**Legionella pneumophila** serogroup 3 prevalence in drinking water survey in Israel (2003–2007)

Rachel Yarom, Rivka Sheinman and Robert Armon

**ABSTRACT**

A 5-year survey (2003 to 2007) was carried out on various drinking water sources in Israel for detection of *Legionella* spp. The most frequently isolated species was *L. pneumophila* serogroup 3 (prevalence 60.44 ± 5.6) followed by *L. pneumophila* serogroup 1 (26.98 ± 5.2) and other serogroups and unidentified species (12.6 ± 9.8). *L. pneumophila* serogroup 1 is the worldwide prevalent serogroup in water sources while serogroup 3 was only occasionally reported. Previous reports on *L. pneumophila* serogroup 1 as the main causative of respiratory disease in Israel revealed a drop from 52 to 15% in incidence during a 5-year survey, with a rise in the incidence of seropositivity to “other Legionellae”, mainly sg. 3. As antigenuria is the main clinical tool to detect Legionellosis among infected patients, the shift from serogroup 1 to serogroup 3 in water sources will go undetected for obvious reasons. The process of serogroups prevalence shift in water sources is an interesting issue that has to be investigated further in order to advance our understanding of *Legionella* epidemiology.

**Key words** | Israel, *Legionella pneumophila*, prevalence, serogroup 3, survey, water

**INTRODUCTION**

*Legionella* bacterium is an opportunistic environmental pathogen frequently isolated from hot drinking water. The main clinical manifestations of *Legionella* infection are acute pneumonia (called Legionnaires' disease) and Pontiac fever (a milder non-pneumonic flu-like syndrome). The bacterium is mainly isolated from domestic hot-water systems (Atlas et al. 1995), cooling towers (Wery et al. 2008), fountains (Hlady et al. 1993), and similar disseminators that tap into public water supply. Other natural sources of *Legionella* include freshwater ponds, creeks, oxidation ponds, etc. (Shelton et al. 2000). The first report on *Legionella* was published by Tatlock in (1944), later rediscovered by McDade during the outbreak among people attending a convention of the American Legion in Philadelphia (July 1976) (McDade et al. 1977). Since then an extensive number of publications have emerged on its distribution, identification, serogrouping, ecology, epidemiology, molecular biology and clinical aspects (Fliermans et al. 1981; Martinelli et al. 2000). Currently there are at least 50 *Legionella* species and approximately 70 serogroups known to us (Grattard et al. 2006). *Legionella pneumophila* serogroups prevalence around the globe reveals that the widely held isolated tap water serogroup is *Legionella pneumophila* serogroup 1 (Helbig et al. 2002; Tateyama et al. 2002; Mika et al. 2005; Harrison et al. 2007; Yu et al. 2008). Specific reports from countries around the Mediterranean Sea (Turkey, Greece, Croatia, Italy, France) also point to *Legionella pneumophila* serogroup 1 as the most prevalent isolate in the drinking water (Alexiou-Daniel et al. 1996; Kuzman 1996; Boccia et al. 2005; Campese et al. 2007; Polat et al. 2007; Chiarini et al. 2008). In Spain, Rivera et al. (2007) reported on higher prevalence of *Legionella* non-serogroup 1 in drinking water; however, their study was limited to health facilities while the present study survey span was much broader.

In Israel, Haifa Public Health laboratory (the only laboratory accredited and certified by the Israeli Ministry...
of Health on *Legionella*) carried out a voluntary 5-year survey on *Legionella* spp. prevalence in drinking water from 2003 to 2007. A variety of locations along the country, such as hospitals, hotels, old aged homes, mental institutions, sick peoples’ homes and other sites, were sampled and tested for the presence of *Legionella* spp. and serogroups in potable water. The present study describes the obtained results and their public health implications.

**MATERIALS AND METHODS**

**Sampling sites**

Geographical area covered by the present survey included the following major cities: Jerusalem, Tel-Aviv, Haifa, Eilat, Acre and Beer-Sheva. Water samples were mainly taken from sites such as hospitals, hotels, old aged homes and mental institutions, all characterised by a large population in contact with hot water from the same source. Other sites comprised certain water sources suspected of *Legionella* spp. contamination by the health authorities (Table 1).

**Sampling numbers and frequency**

The sampling frequency was as follows (year–samples’ number): 2003–484; 2004–340; 2005–468; 2006–942 and 2007–2,290. Positive samples for *Legionella* spp. were sampled again after 1 week. Monthly sampling frequency range was 25 to 216 according to Ministry of Health schedule.

**Sample preparation**

Prior to concentration, water samples (1 L) were dechlorinated by addition of 1 mL sodium thiosulfate (3.5 mg/L stock solution). The water samples were concentrated by centrifugation (6,000 × g) for 10 min, followed by supernatant discharge and precipitate left in the 5 mL water sample. The sample was vortexed (3 × 30 s) and directly plated on isolation medium (150 μL/plate) in duplicate. Dilutions were performed when needed with sterile potassium phosphate buffer (0.1 M, pH 7.0).

**Isolation medium and culture**

The isolation and identification methods were performed according to APHA methods (Anonymous 2005). Briefly: buffered charcoal yeast extract α-ketoglutarate medium

![Table 2 | Drinking water survey for *Legionella* spp. from 2003 to 2007](https://iwaponline.com/wst/article-pdf/10/5/746/416318/746.pdf)

<table>
<thead>
<tr>
<th>Sampling year</th>
<th>No. of samples tested</th>
<th>Positive samples for <em>Legionella</em> spp.</th>
<th>Positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>484</td>
<td>35</td>
<td>7.2</td>
</tr>
<tr>
<td>2004</td>
<td>340</td>
<td>51</td>
<td>15</td>
</tr>
<tr>
<td>2005</td>
<td>468</td>
<td>40</td>
<td>8.5</td>
</tr>
<tr>
<td>2006</td>
<td>942</td>
<td>172</td>
<td>18.2</td>
</tr>
<tr>
<td>2007</td>
<td>2,290</td>
<td>349</td>
<td>15.24</td>
</tr>
</tbody>
</table>

![Table 1 | Sampling distribution according to sites and year](https://iwaponline.com/wst/article-pdf/10/5/746/416318/746.pdf)

<table>
<thead>
<tr>
<th>Year</th>
<th>Hotels</th>
<th>Mental Institutions</th>
<th>Elderly Homes</th>
<th>Hospitals</th>
<th>Others*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>252</td>
<td>19</td>
<td>46</td>
<td>149</td>
<td>18</td>
</tr>
<tr>
<td>2004</td>
<td>71</td>
<td>33</td>
<td>5</td>
<td>87</td>
<td>144</td>
</tr>
<tr>
<td>2005</td>
<td>179</td>
<td>44</td>
<td>2</td>
<td>199</td>
<td>44</td>
</tr>
<tr>
<td>2006</td>
<td>250</td>
<td>16</td>
<td>52</td>
<td>273</td>
<td>351</td>
</tr>
<tr>
<td>2007</td>
<td>1,351</td>
<td>48</td>
<td>48</td>
<td>531</td>
<td>312</td>
</tr>
<tr>
<td>Total</td>
<td>2,103</td>
<td>160</td>
<td>153</td>
<td>1,239</td>
<td>869</td>
</tr>
</tbody>
</table>

*Sites such as: private homes of ill people with pneumonia; commercial centres; ritual baths; garages, etc.

![Figure 1](https://iwaponline.com/wst/article-pdf/10/5/746/416318/746.pdf)

**Figure 1 | *L. pneumophila* serogroups 1 and 3 distribution at specific sites during 2003–2007 survey.**
(BCYEα) (Oxoid, USA) was the base medium for isolation of *Legionella* sp. The medium was supplemented at 50°C before dispersion into Petri dishes with GPVA antibiotics (glycin, Polymyxin B, Vancomycin and Anisomycin) and mixed gently for 5 min. pH was adjusted with sterile 1 N KOH to 6.9 ± 0.05. From each processed sample, 0.15 mL volume was directly plated on BCYEα with a Drigalsky stick and incubated for 3–10 days at 35 ± 0.5°C in a 0.5% CO₂ incubator at 90% humidity. Opaque colonies that had a “ground-glass” appearance following the incubation period were plated again and tested by latex agglutination procedure. Gram staining of typical *Legionella* colonies

Figure 2 | Positive samples for *Legionella* and their monthly distribution (2003–2007).
following second incubation revealed short and hairy-like Gram negative rods (Armon et al. 1990).

**Data analysis**

Positive confirmed colonies on BCYEa media were counted and calculated per mL. Absolute numbers are important to understand the measure of contamination. In the present study, data is presented as positive/negative presence of legionellae and types of Legionella serogrouping without relation to numbers level.

**Legionella species and serogroups identification**

Typical colonies were picked and further analysed for species and serogroups by Legionella Latex Test (DR 0800) (Oxoid, UK) (Uzel et al. 2005). The kit consists of blue latex particles sensitised with specific rabbit antibodies reactive with Legionella pneumophila serogroup 1 antigen, serogroups 2–14 (as a whole group) and the following species and serotypes: L. longbeachae 1 and 2, L. bozemanii 1 and 2, L. dumoffii, L. gormanii, L. jordanis, L. micdadei, L. anisa.

For a more detailed analysis of L. pneumophila serogroups 2–14, Prolex TM kit (Prolab Diagnostics, Canada) was used to differentiate between the specific serogroups.

**RESULTS**

Table 2 summarises the percentage of positive samples for Legionella spp. isolated from various water sources. The years 2003 and 2005 revealed a frequency of 7.2 to 8% of positive samples respectively while 2004, 2006 and 2007 were > 15%.

Table 3 | Prevalence of Legionella serogroups in positive drinking water samples tested during 2003–2007 survey

<table>
<thead>
<tr>
<th>Sampling Year</th>
<th>SG-1 (%)</th>
<th>SG-3 (%)</th>
<th>Other SG-s (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>20</td>
<td>51.4</td>
<td>28.6</td>
</tr>
<tr>
<td>2004</td>
<td>25.5</td>
<td>60.8</td>
<td>13.7</td>
</tr>
<tr>
<td>2005</td>
<td>24</td>
<td>57.1</td>
<td>18.9</td>
</tr>
<tr>
<td>2006</td>
<td>37</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>2007</td>
<td>28.4</td>
<td>69.9</td>
<td>1.7</td>
</tr>
<tr>
<td>(X) ± S.D.</td>
<td>26.98 ± 5.2</td>
<td>60.44 ± 5.6</td>
<td>12.6 ± 9.8</td>
</tr>
</tbody>
</table>

\(X\)—average.

Distribution of positive samples harbouring Legionella pneumophila serogroup 1 and 3 at selected sites that large populations are exposed to is shown in Figure 1. Overall, Legionella pneumophila serogroup 3 was predominant over serogroup 1.

Monthly distribution of all positive water samples for L. pneumophila serogroups and Legionella species along the 5-year survey did not reveal a certain pattern (Figure 2). During the months of May and June of the years 2003 and 2005 there was a sharp drop in positive samples; however, this behaviour cannot be correlated to any variable.

The main finding of the present survey was that Legionella pneumophila serogroup 3 was the most predominant serogroup isolated from these sources, ranging from 51.4 to 69.9% of the positive samples, while Legionella pneumophila serogroup 1 ranged from 20 to 37% (Table 3). Other isolated Legionella pneumophila serogroups were 5, 6, 12 and 14, as well several other Legionella species (not identified). An additional significant result observed along the survey was the decline of “other serogroups” from 29% in 2003 to 0 and 1.7% in 2006 and 2007 respectively (Table 3).

Among the various sites monitored for Legionella pneumophila prevalence, the most frequently contaminated were in the following order: hotels > hospitals > old aged homes > mental institutions > other different sites. These sites encompass many people and, except hotels, all shelter at-risk individuals. All have central drinking water systems (hot and cold) that are poorly maintained and not always...
properly disinfected, therefore promoting regrowth of *Legionella* bacterium.

**DISCUSSION**

*Legionella pneumophila* serogroup 3 was reported by several authors as a recognised pathogen causing severe pneumonia in old people (Sopena et al. 2007), patients from bone marrow transplant unit (Oren et al. 2002) and aerosol therapy procedures (nebulisers for medication delivery) and isolated from pericarditis (Luck et al. 1989), colon infection (Schmidt et al. 1990), extrathoracic organs of an immunosuppressed patient (Watts et al. 1980) and other nosocomial infections (Tram et al. 1990). Isakov et al. (1982) described a first nosocomial case of a 56-year-old man who developed a bilateral pneumonia and hemorrhagic pleural effusion. The diagnosis was confirmed by serological examination resulting in IgM and IgG antibody types to *L. pneumophila* serogroups 1 and 3.

The predominant prevalence of *Legionella pneumophila* serogroup 3 in water in Israel is peculiar in comparison with other countries in the area and around the globe (Cazalet et al. 2008). In a previous study, Boldur et al. (1999) reported on Legionella incidence from 1993 to 1997 as a cause of pneumonia in Israel. Isolation of *Legionella* from different water sources ranged from 7 to 70% with higher numbers in summer. Indirect immunofluorescent assay for 41 serogroups of *Legionella*, as the main diagnostic method used at the time, resulted in *Legionella* sg. 1 to be the most frequent cause of the disease, with an incidence of 52% in 1993 and decreasing to 15% in 1997. Significant to the present study, one of these authors’ observations was on an increase in the incidence of seropositivity to “other *Legionellae*” being “characteristic to Israel”. The authors suggested that larger studies on *Legionella* colonisation in water supplies and in air are needed in order to establish the risk of infection. Based on the Legionellosis reports from Israel, it is clear that *Legionella pneumophila* serotype 3 is the the predominant clinical strain (Anonymous 2009).

This observation raises an important question related to clinical tests carried out to detect *Legionella* in hospitalised persons. The common test for *Legionella* infection in hospitals is based on detection of *L. pneumophila* serogroup 1 antigenuria (Cunha 1998). The main drawback of this test is its inability to detect other serogroups, antigenuria. Therefore, if patients were infected with *L. pneumophila* serogroup 3 the clinical urine test will not reveal its presence. Other possibility is that coupled infections of serogroups 1 or others and serogroup 3 can occur simultaneously (Isakov et al. 1982), but only serogroup 1 will be detected in urine; therefore epidemiological data collected from hospitals based on antigenuria test underestimated the clinical cases where *L. pneumophila* sg. 3 is involved. A further outcome of the present study is the high prevalence of *Legionella pneumophila* serogroup 3 in water samples. Analysing the most recent graphic presentation of Legionellosis cases in Israel (available online in GIDEON expert system, Anonymous 2009), a significant rise from 24 cases in 2003 to 62 cases in 2007 can be observed, with a minor decrease between 2004 and 2005 and a major one between 2007 and 2008. Significant correlation was observed between Legionellosis cases and the prevalence of yearly positive samples (Figure 3). According to the available scientific evidence, it can only be speculated why serogroup 3 was the most prevalent isolate in the present study (2003–2007): 1) it is related to or impacted by a specific water treatment; 2) it is also present in other countries but the detection was insufficient; and 3) Israel has very few direct drinking water sources (Lake Kinneret and groundwater), favouring presence of a specific serogroup. Further studies are needed to understand the ecology of *L. pneumophila* serogroups in water and their direct association with clinical cases.

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**REFERENCES**


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