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Case Report

Detection of Pathogenic Leptospira in Sputum of Leptospirosis Patient with Pulmonary Hemorrhage

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Abstract

Background: The pathogenic *Leptospira* species is the causative agent of leptospirosis, an endemic zoonotic disease in Indonesia. Misdiagnosis of the disease frequently occurs, as confirmatory diagnosis confined to highly specialized laboratories. As well, the pulmonary involvement of leptospirosis with hemoptysis is scarcely reported.

Case Presentation: A 49 years-old male patient was admitted to the district hospital with acute febrile illness and a history of traveling to a malaria-endemic area in Borneo, Indonesia. Based on a chest X-ray result, the patient was clinically suspected to have pulmonary tuberculosis. However, the clinical manifestations of leptospirosis i.e. conjunctival suffusion, calf pain, and oliguria were present, and later hemoptysis was also reported. A clinical diagnosis of leptospirosis with pulmonary involvement was proposed. Immunochromatographic test (ICT)-rapid test for vivax/falciparum malaria and Ziehl-Neelsen (ZN) staining of sputum for tuberculosis results were both negative. Microscopic Agglutination Test (MAT), the IgM anti-*Leptospira* rapid test (lateral flow assay), and PCR amplification of both conventional and real-time (qPCR) were performed using various samples (serum, urine, and sputum). The MAT of acute single serum sample and rapid test were negative. Intriguingly, the PCR showed positive results in sputum and urine samples but not in the serum sample, highlighting the usefulness of leptospiral molecular detection to confirm further diagnosis.

Conclusion: Molecular detection of pathogenic *Leptospira* in sputum samples can be considered for confirmatory diagnosis of leptospirosis patients with pulmonary hemorrhage. Likewise, the urine sample can be used as an option in the examination of severe leptospirosis.

Keywords: *Leptospirosis; Pulmonary Hemorrhage; Hemoptysis; Sputum*
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INTRODUCTION

Leptospirosis is a zoonosis endemic worldwide. The clinical features are mostly a typical acute undifferentiated fever and flu-like syndrome, which often misdiagnosed as other more prevalent (tropical) infectious diseases. About 10-20% of leptospirosis cases are severe icteric cases with multi-organ involvement including lung and kidney[1]. The symptoms of pulmonary

Table 1. The panel group f *Leptospira* serovars used in this study.

Serogroup	Serovar	Strain
Autumnalis	Bangkinang	Bangkinang I
Bataviae	Bataviae	Swart
Canicola	Canicola	Hond Utrecht IV
Djasiman	Djasiman	Djasiman
Grippotyphosa	Grippotyphosa	Moskva V
Hebdomadis	Hebdomadis	Hebdomadis
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Manhao	Manhao	L1-130
Mini	Mini	Sari
Pomona	Pomona	Pomona
Pyrogenes	Pyrogenes	Salinem
Pyrogenes	Robinson	Robinson
Sarmin	Sarmin	Sarmin
Sejroe	Hardjo	Hardjoprajitno
Tarassovi	Rama	316

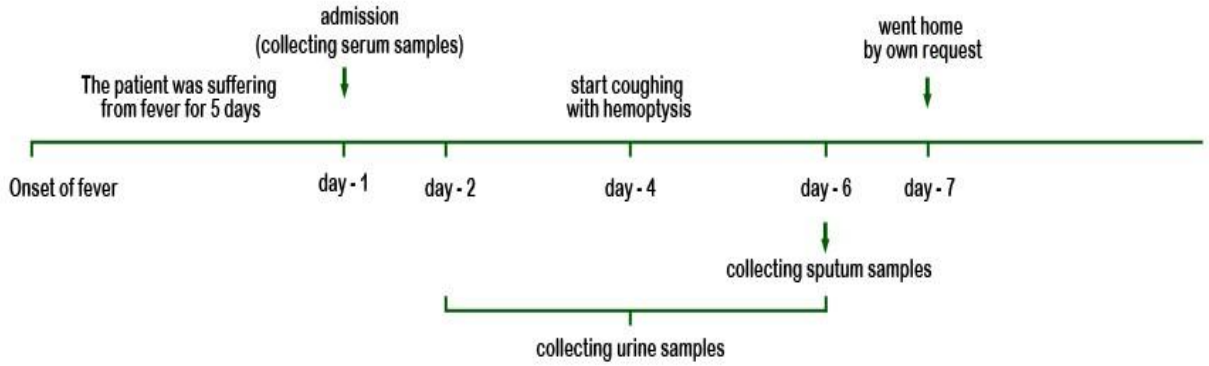

Figure 1. Sample collection procedures for confirmatory diagnostic of leptospirosis patient with pulmonary involvement.

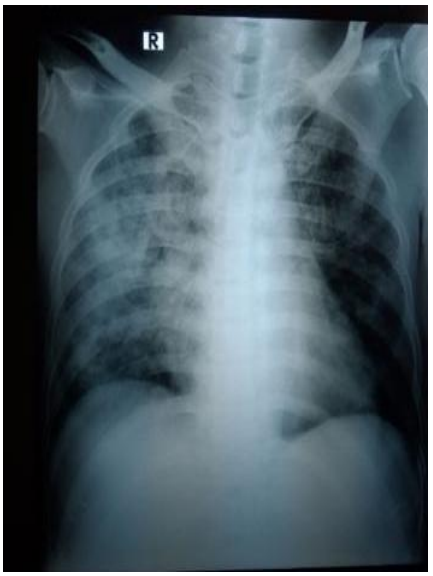

Figure 2. Chest X-ray on admission with bilateral lung infiltrates and localized opacities on upper right lung field suggesting tuberculosis and pulmonary involvement in leptospirosis.

involvement in leptospirosis are widely varied, ranging from cough, dyspnea, hemoptysis, pneumonia-like illness, pulmonary hemorrhage, acute respiratory distress syndrome (ARDS) as well as respiratory failure. Hemoptysis has been reported in 17-50% of leptospirosis patients with pulmonary involvement [2,3]

We report a patient with clinical suspicion of severe leptospirosis with pulmonary hemorrhage. This report was a part of our study in developing an antigen-capture immunoassay for point-of-care (POC) diagnosis for leptospirosis.

CASE REPORTS

A 49 years-old male patient, originating from a malaria-endemic area in Borneo – Indonesia, was admitted to the Demak district hospital with acute febrile illness. He works as a drainage worker and he had an occupational accident in which his palm was pierced with the tip of the pipe steel when he made a metal structure for the drainage work.

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Table 2. Genetic sequence accessing numbers of partial *secY* genes of *Leptospira* obtained from patient sputum and other *Leptospira* strains used in the phylogenetic tree construction.

No	Strain	Species	Accessing number
1	71161S	*L. interrogans*	ON968435
2	Moskva V	*L. kirschneri*	EU358028.1
3	MORU UD009	*L. interrogans*	JF509199.1
4	K5	*L. kirschneri*	EU358044.1
5	IP1506001	*L. borgpetersenii*	MH325388.1
6	TRVL 34056	*L. santarosai*	EU358035.1
7	9160	*L. noguchii*	EU358068.1
8	2014MD FMNH 229014	*L. mayottensis*	KT338851.1
9	2014MD UADBA VS-2474	*L. mayottensis*	KT338850.1
10	B 81/7	*L. kirschneri*	EU358018.1
11	3522 C	*L. kirschneri*	EU358027.1
12	U278	*L. santarosai*	KP862645.1
13	Musa	*L. kirschneri*	EU358020.1
14	LT 1026	*L. interrogans*	EU358017.1
15	Cox	*L. weilii*	EU358009.1
16	RGA	*L. interrogans*	EU357997.1
17	Peludo	*L. noguchii*	EU357960.1
18	Van Tienen	*L. interrogans*	EU357956.1
19	M 7	*L. noguchii*	EU357950.1
20	Jez Bratislava	*L. interrogans*	EU357939.1
21	Hardjoprajitno	*L. interrogans*	CP013147.1
22	Hond HC	*L. interrogans*	EU357984.1
23	136/2/2	*L. interrogans*	EU357970.1
24	bandicoot 343	*L. meyeri*	EU358034.1
25	H6	*L. Inadai*	EU357968.1
26	C72/2017	*L. Interrogans*	MN394913.1

He further developed five days of fever followed by shortness of breath, headache, myalgia, and oliguria. The patient also had conjunctival suffusion, gastrocnemius pain (calf pain), stomach pain, cough, hemoptysis, and dyspnea.

The laboratory results were hemoglobin 12 g/dL, white blood cell count 18,200/μL, platelet count 22,000/μL, blood ureum 148.5 mg/dL, serum creatinine 5.4 mg/dL, aspartate transaminase (AST) 310 U/L, alanine transaminase (ALT) 237 U/L and a non-reactive IgM anti-*Leptospira* rapid-test. Diagnosis of pulmonary tuberculosis was suspected based on a chest X-ray examination. Combining the relevant medical history, laboratory, and radiology findings, a clinical diagnosis of leptospirosis with pulmonary involvement (pulmonary hemorrhage) was made. The samples obtained from the patient were evaluated for three suspected infections including pulmonary tuberculosis, malaria, and leptospirosis. *Falciparum/Vivax* malaria immunochromatographic test (ICT) and Ziehl-Neelsen (ZN) sputum staining were performed and both were negative. Serum specimen was collected on admission day (the patient had been suffering from fever for 5 days before admission), serial urine samples were collected during hospitalization (days 2nd – 6th on admission) and sputum samples were collected when this patient developed hemoptysis (day 6th on admission).

Confirmatory laboratory diagnosis of leptospirosis

A gold standard Microscopic Agglutination Test (MAT) was performed to detect anti-*Leptospira* antibodies in serum. However, in this case, we only obtained a single serum of the patient from the first day of admission. Both conventional and real-time polymerase chain reaction (PCR) tests were performed to detect pathogenic *Leptospira* in serum, urine and sputum samples. The amplicon products of gel-based PCR were sent to the 1st Base agency in Malaysia for sequencing. The sequence accessing number of the patient sample and the *Leptospira* strains used to construct the phylogenetic tree (Figure 2). The phylogenetic tree was developed using MEGA X software with 1000 bootstrap replications. Nodes with bootstrap trusts below 70% are condensed in the presented phylogenetic tree[4].

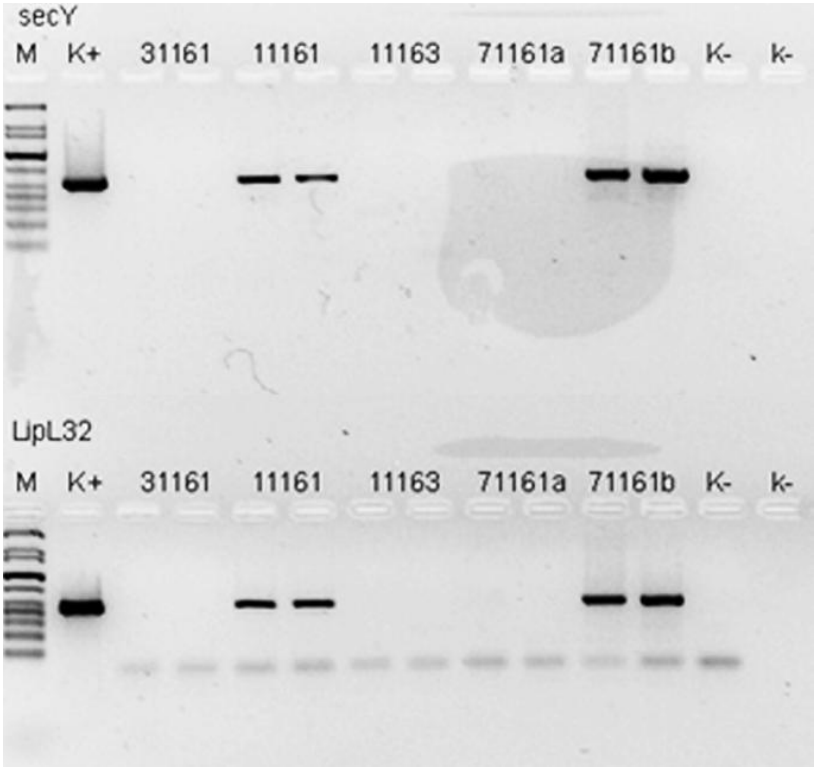

Figure 3. Agarose gel stained with SYBR safe showing PCR products. Lane M: Molecular size marker 100 bp DNA Ladder. Lane K+: Positive control (*L. interrogans* serovar Icterohaemorrhagiae, strain RGA), Lane 31161: serum samples (in duplicate), Lane 11161: urine samples day-1 (in duplicate), Lane 11163: urine samples day-3 (in duplicate), Lane 71161a: sputum samples (batch 1, in duplicate), Lane 271161b: sputum samples (batch 2, in duplicate), and Lane K-: Negative samples

DISCUSSION

In this case report, we describe pulmonary involvement in a leptospirosis patient with a confirmatory diagnosis using a molecular detection of pathogenic leptospiral DNA from sputum and urine samples. The CXR findings were bilateral infiltrates and localized opacities on the right upper lung field, which were commonly observed in pulmonary TB. Previous tuberculosis (TB) treatment history, followed by a chest X-ray (CXR) feature of tuberculosis pattern, has confused clinicians in accurately identifying the etiology of the hemoptysis (Figure 1). Clinical suspicion of pulmonary TB as a possible cause of hemoptysis was ruled out using ZN staining of the sputum. The patient was further clinically diagnosed to have leptospirosis complicated by pulmonary involvement with dyspnea, cough, and hemoptysis later. The incidence of leptospirosis pulmonary hemorrhage has been increasingly reported with varied symptoms, ranging from nonspecific chest pain, cough, dyspnea, and hemoptysis, as well as severe community-acquired pneumonia (CAP) or ARDS[5]. As previously reported, pathogenic *Leptospira* was confirmed using molecular detection (qPCR) in leptospirosis patients with pulmonary involvement with an initial diagnosis of CAP[6]. The mortality rates of leptospirosis with pulmonary hemorrhage can high as 30-60%, although this patient survived upon appropriate clinical management[7,8].

The MAT as a gold standard test should be performed on paired serum samples, however, in this case, we only obtained a single serum of the patient from the first day of admission, approximately 5 days after fever onset. One study mentioned that performing MAT during the early phase of infection or in the first week of infection will not give any diagnostic value, particularly in high-endemic areas[9]. Although considered as the immunological gold standard test, MAT also has low sensitivity especially if performed in the early stages of the disease due to the under-detection of anti-*Leptospira* antibodies. Importantly, using the paired sera of acute and convalescent phases will further increase its sensitivity[10]. The MAT is relevant for both clinical and epidemiological studies only if the seroconversion results are available[9].

The positive PCR results of sputum and urine of the patient PCR indicated that the pathogen had migrated from the bloodstream to the organs, lungs, and kidneys. *Leptospira* dissemination can be fast and effective due to its ability to escape or hijack the host immune system recognition[11]. In this report, PCR determines the presence of DNA of pathogenic *Leptospira* both in the sputum and urine of patient. *SecY* is a housekeeping gene encoding preprotein translocase, which consists of alternating and conserved sequences. Therefore, it has an adequate sequence heterogeneity for *Leptospira* phylogenetic analysis. Furthermore, using the Maximum Likelihood analysis, our results suggested that the isolate belongs to the *Leptospira interrogans* species (Figure 2)[12].

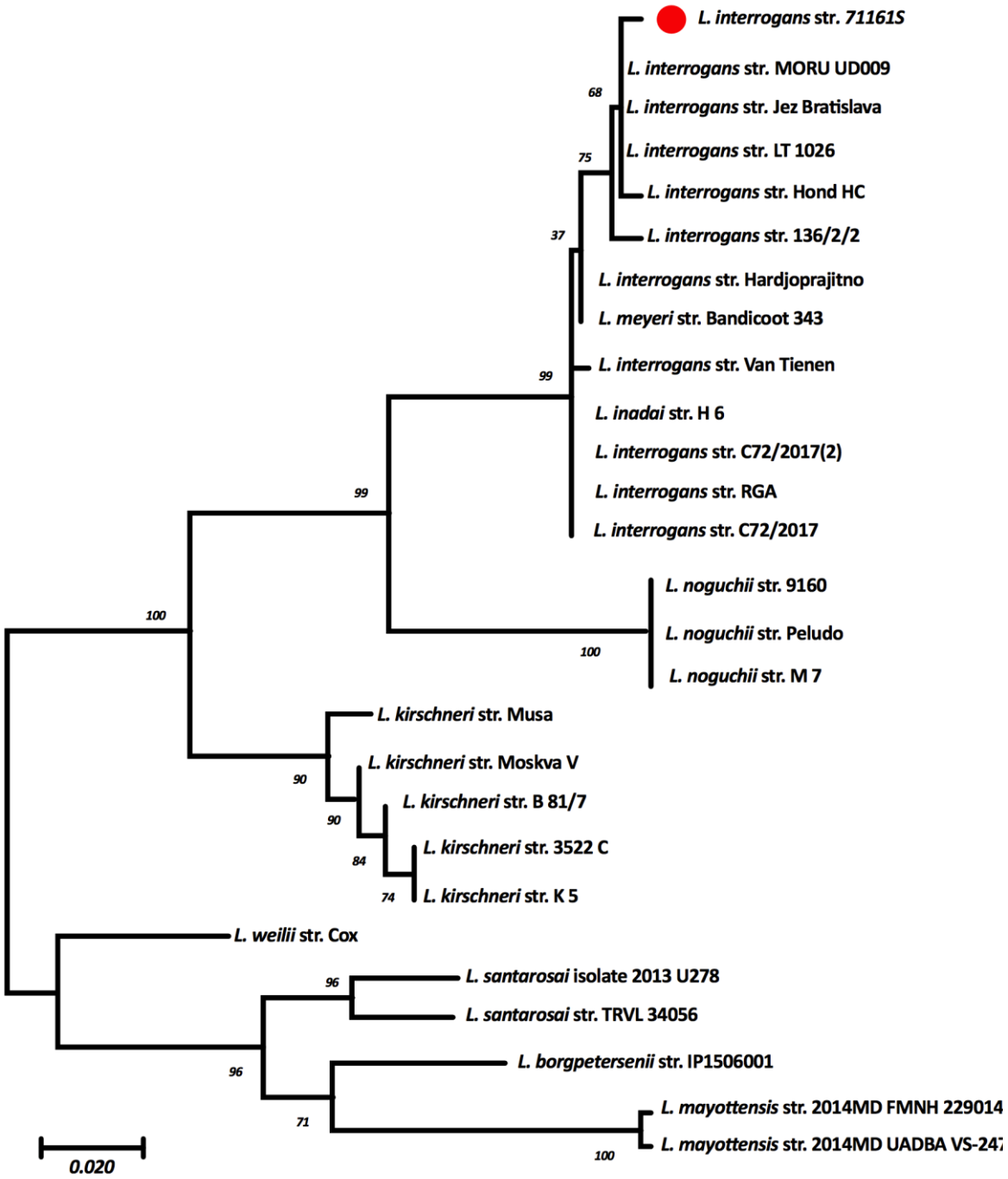

Figure 4. A phylogenetic tree of *Leptospira interrogans* strain 71161S obtained from patient sputum. Evolutionary analyses were performed using partial *secY* genes in relation with other *Leptospiral* strains (5) using MEGA X software. Nodes with bootstrap trusts below 70% are condensed in the presented phylogenetic tree.

CONCLUSION

Our report highlights the importance of considering *Leptospira* molecular detection using sputum samples as a confirmatory diagnostic approach in leptospirosis patients with pulmonary involvement or hemoptysis. Likewise, the urine sample can be used as an option in the examination of severe leptospirosis.

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REFERENCES

1. Haake DA, Levett PN. Leptospira and leptospirosis. Vol. 387, Curr Top Microbiol Immunol. 2015. 65–97 p. doi: 10.1007/978-3-662-45059-8_5.
2. Reller ME, Wunder EA, Miles JJ, Flom JE, Mayorga O, Woods CW, et al. Unsuspected Leptospirosis Is a Cause of Acute Febrile Illness in Nicaragua. PLoS Negl Trop Dis. 2014;8(7):1–8. https://doi.org/10.1371/journal.pntd.0002941
3. Gulati S, Gulati A. Pulmonary manifestations of leptospirosis. Lung India. 2012;29(4):347–53. doi: 10.4103/0970-2113.102822.
4. Bevans AI, Fitzpatrick DM, Stone DM, Butler BP, Smith MP, Cheetham S. Phylogenetic relationships and diversity of bat-associated leptospira and the histopathological evaluation of these infections in bats from Grenada, West Indies. PLoS Negl Trop Dis [Internet]. 2020;14(1):1–15. Available from: http://dx.doi.org/10.1371/journal.pntd.0007940
5. Agrawal VK, Bansal A, Pujani M. A rare case of leptospirosis with isolated lung involvement. Indian J Crit Care Med. 2015;19(3):174–6. doi: 10.4103/0972-5229.152765.
6. Gasem MH, Farida H, Ahmed A, Severin JA, Suryanto A, Isbandrio B, et al. Are pathogenic leptospira species agents of community-acquired pneumonia? case reports of leptospirosis presenting as pneumonia. J Clin Microbiol. 2016;54(1):197–9. doi: 10.1128/JCM.02093-15
7. Isa SE, Onyedibe KI, Okolo MO, Abiba AE, Mafuka JS, Simji GS, et al. A 21-Year-Old Student with Fever and Profound Jaundice. PLoS Negl Trop Dis. 2014;8(1):3. doi:10.1371/journal.pntd.0002534
8. Gasem MH, Kosasih H, Tjitra E, Alisjahbana B, Karyana M, Lokida D, et al. An observational prospective cohort study of the epidemiology of hospitalized patients with acute febrile illness in Indonesia. PLoS Negl Trop Dis [Internet]. 2020;14(1):1–17. Available from: http://dx.doi.org/10.1371/journal.pntd.0007927
9. Budihal SV, Perwez K. Leptospirosis Diagnosis: Competancy of Various Laboratory Tests. J Clin Diagnostic Res. 2014;8(1):199–202. DOI:10.7860/JCDR/2014
10. Niloofa R, Fernando N, De Silva NL, Karunanayake L, Wickramasinghe H, Dikmadugoda N, et al. Diagnosis of Leptospirosis: Comparison between Microscopic Agglutination Test, IgM-ELISA and IgM Rapid Immunochromatography Test. PLoS One. 2015;10(6):1–12. doi: 10.1371/journal.pone.0129236.
11. Picardeau M. Virulence of the zoonotic agent of leptospirosis: Still terra incognita? Nat Rev Microbiol [Internet]. 2017;15(5):297–307. Available from: http://dx.doi.org/10.1038/nrmicro.2017.5
12. Lam JY, Lowid GKK, Cheeid HY. Diagnostic accuracy of genetic markers and nucleic acid techniques for the detection of Leptospira in clinical samples: A meta-analysis. PLoS Negl Trop Dis. 2020;14(2):1–22. https://doi.org/10.1371/journal.pntd.0008074