CORRESPONDING AUTHOR

Judul :

Identification of Legionella pneumophila serogroups as Zoonotic Disease Agen Distributed in Water Sources of East Java

Penulis Utama dan Corresponding Author : Eduardus Bimo Aksono

Nama Jurnal : The Indian Veterinary Journal. Vol.96. No.12. Desember 2019

Bukti :

eduardus bimo aksono h <eduardus-b-a-h@fkh.unair.ac.id>
to Ind

Dear Editor
Indian Veterinary Journal, Chennai

We as the authors of the article entitled "**Identification of Legionella *pneumophila* serogroups as zoonotic diseases agents distributed in environment's water in East Java**" submitted for publication in *Indian Veterinary Journal.* Hereby declare on our own responsibility that all data we have included in the previously mentioned manuscript are original, have not been and will not be published elsewhere.

For whom it may concern (clinical studies and experimental research on laboratory animals). We also state that all procedures and experimental protocols comply with the current guidelines

Corresponding Author,

Eduardus Bimo

Airlangga University, Surabaya – Indonesia.
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36 of 45

Acknowledgement Letter # 207/19 External Inbox x

Ind Vet Journal <ivj83@yahoo.com> Tue, Jun 4, 2019, 12:16 PM

to me

ACKNOWLEDGEMENT

Reg. No: 207/19 Dated : 4/06/2019

Dear Dr. Eduardus Bimo Aksono,

We acknowledge the receipt of the following articles entitled "Identification of Legionella Pneumophila Serogroups as Zoonotic Disease Agent Distributed in Environtment's Water in East Java." (Eduardus Bimo Aksono, et al.).

For any further correspondence, please always quote the Registration Number of the Article.

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1 of 630

Sir / Madam,

Revise the paper according to the referee's comments and corrections marked on the manuscript. Resubmit the revised article as per IVJ format for further action.

Sincerely

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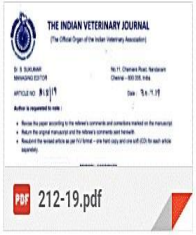

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Revised Guidelines...

THE INDIAN VETERINARY JOURNAL

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Dr. S. SUKUMAR
MANAGING EDITOR

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ARTICLE NO: 212/19 Date : 30.7.19

Author is requested to note :

- Revise the paper according to the referee's comments and corrections marked on the manuscript.
- Return the original manuscript and the referee's comments sent herewith.
- Resubmit the revised article as per IVJ format – one hard copy and one soft (CD) for each article separetely.

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1) The abstract should not exceed 100 words.
2) Introduction without subtitle to be Confined to 5lines.
3) References should follow IVJ format. It should be arranged in alphabetical order. Correct year should be furnished
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Acceptance Letter # 207/19

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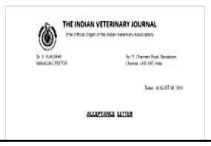

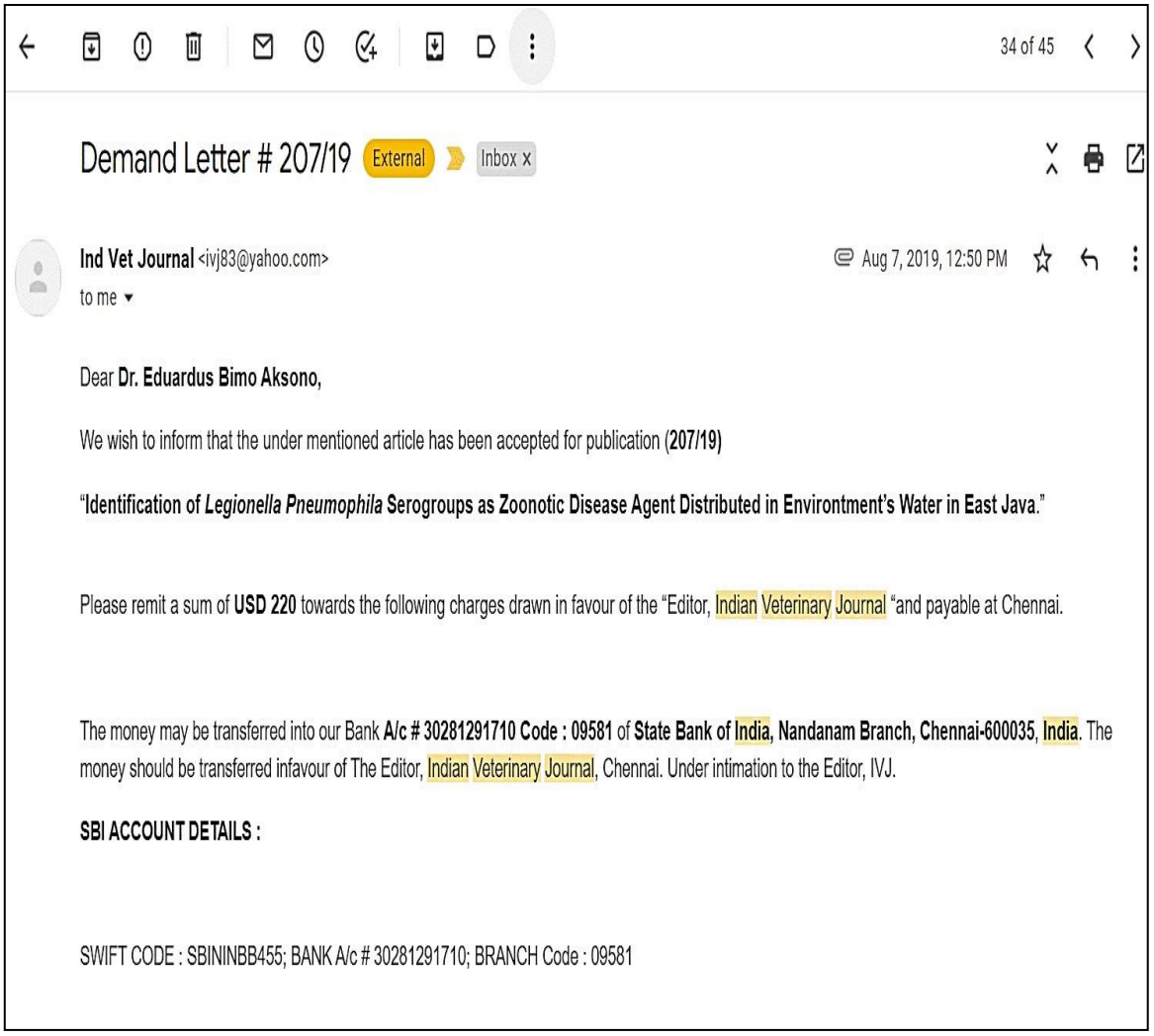

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to me

Aug 7, 2019, 12:50 PM

Dear **Dr. Eduardus Bimo Aksono,**

We wish to inform that the under mentioned article has been accepted for publication (**207/19**)

"**Identification of *Legionella Pneumophila* Serogroups as Zoonotic Disease Agent Distributed in Environtment's Water in East Java.**"

Please remit a sum of **USD 220** towards the following charges drawn in favour of the "Editor, Indian Veterinary Journal "and payable at Chennai.

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MANAGING EDITOR

No.11, Chamiers Road, Nandanam,
Chennai – 600 035, India.

Dated : AUGUST 08, 2019

ACCEPTANCE LETTER

The following article has been accepted and will be published in DECEMBER, 2019 issue of Indian Veterinary Journal.

Article No.	Title	Author (s)
207/19	Identification of *Legionella Pneumophila* Serogroups as Zoonotic Disease Agent Distributed in Environment's Water in East Java	Eduardus Bimo Aksono, Kadek Rachmawati, Retno Bijanti, Heriyda Pertiwi

Sd/-

Managing Editor,
Indian Veterinary Journal

To,

Dr. Eduardus Bimo Aksono
Institute of Tropical Disease,
Faculty of Veterinary Medicine,
Faculty of Vocational Studies
Universitas Airlangga -
Kampus C Mulyorejo
Surabaya, Indonesia. 60115
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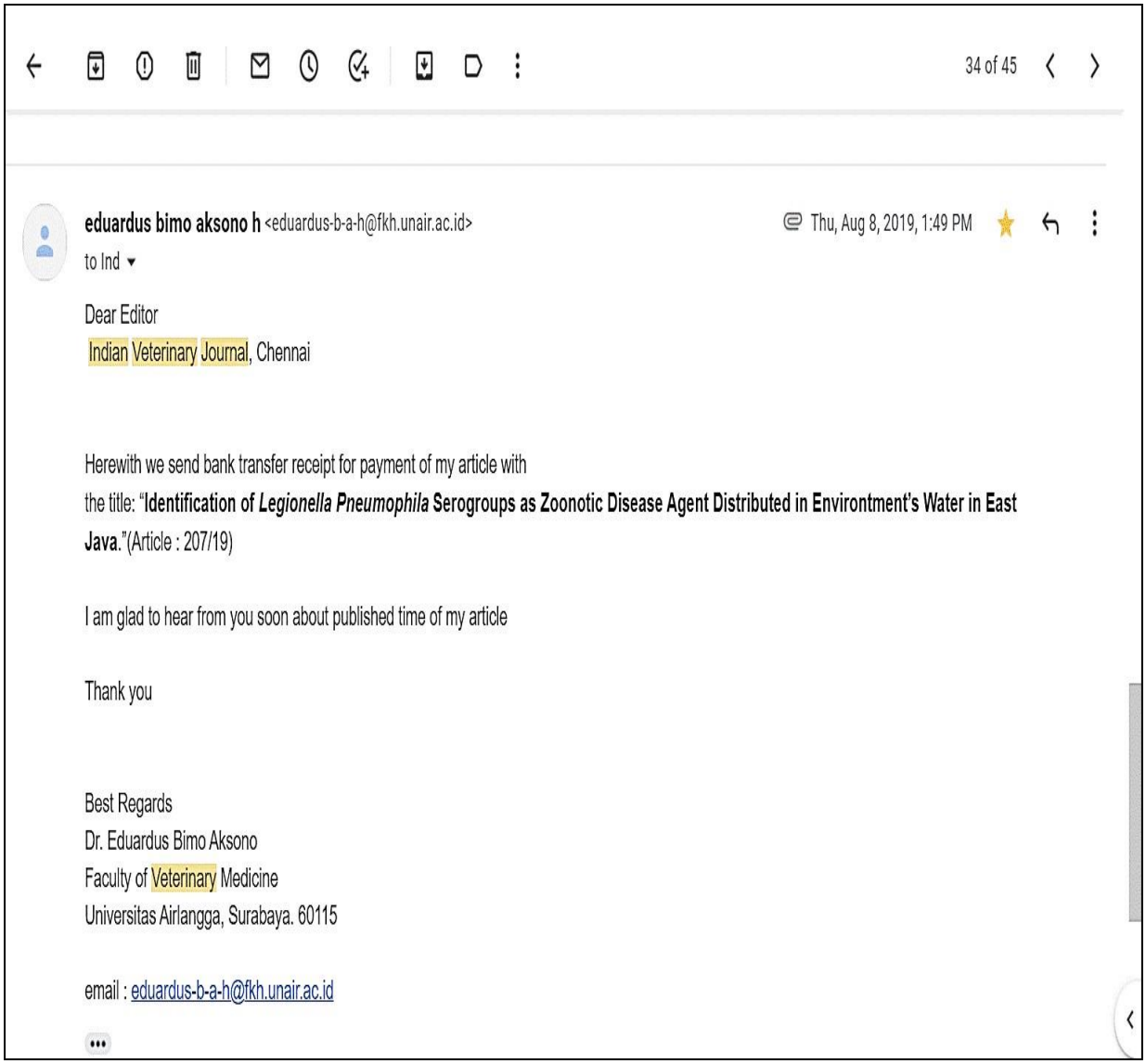

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Thu, Aug 8, 2019, 1:49 PM

to Ind

Dear Editor
Indian Veterinary Journal, Chennai

Herewith we send bank transfer receipt for payment of my article with
the title: "**Identification of *Legionella Pneumophila* Serogroups as Zoonotic Disease Agent Distributed in Environtment's Water in East Java.**"(Article : 207/19)

I am glad to hear from you soon about published time of my article

Thank you

Best Regards
Dr. Eduardus Bimo Aksono
Faculty of Veterinary Medicine
Universitas Airlangga, Surabaya. 60115

email : eduardus-b-a-h@fkh.unair.ac.id

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Demand Letter # 207/19

Ind Vet Journal <ivj83@yahoo.com> Wed, Aug 7, 2019 at 12:50 PM
Reply-To: Ind Vet Journal <ivj83@yahoo.com>
To: "eduardus-b-a-h@fkh.unair.ac.id" <eduardus-b-a-h@fkh.unair.ac.id>

Dear **Dr. Eduardus Bimo Aksono,**

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Identification of *Legionella Pneumophila* Serogroups as Zoonotic Disease Agent Distributed in Environtment's Water in East Java

Eduardus Bimo Aksono, Kadek Rachmawati, Retno Bijanti, Herinda Pertiwi

Institute of Tropical Disease, Faculty of Veterinary Medicine, Faculty of Vocational Studies
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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 detected using polymerase chain reaction and then it was analyzed by phylogenetic tree. For the 60 water samples collected in East Java, 12% of the samples were positively contaminated by L. pneumophila. The phylogenetic tree showed that *L.pneumophila* contaminating well water isolated from Surabaya and tap water isolate from Sidoarjo was closer to *L.pneumophila* serogroups 2;3;4; 6;9;10;12 from Brazil, China, Spain and Australia. *L.pneumophila* contaminating the ice cubes from Sidoarjo was closer to serogroups 1;7;8;11;13;4, while the bacteria contaminating in 2 well water isolate from Surabaya classified into their own group.

Keywords: bacteria, environtment's water, *L.pneumophila*, serogroup,

Legionella spp. are gram-negative, found everywhere in nature, and spread aerogenically until 300 m radius. These bacteria are zoonotic disease agent, infect the lungs and cause symptoms of pneumonia and they can be a life-threatening after inhalation of contaminated small droplets of water in the air from various water sources (Ghotaslou *et al*, 2013). These bacteria grow well in any environments; 56% in domestic hot water system, 44% in cooling water system and the ideal condition is in the pH 6.9 and temperature 35-37^{o}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 offices building and hotels. That is why the illness caused by the bacteria is also called "sick building syndrome".

materials and Methods

60 samples of clean water (each 200ml) were collected from the environments in Surabaya-Sidoarjo-Kediri East Java isolated from well water (25 samples), tap water (5 samples), ice cubes (5 samples), hospital water (16 samples), and hotel water (9 samples). Samples were put in a sterile vial then filtered using a Millipore membrane 0.22 μm, after that they were put in a 50 ml conical tube and rinsed with 1 ml PBS vortex for 10 minutes. 1 ml was taken and transferred into into eppendorf tubes. Then, it was centrifuged at 13000

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rpm for 3 minutes. The supernatant was discarded and pellet was ready to be used for DNA extraction using a DNA extraction kit (QIAamp®DNA mini kit Qiagen) following the manufacturer's instructions. Phylogenetic analysis did by using software Genetix Mac Ver. 10.0

Results and Discussion

Table 1. Results of *Legionella* bacteria detection in water samples from East Java by PCR

Samples Origin	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*

Table 1 and figure 1 showed that using PCR method, from the 60 water samples collected in East Java, 12% of the samples (7/60) were positively contaminated by L. pneumophila. In details, there was 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).

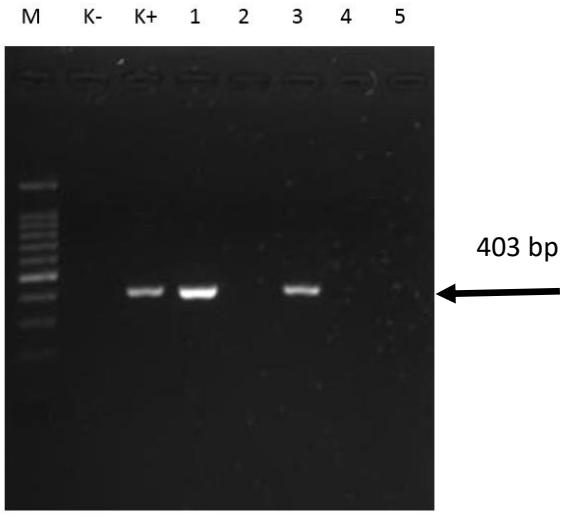

Figure 1. Results of PCR *L.pneumophila* in water samples from East Java by electrophoresis gel in 2% (403 bp). M (marker); K+ (positive control); K-(negative control); 1 (positive from tap water); 3 (positive from ice cubes)

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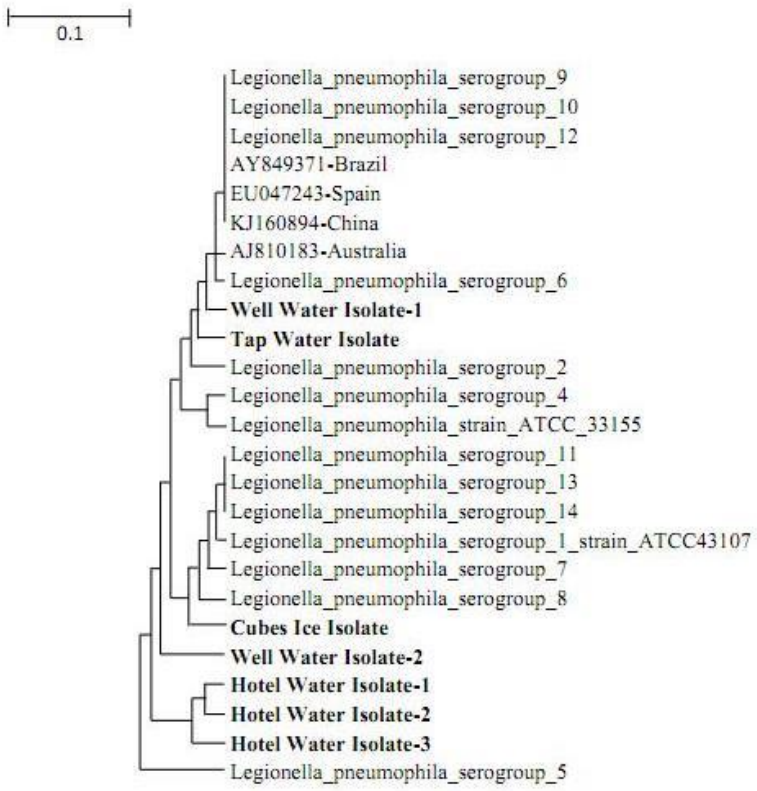

Figure 2. Results of phylogenetic analysis of *L.pneumophila* from water samples in the environments of East Java

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

The result showed that 33.33% of the hotel water samples were contaminated by *L. Pneumophila*. There was 8% contamination found from well water samples, 2% contamination found from tap water samples and 2% contamination found from ice cubes. Eventhough the *Legionella* bacteria contamination was quite high, it was only found a little in quantity (>1,000 CFU/liter) (Qin *et al*, 2012) and it is not dangerous in the water, including in tap water (Aksono and Hermadi, 2017). However, they may multiply fast in inundated water.

The infection is transmitted through water spray or aerosol containing *Legionella* microbes. The infected person will fell ill after five or six days from the infection. *Legionella*

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infection risk in family housing is less than in big building. In family housing, the infection found only 6-30% (Byrne *et al*, 2018). Prevention of *L.pneumophila* transmission could be conducted by added chlorine >2 mg/liter of water (Moran-Gilad *et al*, 2014; Sánchez-Busó *et al*, 2015).

In this research, *L.pneumophila* serogroups distribution from the natural water (such as well water) was different from artificial 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, temperature and pH level). There are various characteristics of water sources, while cooling tower and drinking water system tend to have similar characteristics due to same water treatments. *Legionella* has high prevalence, fast intracellular growth, and diverse genetics in hot water source (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 amoeba host which adapts in different environment. It has been reported that the growth of *L. pneumophila* in amoeba host depends on the bacterial genetic (Buse *et al*, 2012; Dey *et al*, 2009).

Summary

L.pneumophila serogroups distribution isolated from environment water in East Java had a closer corelation with serogroups from Brazil, China, Spain and Australia altough some sample was catagorized including in the local serogroups.

Acknowledgement

We would like to express our gratitude to Rector of Universitas Airlangga and the Ministry of Research, Technology and Higher Education for PDUPT research grants given in 2018.

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