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Published

2025

Journal Title

The Journal of Infectious Diseases

Version

Version of Record (VoR)

DOI

10.1093/infdis/jiaf407

The Journal of Infectious Diseases

MAJOR ARTICLE

Seroprevalence of Antibodies Against Legionella Species in Northeastern Australian Blood Donors, 2016 and 2023

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Background. In 2021–2022, Queensland, Australia observed an increase in Legionnaire's disease cases, predominantly due to *Legionella longbeachae*. This study assessed seroprevalence at time points 2016 and 2023, representing before and after the higher incidence and explored if demographic, environmental and geographical factors associated with legionellosis seroprevalence.

Methods. A total of 1001 human plasma samples (496 from 2016/505 from 2023) were analysed for the presence of Legionella antibodies (IgG) using indirect immunofluorescence assays. Primary screens detected IgG to *L. pneumophila* serogroups (SG) 1–6, SG 7–14, or "other" *Legionella* spp. Samples positive for "other" underwent secondary screening for *L. longbeachae* SG 1 and 2. A chi-square test assessed associations between seroprevalence and demographics, while a generalized linear model evaluated rainfall, temperature, and land cover associations.

Results. While total *Legionella* seroprevalence remained stable (32.46% vs 32.28%) between 2016 and 2023, we observed a decrease in *L. pneumophila* (SG 1–6: 19%–13% [$P = .0182$] and SG 7–14: 24%–18% [$P = .0257$]) and an increase in *L. longbeachae* (1%–3% [$P = .0355$]) seropositivity. *L. pneumophila* seroprevalence positively associated with higher rainfall and land cover, with croplands and urban areas showing increased prevalence.

Conclusions. Between 2016 and 2023, total Legionella seroprevalence remained unchanged. However, rainfall and specific land cover types were positively associated with seropositivity for certain *Legionella* spp. This study highlights the importance of assessing Legionella exposure risks in high-risk areas, particularly for vulnerable individuals such as the elderly, immunosuppressed, or those with co-morbidities.

Keywords. IgG antibodies; seropositive; legionella; pneumonia; risk-factors.

Legionella (L.) is a genus of Gram-negative bacteria, with over 60 species currently identified [1]. A number of these bacteria such as *L. pneumophila*, are known to colonize human-made water systems, including cooling towers, hot water systems, plumbing fixtures and drinking water [2, 3]. Others, like *L. longbeachae*, are commonly found in potting soil [4], with studies speculating pine bark may be the source [5]. These two species are of particular public health concern because of their propensity to infect humans, causing similar clinical symptoms. As such, each species poses a distinct threat within different environments. *L. pneumophila* is divided into 15 serogroups (SG) [6], with SG 1, 4 and 6 most frequently associated with human infections such as Legionnaires' disease, and responsible for the majority of reported legionellosis cases globally [7, 8]. *L. longbeachae* conversely has two known SG, with serogroup 1 being the most clinically significant [9].

Legionella infects humans via inhalation and is not spread person to person [10]. Smokers, immunocompromised individuals, and those with existing conditions are at higher risk [2]. Infection, known as legionellosis, includes Pontiac fever—a mild, self-limiting illness with myalgia, headache, and fatigue [11], and Legionnaires' disease, a more severe pneumonia with fever, chills, cough, and shortness of breath [12].

Although relatively uncommon, Legionnaire's disease may be life-threatening. Legionella infections are thought to be responsible for approximately 5%–15% of all community acquired pneumonias [13], and approximately 10% of people who contract Legionnaire's disease will die despite receiving medical treatment [14, 15]. In 2023–2024, outbreaks of Legionnaires' disease occurred in Poland (14% mortality rate) [16], Lithuania (29% mortality rate) [17], Richmond, California, (33% mortality; only 2/6 cases) [18]. Additional outbreaks in Scotland, Texas,

Received 21 October 2024; editorial decision 29 July 2025; published online 4 September 2025

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The Journal of Infectious Diseases®

https://doi.org/10.1093/infdis/jiaf407

and Italy had varying fatality rates, typically ranging from 3% to 14%, with vulnerable populations most affected [19].

From 2023 to 2024, Australia had 2 significant outbreaks of Legionnaire's disease. The first was in Sydney's central business district, where eleven cases were positive for *L. pneumophila* serogroup 1. Three of these cases were linked to a local water-cooling tower [20]. Since July 2024, Melbourne has experienced an explosive Legionella outbreak with a total of 114 confirmed cases and 5 suspected cases notified between July 26th and August 20th [21]. Two deaths have been reported. The contamination source was identified, and the cooling tower was decontaminated to prevent further spread. This *L. pneumophila* outbreak is the most severe in over two decades, underscoring legionellosis as a continuing public health concern.

In Australia, Legionnaire's disease is a notifiable condition [22]. In late 2021 and early 2022, the National Notifiable Disease Surveillance System captured an increase in the number of reported legionella cases in Queensland [23], which was due to an increase in *L. longbeachae* infections [24]. *L. longbeachae* notifications in 2022 were 3 times the 5-year average [23, 24]. The cause of the increase is unknown, but it is hypothesized that climatic factors—such as flooding during this period—may have contributed, as legionellosis outbreaks have previously been linked to heavy rainfall or floods [25, 26]. Further, because the surveillance system in Australia does not capture cases of Pontiac fever, the increase in disease due to *L. longbeachae* may be underestimated.

This study investigated demographic, climatic, and environmental characteristics of seropositive samples in Northeastern Australia before and after the 2021–2022 notification increase. We assessed L. spp. seroprevalence in Lifeblood (Australian Red Cross blood donation service; https://www.lifeblood.com.au) plasma samples and examined associations with demographic and environmental variables, analysing species groups separately due to their distinct habitats.

METHODS

Samples and Ethics

In the absence of Australian seroprevalence studies for *L. longbeachae*, sample size calculation used the estimate of global *Legionella* spp. seroprevalence of 8% as identified by Graham et al. [27] among studies published between 2010 and 2017 [27]. To detect a doubling of seroprevalence between the two time points of sample collection for this study, with 95% confidence and 95% power, 423 samples per time point were required.

Human plasma samples from 2016 ($n = 496$) were obtained from archived specimens used in a previous independent seroprevalence study (Lifeblood Human Research Ethics: Faddy 230713; Hoad 2017). Samples from 2023 ($n = 505$) were similarly sourced from Lifeblood donations. All donations were screened and found negative for HIV, Hepatitis B and C, HTLV, and syphilis. Most donors resided in Queensland, with a few from northeastern New South Wales.

Donor age, gender, and Statistical Area Level 3 (SA3; geographical units defined by the Australian Bureau of Statistics by grouping multiple SA2 areas) of residence were obtained from Lifeblood records prior to sample de-identification. No clinical information was provided (Supplementary Table 6).

Ethical approval for the use of plasma samples was provided by Lifeblood Human Research Ethics Committee (Ref No: 2023#10-LNR, 16 March 2023) and Griffith University Human Research Ethics Committee (GU Ref No: 2023/234, 6 April 2023). Written informed consent was obtained from all subjects involved in the study at the time of blood donation.

Indirect Immunofluorescence Assay

Immunofluorescence assay (IFA) is regarded as the standard serological reference test for Legionella antibody detection in a patient's plasma [28]. Primary detection of anti-*Legionella* IgG antibodies was accomplished using the Legionella SP IFA IgG, PLESPG kit (Vircell) as per manufacturer's instructions. This kit tests for the presence of IgG antibodies against *L. pneumophila* SG 1–6, *L. pneumophila* SG 7–14 and "*other*" *Legionella* spp. defined collectively as, *L. bozemanii* SG 1 and 2, *L. longbeachae* SG 1 and 2, *L. dumoffii*, *L. gormanii*, *L. micdadei* and *L. jordanis* (Figure 1).

Samples that were IgG positive under the category "*other*" within the primary assay were secondarily screened using a separate indirect IFA (Biocell Diagnostics) for the detection of *L. longbeachae* SG 1,2 IgG antibodies (Figure 1).

Environmental and Climate Variables

Temperature and precipitation variables were extracted from the ERA5-Land climate database to a resolution of 9 km grid spacing for each SA3 location in 2016 and 2023 [29]. Variables with a correlation coefficient ≥0.7 were discarded (Supplementary Figure 1) to avoid issues related to multicollinearity, which can negatively impact the model's performance and interpretation. The variables of mean, maximum, median, coefficient of variance and minimum annual rainfall in mm, and mean annual temperature in °C were included in the statistical analysis.

Total Land Cover Classification area for each SA3 was obtained from the Moderate Resolution Imaging Spectroradiometer Land Cover Type Version 6.1 product (Terra and Aqua combined), which provides annual global maps at 500 m resolution [30]. Classifications follow the MCD12Q1 International Geosphere-Biosphere Programme scheme, categorizing land into 17 classes by predominant characteristics (see Supplementary Table 1). Each land cover class was represented as a continuous variable denoting the total area (in km^2) of that class within the SA3 region. This allowed inclusion of multiple classes per region.

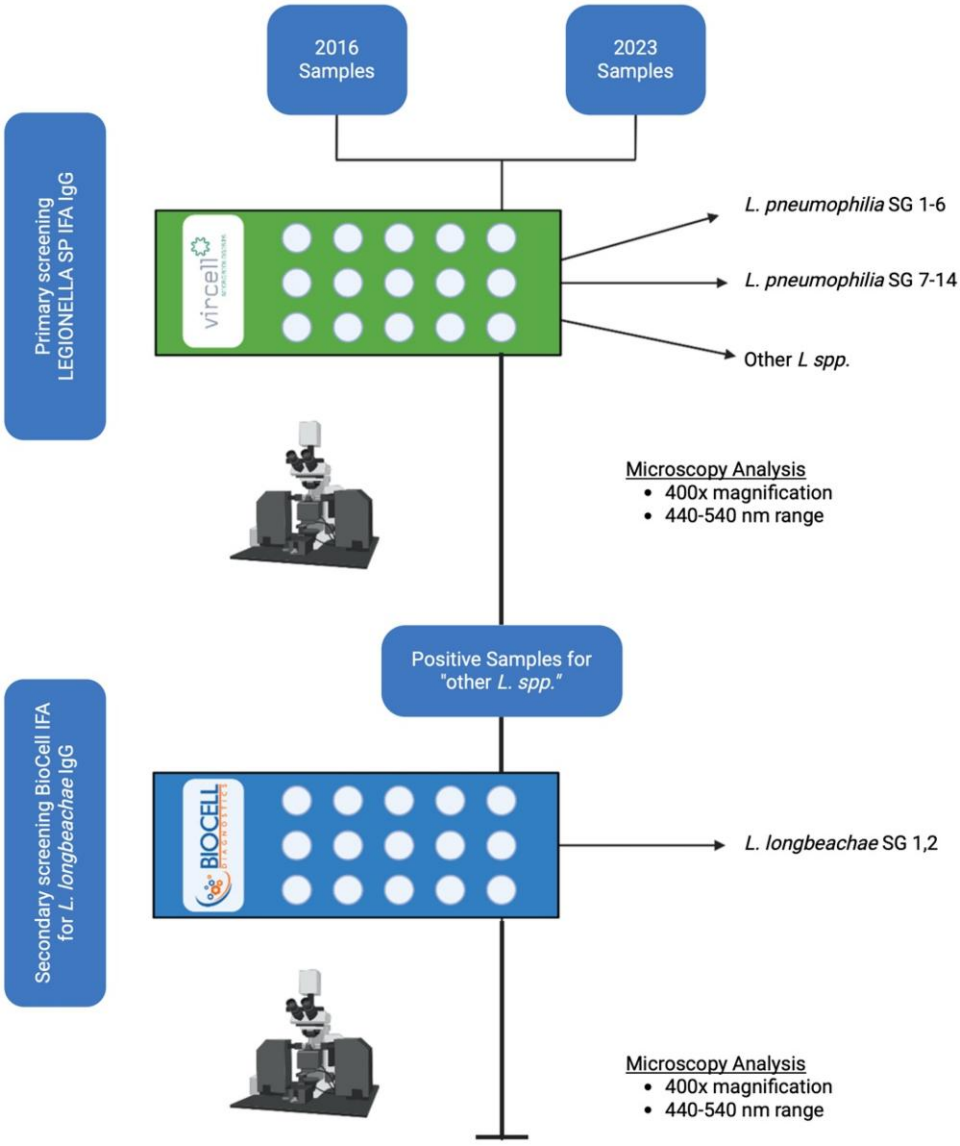

Figure 1. Flowchart illustrating the chain of events of primary and secondary assays. The figure illustrates the chain of events for sample testing within this study, illustrating testing categories during both the primary and secondary screening of samples.

Location

Files containing SA3 location data from 2021 were obtained from the Australian Bureau of Statistics and were utilized in the software QGIS 3.32 to generate accurate geospatial maps [31].

Statistical Analyses

Categorical data such as gender, SA3 location and donation year were analysed using a Chi-square test. Statistically significant Chi-square test results were analysed post hoc using Cramer's V. The continuous variable age was analysed using one-way ANOVA. Confidence intervals were calculated by the published Clopper & Pearson method using R.

Generalized linear models (GLM) were used to assess associations between Legionella seroprevalence and climate or environmental variables. Response variables included percentage seropositivity for *L. pneumophila* SG 1–6, SG 7–14, *L. other*, and *L. longbeachae* SG 1,2 (Supplementary Table 2, 3, 4, and 5). Predictors included climate metrics (annual mean, max, min, median, and coefficient of variation for precipitation; mean, max, min temperature), tested in raw and log-transformed forms. Land cover variables were analysed in a separate GLM, covering areas of croplands, urban areas, vegetation mosaics, grasslands, shrublands, savannas, water bodies, and evergreen forests, also using raw and log-transformed values.

To address spatial autocorrelation, seroprevalence data were aggregated by SA3 region. Environmental variables were matched

Table 1. Demographic breakdown of samples by greater capital city statistical area listing donation year, number of samples, age groups and the male % of samples. Bold values in Age Group represent the number of individuals out of 1001.

		Age Group						
Greater Capital City Statistical	Number of Samples	<25	25–34	35–44	45–54	55–64	65+	Male (%)
2016								
Greater Brisbane	177	32	39	32	36	30	8	47.46
Rest of Queensland	265	47	42	45	53	47	31	52.08
Rest of New South Wales	54	5	6	9	12	17	5	55.56
2023								
Greater Brisbane	220	22	47	37	46	35	33	56.36
Rest of Queensland	285	18	43	39	69	68	48	55.09
Rest of New South Wales	0	0	0	0	0	0	0	0
Total	**1001**	**124**	**177**	**162**	**216**	**197**	**125**	**53.25**

Table 2. Comparison of seropositivity for Legionella spp. in blood donors 2016 and 2023

	2016 ($n = 496$)		2023 ($n = 505$)			
	N Positive	Proportion Positive [95% CI]	*N* Positive	Proportion Positive [95% CI]	*P* value	Cramer's V
Legionella spp[a]	161	32.46 [28.35, 36.78]	163	32.28 [28.21, 36.55]	.9508	0.00195
L. pneumophila SG 1–6	94	18.95 [15.6, 22.68]	67	13.27 [10.43, 16.54]	.0182	0.0774
L. pneumophila SG 7–14	121	24.40 [20.68, 28.42]	93	18.42 [15.13, 22.08]	.0257	0.0729
L. other	90	18.15 [14.85, 21.82]	114	22.57 [19.00, 26.47]	.0967	0.0550
L. longbeachae SG 1,2	4	0.81 [0.22, 2.05]	14	2.78 [1.52, 4.61]	.0355	0.0740

[a]*Legionella* spp. was calculated as the total number of samples that tested positive for one or more *Legionella* species during the primary screening (*L. pneumophila* SG 1–6, SG 7–14, and *L. other*). The rows for *L. pneumophila* SG 1–6, SG 7–14, *L. other*, and *L. longbeachae* represent the number of samples that tested positive for at least one serogroup or species within each respective category. CI were calculated by the published Clopper & Pearson method.

to SA3 regions. A random SA3 effect was considered but omitted due to low regional variability. Donation year (2016, 2023) was included as a categorical variable to account for temporal trends. Models were implemented in R (v2023.12.1 build 402) using glm() with a Gaussian link. Assumptions were assessed, including multicollinearity via variance inflation factors; highly correlated variables were excluded from final models.

RESULTS

Sample Demographics

A total of 1001 samples that covered a range of ages/genders were included in this study (Supplementary Table 6). Of the 1001 samples tested, most donors from both years (89% from 2016 and 100% from 2023) had an address listed in Queensland, with only 54 donors in 2016 having an address listed in New South Wales (Table 1).

Seropositivity for Anti-Legionella IgG by Year, Age and Gender

When comparing the percentage of total samples that tested positive for one or more *L.* spp. between 2016 and 2023, the primary assay showed no change in seroprevalence of total Legionella IgG (32% at both time points; $P = .9508$) (Table 2). There was a decrease in *L. pneumophila* SG 1–6 seroprevalence from 2016 to 2023 (19%–13%, $P = .0182$) and *L. pneumophila* SG 7–14 seroprevalence (24%–18% of samples, $P = .0257$) and a slight increase in seroprevalence of *L. other* (18%–23% of samples, $P = .0967$). Overall, *L. pneumophila* decreased across both time points (43%–31% of samples, $P = .0149$). There was also an increase in the seroprevalence of *L. longbeachae* SG 1, 2 between 2016 and 2023 (1%–3%, $P = .0355$; Table 2). No statistically significant relationship was found between seroprevalence and gender or age for any of the species of Legionella (data not shown).

Seropositivity for Anti-Legionella IgG by Sa3 Location

Examining the seropositivity of species by year and location (Seventy-six SA3 locations), it was found that the highest seropositive regions for *L. pneumophila* and *L. other* in 2016 were Richmond Valley—Coastal whereas in 2023 it was Rockhampton. In contrast *L. longbeachae* SG 1,2 appeared evenly distributed in 2016 with only four positive samples detected, and in 2023, it was highest in Rockhampton and Biloela.

Specifically, by comparing the 161 positive samples for *L. pneumophila* SG 1–6 across both years, the highest regions in 2016, for seropositivity against *L. pneumophila* SG 1–6 were Richmond Valley—Coastal (11/161; 6.83% [95% CI: 3–11]), Bundaberg (7/161; 4.35%; [95% CI: 2–8] and Caloundra 7/161; 4.35%; [95% CI: 2–8]) (Figure 2). In 2023, the highest seropositive regions for *L. pneumophila* SG 1–6 were Rockhampton ($n = 8/161$; 4.97% [95% CI: 2–9]) and Biloela (6/161; 3.73% [95% CI: 1–7]) (Figure 2).

In 2016 *L. pneumophila* SG 7–14 showed similar trends with Richmond Valley—Coastal (13/214; 6.07% [95% CI: 3–10]) and

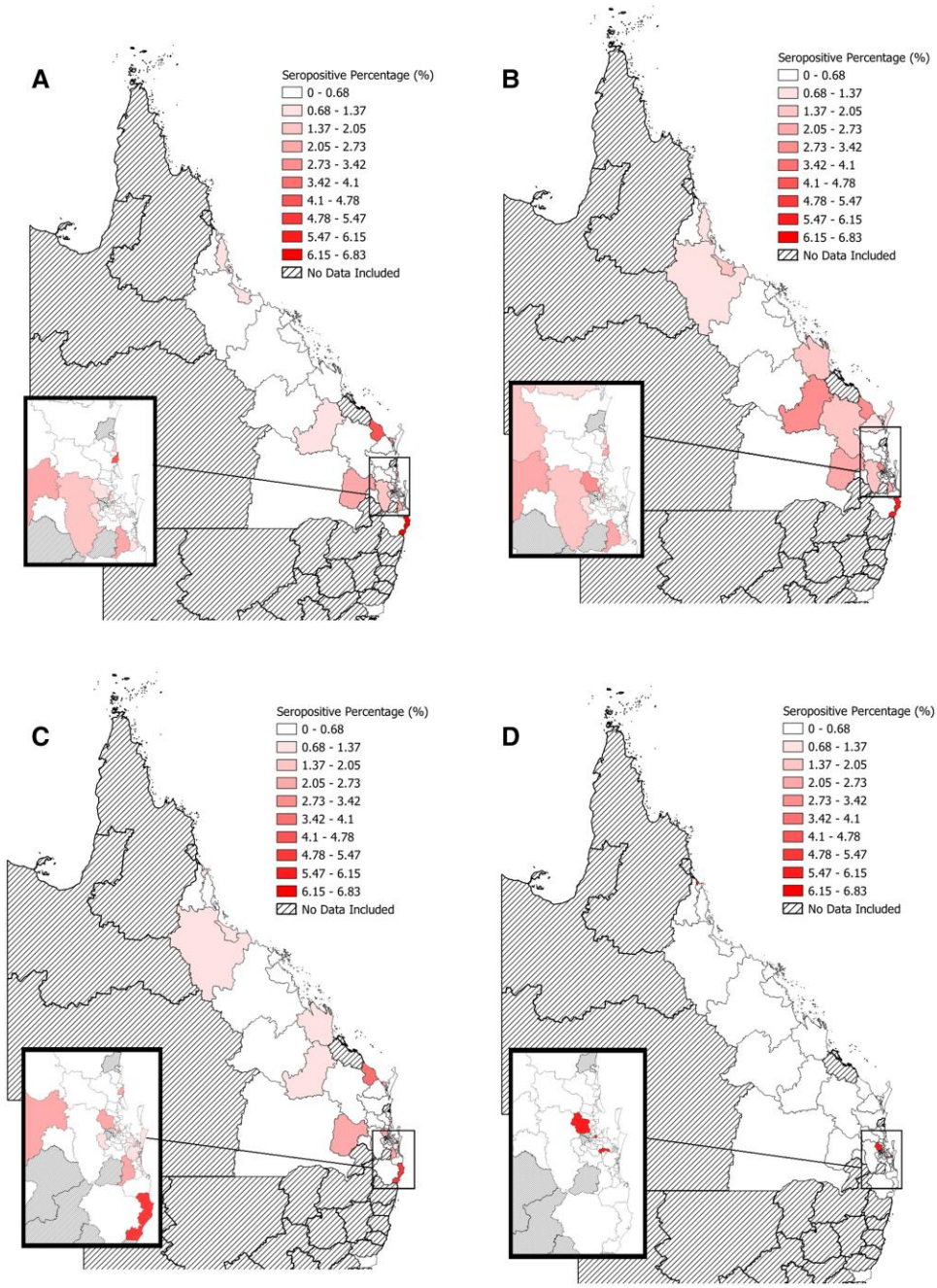

Figure 2. Legionella species seropositive proportions by region 2016 and 2023. 2016, (A–D); 2023 (E–H). (A): *L. pneumophila* SG 1–6 in 2016, (B): *L. pneumophila* SG 7–14 in 2016, (C): *L. other* in 2016, (D): *L. longbeachae* SG 1,2 in 2016. (E): *L. pneumophila* SG 1–6 in 2023, (F): *L. pneumophila* SG 7–14 in 2023, (G): *L. other* in 2023, (H): *L. longbeachae* SG 1,2 in 2023. The figure shows a geospatial map showing seropositive results for each SA3 location. Numbers within the legend are representative of the total number of seropositive results for that SA3 location as a percentage of total seropositive values for each species category.

Bundaberg (7/214; 3.27% [95% CI: 1–6]) remaining highest (Figure 2). Whereas in 2023 *L. pneumophila* SG 7–14 was highest in Rockhampton (12/214; 5.61%; [95% CI: 3–9]) and Burnett (9/214; 4.21 [95% CI: 2–7]) (Figure 2).

In 2016, the highest seropositive regions for *L. other* were again Richmond Valley—Coastal (10/204; 4.90% [95% CI: 2–8]) and Bundaberg (7/204; 3.43% [95% CI: 1–6]) (Figure 2). Whereas in 2023, the highest seropositive regions for *L. other* were Rockhampton (16/204; 7.84% [95% CI: 4–12]) and Burnett ($n = 11/204$; 5.39% [95% CI: 3–9]) (Figure 2).

In 2016, there were only four cases of *L. longbeachae* SG 1,2 (Figure 2). While in 2023, the highest seropositive regions were

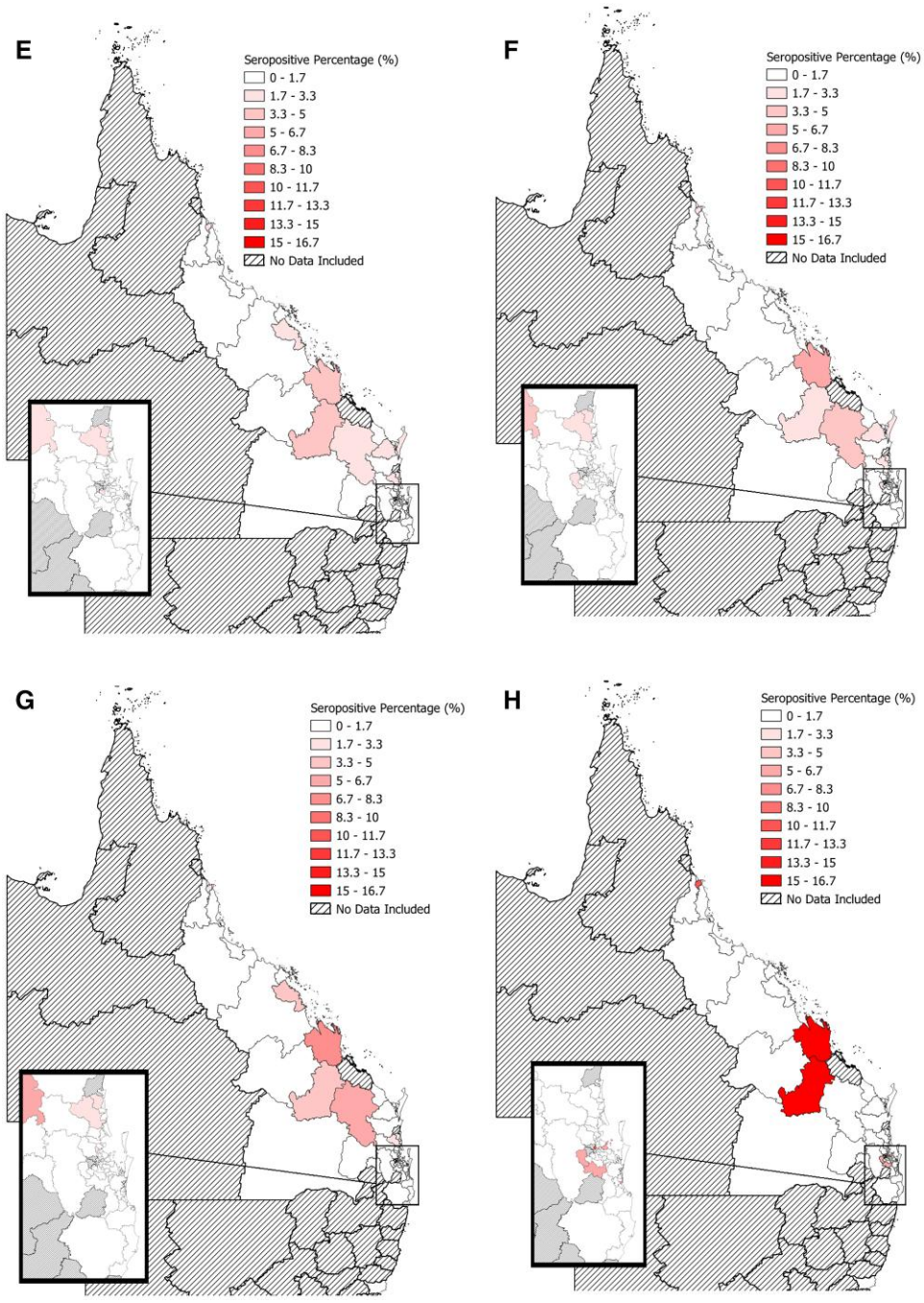

Figure 2. Continued

Rockhampton (3/18; 16.67% [95% CI: 5–39]), and Biloela (3/18; 16.67%; [95% CI: 5–39]) (Figure 2).

Using a chi-square test, there was no significant relationship in 2016 or 2023 between SA3 location and seropositive proportions for any of the serotypes tested. [*L. pneumophila* SG 1–6 2016 ($P = .3439$), 2023 ($P = .6453$); *L. pneumophila* SG 7–14 2016 ($P = .5659$), 2023 ($P = .5348$), *L. other* 2016 ($P = .5411$); 2023 ($P = .1303$) and *L. longbeachae* SG 1,2 2016 ($P = .8482$), 2023 ($P = .7807$)].

Seropositivity for Anti-Legionella IgG by Climate Variables

The individual species models used to assess the impact of climate variables on seroprevalence found that whereas most were not correlated with seropositivity there was significant association between annual median rainfall and *L. pneumophila* SG 1–6 (Table 3) as well as *L. pneumophila* SG 7–14 (Table 4).

Table 3. General linear model results for *L. pneumophila* SG 1–6 associated with climate variables

Term	Estimate	Standard Error	*P* value	Confidence Low	Confidence High
Intercept	−0.518	0.684	.550	−2.21	1.18
Annual Max Rainfall (mm)	0.00896	0.0127	.481	−0.0159	0.0338
Annual Median Rainfall (mm)	0.00562	0.00178	.00191**	0.00213	0.00911
Annual Coefficient of Variance	0.000329	0.00449	.942	−0.00847	0.00913
Annual Mean Temperature (°C)	0.264	0.309	.394	−0.342	0.871

Statistically significant *P* values are depicted by ** when $P < 0.01$.

Seropositivity for Anti-Legionella IgG by Land Cover Class

Several land cover categories, particularly croplands and urban areas, showed statistically significant associations ($P < 0.05$) with anti-*Legionella* seropositivity across the *Legionella* species tested (Table 5). Specifically, for *L. pneumophila* SG 1–6, significant associations were observed for croplands, natural vegetation mosaics (Mosaics), grasslands, and urban and built-up areas (Supplementary Table 2). For *L. pneumophila* SG 7–14, croplands and urban and built-up areas were significant (Supplementary Table 3), while for *L. other*, open shrublands, grasslands, croplands, and urban and built-up areas were associated with seropositivity (Supplementary Table 4).

The model included each land cover class as a separate predictor, rather than using a single reference category. As such, the estimates in Table 5 represent the independent association of each land cover type with *Legionella* seropositivity, adjusting for the presence of other land cover categories. Positive estimates indicate a higher likelihood of seropositivity in that land cover class, while negative estimates suggest a lower likelihood. For example, croplands and urban areas were consistently associated with increased seropositivity, whereas natural vegetation mosaics and grasslands were associated with reduced seropositivity for *L. pneumophila* SG 1–6.

L. longbeachae SG 1,2 was excluded from this analysis due to insufficient statistical power.

DISCUSSION

The aim of this study was to highlight demographic, environmental and temporal factors associated with Legionella IgG seropositivity to further understand environmental risk and to assess the impact that flooding events may have had on Legionella seropositivity at a population level. We hypothesized that there would be a higher seroprevalence of *L. longbeachae* antibodies detected in samples collected in 2023 compared with those gathered in 2016 [22]. The basis of our hypothesis was threefold: (1) there was an increase in flooding incidences across Queensland, particularly during 2021–2022 and *Legionella* spp. are known to be prevalent in water and soil environments; (2) Although the literature on long-term Legionella antibody presence is sparse, previous articles have suggested that detectable titers of Legionella antibodies in instances of prolonged exposure can be measurable for up to 10 years [27, 32]; (3) There was an increase in reported clinical cases of *L. longbeachae*, with a rise from 46 cases in 2021 to 106 in 2022 [33]. While total Legionella IgG seroprevalence was not different between 2016 and 2023, we detected a shift in specific species' seroprevalence. All species of *L. pneumophila* showed a decrease between these two time points, while *L. longbeachae* SG 1,2 seroprevalence increased. However, the number of positives detected was considerably lower than that of *L. pneumophila*. These results indicate that overall exposure to *Legionella* spp remained relatively constant over this time period but that exposure to *L. longbeachae* increased. This increase in seroprevalence could point to changing human behaviors that increase exposure to this species such as gardening or increased contact with potting soils [2]. We also observed an increase in *L. other* from 2016 to 2023. It is important to note that *L. other* is an amalgamation of *L. bozemanii* SG 1 and 2, *L. longbeachae* SG 1 and 2, *L. dumoffii*, *L. gormanii*, *L. micdadei*, and *L. jordanis*. Although all these species are found in water and soil, we cannot determine whether the increase is due to specific *Legionella* species or *L. longbeachae* within the group.

While there have been limited studies assessing the seroprevalence of Legionella antibodies in a cross section of the population, a systematic review of seroprevalence analysing 57 papers from 1990 to 2017 found an overall seroprevalence of 13.7%—including papers assessing populations of higher exposure risk [27]. When restricted to only studies of the general population, the seroprevalence of Legionella was 10.5%. Our results show a higher seroprevalence than many of the studies analysed in the systematic review. Whereas we have no clear reason why this might be the case, we do note that our study included a broader range of *Legionella* spp (*L. pneumophila SG 1–14*, and other) than most studies, giving our study a higher change of detecting seropositivity. Only one study in this systematic review specifically reported on *L. longbeachae*. This was in the Jeollanam-do Province, Korea [34].

There have been more studies focused on the incidence and causative spp of Legionella clinical cases globally. Scotland has reported an increase in cases of Legionnaires' disease caused by *L. longbeachae* since 2008, with peaks in 2013 and 2014 [35], which seems to be related to gardening with long exposure to soil and growing media [36, 37]. New Zealand has seen a recent shift, with infections from 2010 to 2020 being dominated by

Table 4. General linear model results for *L. pneumophila* SG 7–14 associated with climate variables

Term	Estimate	Standard Error	*P* value	Confidence Low	Confidence High
Intercept	−0.515	0.308	.0968	−1.12	0.0893
Annual Max Rainfall (mm)	−0.000209	0.000241	.389	−0.000682	0.000265
Annual Median Rainfall (mm)	0.00356	0.00177	.0464*	0.0000827	0.00703
Annual Coefficient of Variance	0.000528	0.000562	.349	−0.000574	0.00163
Annual Mean Temperature (°C)	0.0219	0.0143	.127	−0.00611	0.0498

Statistically significant *P* values are depicted by * when $P < .05$.

Table 5. Significant general linear model results for *L.* spp. associated with land cover categories

	Term	Estimate	Standard Error	*P* value	Confidence Low	Confidence High
L. pneumophila 1–6	Mosaics	−0.00472	0.00213	.0279	−0.00889	−0.000551
	Grasslands	−0.00496	0.00245	.0448	−0.00976	−0.000154
	Croplands	0.00800	0.00215	.000280	0.00378	0.0122
	Urban & built-up areas	0.00826	0.00251	.00122	0.00334	0.0132
L. pneumophila 7–14	Croplands	0.00700	0.00228	.00250	0.00253	0.0115
	Urban & built-up areas	0.00855	0.00266	.00155	0.00335	0.0138
L. other	Open Shrublands	0.00722	0.00183	.000118	0.00363	0.0108
	Grasslands	−0.00527	0.00246	.0340	−0.0101	−0.000440
	Croplands	0.00486	0.00217	.0264	0.000609	0.00910
	Urban & built-up areas	0.00844	0.00252	.00102	0.00350	0.0134

L. longbeachae, largely attributed to improvements in diagnostic methods, switching from serology and culture to PCR-based testing [38]. Peaks in *L. longbeachae* cases were identified in spring and summer, particularly in the >60 years age group, where gardening exposure was high [27].

Although cases of legionellosis are more often diagnosed in men than women [39–42], our analysis found no relationship between outcomes of seroprevalence across all species tested with gender. This is in agreement with other seroprevalence studies compiled in a meta-analysis of the seroprevalence of antibodies from 7 and 6 studies of male and female cases, respectively [27]. The reason behind this variation is unclear; however, there have been studies to suggest this occupation and lifestyle factors (such as smoking and alcohol consumption) may play a role [43, 44]. Additionally, previous studies have indicated that *Legionella* spp. are a cause of pneumonia within all age groups [45], consistent with our findings that show no association between age and seroprevalence; however, it must be noted that our study was limited to adults >18 years of age.

Further analysis of the rainfall and temperature data in each of the SA3 location found a relationship between seropositive outcomes for *L. pneumophila* SG 1–6, *L. pneumophila* SG 7–14 and annual median rainfall. This implies that a particular range or threshold of rainfall values influences seropositive outcomes while extreme values, such as maximum or minimum values do not have as strong an impact (Supplementary Figure 1). It has been previously noted that median rainfall has affected the incidence rates of Legionella induced pneumonia [46] and that an increase in total annual rainfall has been associated with an increase in risk of legionellosis [25].

Our data showed seropositivity was associated with certain SA3 Land Cover types, particularly Croplands and Urban areas, across all *Legionella* spp. tested. While literature is limited, a few studies have reported similar patterns. For example, Grabowska-Grucza & Kiersztyn, found that the diversity of the microbiome taxa in eutrophic lakes depends on seasons and contributes to an increase in the proportion of potentially pathogenic *Legionella* spp [47]. Another study by Peabody et al. collected monthly water samples from various land use sites in British Columbia, Canada. Metagenomic sequencing showed the highest relative abundance of Legionella in minimally impacted areas, including upstream of agriculture and a river feeding a drinking reservoir [48]. Interestingly this study did not find a correlation between urban environments and isolation of Legionella genomic material whereas our study found a positive association between urban environments and seroprevalence. However, it is important to note that seroprevalence is vastly different to evidence of the legionella pathogen. Taken together this demonstrated that land use is an important factor that contributes to the proliferation of *Legionella* spp.

This study, like most, is not without limitations. It assumes exposure occurred within the SA3 of residence, which may misattribute seropositivity if individuals were exposed elsewhere. The GLM models used to analyse geospatial and climate data are also sensitive to data that are not normally distributed. Efforts were taken to control for this by taking a log of Land Cover variables, however, it is possible that this could have influenced the

outcomes of these analyses. Furthermore, literature on land cover and Legionella seroprevalence is limited, especially for *L. longbeachae*. Due to only 18 positive samples across both years, this species was excluded from environmental analysis, as the low sample size lacked sufficient statistical power for meaningful conclusions. Finally, the demographic distributions for 2016 and 2023 are not perfectly matched, as the data were derived from random blood donors rather than a controlled sampling process. In addition, these samples were positive for multiple *L.* spps or SG, suggesting possible co-exposure that may reflect real-world environmental exposure to diverse strains. As a result, the outcomes are not entirely independent, which limits species-specific interpretation.

Overall, our study demonstrated that there was an increase in *L. longbeachae* SG 1,2 between 2016 and 2023. The increase in seroprevalence was correlated with an increase in annual rainfall, suggesting a potentially important risk factor for all the transmission of all Legionella SG in Australia [46]. This knowledge provides an insight into the risks associated with *Legionella* spp. exposure and as such should be considered by healthcare professionals when assessing higher risk locations for exposure particularly with vulnerable individuals such as the elderly, immunosuppressed or those with significant co-morbidities.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We would like to thank Dr Lauren Hartley for assistance with immunofluorescence scanners and Dr Thilini N Perera at Lifeblood for assistance in handling and collecting the samples for this study. We would also like to thank Michael Redlinger for his aid and expertise on GIS mapping software and helping navigate QGIS. Australian Governments fund Australian Red Cross Lifeblood for the provision of blood, blood products and services to the Australian community.

Author Contributions. Study design: L. J. H., M. K. Y., H. M. F. Study optimization: J. L., M. F. H., W. F., P. A. R. Specimen Collection: H. M. F., L. R., E. R. Data collection: J. L., W. F., H. M. F., L. R., E. R. Data Analysis and interpretation: J. L., E. B. S., M. K. Y. Writing: J. L., W. F., L. J. H., P. A. R., M. K. Y. Manuscript review and editing: L. J. H., M. K. Y., H. M. F., W. F., P. A. R., E. B. S., E. R., L. R. Supervision: L. J. H.

Financial support. This project received no specific funding. This project has not been presented in any previous conferences or meetings.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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