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Identification of *Legionella Pneumophila* Serogroups as Zoonotic Disease Agent Distributed in Water Sources of East Java

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Abstract

The aims of this study was to understand the differences of *L.pneumophila* serogroups distribution in well water, tap water, ice cubes, hospital water and hotel water in East Java-Indonesia. A total of 60 water samples were tested by polymerase chain reaction and then it was analyzed by phylogenetic tree. Out of the 60 water samples collected, 12% of the samples were contaminated with *L. pneumophila.* The phylogenetic tree revealed *L.pneumophila* contamination in well water from Surabaya and tap water from Sidoarjo and the ice cubes from Sidoarjo, while the bacterial contamination in 2 well water isolate from Surabaya classified into their own group.

Key words: *L.pneumophila*, serogroup contamination distribution.

Legionella spp. are gram-negative bacteria, found in nature, and spreads by air upto 300 m radius. These bacteria are zoonotic and, infect the lungs leading to pneumonia which can be life-threatening, by inhalation of contaminated droplets of water (Ghotaslou *et al*, 2013). These bacteria thrives well in any environments; 56% in domestic hot water system, 44% in water cooling system and their ideal conditions are at the pH 6.9 and temperature 35-37°C with availability of ferric oxide as nutrients in the water. The bacteria are prevalent in any buildings with cooling and hot water system such as in office building and hotels. That is why the illness caused by the bacteria is also called "sick building syndrome".

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Materials and Methods

60 samples of water (200ml) were collected from different environments in Surabaya-Sidoarjo-Kediri East Java such as well water (25), tap water (5), ice cubes (5), hospital water (16), and hotel water (9). Samples were put in a sterile vials then filtered using a Millipore membrane 0.22 µm, and they were put in a 50 ml conical flask and rinsed with 1 ml Phasphate Buffer solution (PBS) vortex for 10 minutes. 1 ml of this was transferred into into

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Table I. Presence of *Legionella* bacteria in water samples from East Java by PCR

Origin of Samples	Results		Species
	Negative	Positive	
Well water	23/25	2/25	*L. pneumophila*
Tap water	4/5	1/5	*L. pneumophila*
Ice cubes	4/5	1/5	*L. pneumophila*
Hospital water	16/16	0/16	*L. pneumophila*
Hotel water	6/9	3/9	*L. pneumophila*
TOTAL	53/60	7/60	*L. pneumophila*

eppendorf tubes, and centrifuged at 13000 rpm for 3 minutes. The supernatant was discarded and the sediment was used for DNA extraction using DNA extraction kit (QIAamp®DNA mini kit Qiagen) as per the manufacturer's instructions. Phylogenetic analysis was done by using software Genetix Mac Ver. 10.0

Results and Discussion

Table I showed that using PCR method for the 60 water samples collected in East Java, 12% of the samples (7/60) were contaminated by *L. pneumophila.* In details, 8% of the well water samples (2/25), 2% of the tap water samples (1/5), 2% of the ice cubes samples(1/5), 0% of the hospital water samples (0/16) and 33.33% of the hotel water samples (3/9) were positive for *L. pnuemophila.*

Results of Phylogenetic analysis showed that *Legionella pneumophila* contamination from well water was 1 from Surabaya and in tap water from Sidoarjo which were closer to *L.pneumophila* serogroup 2, 3, 4, 6, 9, 10, 12, isolates reported from Brazil, China, Spain and Australia. *L.pneumophila* contamination in the ice cubes from Sidoarjo were closer to serogroup1, 7, 8, 11, 13, 14, while the bacteria contaminating well water were 2 and water from hotels of Surabaya (hotel water isolate 1, 2 and 3).

The *Legionella* bacteria contamination was found in little quantity (>1,000 CFU/liter) (Qin *et al,* 2012) in tap water and it is not dangerous, (Aksono and Hermadi, 2017). However, they can multiply fast in flood waters.

The infection is transmitted through water spray or aerosol contaminated with *Legionella* microbes. The infected person will fall ill after five or six days from the exposure. *Legionella* infection risk is less in small houses than the big building. In family housing, the infection was found only 6-30%. Prevention of *L.pneumophila* transmission could be contained by added chlorine >2 mg/liter of water (Moran-Gilad *et al,* 2014;Sánchez-Busó*et al,* 2015).

In this research, *L.pneumophila* distribution of serogroups from the natural water (such as well water) was different from different water source (such as water tank and drinking water system in hotels). These results are in line with the previous research conducted in Japan and Canada (Amemura-Maekawa *et al,* 2012; Reimer *et al,* 2009), they may correlated with water source characteristic differences (such as,

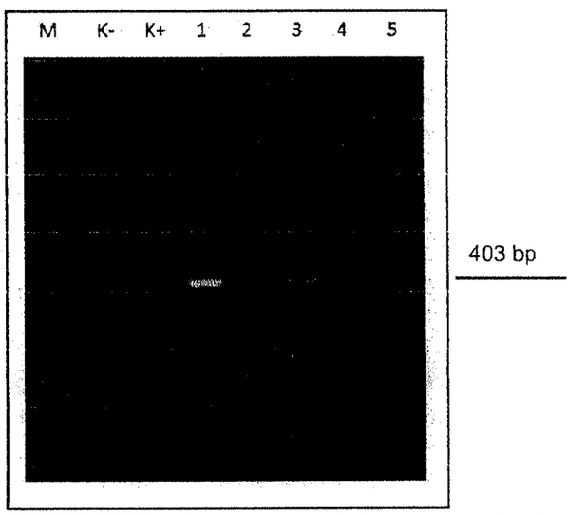

Fig 1. Results of PCR *L.pneumophila* in water samples from East Java by electrophoresis gel in 2% (403 bp). M (marker); K+ (positive control); K-(negative control); 1 (positive from tap water); 3 (positive from ice cubes).

temperature and pH level). There are various types of water sources, while cooling tower and drinking water system tend to have similar characteristics due to same water treatments. *Legionella* has high prevalence, due to their faster intracellular growth, and in diverse hot water sources (Qin *et al*, 2013).

L. pneumophilla isolated from hot water tank in hotels had higher genetic diversity, compared with natural water and drinking water. This result may be related to growth in amoeba which adapts in different environment. It has been reported that the growth of *L. Pneumophila* in amoebae host depends on the bacterial genetics (Buse *et al*, 2012; Dey *et al*, 2009).

Summary

L.pneumophila serogroups isolated from environment water in East Java had a closer corelation with serogroups from Brazil, China, Spain and Australia and some samples were comparable to the local serogroups.

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