) Count of *Legionella pneumophila* per processed volume for qPCR[b] Count of *Legionella pneumophila* per processed volume for cultivation[b] Limits of detection (LOD) for qPCR Limits of detection (LOD) for cultivation Limit of quantification (LOQ) for qPCR Limit of quantification (LOQ) for cultivation Recovery rates for qPCR (whole process, nucleic acid extraction, PCR detection) Recovery rates for cultivation

[a]This list does not include all quality control and assurance elements necessary for qPCR and cultivation analyses of environmental samples.

[b]When counts were unavailable, reported concentrations were extracted.

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measures reported for qPCR and culture methods in the included studies. This tool focused on assessing key aspects of quality control and assurance of qPCR analyses, including whether studies reported methods for generating standard curves for qPCR, control measures for nucleic acid extraction, PCR detection, and inhibition. For cultivation methods, we verified adherence to standardized protocols.

3 Statistical analyses

3.1 Variability of the qPCR-to-culture ratio within a building

To estimate how much the ratio between the results of the two methods (qPCR and cultivation) varies within each building, we adapted the statistical method previously developed by Sylvestre, Dorner [22]. This method involves comparing two sets of results that follow a specific probability distribution, known as a Poisson–lognormal distribution. This model assumes that counts x (CFU for culture and genome copies for qPCR) are randomly (Poisson) distributed in each water sample of volume V and concentration c. The probability of finding x organisms in the water sample is thus:

$$P(x; c, V) = \frac{(cV)^x e^{-cV}}{x!} \tag{1}$$

Organisms have a concentration c, and their expected number in a sample volume V is cV. The concentration c is likely to vary in time or space. For MPN methods, Eq. 1 was used as an approximation method because primary studies did not report counts of positive and negative wells. When such data are reported, the MPN method uncertainty can be modelled more accurately using the binomial and Poisson distributions [23].

This variation in concentration c can be described by a lognormal distribution, which has the following probability density function:

$$f(c; \mu, \sigma) = \frac{1}{c\sigma\sqrt{2\pi}} \exp\left(-\frac{(\ln(c) - \mu)^2}{2\sigma^2}\right) \tag{2}$$

where μ and σ are the mean and standard deviation of the underlying normal distribution on the logarithmic scale. If Eq. 2 describes the variation of c in Eq. 1, then the count, x, follows a Poisson–lognormal distribution. The goodness-of-fit of the Poisson–lognormal distribution was compared to the one of an alternative model, the Poisson–gamma distribution, using the marginal deviance information criterion (mDIC) [24]. Results indicated that the lognormal distribution better described concentration variations for qPCR data (S1 Table) and cultivation data (S2 Table); therefore, the Poisson–lognormal distribution was chosen for modelling *L. pneumophila* concentrations and their ratios. As the *mip* gene, which is used to quantify *L. pneumophila* via qPCR, exists in a single copy within the genome of *L. pneumophila* [25], methods can be compared by directly taking the ratios of CFUs to gene copies. CFU and genome copies were estimated from reported concentrations, processed sample volumes, limits of quantification (LOQ), and limits of detection (LOD). Due to insufficient reporting, the impact of analytical recovery rates on estimated concentrations was ignored.

The distribution of the ratio of two lognormal random variables is also lognormally distributed with a mean of:

$$\mu_{ratio} = \mu_{PCR} - \mu_{Culture} \tag{3}$$

and a variance of:

$$\sigma^2_{ratio} = \sigma^2_{PCR} + \sigma^2_{Culture} - 2\sigma_{PCR,Culture} \tag{4}$$

Here, $\sigma_{PCR,Culture}$ is the covariance between qPCR and cultivation results on the log scale. The empirical covariance was estimated using the logarithm of the concentrations measured by qPCR and culture methods. By considering the covariance between the lognormal variables, Eq. 4 accounts for the dependency between the qPCR and culture results and adjusts the variance of the ratio accordingly.

Models were implemented using the Markov chain Monte Carlo (MCMC) method in a Bayesian framework. A uniform prior ranging from -10^2 to 10^2 and an exponential prior with a rate of 1.0 were specified for the location parameter μ and the shape parameter σ, respectively, for each lognormal distribution (qPCR and culture). Cumulative distribution functions (CDFs) were used to illustrate distributions of *L. pneumophila* concentrations and qPCR-to-culture ratios, indicating the expected frequency of observing a ratio below a particular level. The best-fit curve was computed from the median values of the posterior distribution of each parameter. The uncertainty of the fit was represented with a 95%

uncertainty interval obtained from the 2.5% and 97.5% percentiles of the posterior distribution of the parameters. The analysis was conducted in R (version 4.3.0). The database and R code used for data analysis and visualization can be found in the GitHub repository: http://bit.ly/4fMkqGM.

3.2 Meta-analysis models

Statistical meta-analysis models were used to compare mean qPCR-to-culture ratios across multiple studies and obtain an overall distribution of the mean ratios. The geometric mean and arithmetic mean ratios were chosen as summary estimates, as they provide complementary information for interpreting the data. The mean $\log_{10}$ ratio, equivalent to the geometric mean ratio on the arithmetic scale, represents the median when the ratio is a lognormal random variable. Given the high skewness of qPCR-to-culture ratio distributions, the arithmetic mean, which is more sensitive to high ratios, was also computed for the meta-analysis. This summary descriptor complements the geometric mean, which can suppress the impact of high ratios. Results were illustrated using the *metafor* package version 3.0-2 [26] in R (version 4.3.0).

3.2.1 Geometric mean ratio. Here, the meta-analysis is conducted for the mean log ratio on the natural log scale $\mu_{ratio, 1}$, $\mu_{ratio, 2}$, $\ldots$ $\mu_{ratio, k}$ and known standard errors of these means $\sigma_{\mu ratio, 1}$, $\sigma_{\mu ratio, 2}$, $\ldots$ $\sigma_{\mu ratio, k}$. The location parameter of the lognormal distribution of the ratio is represented by μ_{ratio}, and the standard error is given by:

$$\sigma_{\mu_{ratio, k}} = \frac{\sigma_{ratio, k}}{\sqrt{n}} \tag{5}$$

where $\sigma_{ratio, k}$ is the scale parameter of the lognormal distribution of the ratio, and n is the sample size.

3.2.2 Arithmetic mean ratio. In this case, the meta-analysis is conducted on the ratio $\bar{r}$, which can be obtained from lognormal parameters as follows:

$$\bar{r} = e^{\mu_{ratio} + \frac{\sigma_{ratio}^2}{2}} \tag{6}$$

An estimator of the standard error (e.s.e.) of $\ln(\bar{r})$ can be derived following the approach presented by Olsson [27]. That is:

$$\text{e.s.e}\left(\widehat{\ln(\bar{r})}\right) = \sqrt{\frac{\sigma_{ratio}^2}{n} + \frac{\sigma_{ratio}^4}{2(n-1)}} \tag{7}$$

However, it is more convenient to express the e.s.e. of $\bar{r}$ on the $\log_{10}$-scale, as $\bar{r}$ is commonly presented in this format. Since $y = -\log_{10}(x)$ is a continuous monotonic decreasing function, it follows that the e.s.e for $\log_{10}(\bar{r})$ is:

$$\text{e.s.e}\left(\widehat{\log_{10}(\bar{r})}\right) = \log_{10}\left(e^{\text{e.s.e.}\left(\widehat{\ln(\bar{r})}\right)}\right) \tag{8}$$

The proof of Eq. 8 is provided in S1 Proof.

3.2.3 Random-effects. A random-effects model assumes that the studies included in the meta-analysis are randomly drawn from a broader pool of potential studies. These studies are assumed to represent a population described by a single underlying distribution. This approach allowed us to account for both within-study and between-study variability. In the

first stage of the model, the $\log_{10}$-transformed mean ratio (either geometric or arithmetic mean) from each study is given a weight based on its variance. These weights reflect the confidence in each set of observations of a study; the lower the variance, the higher the weight. Given the central limit theorem, the uncertainty around the estimated log mean ratio of each study is assumed to be normally distributed. For the geometric mean ratio of a study k, that is:

$$\mu_{ratio,k} \sim N\left(\theta_{ratio,k}, \sigma^2_{\mu_{ratio,k}}\right) \tag{9}$$

where $\mu_{ratio,k}$ is the inferred parameter of the parametric model of the ratio distribution (Eq. 3), $\theta_{ratio,k}$ is the true mean log ratio, and $\sigma^2_{\mu_{ratio,k}}$ is the within-study variance (Eq. 5), which represents the sampling uncertainty within each study.

The second stage of the model introduces a random effect to account for differences in $\log_{10}$-transformed mean ratios between studies. We modelled this random effect with an exponential distribution or a lognormal distribution. For an exponential distribution with parameter λ, the second stage is:

$$\theta_{ratio,k} \sim Exp(\lambda) \tag{10}$$

Here, λ represents the between-study variance, capturing the variability in the true mean ratios ($\theta_{ratio,k}$) across studies. The parameter of this distribution was inferred with MCMC methods. We specified an uninformative prior for the parameter, as proposed by Higgins, Thompson [28]. The exponential distributions were illustrated with CDFs.

3.2.4 Subgroup analyses. Subgroup analyses were conducted to assess potential sources of variability in the mean ratios across various subgroups. The pooled estimates, representing the aggregated outcomes from each subgroup, were obtained using a Bayesian random-effects model. In this model, the within-study mean and the random effect were assumed to be normally distributed. Given the substantial uncertainty associated with subgroup pooled estimates due to small sample sizes, we focused on exploring the overall patterns and trends across the subgroups rather than conducting formal significance tests between them.

4 Results

4.1 Data collection and reporting

Of the 1256 records screened, 192 full-text articles were assessed for eligibility, and 17 were selected. Seven of these studies reported site-specific data [10,13,15,29–31,32] (Fig 1), and then ten studies reported the data from the studied locations as pooled, limiting our capacity to assess the influence of distinct water system characteristics and water quality parameters on *L. pneumophila* qPCR-to-culture ratios. Two studies [14,33], five data sets from Lee, Lai [15], and three data sets from Sauget, Richard [29] were excluded for having less than 20% quantifiable samples. All but two studies were conducted in European countries. One selected study, Lee, Lai [15], reported site-specific data from multiple potable water systems in different countries. The study of Sauget, Richard [29] reported room-specific data from three different buildings located on a hospital campus. For statistical analyses, the data were grouped by building floor because combining data from multiple floors resulted in multimodal distributions, which hindered simple parametric modeling.

Several chemical disinfection strategies were used across studies, with the predominant approach being chlorine disinfection (Table 2). The water samples tested exhibited a range in average temperature of 22–59 °C. Sampling strategies vary significantly across studies. The number of sampling locations per building ranged from as few as one to as many as 36, with
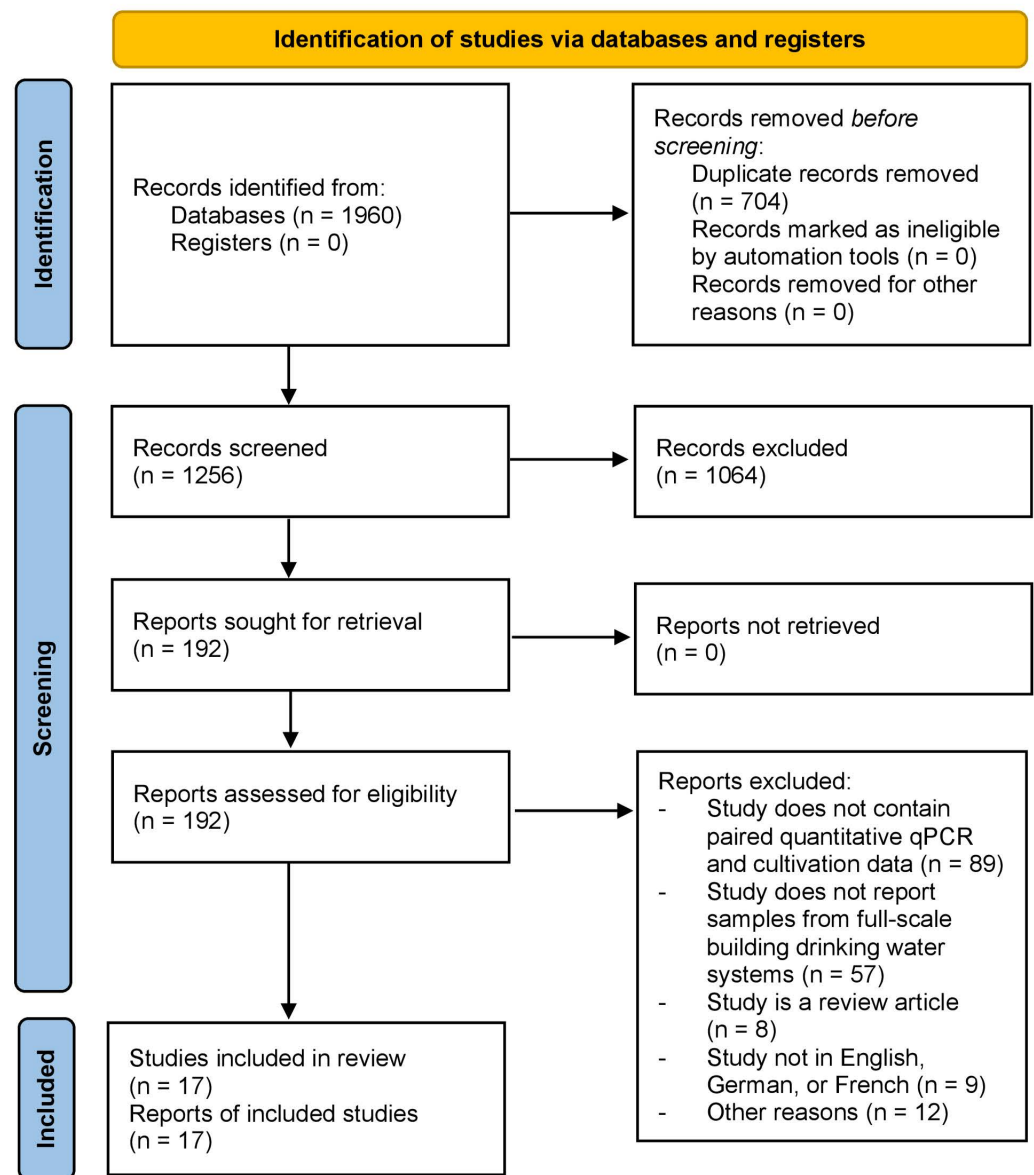
Fig 1. PRISMA flow diagram for the systematic review.

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most studies monitoring less than five sampling points. Approximately ten samples were typically collected at each sampling location, and most studies collected flushed samples (i.e., water has been intentionally run through the outlet being sampled before sampling). The volume analyzed for the quantification of *L. pneumophila* varied considerably, ranging from 27 mL to 1 L, for both culture-based and qPCR methods. The reported lower limit of detection ranged from 1 to 250 CFU L^{-1} or MPN L^{-1} for culture-based methods and 80–2000 genome copies L^{-1} for qPCR methods. Typically, the proportion of samples above the detection limit exceeded 70% when using qPCR. This proportion averaged 60% with culture-based methods, though this was as low as 20% for some sites.

Table 2. Characteristics of building potable water systems, sampling strategy, and Legionella pneumophila enumeration methods for evaluating site-specific qPCR-to-culture ratio.

Reference, site	$\bar{t}$ (°C)	Treatment	Sampling strategy			Volume analyzed		Detection limit	
			Sampling point	Sample per point	Method	Culture (L)	qPCR (L)	Culture (# L⁻¹)	qPCR (GC L⁻¹)
Bonetta et al. (2018)	22.1	None	14	5	Flushed	1.000	1.000	1ᵃ	80
Bonetta et al. (2018)	38.0	NEOW	14	5	Flushed	1.000	1.000	1ᵃ	80
Grimard-C. et al. (2022)	31.1	Chlorine	21	6	First-draw	0.100	0.700	10	10
Grimard-C. & Prévost (2023)	–	Remedial flush	6	1–3	First-draw	0.100	0.700	10	10
Grimard-C. & Prévost (2023)	–	Chlorine	6	1–3	First-draw	0.100	0.700	10	10
Joly et al. (2006), Grenoble	–	N/S	N/S	N/S	N/S	0.800	0.200	50	30
Lee et al. (2011), France DW1	42.7	None	5	3–10	Flushed	0.027	0.027	190	190
Lee et al. (2011), France DW2	47.9	None	4	3–10	Flushed	0.027	0.027	190	190
Lee et al. (2011), HPA SH	50.9	Chlorine diox.	4	4–13	Flushed	0.027	0.027	190	190
Lee et al. (2011), Italy Scre	38.7	N/S	1	11	Flushed	0.027	0.027	190	190
Lee et al. (2011), Italy Ed 1	34.7	N/S	2	15	Flushed	0.027	0.027	190	190
Lee et al. (2011), Italy Pad 5	37.9	N/S	3	11–12	Flushed	0.027	0.027	190	190
Lee et al. (2011), Spain DW1	50.2	Copper/silver	5	8–9	Flushed	0.027	0.027	190	190
Lee et al. (2011), Spain DW2	44.8	Chlorine	5	9	Flushed	0.027	0.027	190	190
Lee et al. (2011), Spain DW3	51.3	Chlorine	5	9	Flushed	0.027	0.027	190	190
Lee et al. (2011), Switz. 100	57.5	None	3	9–10	Flushed	0.027	0.027	190	190
Lee et al. (2011), Switz. 110	57.3	None	3	9–10	Flushed	0.027	0.027	190	190
Lee et al. (2011), Switz. 120	53.9	None	3	9–10	Flushed	0.027	0.027	190	190
Morio et al. (2008) [32]	50.1	Chlorine	N/S	N/S	N/S	0.020	0.200	250	100
Sauget et al. (2023), Build. 1, Floor 2	54.1	N/S	14	1–2	Flushed	0.100	1.000	10	80
Sauget et al. (2023), Build. 1, Floor 3	52.0	N/S	14	1–2	Flushed	0.100	1.000	10	80
Sauget et al. (2023), Build. 2, Floor 2	59.0	N/S	36	1–3	Flushed	0.100	1.000	10	80
Sauget et al. (2023), Building 3	43.2	N/S	12	1	Flushed	0.100	1.000	10	80

ᵃThis detection limit is self-reported in the study. It may have been developed assuming 100% recovery from filtration, but recoveries were not reported.

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4.2 Quality assessment and risk of bias

The quality assessment of the selected studies highlighted areas of uncertainty, potentially introducing bias into the reported qPCR concentrations. Of the 17 studies, one reported recovery rates for the whole process, five reported recovery rates for nucleic acid extraction, three reported doing positive/negative controls for nucleic acid extraction, 10 reported doing positive/negative controls for PCR detection, 11 described methods for generating standard curves, and 14 reported using inhibition controls (S4 Table). For cultivation analyses, all studies reported adhering to standardized protocols (S5 Table).

4.3 Temporal variations in qPCR-to-culture ratios

We observed substantial variability in qPCR-to-culture ratios, with most sites demonstrating ratios spanning an approximate 3.0-log (Fig 2). The lower tail of the lognormal distribution of the ratio began to rise within a qPCR-to-culture ratio of 0.1–1.0 for all data sets except the remedial flushing and shock chlorination data reported by Grimard-Conea and Prévost [30], where minimum predicted ratios were about 10. In practice, ratios below 1.0 are possible because bacterial aggregates, VBNC cells, incorporation into amoebas, or false positives in culture-based methods. However, the model tends to predict a higher probability of such low
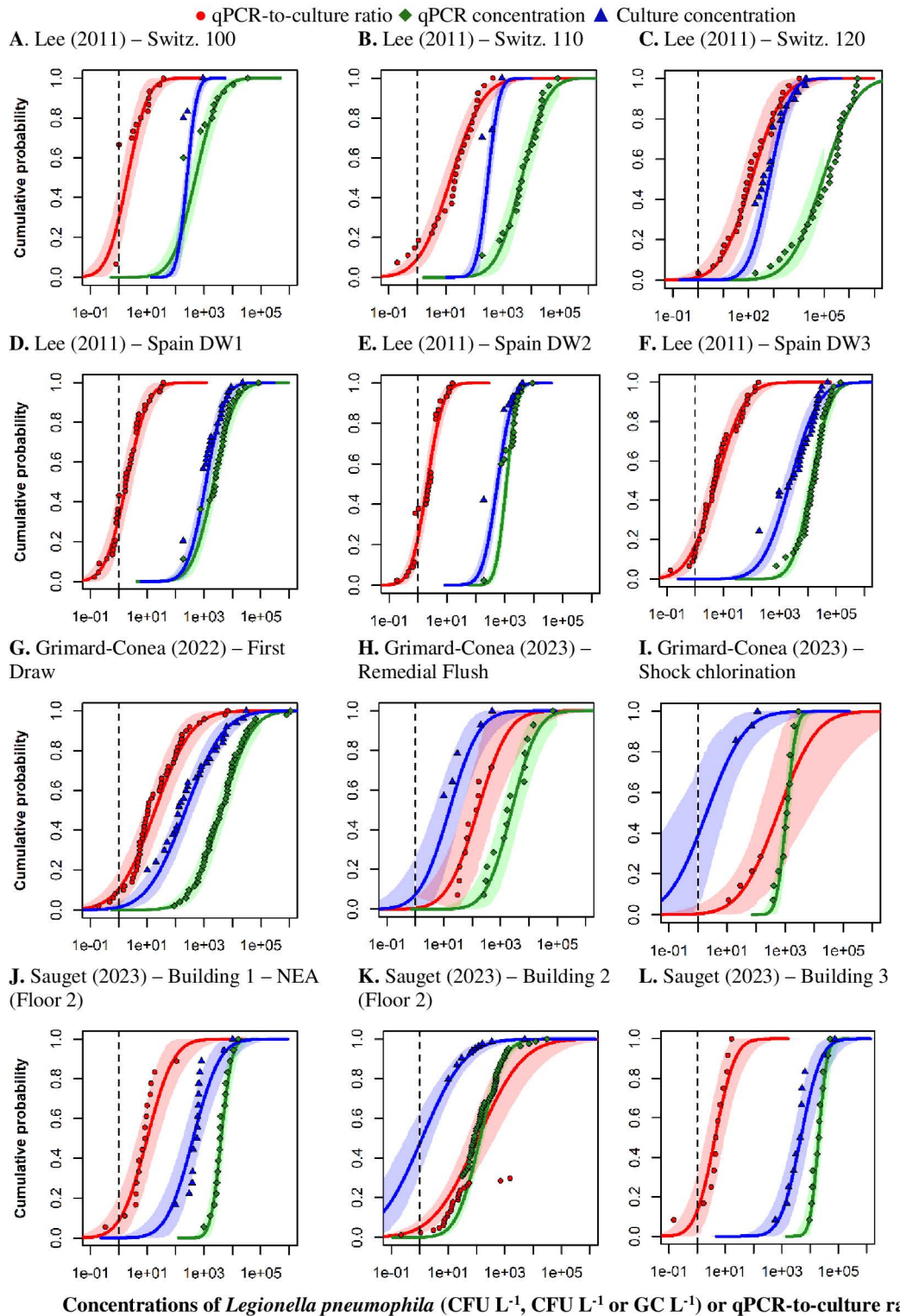
Fig 2. Cumulative distribution function of the lognormal distribution (with 95% uncertainty interval) for building-specific qPCR and culture concentrations of *Legionella pneumophila* and their ratio in water samples from selected building potable water systems. Only 12 of the 19 sites are shown to illustrate the main trends.

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ratios when data sets contain multiple cultivation and qPCR results that are below the detection limits.

The upper tail of the distribution varied among studies. Most sites exhibited maximum ratios between 1.0 and 2.0-log, while a few presented maximum ratios exceeding 3.0-log. In many studies, a small number of samples with high qPCR-to-culture ratios considerably influenced the arithmetic mean ratio (Table 3). This analysis demonstrates that the distribution of the concentration—either derived from qPCR or culture-based measurements—can dominate the dispersion of the ratio distribution (Fig 2). When culture-based measurements returned a high number of non-detects, the qPCR concentration distribution predominantly influenced the distribution of the ratio (Fig 2A, 2B, 2G). In other cases, the culture-based concentration distribution dictated the distribution of the ratio (Fig 2F, 2L). As shown in Table 3, the correlation coefficient also affects the dispersion of the ratio distribution. This finding highlights the need to account for the interdependence between the two methods to characterize the ratio distribution accurately.
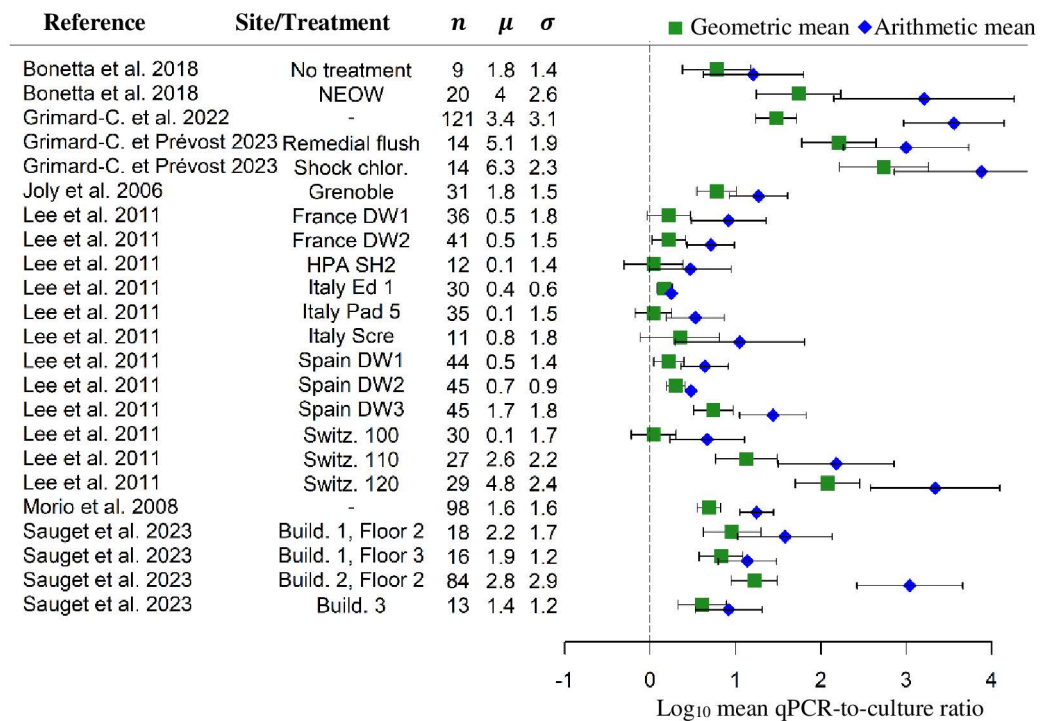
4.4 Between-study variability in mean qPCR-to-culture ratios

For reviewed studies reporting site-specific data, the geometric mean qPCR-to-culture ratios display considerable variability, ranging from 0.0 to 2.1-$\log_{10}$ (Fig 3). The exponential

Table 3. Statistical analysis of site-specific qPCR-to-culture ratios, qPCR concentrations, and culture concentrations for *Legionella pneumophila* in water samples collected in building potable water systems.

Reference, site	n			Lognormal parameters							Pearson corr. (ρ)	qPCR-to-culture ratio	
	Total	% of +ve qPCR	% of +ve culture	qPCR		Culture		Ratio				Geometric mean	Arithmetic mean
				$\widehat{\mu}$	$\widehat{\sigma}$	$\widehat{\mu}$	$\widehat{\sigma}$	$\widehat{\mu}$	$\widehat{\sigma}$	$\widehat{\sigma}_\rho$			
Bonetta et al. (2018) Pre-inter.	9	100	100	10.3	1.3	8.5	1.2	1.8	1.9	1.4	0.47	6.0E + 00	1.7E + 01
Bonetta et al. (2018) Post-inter.	20	60	60	4.9	0.8	0.8	2.5	4.0	2.6	2.6	0.01	5.9E + 01	2.1E + 03
Grimard-C. et al. (2022) First draw	62	100	69	7.7	2.2	4.2	3.4	3.4	4.1	3.1	0.45	3.0E + 01	3.7E + 03
Grimard-C. & Prévost (2023) Flush	14	100	50	7.8	1.6	2.7	1.9	5.1	2.5	1.9	0.42	1.6E + 02	1.0E + 03
Grimard-C. & Prévost (2023) Shock	14	100	20	7.0	0.6	0.6	2.3	6.3	2.6	2.3	0.15	5.5E + 02	7.7E + 03
Joly et al. (2006), Grenoble	31	100	87	8.4	1.3	6.6	1.9	1.8	1.9	1.5	0.69	6.0E + 00	1.9E + 01
Lee et al. (2011), France DW1	36	67	47	6.5	1.9	6.0	0.7	0.5	2.0	1.8	0.30	1.0E + 00	8.0E + 00
Lee et al. (2011), France DW2	33	91	79	7.0	1.8	6.4	1.1	0.5	2.1	1.5	0.53	1.0E + 00	5.0E + 00
Lee et al. (2011), HPA SH2	12	83	50	6.5	1.0	6.4	1.5	0.1	1.8	1.4	0.53	1.0E + 00	2.0E + 00
Lee et al. (2011), Italy Scre	11	100	64	7.7	0.9	6.9	1.7	0.8	1.8	1.8	0.24	2.0E + 00	1.2E + 01
Lee et al. (2011), Italy Ed 1	30	100	67	6.7	0.4	6.3	0.7	0.4	0.8	0.6	0.35	1.0E + 00	1.0E + 00
Lee et al. (2011), Italy Pad 5	35	89	69	7.3	1.8	7.2	1.6	0.1	2.4	1.5	0.57	1.0E + 00	3.0E + 00
Lee et al. (2011), Spain DW1	44	89	80	7.6	1.5	7.0	1.2	0.5	1.9	1.4	0.45	1.0E + 00	4.0E + 00
Lee et al. (2011), Spain DW2	45	100	58	7.0	0.6	6.2	0.9	0.7	1.1	0.9	0.27	2.0E + 00	3.0E + 00
Lee et al. (2011), Spain DW3	45	100	76	9.5	1.2	7.7	1.8	1.7	2.2	1.8	0.33	5.0E + 00	2.8E + 01
Lee et al. (2011), Switz. 100	30	43	20	6.1	1.4	5.5	0.6	0.6	1.3	1.3	0.49	1.0E + 00	4.0E + 00
Lee et al. (2011), Switz. 110	27	93	30	8.3	1.7	5.6	0.7	2.6	1.8	2.1	− 0.47	1.3E + 01	1.5E + 02
Lee et al. (2011), Switz. 120	29	97	38	11.3	2.5	6.5	1.5	4.8	3.9	2.2	0.42	1.2E + 02	2.2E + 03
Morio et al. (2008)	98	55	31	6.1	1.5	4.5	1.5	1.6	2.1	1.6	0.36	5.0E + 00	1.8E + 01
Sauget et al. (2023), Build. 1, Floor 2	18	100	88	8.2	0.7	6.0	1.7	2.2	1.8	1.7	0.24	9.0E + 00	3.8E + 01
Sauget et al. (2023), Build. 1, Floor 3	16	100	100	8.6	1.1	6.6	1.7	1.9	2.2	1.2		0.80	6.7E + 00
Sauget et al. (2023), Build. 2, Floor 2	84	71	29	4.8	1.5	0.1	3.2	2.8	4.7	2.9		0.21	1.6E + 01
Sauget et al. (2023), Building 3	13	100	100	9.9	0.5	0.4	1.3	1.4	1.4	1.2		0.27	4.0E + 00

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Reference	Site/Treatment	n	μ	σ	
Bonetta et al. 2018	No treatment	9	1.8	1.4	
Bonetta et al. 2018	NEOW	20	4	2.6	
Grimard-C. et al. 2022	-	121	3.4	3.1	
Grimard-C. et Prévost 2023	Remedial flush	14	5.1	1.9	
Grimard-C. et Prévost 2023	Shock chlor.	14	6.3	2.3	
Joly et al. 2006	Grenoble	31	1.8	1.5	
Lee et al. 2011	France DW1	36	0.5	1.8	
Lee et al. 2011	France DW2	41	0.5	1.5	
Lee et al. 2011	HPA SH2	12	0.1	1.4	
Lee et al. 2011	Italy Ed 1	30	0.4	0.6	
Lee et al. 2011	Italy Pad 5	35	0.1	1.5	
Lee et al. 2011	Italy Scre	11	0.8	1.8	
Lee et al. 2011	Spain DW1	44	0.5	1.4	
Lee et al. 2011	Spain DW2	45	0.7	0.9	
Lee et al. 2011	Spain DW3	45	1.7	1.8	
Lee et al. 2011	Switz. 100	30	0.1	1.7	
Lee et al. 2011	Switz. 110	27	2.6	2.2	
Lee et al. 2011	Switz. 120	29	4.8	2.4	
Morio et al. 2008	-	98	1.6	1.6	
Sauget et al. 2023	Build. 1, Floor 2	18	2.2	1.7	
Sauget et al. 2023	Build. 1, Floor 3	16	1.9	1.2	
Sauget et al. 2023	Build. 2, Floor 2	84	2.8	2.9	
Sauget et al. 2023	Build. 3	13	1.4	1.2	

Fig 3. Forest plots of site-specific qPCR-to-culture ratios for *Legionella pneumophila* in building potable water systems for arithmetic mean ratios and geometric mean ratios. μ and σ are the parameters of the lognormal distribution of the ratio, and n is the sample size. Horizontal lines represent 95% confidence intervals on mean values. Arrows indicate that confidence intervals exceed a log-ratio of 4.0.

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distribution of the random effect illustrates how the geometric or arithmetic mean ratios varies across studies (Fig 4). It predicts geometric mean qPCR-to-culture ratios below 10:1 (1.0-$\log_{10}$) for approximately 80% of the sites. This suggested that qPCR and cultivation results were similar in most locations. For the remaining 20% of the sites, the geometric mean ratio was predicted to fall between 1.0 and 2.0-$\log_{10}$. The distribution of arithmetic mean ratios were more skewed, with 50% of the sites presenting mean ratios above 1.0-$\log_{10}$ and considerable uncertainty, indicating that high ratios were expected at these sites.

4.5 Impact of viability-qPCR on qPCR-to-culture ratios

For the three studies employing viability-qPCR, arithmetic and geometric mean qPCR-to-culture ratios display lower values than standard qPCR (Fig 5, S1 Fig, S3 Table). Across all studies, viability-qPCR reduced geometric mean ratios by 0.2-log and arithmetic mean ratios by 0.5-log. For the studies conducted by Bonetta, Pignata [31] and Yáñez, Nocker [34], arithmetic mean ratios were more uncertain for standard qPCR than viability-qPCR, suggesting that viability-qPCR can reduce the variability in ratios.

4.6 Impact of water temperature on qPCR-to-culture ratios

The analysis of the impact of temperature on qPCR-to-culture data was limited to one study [15], as this was the only study that consistently reported sample-specific temperature data. The influence of hot water temperature on qPCR-to-culture ratios was examined using data
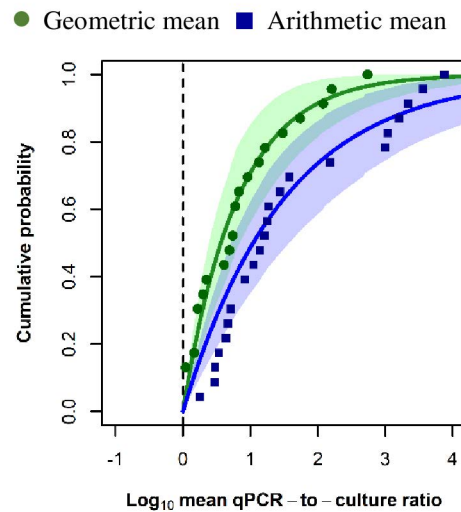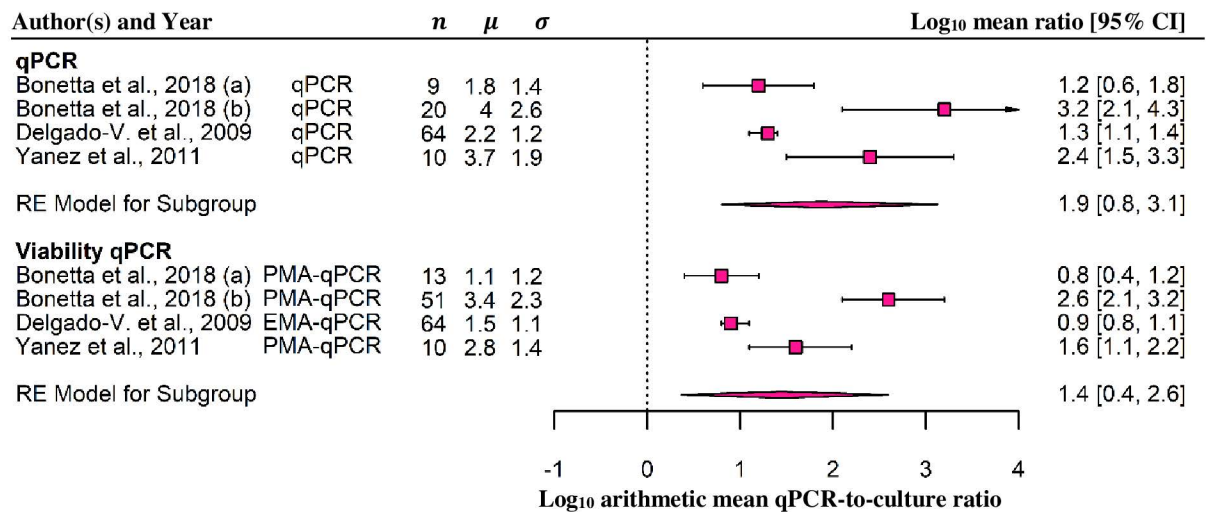
Fig 4. Cumulative distribution function of the random-effects distribution (with 95% uncertainty interval) predicted using an exponential random-effects model for geometric mean qPCR-to-culture ratios (green) and arithmetic mean qPCR-to-culture ratios (blue) for *Legionella pneumophila* in building potable water systems.

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Author(s) and Year		n	μ	σ	Log$_{10}$ mean ratio [95% CI]
qPCR					
Bonetta et al., 2018 (a)	qPCR	9	1.8	1.4	1.2 [0.6, 1.8]
Bonetta et al., 2018 (b)	qPCR	20	4	2.6	3.2 [2.1, 4.3]
Delgado-V. et al., 2009	qPCR	64	2.2	1.2	1.3 [1.1, 1.4]
Yanez et al., 2011	qPCR	10	3.7	1.9	2.4 [1.5, 3.3]
RE Model for Subgroup					1.9 [0.8, 3.1]
Viability qPCR					
Bonetta et al., 2018 (a)	PMA-qPCR	13	1.1	1.2	0.8 [0.4, 1.2]
Bonetta et al., 2018 (b)	PMA-qPCR	51	3.4	2.3	2.6 [2.1, 3.2]
Delgado-V. et al., 2009	EMA-qPCR	64	1.5	1.1	0.9 [0.8, 1.1]
Yanez et al., 2011	PMA-qPCR	10	2.8	1.4	1.6 [1.1, 2.2]
RE Model for Subgroup					1.4 [0.4, 2.6]

Log$_{10}$ arithmetic mean qPCR-to-culture ratio

(a) No water treatment, (b) On-site water treatment with a neutral electrolyzed oxidizing water (NEOW) device

Fig 5. Forest plots of qPCR-to-culture ratio and viability-qPCR-to-culture ratio for *Legionella pneumophila* in water samples from building potable water systems. Subgroup analyses were carried out for arithmetic mean ratios. μ and σ are the parameters of the lognormal distribution of the ratio, and n is the sample size. Horizontal lines represent 95% confidence intervals on mean values. Pooled estimates were obtained using a random-effect model.

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sets from sites Switzerland 100, 110, and 120. During the monitoring period, no chemical residuals, on-site chemical treatment, or shock disinfection were applied at these three sites (V. Gaia, personal communication, April 2023). qPCR-to-culture ratios increased appreciably across the three sites as the temperature became more variable with intermittently lower temperatures, as shown in the CDFs (Fig 6). At site 100, the water temperature from $30 - 60$ sec

A. qPCR-to-culture ratio   **B.** Water temperature

● Switz. 100 ◆ Switz. 110 ▲ Switz. 120
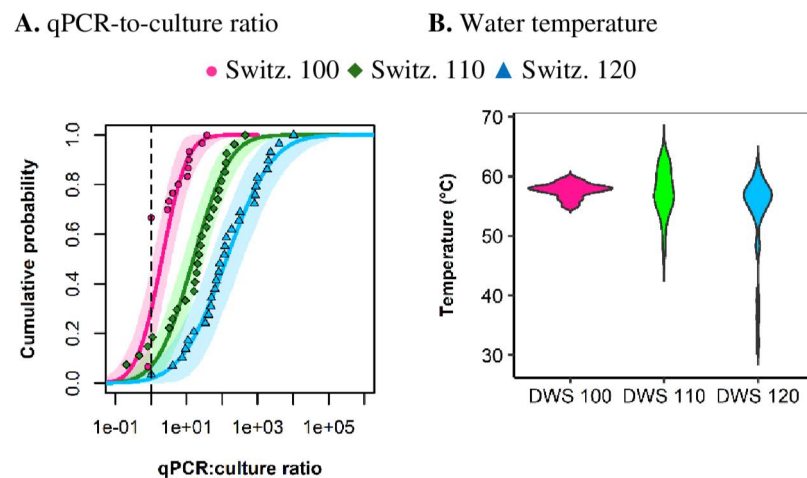
Fig 6. A. Cumulative distribution function of the lognormal distribution (with 95% uncertainty interval) for qPCR-to-culture ratios of *Legionella pneumophila* in flushed water samples from non-chlorinated building potable water systems. Samples were collected weekly for ten weeks at three different sampling locations for each water system. B. Violin plots showing sample-specific temperature measurements.

flush samples consistently stayed above 55°C. Conversely, the other sites recorded lower temperatures, with values as low as 47°C at site 110 and 32°C at site 120.

5 Discussion

This review considered the complex interplay between qPCR and culture-based methods by modelling published data sets reporting *L. pneumophila* concentrations measured in building potable water systems. We found that, predominantly, qPCR yields higher measurements than culture-based methods, a difference influenced by several factors. Higher measurements with qPCR compared to cultivation likely arise from the detection of unculturable cells and extracellular DNA [12]. The detection of all genetic material could overestimate health risks, placing an unnecessary burden on building managers. Conversely, conventional culture-based methods may underestimate risks due to cultivation bias, or by not adequately quantifying bacterial aggregates or VBNC cells, even though thermally induced VBNC *L. pneumophila* can have lower virulence compared to culturable *L. pneumophila* [35]. This divergence between qPCR and cultivation results may further be influenced by analytical strategies and environmental variables [15], including water temperature, disinfectant levels, and water stagnation, which can be affected by operational parameters like boiler settings as well as flushing and sampling strategies. Given this complexity, caution is necessary when converting qPCR data for use in QMRA.

5.1 Ecology of *Legionella pneumophila* in building potable water systems

The temporal analysis of qPCR-to-culture ratios within building potable water systems shows that while some water samples have comparable concentrations in both qPCR and cultivation, others exhibit much higher concentrations when measured by qPCR compared to cultivation. Typically, the temporal distribution is right-skewed with a long tail towards high ratios, suggesting that a small proportion of samples is characterized by high concentrations of *L. pneumophila* nucleic acids not associated with culturable bacteria. The water temperature is one

potential explanation for variability in qPCR-to-culture ratios, as high water temperatures may reduce culturability while minimally influencing qPCR detection. Sample-specific temperature data from Lee, Lai [15] enabled us to investigate how temperature impacts variations in qPCR-to-culture ratios in non-chlorinated hot water systems from three hospitals in Switzerland. At site Switzerland 110, qPCR concentrations displayed high variations of approximately 3.0-$\log_{10}$, while culture concentrations were predominantly at the LOD. These results suggest that *L. pneumophila* enter the hot-water heater through recirculation lines or potentially grow within the water heater but are then inactivated by the thermal barrier in the water heater, hence a high qPCR-to-culture ratio. In contrast, site Switzerland 120 demonstrated high variability in qPCR concentrations, but multiple culture-positive results were recorded. This observation points toward potential *L. pneumophila* growth in the hot-water distribution system or a compromised disinfection capacity of the hot-water heater. This hypothesis is supported by water sample temperatures measured at this site, which sporadically fell within the ideal temperature for *L. pneumophila* growth (25–43°C) [4]. Notably, a full-scale study by Bédard, Boppe [36] demonstrated that implementing corrective measures to maintain a water temperature of 55 °C throughout the hot-water system of a large building resulted in a gradual reduction in median concentrations of *L. pneumophila*, as measured by both qPCR and cultivation.

Remedial control measures, such as thermal and chemical shock treatments, can impact qPCR-to-culture ratios. In the full-scale study conducted by Grimard-Conea and Prévost [30], shock chlorination and remedial flushing strategies resulted in culturable *L. pneumophila* concentrations below the LOD, while qPCR still indicated concentrations of approximately 10^3 genome copies per liter. Additionally, under bench-scale conditions, Delgado-Viscogliosi, Solignac [12] demonstrated that exposing *L. pneumophila* to 70°C water for 60 min reduced cultivability by more than 6.0-$\log_{10}$. In contrast, nucleic acid integrity was only affected by approximately 0.2-$\log_{10}$. Despite clear evidence that such measures impact ratios, there were an insufficient number of studies to provide conclusive quantitative estimates of this impact.

5.2 qPCR data integration into *L. pneumophila* risk assessment

A conversion from genome copies to CFU is necessary for QMRA because available *L. pneumophila* dose–response models predict health risks for inhaled doses in CFU [2,3]. To integrate qPCR data into QMRA, previous studies converted genome copies to an estimate of viable pathogen counts using a constant qPCR-to-culture ratio [37]. This approach can be effective if qPCR-to-culture ratios remain relatively stable over time. However, a stable ratio is unlikely for *L. pneumophila* in building potable water systems. These systems provide variable conditions, with possible inactivation of *L. pneumophila* in a point-of-entry treatment system (e.g., hot water heater) but also possible (re)growth in the plumbing system before the point of use.

Our meta-analysis indicates that qPCR-to-culture ratios tend to be low and exhibit significant variability. For about 80% of the building potable water systems, predicted geometric mean qPCR-to-culture ratios were less than 10, with predicted ratios approaching 1:1 at all sites. Therefore, to avoid underestimating the concentration of viable *L. pneumophila*, applying a 1:1 adjustment factor appears necessary when converting a *L. pneumophila* qPCR result into a cultivation result to quantify *L. pneumophila* concentrations in building potable water for QMRA. Using higher constant qPCR-to-culture ratios like 10:1, 100:1, or 1000:1 to adjust monitoring results could mask *L. pneumophila* growth events, thereby underestimating the health risks associated with these systems. This recommendation applies exclusively to *L. pneumophila*; this review did not target other *Legionella* species. While some conservative risk assessments might apply *L. pneumophila* dose–response models for other *Legionella* species, the interpretation of health risks associated with *Legionella* species is currently limited due to the absence of dose–response models for species beyond *L. pneumophila* and *L. longbeachae* [2,3].

The recommended 1:1 factor might significantly overestimate health risks in scenarios where point-of-entry treatment systems, such as thermal disinfection or on-site chemical disinfection, reduce the culturability or viability of *L. pneumophila*, with minimal to no impact on DNA concentrations. Directly using qPCR concentrations to estimate risks in such systems may result in an excessive number of sites being erroneously classified as hazardous, triggering unnecessary interventions and imposing undue burdens on facility managers. However, determining an adjustment factor that accurately captures the impact of disinfection from point-of-entry treatment systems is challenging. This difficulty arises because *L. pneumophila* can grow within a hot water line post-disinfection or at a fixture, potentially leading to lower qPCR-to-culture ratios compared to those measured directly from the effluent of the hot water heater. Consequently, relying on a qPCR-to-culture ratio based solely on the inactivation efficacy of the point-of-entry treatment system to adjust qPCR data for QMRA may mask *L. pneumophila* growth events. More accurate estimates of potential health risks in such systems could be obtained by pairing measurements from qPCR methods with those from culture-based or viability-qPCR methods. Therefore, qPCR should, at this stage, be viewed as a complementary tool for risk assessment and building investigations rather than as a replacement for cultivation.

Nonetheless, data on qPCR-derived *L. pneumophila* concentrations can offer insights into water safety beyond what is possible using cultivation data alone. For example, elevated or sudden increases in qPCR-based concentrations can infer *L. pneumophila* growth within the system. This information can become valuable when considered within the context of the site. For instance, it can highlight areas requiring intervention such as disinfection, elimination of dead ends, improvement in temperature controls, maintenance, or redesign. For population segments more vulnerable to developing severe Legionnaires' disease, including the elderly, immunocompromised individuals, smokers, and those with chronic lung conditions, qPCR results may be valuable for identifying high-risk systems.

Viability-qPCR can reduce and stabilize qPCR-to-culture ratios by excluding non-viable cells [34,38]. The subgroup analysis conducted in this study revealed that, on average, viability-qPCR leads to a reduction of approximately 0.2-log in the geometric mean and 0.5-log in the arithmetic mean when compared to standard qPCR. This reduction appears relatively low, considering the range of mean ratios expected from the meta-analysis. A partial explanation is that the viability-qPCR methods are conservative (i.e., only targeting extensively damaged cells) [39]. Viability-qPCR may have limited utility in cases where minor differences with standard qPCR results are expected, such as routine monitoring of conventional cold tap water systems, as observed for *Legionella* species by Hozalski, Zhao [40]. However, it is expected to provide useful insights in specific cases, such as validating the disinfection efficacy of on-site treatment (e.g., thermal barrier of the boiler, inline chlorination or shock disinfection), where most *L. pneumophila* detected by standard qPCR are likely non-viable. Routine application of viability-qPCR would benefit from an extensive data set documenting the conditions (e.g., exposure to heat or disinfectants) that sufficiently damages *Legionella* to affect viability detection, and simply more comparative data from full-scale systems. Further research into the conditions under which VBNC *L. pneumophila* can regain virulence and the factors influencing their transition back to a culturable state could also be beneficial to improve risk assessment accuracy.

5.3 Improved reporting in qPCR-to-culture comparison studies

The lack of comprehensive data reporting limited our ability to accurately quantify uncertainties associated with concentrations of *L. pneumophila* measured by qPCR and culture-based methods. In all studies, only estimated concentrations (not raw data) were reported. However,

for modelling temporal variations in pathogen concentrations, it is preferable to use original lab observations (counts and processed volumes). This approach allows for incorporating statistical uncertainties into the analysis [41,42]. In our study, we estimated cultivation counts from reported concentrations and sample volumes, but this method can underestimate uncertainties when samples undergo dilution or subsampling. To accurately back-calculate counts from reported cultivation concentrations, it is necessary to have access to dilution factors, and volumes plated for plating methods, positive and negative well counts for MPN methods, and elution and template volumes for qPCR methods.

LODs from qPCR and culture-based methods in reviewed studies sometimes differed by orders of magnitude. Minimizing differences between LODs would be beneficial as it would enhance the accuracy of qPCR-to-culture ratios at low concentrations. Ideally, when qPCR results are positive, corresponding culturable concentrations should be detectable. More pairing of positive results could increase the covariance between qPCR and cultivation, reducing the variability in the ratio distribution (see Eq. 4). This adjustment might correct for the tendency of the lower tail of the ratio distribution to predict ratios below 1.0, which may facilitate the adoption of less conservative conversion factors than 1:1 in some situations.

Comparing qPCR and cultivation data can be further complicated by differences in analytical recovery rates between the two methods. However, we were unable to evaluate this impact due to insufficient reporting. Whole-process recovery rates for the cultivation of *L. pneumophila* in drinking water samples have been found to range from 30–90% for direct plating [43-44] and 12–48% for pre-treated samples [45,44]. For qPCR, reported recovery rates have ranged from 42–98% for the whole process [46,47] and 57–123% specifically for DNA extraction [11,48]. Although the direct impact of these variations on the qPCR-to-culture ratio may be relatively minor compared to certain environmental factors, the effect of this methodological factor remains uncertain and warrants further investigation.

On a broader scale, while the study by Lee, Lai [15] adhered to the AFNOR NF-T90-471:2010 standard for qPCR testing, other studies reviewed did not follow an established qPCR standard. Many studies only partially reported specific controls and reporting elements recommended in the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines [49] or the Environmental Microbiology Minimum Information (EMMI) guidelines [50]. Adopting these guidelines in future research studies would improve the reliability and reproducibility of qPCR results.

Without standardized methods for both cultivation and qPCR, consistent correlations between qPCR and cultivation concentrations in building potable water systems remain uncertain [20]. However, even with standardized methods, variations in environmental factors such as water temperature can still impact culturability and affect correlations in site-specific temporal analyses. To quantify the influence of these environmental variables on the relationship, mathematical models, such as qPCR-to-culture ratio distributions and multiple regression models can be applied, provided that site-specific contextual data are reported. The parameters in Table 1 provide a preliminary framework for more standardized reporting.

6 Conclusions

This meta-analysis examined the qPCR-to-culture concentration ratio for *L. pneumophila* in building potable water systems, aiming to guide the interpretation of qPCR data for QMRA. The key conclusions drawn from this study are as follows:

- Our analysis confirms that the Poisson−lognormal distribution can effectively describe system-specific variations in *L. pneumophila* concentrations measured by qPCR and culture-based methods.

- The relationship between qPCR and culture methods, modeled as the ratio between two correlated lognormal random variables, showed substantial variability within systems, with ratios typically ranging from 1:1 to 100:1. This variability can be attributed to environmental factors such as water temperature and methodological considerations, including detection limits.

- Across the literature, the random-effects meta-analysis model indicated that for approximately 80% of systems, geometric mean qPCR-to-culture ratios are less than 10:1. However, due to occasional high ratios, the arithmetic mean ratios exceed 100:1 for about 20% of the systems.

- Given the observed variability and frequent occurrences of ratios close to 1:1, implementing a default 1:1 conversion factor for converting qPCR data to culturable concentrations in QMRA is recommended. This strategy avoids underestimating culturable concentrations due to potential *L. pneumophila* regrowth within the building.

- In cases of heavy *L. pneumophila* contamination before point-of-entry treatment systems, directly incorporating qPCR data into QMRA might overestimate health risks by detecting large amounts of DNA from non-infectious bacteria. Viability-qPCR, which targets DNA within intact cells, could provide a more accurate alignment of concentration estimates with actual health risks associated with *L. pneumophila*. However, these techniques are not yet widely implemented in qPCR-based applications.

- More standardized, site-specific reporting can facilitate detailed analysis of environmental and methodological variables influencing qPCR-to-culture ratios. This includes studying conditions that are likely to yield high proportions of non-viable and viable but non-culturable (VBNC) *L. pneumophila*.

Supporting information

S1 Fig. Forest plots of qPCR-to-culture ratio and viability-qPCR-to-culture ratio for *Legionella pneumophila* in water samples from drinking water systems. Subgroup analyses were carried out for geometric mean ratios. Horizontal lines represent 95% confidence intervals on mean values. Pooled estimates were obtained using the random-effect model.(DOCX)

S1 Proof. This section presents the proof for the transformation of the e.s.e on the $\log_{10}$ scale (Equation 8)**.**
(DOCX)

S1 Table. Comparison of Poisson gamma (PGA) distribution to the Poisson lognormal (PLN) distribution for predicting site-specific concentrations of *Legionella pneumophila* measured by qPCR in building drinking water systems. The marginal deviance information (mDIC) and its standard error (SE) are listed for each site. The lowest mDIC indicates the best model fit. A difference of 3.0 and more is considered significant. Bold mDIC values indicate best fit models. (DOCX)

S2 Table. Comparison of Poisson gamma (PGA) distribution to the Poisson lognormal (PLN) distribution for predicting site-specific concentrations of *Legionella pneumophila* measured by culture in building drinking water systems. The marginal deviance information (mDIC) and its standard error (SE) are listed for each site. The lowest mDIC indicates the best model fit. A difference of 3.0 and more is considered significant. Bold mDIC values indicate best fit models.
(DOCX)

S3 Table. Comparison of sample statistics and parameter values of lognormal distributions of the qPCR-to-culture ratio, qPCR concentrations and culture concentrations for measurement of *L. pneumophila* with standard qPCR and viability qPCR in paired water samples collected in potable water systems from reviewed studies.
(DOCX)

S4 Table. Selected quality control and assurance elements for qPCR analyses reported in reviewed studies.
(DOCX)

S5 Table. Standard for cultivation of *Legionella pneumophila* reported in reviewed studies.
(DOCX)

S6 Table. Characteristics of building potable water systems, sampling strategy and *Legionella pneumophila* enumeration methods for evaluating pooled qPCR-to-culture ratio from multiple buildings.
(DOCX)

S1 Text. Protocol for the systematic review and meta-analysis.
(DOCX)

S1 PRISMA Checklist.
(DOCX)

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Visualization: Emile Sylvestre.

Writing – original draft: Emile Sylvestre.

Writing – review & editing: Emile Sylvestre, William J. Rhoads, Timothy R. Julian, Frederik Hammes.

References

1. Phin N, Parry-Ford F, Harrison T, Stagg HR, Zhang N, Kumar K, et al. Epidemiology and clinical management of Legionnaires' disease. Lancet Infect Dis. 2014;14(10):1011–21. https://doi.org/10.1016/S1473-3099(14)70713-3 PMID: 24970283

2. Armstrong TW, Haas CN. A quantitative microbial risk assessment model for Legionnaires' disease: animal model selection and dose-response modeling. Risk Anal. 2007;27(6):1581–96. https://doi.org/10.1111/j.1539-6924.2007.00990.x PMID: 18093054

3. Armstrong TW, Haas CN. Legionnaires' disease: evaluation of a quantitative microbial risk assessment model. J Water Health. 2008;6(2):149–66. https://doi.org/10.2166/wh.2008.026 PMID: 18209278

4. National Academies of Sciences E, Medicine. Management of Legionella in water systems. National Academies Press; 2020.

5. Donohue MJ, Pham M, Brown S, Easwaran KM, Vesper S, Mistry JH. Water quality influences *Legionella pneumophila* determination. Water Res. 2023;238:119989. https://doi.org/10.1016/j.watres.2023.119989 PMID: 37137207

6. Ahmed S, Liwak-Muir U, Walker D, Zoldowski A, Mears A, Golovan S, et al. Validation and in-field testing of a new on-site qPCR system for quantification of Legionella pneumophila according to ISO/TS 12869:2012 in HVAC cooling towers. J Water Health. 2019;17(2):237–53. https://doi.org/10.2166/wh.2019.252 PMID: 30942774

7. He Z, Zhang J, Jia H, Zhang S, Sun X, Nishawy E, et al. Correction to: Genome-wide identification and analyses of ZmAPY genes reveal their roles involved in maize development and abiotic stress responses. Mol Breed. 2024;44(6):44. https://doi.org/10.1007/s11032-024-01482-9 PMID: 38868574

8. Anonymous. ISO/TS 12869:2012: Water quality—Detection and Quantification of Legionella spp. and/or Legionella pneumophila by Concentration and Genic Amplification by Quantitative Polymerase Chain Reaction (qPCR). Geneva: International Organisation for Standardisation; 2012.

9. AFNOR. NF T90-471: Water quality. Detection and quantification of Legionella and/or Legionella pneumophila by concentration and genic amplification by polymerase chain reaction (PCR); 2010.

10. Joly P, Falconnet P-A, André J, Weill N, Reyrolle M, Vandenesch F, et al. Quantitative real-time Legionella PCR for environmental water samples: data interpretation. Appl Environ Microbiol. 2006;72(4):2801–8. https://doi.org/10.1128/AEM.72.4.2801-2808.2006 PMID: 16597985

11. Collins S, Stevenson D, Walker J, Bennett A. Evaluation of Legionella real-time PCR against traditional culture for routine and public health testing of water samples. J Appl Microbiol. 2017;122(6):1692–703. https://doi.org/10.1111/jam.13461 PMID: 28376265

12. Delgado-Viscogliosi P, Solignac L, Delattre J-M. Viability PCR, a culture-independent method for rapid and selective quantification of viable Legionella pneumophila cells in environmental water samples. Appl Environ Microbiol. 2009;75(11):3502–12. https://doi.org/10.1128/AEM.02878-08 PMID: 19363080

13. Grimard-Conea M, Deshommes E, Doré E, Prévost M. Impact of recommissioning flushing on *Legionella pneumophila* in a large building during the COVID-19 pandemic. Front Water. 2022;4:959689.

14. Donohue MJ. Quantification of *Legionella pneumophila* by qPCR and culture in tap water with different concentrations of residual disinfectants and heterotrophic bacteria. Sci Total Environ. 2021;774:145142. https://doi.org/10.1016/j.scitotenv.2021.145142 PMID: 33610980

15. Lee JV, Lai S, Exner M, Lenz J, Gaia V, Casati S, et al. An international trial of quantitative PCR for monitoring Legionella in artificial water systems. J Appl Microbiol. 2011;110(4):1032–44. https://doi.org/10.1111/j.1365-2672.2011.04957.x PMID: 21276147

16. Nisar M, Ross K, Brown M, Bentham R, Best G, Whiley H. Detection and quantification of viable but non-culturable Legionella pneumophila from water samples using flow cytometry-cell sorting and quantitative PCR. Front Microbiol. 2023;14:32.

17. Haas C. Statistics of enumerating total coliforms in water samples by membrane filter procedures. Water Res. 1986;20(4):525–30. https://doi.org/10.1016/0043-1354(86)90203-4

18. Haas CN, Heller B. Test of the validity of the Poisson assumption for analysis of most-probable-number results. Appl Environ Microbiol. 1988;54(12):2996–3002. https://doi.org/10.1128/aem.54.12.2996-3002.1988 PMID: 3223765

19. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Int J Surg. 2021;88:105906. https://doi.org/10.1016/j.ijsu.2021.105906 PMID: 33789826

20. Whiley H, Taylor M. Legionella detection by culture and qPCR: comparing apples and oranges. Crit Rev Microbiol; 2014:1–10.

21. Aydin O, Yassikaya MY. Validity and reliability analysis of the PlotDigitizer software program for data extraction from single-case graphs. Perspect Behav Sci. 2021;45(1):239–57. https://doi.org/10.1007/s40614-021-00284-0 PMID: 35342869

22. Sylvestre É, Dorner S, Burnet J-B, Smeets P, Medema G, Cantin P, et al. Changes in Escherichia coli to enteric protozoa ratios in rivers: implications for risk-based assessment of drinking water treatment requirements. Water Res. 2021;205:117707. https://doi.org/10.1016/j.watres.2021.117707 PMID: 34619609

23. Haas CN, Rose JB, Gerba CP. Quantitative microbial risk assessment; 2014. https://doi.org/10.1002/9781118910030

24. Quintero A, Lesaffre E. Comparing hierarchical models via the marginalized deviance information criterion. Stat Med. 2018;37(16):2440–54. https://doi.org/10.1002/sim.7649 PMID: 29579777

25. Engleberg N, Carter C, Weber D, Cianciotto N, Eisenstein B. DNA sequence of mip, a *Legionella pneumophila* gene associated with macrophage infectivity. Infect Immunity. 1989;57(4):1263–70.

26. Viechtbauer W. Conducting meta-analyses in R with the metafor package. J Stat Softw. 2010;36(3):1–48.

27. Olsson U. Confidence intervals for the mean of a log-normal distribution. J Stat Educ. 2005;13(1). https://doi.org/10.1080/10691898.2005.11910638

28. Higgins JPT, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. J R Stat Soc Ser A Stat Soc. 2009;172(1):137–59. https://doi.org/10.1111/j.1467-985X.2008.00552.x PMID: 19381330

29. Sauget M, Richard M, Chassagne S, Hocquet D, Bertrand X, Jeanvoine A. Validation of quantitative real-time polymerase chain reaction for detection of Legionella pneumophila in hospital water networks. J Hosp Infect. 2023;138:74–80. https://doi.org/10.1016/j.jhin.2023.06.010 PMID: 37353007

30. Grimard-Conea M, Prévost M. Controlling *Legionella pneumophila* in showerheads: combination of remedial intervention and preventative flushing. Microorganisms. 2023;11(6):1361. https://doi.org/10.3390/microorganisms11061361 PMID: 37374862

31. Bonetta S, Pignata C, Bonetta S, Meucci L, Giacosa D, Marino E, et al. Effectiveness of a neutral electrolysed oxidising water (NEOW) device in reducing Legionella pneumophila in a water distribution system: a comparison between culture, qPCR and PMA-qPCR detection methods. Chemosphere. 2018;210:550–6. https://doi.org/10.1016/j.chemosphere.2018.07.053 PMID: 30029147

32. Morio F, Corvec S, Caroff N, Le Gallou F, Drugeon H, Reynaud A. Real-time PCR assay for the detection and quantification of *Legionella pneumophila* in environmental water samples: utility for daily practice. International Journal of Hygiene and Environmental Health. 2008 Jul 15;211(3-4):403–11. https://doi.org/10.1016/j.ijheh.2007.06.002

33. Mapili K, Pieper KJ, Dai D, Pruden A, Edwards MA, Tang M, et al. Legionella pneumophila occurrence in drinking water supplied by private wells. Lett Appl Microbiol. 2020;70(4):232–40. https://doi.org/10.1111/lam.13273 PMID: 31904109

34. Yáñez MA, Nocker A, Soria-Soria E, Múrtula R, Martínez L, Catalán V. Quantification of viable *Legionella pneumophila* cells using propidium monoazide combined with quantitative PCR. J Microbiol Methods. 2011;85(2):124–30. https://doi.org/10.1016/j.mimet.2011.02.004 PMID: 21329735

35. Cervero-Aragó S, Schrammel B, Dietersdorfer E, Sommer R, Lück C, Walochnik J, et al. Viability and infectivity of viable but nonculturable *Legionella pneumophila* strains induced at high temperatures. Water Res. 2019;158:268–79. https://doi.org/10.1016/j.watres.2019.04.009 PMID: 31048196

36. Bédard E, Boppe I, Kouamé S, Martin P, Pinsonneault L, Valiquette L, et al. Combination of heat shock and enhanced thermal regime to control the growth of a persistent *Legionella pneumophila* strain. Pathogens. 2016;5(2):35. https://doi.org/10.3390/pathogens5020035 PMID: 27092528

37. McBride G, Stott R, Miller W, Bambic D, Wuertz S. Discharge-based QMRA for estimation of public health risks from exposure to stormwater-borne pathogens in recreational waters in the United States. Water Res; 2013.

38. Lizana X, López A, Benito S, Agustí G, Ríos M, Piqué N, et al. Viability qPCR, a new tool for Legionella risk management. Int J Hyg Environ Health. 2017;220(8):1318–24. https://doi.org/10.1016/j.ijheh.2017.08.007 PMID: 28882518

39. Hammes F, Berney M, Egli T. Cultivation-independent assessment of bacterial viability. Adv Biochem Eng Biotechnol. 2011;124:123–50. https://doi.org/10.1007/10_2010_95 PMID: 21069588

40. Hozalski RM, Zhao X, Kim T, LaPara TM. On-site filtration of large sample volumes improves the detection of opportunistic pathogens in drinking water distribution systems. Appl Environ Microbiol. 2024;90(2):e0165823. https://doi.org/10.1128/aem.01658-23 PMID: 38236032

41. Schmidt PJ, Acosta N, Chik AHS, D'Aoust PM, Delatolla R, Dhiyebi HA, et al. Realizing the value in "non-standard" parts of the qPCR standard curve by integrating fundamentals of quantitative microbiology. Front Microbiol. 2023;14:1048661. https://doi.org/10.3389/fmicb.2023.1048661 PMID: 36937263

42. Chik AHS, Schmidt PJ, Emelko MB. Learning something from nothing: the critical importance of rethinking microbial non-detects. Front Microbiol. 2018;9:2304. https://doi.org/10.3389/fmicb.2018.02304 PMID: 30344512

43. Villari P, Motti E, Farullo C, Torre I. Comparison of conventional culture and PCR methods for the detection of *Legionella pneumophila* in water. Lett Appl Microbiol. 1998;27(2):106–10. https://doi.org/10.1046/j.1472-765x.1998.00389.x PMID: 9750332

44. Blanky M, Rodríguez-Martínez S, Halpern M, Friedler E. *Legionella pneumophila*: from potable water to treated greywater; quantification and removal during treatment. Sci Total Environ. 2015;533:557–65. https://doi.org/10.1016/j.scitotenv.2015.06.121 PMID: 26188406

45. Boulanger CA, Edelstein PH. Precision and accuracy of recovery of *Legionella pneumophila* from seeded tap water by filtration and centrifugation. Appl Environ Microbiol. 1995;61(5):1805–9. https://doi.org/10.1128/aem.61.5.1805-1809.1995 PMID: 7646019

46. Behets J, Declerck P, Delaedt Y, Creemers B, Ollevier F. Development and evaluation of a Taqman duplex real-time PCR quantification method for reliable enumeration of Legionella pneumophila in water samples. J Microbiol Methods. 2007;68(1):137–44. https://doi.org/10.1016/j.mimet.2006.07.002 PMID: 16914218

47. Omiccioli E, Schiavano GF, Ceppetelli V, Amagliani G, Magnani M, Brandi G. Validation according to ISO/TS 12869:2012 of a molecular method for the isolation and quantification of Legionella spp. in water. Mol Cell Probes. 2015;29(2):86–91. https://doi.org/10.1016/j.mcp.2014.12.004 PMID: 25595345

48. Collins S, Jorgensen F, Willis C, Walker J. Real-time PCR to supplement gold-standard culture-based detection of Legionella in environmental samples. J Appl Microbiol. 2015;119(4):1158–69. https://doi.org/10.1111/jam.12911 PMID: 26218315

49. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611–22. https://doi.org/10.1373/clinchem.2008.112797 PMID: 19246619

50. Borchardt MA, Boehm AB, Salit M, Spencer SK, Wigginton KR, Noble RT. The Environmental Microbiology Minimum Information (EMMI) guidelines: qPCR and dPCR quality and reporting for environmental microbiology. Environ Sci Technol. 2021;55(15):10210–23. https://doi.org/10.1021/acs.est.1c01767 PMID: 34286966