

The investigation of suspected Legionellosis cases in family aggregation

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Abstract

Taiwan CDC received report of a suspected Legionellosis case on December 30, 2005. During the outbreak investigation and specimen collection, it was suspected that there is an aggregate of cases within the family. After further investigation, the investigation confirmed the index case to be legionellosis. The source of infection was the non-filter drinking fountain in the house. The investigation also eliminated the possibility of family aggregate of legionellosis. The non-filter drinking fountain contained *Legionella pneumophila* serogroup 8. Furthermore, pulsed-field gel electrophoresis (PFGE) was used to compare environmental strains between historic isolates and the investigation's strain. The study found the patterns of PEGE were different. The results showed there are

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more than 2 types of *L. pneumophila* serogroup 8 in Taiwan.

Key words: *Legionella pneumophila* serogroup 8 (Lp8), pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR)

Introduction

In 1976, veterans in USA had an outbreak of pneumonia after gathering in a hotel in Philadelphia. The pathogen, named *Legionella pneumophila* (Lp), was identified from tissues of cases by the United States Centers for Disease Control and Prevention [1, 2]. Among community-acquired pneumonias, 2-15% is caused by Lp [3-7]. In Taiwan, 9% of hospitalized pneumonia patients are infected by Lp [8]. In addition, according to Tsai et al [9], 27.9% of the 618 cooling water towers in Taipei area were positive for pathogens, and Lp accounted for 86.3%. The most dominant Lp was *Legionella pneumophila* serogroup 1 (Lp1), consisted of 59.3% of the positive specimens.

On December 30, 2005, the Taiwan Centers for Disease Control (CDC) received report of a suspected case of Lp infection from a health bureau. During the outbreak investigation and specimen collection, it was suspected that there is an aggregate of cases within the family. Further investigation was conducted to clarify whether the suspected case truly had legionellosis, presence of other suspected cases or contacts, epidemiological relationship among cases, whether there was a cluster, and the infection source. This article describes the results of the investigation.

Material and methods

Case definition: the definition of confirmed cases, according to Taiwan CDC, must have compatible clinical symptoms and laboratory evidences. Compatible clinical symptoms include: malaise, chills, myalgia, headache, fever, dizziness,

cough, nausea, and abdominal pain, with pneumonia as the major symptom and may be combined with encephalopathy, diarrhea, or multiple organ system involvement. Confirmed cases should have one of the following laboratory evidence: positive for urine antigen, more than 4-fold rising in serum antibody within 4-12 weeks and the titer is more than 128, or positive for Lp culture [10].

Steps to confirm cases: first, Legionnaire's disease is a category 3 notifiable disease; suspected or confirmed cases should be reported to local health bureau within 7 days. After receiving reports, local health bureau should confirm whether the suspected cases have compatible clinical symptoms, and report to the CDC. Second, specimens from suspected cases should be gathered, including urine, sputum, and two serum specimens, for laboratory examination. Third, structured questionnaire should be used for case investigation. In addition, environmental specimens should be taken for nosocomial infections or in case of clusters of two or more cases. Fourth, environmental specimens should include water cooling tower of air conditioning system, hot water supply system, and drinking water supply system to prove the existence of Lp in the environment. Fifth, environmental specimens should match with the results from testing of cases. Sixth, analyzing demographic data of cases, dates of onset, locations of disease onset and examination results according to the data gathered above, in addition to the relationship between contacts to understand whether the event is a sporadic case or part of a cluster. The source of infection should be identified for disease control.

Subjects: the suspected case and four family members (his wife, son, daughter, and an Indonesian maid) were interviewed in person. A total of five questionnaires were administered.

Investigation tools: Legionnaire's disease case investigation questionnaire in

the handbook for prevention of infectious disease by the CDC was used. Information collected included demographic data of the case, medical treatment history, exposures (including hospital visit, places of stay other than home, or visits to public places during the ten days before disease onset, air conditioning at home and office, method of bathing, environment surrounding the home and working places, daily habits, and contacts were gathered.

Environmental specimens collection: aseptic polyethelene tubes with water-proof screw caps or aseptic bags were used. If oxidizing disinfectant was suspected to be present in water specimens, inactivating agents, potassium thiosulfate or sodium thiosulfate, were added. Specimens were sent to the laboratory within two days. Temperature was kept between 18°C and 6°C. Specimens were kept away from heat and light. Collecting methods of each environmental specimen are as the following:

Water cooling tower: 100 mL of water collected approximately 10 cm below surface using aseptic containers or bags. Precipitates were avoided.

Hot water basin: two water specimens were collected. 1. 100 mL of water was taken immediately after turning on (labeled 1). 2. 100 mL was collected after water had been flowing for 15-30 seconds (labeled 2).

Faucets, water fountains, and shower nozzles: Bio-film in the facets was collected using aseptic cotton or non-woven swabs, and kept in aseptic bags or screw cap containers before taking 100 mL water samples. Note: filters of facets should be removed before sample collection.

Environmental specimen collection: specimens were collected from the shower nozzle, faucets in the bathroom, sinks, and the kitchen, water filtration system in the kitchen, and non-filter drinking fountain by two cotton swabs using the method described above. A total of 18 samples were collected. In addition, 14

water samples (100 mL each) from 7 places including the shower nozzle, faucets in the bathroom, sinks and the kitchen, water filtration system in the kitchen, and non-filter drinking fountain. A total of 32 environmental samples were collected.

Specimen examination: serum, urine and sputum samples were collected from the suspected case. Serum samples were tested by indirect fluorescent antibody test (IFA) kit from Zeus Scientific Inc, USA for titer of anti-*Legionella pneumophila* antibody. Urine specimens were tested by *Legionella* Urine Antigen Elisa Kit, BINAX, Inc., USA for type I Lp antigen. Sputum specimens were cultured for microorganism and biochemical analysis of Lp antigen identification by *Legionella* Latex Agglutination Test Kit, Oxoid Limited, Basingstoke, Hampshire, England and direct fluorescent antibody test, DFA, Zeus Scientific, Inc., USA. Positive strains were further analyzed by pulsed-field gel electrophoresis (PFGE).

Specimen collection from the patient: sputum, urine and serum specimens were collected on January 6 from the suspected patient. Serum specimens were collected again on January 17. In addition, two serum samples of the 4 contacts were taken on January 25 and February 7, 2006. A total of 12 samples were sent to the CDC for analysis.

Results

History of the suspected case: the case was an 83-year-old married men who had been bed-ridden for 8 year after a stroke. He had no travel history in the past 8 years because of his limited mobility. Daily life management was mainly performed by the Indonesian maid. The case smoked when he was young, but had quit for the last 20 years. He was not a heavy drinker. Patient had a history of hypertension. On December 10, 2005, the case started to have symptoms

including sneezing and mild fever. Over-the-counter drugs were taken, but the patient's fever increased. The case was taken to the hospital on December 17 and was hospitalized. He was then transferred to the intensive care unit on December 29. The hospital reported the case on December 30. The clinical symptoms included malaise, fever, dizziness, and cough, and symptoms of pneumonia. These symptoms were compatible with Legionnaire's disease.

Histories of contacts: None of the four contacts had symptoms between December 1, 2005 and January 8, 2006.

Examination results: among the 14 clinical samples, *L. pneumophila* serogroup 8 (Lp8) was isolated from sputum samples of the suspected case. The urine was negative. Titers of the serum samples were 256 and 1024, respectively, showing a four-fold increase. In addition, the daughter of the suspected case had serum titer of 128, and she had mild fever and cough in early December. She did not seek medical treatment because of the mildness of her symptoms, but a family cluster was suspected. The second serum sample still had a titer of 128. The rest of the three persons had serum titer of 16. Among the 32 environmental samples, other than the four samples taken from the drinking fountain which had Lp8 isolated, all were negative (Table 1).

PFGE analysis showed that the Lp8 strains isolated from clinical and environmental samples were the same (Table 1, Fig. 1 and 2).

The Lp8 strains from clinical and environmental samples were further tested for 6 toxin genes including *lvh* and *rtx* by polymerase chain reaction (PCR), based on methods described by Samrakandi et al [11]. The results showed that the Lp8 strains from clinical and environmental samples were positive for the *rtxA* gene, but negative for the *lvh* gene (Table 2, Fig. 3).

Results of the case: based on epidemiological and laboratory evidence, the

suspected case was reclassified to be a confirmed case, and the infection source was the unfiltered drinking fountain. Cluster of Legionnaire's disease among the four contacts were ruled out.

Discussion

After Bissett first isolated Lp8 from the lung tissue of patients in 1983 [12], Lp8 was again isolated in 1987 by Senn from a 18-year-old patient [13]. In 1990, Lp1 and Lp8 were isolated simultaneously by Aubert from a 10-year-old body infected in a hospital. Lp1 and Lp8 were also isolated from samples of the hot water system [14]. In 1992, Pelaz reported analysis of Lp isolated in Spain between 1983 and 1990; 92.5% was Lp1, and 2 were Lp6 and another 2 were Lp8. In the environment, the majority were Lp1(53.6%), followed by Lp8 (27.6%), Lp 3 (9.4%) and Lp 6 (7.2%) [15]. In 1993, among the 6 community-acquired cases reported by Pelaz, Lp1 was the major culprit, but Lp3, 4, 6, 8, and 10 also caused infections [16]. In 2005, Borella reported that 75% of the hot water system in hotels in Italy was contaminated, and 87% was contaminated by *Legionella*. Among these, Lp1 accounted for 45.8%, and others were Lp2-14 [17]. According to the above literature, Lp8 could be isolated from the environment, cause disease in humans, and survive in hot water systems. In Taiwan, people do not drink water directly from the tap, instead, people boil water before drinking. Hence, transmission of disease through water is rare. Lp could survive in 5-65°C, with optimal temperature of 35-45°C. This article showed that bio-film formed in drinking fountain could be contaminated by Lp8 and cause disease. We suggest that drinking fountains, including the outlet and inner parts, should be cleaned frequently, especially when the fountain is a non-filter one.

The case was confirmed to be infected by Lp8 through non-filter drinking

fountain by epidemiological investigation and laboratory evidence. In addition, results of PFGE showed that Lp8 isolated from the case was different from historic strains, showing the existence of at least two Lp8 strains in Taiwan.

Everybody may be infected by Lp, but most healthy people have immunity. Research had shown that more than half of the Lp infections cause pneumonia. The older the patients are, the more severe the disease might be (most patients are older than 50 years). Smokers, patients with diabetes, chronic obstructive pulmonary disease, renal disease, malignant tumor, or immune-compromise, especially those who receive corticosteroids therapy or organ transplantation, are most susceptible to Legionnaire's disease. Male to female ratio is approximately 2.5:1 [18]. There were 5 people in the case's family, and they all drank water from the same drinking fountain. However, only the case had symptoms. The reasons might be advanced age, male, a history of hypertension, and stroke. The strains were only positive for *rtxA* toxin gene, suggesting decreased virulence. The case might be infected because he is more susceptible host. The daughter of the case had only mild symptoms. Because her serum titers were both 128, showing no four-fold increase and on pathogens were identified, it is not possible to confirm Lp infection. The daughter might have been infected but did not develop illness because she was young, female, and the strain had decreased virulence. A family cluster requires at least two confirmed cases within a family. In this incident, there was only one case confirmed, hence a family cluster was ruled out.

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Table 1. Examination results of clinical and environmental samples

Sample source	sample number	culture results		strains	PFGE types
		amount ^a	strain code ^b		
Environmental water					
Bathroom shower nozzle	2	0			
Bathroom faucet water	2	0			
Sink faucet water	2	0			
Kitchen faucet water	2	0			
Cooling water tower	2	0			
Kitchen UV filtration	2	0			
Drinking fountain	2	0			
Aseptic cotton swabs					
Bathroom shower nozzle	2	0			
Bathroom faucet (cold)	2	0			
Bathroom faucet (hot)	2	0			
Kitchen faucet (cold)	2	0			
Kitchen faucet (hot)	2	0			
Kitchen faucet	2	0			
Kitchen UV filtration	2	0			
Drinking fountain (cold)	2	4	EN1-1 EN1-2 EN1-3 EN1-4	SG8 SG8 SG8 SG8	P1 P1 a P1 P1
Drinking fountain (hot)	2	2	EN1-7 EN1-8	SG8 SG8	P1 P1
Old environmental strain		1	OEN 5-1	SG8	P2
Old environmental strain		1	OEN 6-1	SG8	P2
ATCC 35096		1	ATCC	SG8	P3
Sputum					
Clinical samples	1	1	Case	SG8	P1
Total	33	10			

a suspected strains isolated from culture plate for analysis:

1 Case, 6 EN, 2OEN, 1ATCC

bcode for strain analysis

Table 2. Detection of *lvh* and *rtxA* toxin genes in clinical and environmental strains by PCR

PFGE Types	serum types	strain code	toxic gene <i>lvh</i>	toxic gene <i>rtxA</i>	strain source
P1	SG8	EN1-1	-	+	this study
P1a	SG8	EN1-2	-	+	this study
P1	SG8	EN1-3	-	+	this study
P1	SG8	EN1-4	-	+	this study
P1	SG8	EN1-7	-	+	this study
P1	SG8	EN1-8	-	+	this study
P1	SG8	Case	-	+	this study

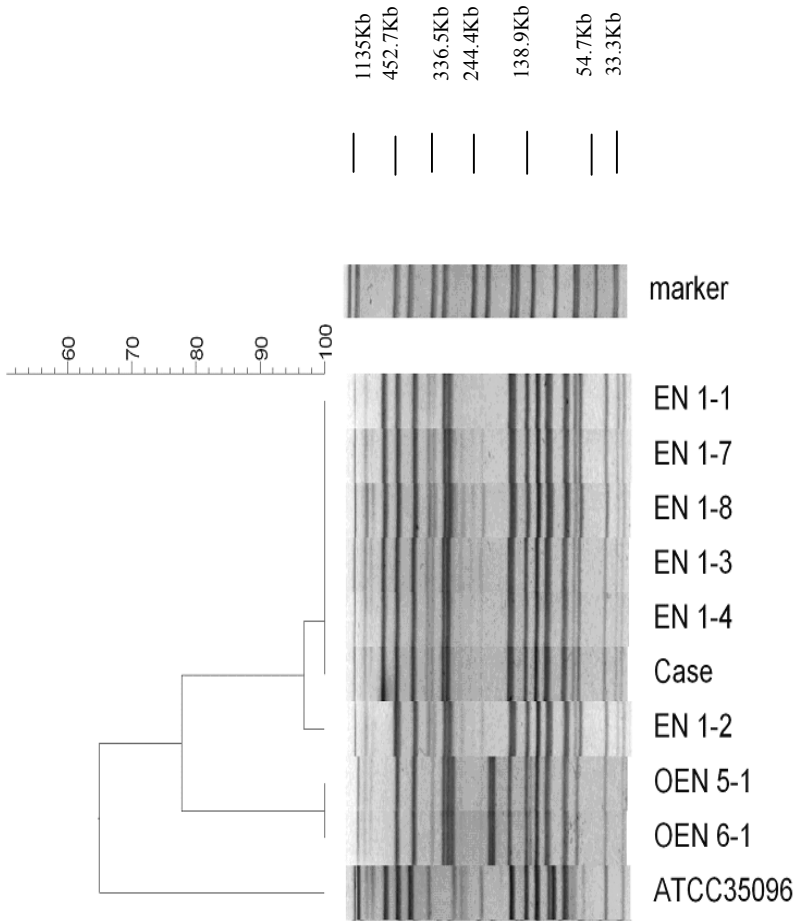


Figure 1. Dendrogram of clinical and environmental strains by PFGE analysis

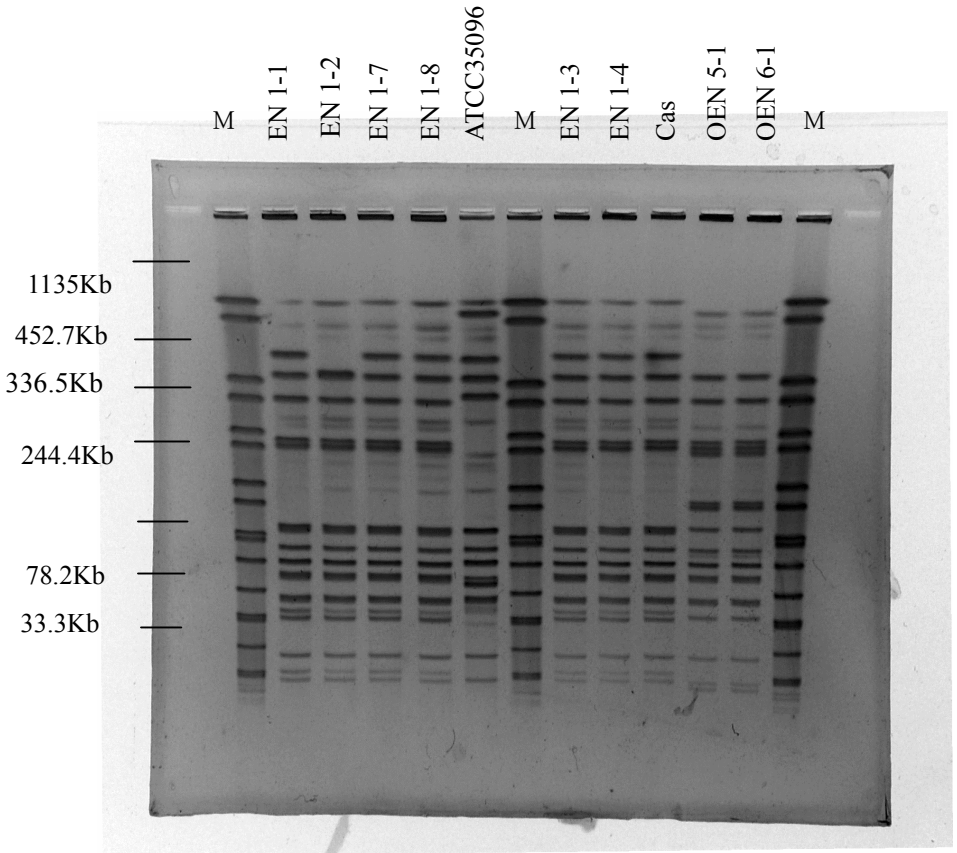
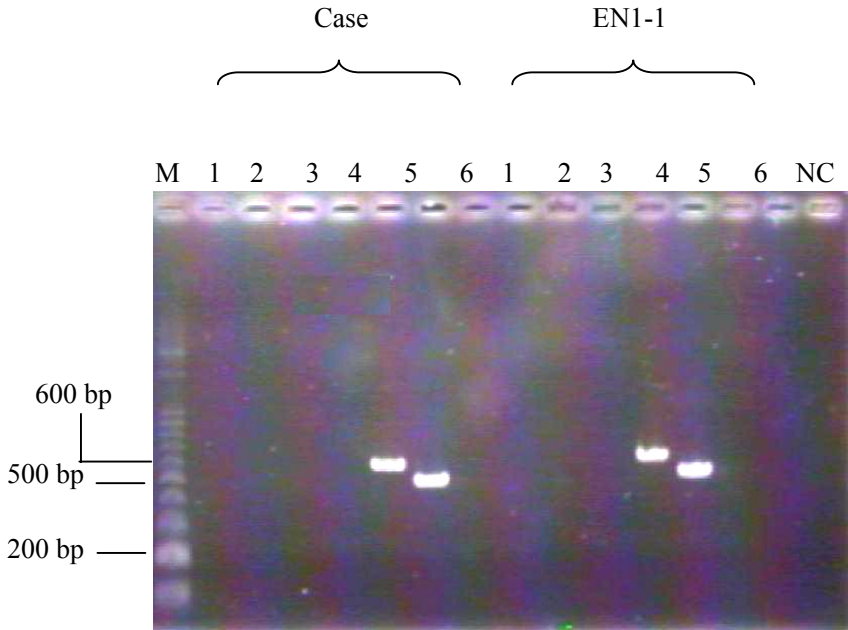


Figure 2. Gel picture of clinical and environmental strains by PFGE analysis



Lane designations:

<i>lvh1/prpA-lvh2/prpA</i>	258 bp
<i>lvh3/lvhB3-lvh4/lvhB4</i>	1006 bp
<i>lvh5/lvhB8-lvh6/lvhB9</i>	293 bp
<i>lvr1/lvrE-lvr2/lvrE</i>	422 bp
<i>rtx1/rtxA-rtx2/rtxA</i>	630 bp
<i>rtx3/rtxA-rtx4/rtxA</i>	542 bp

Figure 3. Gel picture of toxic gene analysis of clinical and environmental strains by PCR