


# Are Pathogenic *Leptospira* Species Agents of Community-Acquired Pneumonia? Case Reports of Leptospirosis Presenting as Pneumonia

M. Hussein Gasem,<sup>a,b</sup>  Helmia Farida,<sup>c</sup> Ahmed Ahmed,<sup>d</sup> Juliŕte A. Severin,<sup>e</sup> Agus Suryanto,<sup>a</sup> Bambang Isbandrio,<sup>b,c</sup> Henri A. Verbrugh,<sup>e</sup> Rudy A. Hartskeerl,<sup>d</sup> Peterhans J. van den Broek<sup>f</sup>

Department of Internal Medicine, Faculty of Medicine Diponegoro University—Dr. Kariadi Hospital, Semarang, Central Java, Indonesia<sup>a</sup>; National Reference Laboratory for *Leptospira*, Dr. Kariadi Hospital, Semarang, Central Java, Indonesia<sup>b</sup>; Department of Microbiology, Faculty of Medicine Diponegoro University—Dr. Kariadi Hospital, Semarang, Central Java, Indonesia<sup>c</sup>; KIT Biomedical Research, WHO/FAO/OIE and National Leptospirosis Reference Center, Royal Tropical Institute, Amsterdam, North Holland, The Netherlands<sup>d</sup>; Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, South Holland, The Netherlands<sup>e</sup>; Department of Infectious Diseases, Leiden University Medical Center, Leiden, South Holland, The Netherlands<sup>f</sup>

**We report four Indonesian cases meeting the clinical and radiological criteria for community-acquired pneumonia and other findings suggestive of leptospirosis. Quantitative PCR (qPCR) analyses of serum and urine samples and serology confirmed the diagnosis of leptospirosis in each. Results of qPCR analysis of throat swabs were concordant with those obtained with acute-phase serum samples, which suggests its potential for use as a noninvasive diagnostic tool for leptospirosis.**

**P**ulmonary involvement in leptospirosis varies from atypical symptoms to severe pulmonary hemorrhage. However, pneumonia is not commonly associated with leptospirosis (1). We report four cases of laboratory-confirmed leptospirosis clinically presenting with community-acquired pneumonia (CAP) during a CAP study involving 148 subjects in Semarang, Indonesia, from October 2007 to April 2009. These patients had other findings suggesting leptospirosis (2) and were positive by commercial rapid tests.

For confirmation of leptospirosis, microscopic agglutination test (MATs) and enzyme-linked immunosorbent assays (ELISAs) of (paired) serum samples were performed (3) and qPCR analyses of sputum, throat swab, serum, and urine samples were performed (4). Throat swab, first serum, and urine samples were taken on the day of admission; convalescent-phase serum samples were taken 2 to 4 weeks after admission. Samples were refrigerated at  $-80^{\circ}\text{C}$ . DNA was extracted with NucliSens easyMAG (bioMérieux, Zaltbommel, The Netherlands). Negative controls were used. Infection with common CAP pathogens, including *Chlamydia pneumoniae*, *C. psittaci*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, influenza virus, parainfluenza virus, respiratory syncytial virus, adenovirus, rhinovirus, coronaviruses (2293E, OC43, and NL63), metapneumovirus, *Streptococcus pneumoniae*, and other bacteria, was investigated with a wide range of microbiological tests. Written informed consent for the publication of this case series was obtained from the individuals described.

**Case 1.** A 65-year-old woman presented in a tertiary-care hospital in Semarang, Indonesia, with 4 days of fever ( $39^{\circ}\text{C}$ ), a dry cough, and dyspnea (respiratory rate [RR], 32 breaths/min), conjunctival suffusion, and crackles in her lungs. Her white blood cell (WBC) count was  $12,600/\mu\text{l}$ , and her thrombocyte count (TC) was  $20,000/\mu\text{l}$ . A chest X-ray (CXR) showed lung infiltrates. CAP was diagnosed with a pneumonia severity index (PSI) score (5) of 135. She recovered after therapy with ceftriaxone. Diagnostic tests for other CAP pathogens were all negative. An ELISA for leptospirosis showed significant increases in IgM and total immunoglobulin (IgT). A MAT showed seroconversion for serovar Bataviae. Real-time quantitative PCR (qPCR) assays of acute-phase serum, urine, and throat swab samples were positive, but a qPCR assay of

convalescent-phase serum was negative (Table 1). Amplicons had a melting temperature ( $T_m$ ) of  $80.5^{\circ}\text{C}$ .

**Case 2.** A 58-year-old male had 4 days of fever, a dry cough, dyspnea (RR, 28 breaths/min), icteric sclerae, and crackles over the right lower lung. His WBC count was  $6,800/\mu\text{l}$ , his TC was  $101,000/\mu\text{l}$ , and his creatinine level was  $1.68\text{ mg/dl}$ . A CXR confirmed lung infiltrates. CAP was diagnosed with a PSI score of 78. He recovered after therapy with ceftriaxone. An ELISA showed increased IgM and IgT titers; a MAT showed seroconversion for serovar Bataviae. qPCR assays of acute-phase serum and throat swab samples were positive ( $T_m$   $80.5^{\circ}\text{C}$ ), but qPCR assays of urine and convalescent-phase serum samples were negative. Other microbiology tests were negative, except for a rhinovirus PCR assay.

**Case 3.** A 72-year-old male had 7 days of fever, a dry cough, dyspnea, icteric sclerae, petechiae, and gum bleeding. Crackles were heard over the lungs. His WBC count was  $11,700/\mu\text{l}$ , his TC was  $45,000/\mu\text{l}$ , his serum alanine aminotransferase level was  $104\text{ U/liter}$ , his total bilirubin level was  $4.01\text{ mg/dl}$ , and his creatinine level was  $1.68\text{ mg/dl}$ . A CXR showed lung infiltrates. CAP was diagnosed with a PSI score of 92. He recovered after therapy with cefotaxime followed by oral doxycycline. A MAT showed seroconversion for serovar Bataviae. *Leptospira* qPCR assays of acute-phase serum and throat swab samples were positive ( $T_m$   $80.5^{\circ}\text{C}$ ), but those of urine, sputum, and convalescent-phase serum samples were negative. Serology tests also showed seroconversion of IgM antibody to *C. pneumoniae*; however, this finding was con-

Received 4 August 2015 Returned for modification 2 September 2015

Accepted 23 October 2015

Accepted manuscript posted online 28 October 2015

**Citation** Gasem MH, Farida H, Ahmed A, Severin JA, Suryanto A, Isbandrio B, Verbrugh HA, Hartskeerl RA, van den Broek PJ. 2016. Are pathogenic *Leptospira* species agents of community-acquired pneumonia? Case reports of leptospirosis presenting as pneumonia. *J Clin Microbiol* 54:197–199.  
doi:10.1128/JCM.02093-15.

**Editor:** P. Bourbeau

Address correspondence to M. Hussein Gasem, mhgasem@yahoo.com.

M.H.G. and H.F. contributed equally to this article.

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TABLE 1 Diagnostic microbiology of the cases with CAP and leptospirosis at Dr. Kariadi Hospital

Parameter	Case 1	Case 2	Case 3	Case 4
Time (days) from admission to symptom onset	4	4	7	4
PCR assay result				
Serum sample 1	Positive	Positive	Positive	Positive
Serum sample 2	Doubtful	Negative	Negative	Not available
Urine sample	Positive	Negative	Negative	Negative
Sputum sample	Not available	Not available	Negative	Not available
Throat swab sample	Positive	Positive	Positive	Positive
Serology result				
Specific IgM				
Serum sample 1	1/20	1/10	1/20	1/10
Serum sample 2	1/160	1/40	1/10	Not available
Specific Total Ig				
Serum sample 1	1/20	<1/10	<1/320	1/10
Serum sample 2	1/5,120	1/160	1/40	Not available
MAT result <sup>b</sup>				
Serum sample 1	Negative	Negative	Negative	Negative
Serum sample 2	1/320	1/80	1/40	Not available
Other respiratory pathogens	Negative	Rhinovirus	Negative	Negative

<sup>a</sup> Serum sample 1 and urine and sputum samples were taken on the day of admission; serum sample 2 was taken 2 to 4 weeks after admission.

<sup>b</sup> For serovar Bataviae strain Swart.

sidered inconclusive since the clinical presentation did not support *C. pneumoniae* pneumonia and PCR assays for *C. pneumoniae* and *C. psittaci* were negative.

**Case 4.** A 33-year-old male had 4 days of fever, a nonproductive cough, dyspnea, icteric sclerae, and conjunctival suffusion. Calf tenderness was found; crackles were heard in both lungs. His WBC count was 12,300/ $\mu$ l, his TC was 21,000/ $\mu$ l, his blood urea level was 288.0 mg/dl, his creatinine level was 7.02 mg/dl, and his total bilirubin level was 4.85 mg/dl. A CXR showed infiltrates in his lungs. CAP was diagnosed with a PSI score of 83. He recovered after therapy with ceftriaxone. A MAT and an ELISA of the single serum sample were inconclusive. qPCR assays of serum and throat swab samples were positive. Other microbiology tests were negative.

In all four cases, results of qPCR assays of throat swab samples correlated with those of acute-phase serum samples but not with those of sputum or convalescent-phase serum samples, implying the presence of *Leptospira* in the upper respiratory tract in the acute phase of the disease. All qPCR products from throat swab, serum, or urine samples had a  $T_m$  of 80.5°C, consistent with *Leptospira interrogans* serovar Bataviae reference strain Swart and, in turn, were in concordance with the observed seroconversions for serovar Bataviae.

Leptospire from pulmonary tissue may reach the throat via expectorated sputum, but only one patient produced sputum that was, however, negative by qPCR assay. Further research is needed to confirm the consistent presence of leptospire in the throat in the acute phase.

Our findings suggest that pathogenic *Leptospira*, detected by qPCR assay, may be causative of CAP. The finding of rhinovirus in case 2 indicates a coinfection, which is a common finding among CAP patients (6).

*Leptospira* bacteria appear in the urine 7 days after the onset of

the disease but are occasionally found in early urine samples (7). Consistently, a qPCR assay of one of the four urine samples was positive. While qPCR analysis of acute-phase urine samples lacks the sensitivity required for early diagnosis, throat swabs consistently provided confirmation at this stage of the disease. Hence, qPCR analysis of early throat swab samples might present a potentially interesting novel noninvasive diagnostic approach.

Pulmonary radiographic alterations, usually bilaterally, in leptospirosis have been frequently reported before. These patterns are not specific and can be ascribed to pulmonary hemorrhages or pneumonia in general (7). The patients described here may have suffered from leptospirosis with pulmonary involvement of pneumonia or pulmonary hemorrhage and therefore were diagnosed as having CAP at admission. However, leptospirosis with pulmonary hemorrhages has a high mortality rate (30 to 60%) (1), whereas our four patients survived and did not have bleeding manifestations, except for case 3. Thus, the CXR patterns in our cases could be regarded more as pneumonia. CAP due to leptospirosis was reported previously, but the diagnosis was supported by a MAT only (8).

In conclusion, patients with leptospirosis may present with signs and symptoms compatible with CAP. In areas where leptospirosis is endemic, the disease should be included in the differential diagnosis of patients with CAP. qPCR analysis of throat swab samples is a potential novel tool for the early diagnosis of leptospirosis.

#### ACKNOWLEDGMENTS

The clinical study of CAP from which the four patients described here were derived was funded by Royal Netherlands Academy of Arts and Sciences (KNAW) SPIN Postdoc Programme no. 06-PD-17 and the Laboratory of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, The Netherlands. The PCR and serology tests for

leptospires were funded by the Royal Tropical Institute (KIT), Amsterdam, The Netherlands. All authors declare no conflict of interest.

We thank Marga G. A. Goris and Hans J. van Linden of KIT, Amsterdam, The Netherlands, for technical support.

#### FUNDING INFORMATION

KNAW provided funding to M. Hussein Gasem under grant number 06-PD-17.

The clinical study of community-acquired pneumonia, from which these four patients were derived, was funded by Royal Netherlands Academy of Arts and Sciences (KNAW) SPIN postdoc program no. 06-PD-17. The microbiology tests for CAP were funded by the Laboratory of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands. PCR and serology tests for the leptospires were funded by the Royal Tropical Institute (KIT), Amsterdam, The Netherlands.

#### REFERENCES

1. Dolhnikoff M, Mauad T, Bethlem EP, Carvalho CRR. 2007. Pathology and pathophysiology of pulmonary manifestations in leptospirosis. *Braz J Infect Dis* 11:142–148. <http://dx.doi.org/10.1590/S1413-86702007000100029>.
2. Anonymous. 2009. Informal expert consultation on surveillance, diagnosis and risk reduction of leptospirosis, Chennai, India, 17–18 September 2009. World Health Organization Regional Office for Southeast Asia, New Delhi, India. [http://www.searo.who.int/entity/emerging\\_diseases/topics/Communicable\\_Diseases\\_Surveillance\\_and\\_response\\_SEA-CD-217.pdf](http://www.searo.who.int/entity/emerging_diseases/topics/Communicable_Diseases_Surveillance_and_response_SEA-CD-217.pdf).
3. Goris MGA, Leeftang MMG, Boer KR, Goeijenbier M, Gorp ECMv, Wagenaar JFP, Hartskeerl RA. 2012. Establishment of valid laboratory case definition for human leptospirosis. *J Bacteriol Parasitol* 3:132.
4. Ahmed A, Engelberts MFM, Boer KR, Ahmed N, Hartskeerl R. 2009. Development and validation of a real-time PCR for detection of pathogenic leptospira species in clinical materials. *PLoS One* 4:e7093. <http://dx.doi.org/10.1371/journal.pone.0007093>.
5. Fine MJ, Smith MA, Carson CA, Mutha SS, Sankey SS, Weissfeld LA, Kapoor WN. 1996. Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis. *JAMA* 275:134–141.
6. Reechaipichitkul W, Lulitanond V, Sawanyawisuth K, Lulitanond A, Limpawattana P. 2005. Etiologies and treatment outcomes for out-patients with community-acquired pneumonia at Srinagarind Hospital, Khon Kaen, Thailand. *Southeast Asian J Trop Med Public Health* 36:1261–1267.
7. Wilkins TR, Wilkins RL. 2005. Clinical and radiographic evidence of pneumonia. *Radiol Technol* 77:106–110.
8. Perani V, Farina C, Maggi L, Michetti G, Moiola F, Pizzocaro P, Pugliese C. 1998. Pneumonia due to *Leptospira* spp.: results of an epidemiological and clinical study. *Int J Tuberc Lung Dis* 2:766–770.