Maël Bessaud,*
Christophe N. Peyrefitte,*†
Boris A.M. Pastorino,*
Fabienne Tock,* Olivier Merle,*
Jean-Jacques Colpart,‡
Jean-Sébastien Dehecq,§
Romain Girod,* Marie-Christine
Jaffar-Bandjee,¶ Pamela J. Glass,¶
Michael Parker,# Hugues J. Tolou,#
and Marc Grandadam*

*Institut de médecine tropicale du Service
de santé des armées, Marseille, France;
†Walter Reed Army Institute of Research,
Silver Spring, Maryland, USA; ‡Agence de
la biomédecine, Lyon, France; §Direction
régionale des affaires sanitaires et
sociales, Saint-Denis, Île de la Réunion,
France; ¶Centre hospitalier départemental
Félix-Guyon, Saint-Denis, Île de la Réunion,
France; and #United States Army
Medical Research Institute for Infectious
Diseases, Fort Detrick, Maryland, USA

References

1. World Health Organization. Chikungunya
in La Réunion Island (France) 2006 Feb 17
[cited 2006 Aug 1]. Available from http://
www.who.int/csr/don/2006_02_17a/en/

2. La Réunion-Mayotte CIRE. Epidémie de
chikungunya à la Réunion. Point hebdoma-
daire, semaine 13. 2006 Apr 6 [cited
2006 Aug 7]. Available from http://www.
inv.sante.fr/presse/2006/le_point_sur/chik
ungunya_reunion_070406/chikungunya_s1
3.pdf

3. Peyrefitte CN, Pastorino BAM, Bessaud M,
Gravier P, Tock F, Cousin-Pinari P, et al.
Dengue type 3 virus, Saint Martin,
2003–2004. Emerg Infect Dis.
2005;11:757–61.

4. Pastorino B, Muysembe-Tamfum JJ,
Bessaud M, Tock F, Tolou H, Durand JP, et al.
Epidemic resurgence of chikungunya
virus in Democratic Republic of the Congo:
identification of a new Central African
strain. J Med Virol. 2004;74:277–82.

5. Chartel R, Lambererie X. Réunion
sequence. ProMed. 2006 Mar 23. Available
from http://www.promedmail.org, archive
no. 20060323.0896.

6. Powers AM, Braut AC, Tesh RB, Weaver
SC. Re-emergence of chikungunya and
o’nyong-nyong viruses: evidence for dis-
tinct geographical lineages and distinct evo-
lutionary relationships. J Gen Virol.
2000;81:471–9.

7. Edelman R, Tacket CO, Wasserman SS,
Bodinson SA, Perry JG, Mangiafico JA.
Phase II safety and immunogenicity study
of live chikunguya virus vaccine TSI-
GSD-218. Am J Trop Med Hyg. 2000;62:
681–5.

8. Vaucel M. Affections dues aux virus:
chapitre VII. Affections à virus neu-
rotropes. In: Mécine tropicale. Paris:
Editions Médicales Flammarion; 1966. p.
1111–7.

9. Jupp PG, McIntosh BM, dos Santos I,
DeMoor P. Laboratory vector studies on six
mosquito and one tick species with chikun-
gunya virus. Trans R Soc Trop Med Hyg.
1981;75:15–9.

10. Pastorino B, Bessaud M, Grandadam M,
Murri S, Tolou HJ, Peyrefitte CN.
Development of a TaqMan RT-PCR assay
without RNA extraction step for the detec-
tion and quantification of African chikun-
gunya viruses. J Virol Methods.
2005;124:65–71.

Address for correspondence: Marc Grandadam,
Unité de Virologie Tropicale, IMTSSA, BP 46,
13 998 Marseille Armées, France; email:
publi.viro@laposte.net

Legionnaires’ Disease and Travel in Europe

To the Editor: The European Working Group for Legionella
Infections (EWGLINET) conducts epidemiologic surveillance of
Legionnaires’ disease cases associated
with travel (1) and provides epi-
demiologic typing markers of
Legionella pneumophila among refer-
cent laboratory studies in collaborat-
ing countries. The procedures and criteria
of notification are found in the
Guidelines for Control and Prevention
of Travel Associated Legionnaires’
Disease (2). However, establishing
the association of ≥1 case of this dis-
ease and a specific tourist accommo-
dation site is difficult because of low
attack rates and dispersal of people
from the source of infection during
the incubation period.

Collaboration promoted by this
working group encourages the exchange of data instead of cultures.

This distinction is critical when
research is conducted on travel-assoc-
ated Legionnaires’ disease, in which
strains from patients and environmen-
tal sources of infection studied are
in different laboratories.

The value of such information
is shown in a complex case study that
was recently investigated. During July
and August 2005, two patients with
Legionnaire’s disease living in 2
countries in Europe were reported
to EWGLINET. Patient 1 was a 45-year-
old woman who traveled in France
and Spain July 1–6, 2005. Her symp-
toms started on July 6, 2005, when
she was in Girona, Spain, where she
was hospitalized. Patient 2 was a 56-
year-old woman who traveled in
Spain and France August 16–21,
2005. Her symptoms started on
August 8, 2005, when she was in
France, where she was hospitalized.
Both patients tested positive for L.
pneumophila serogroup 1 by specific
urinary antigen test and culture, but
they recovered and were discharged.

After routine notification to EWG-
LINET, it was established from the
list of accommodation sites provided
by the 2 patients that they each had
stayed for 1 night at the same hotel in
a French city within a 45-day interval.
This finding led us to identify a clus-
ter according to the definition in use
(2 cases associated with the same
accommodation within 2 years) (2).
However, patient 2 spent 1 day in
August in Zaragoza, Spain, during
which an outbreak of Legionnaires’
disease in the city affected 30 persons.
Thus, illness in patient 2 could have
been associated with the Zaragoza
outbreak. Alternatively, both patients
could have contracted the illness inde-
dependently at different sites. Before
onset, patient 1 stayed 5 days in her
private residence in Girona and
patient 2 visited 3 other hotels.

As soon as cultures from the 2
patients were available, the National
Reference Laboratories of France and
Spain shared their respective micro-
biologic results. Since both strains were identified as *L. pneumophila* serogroup 1, we performed sequence-based typing (SBT) (3) of 6 genes (*flaA, pilE, asd, mip, mompS*, and *proA*) by using the protocol and database of EWGLINET. Both isolates showed identical SBT patterns (2,3,18,15,2,1).

Isolates from 4 patients in the Zaragoza outbreak were identified at the Spanish Reference Laboratory as *L. pneumophila* serogroup 1 (Philadelphia monoclonal antibody type) and had identical SBT patterns (3,4,1,1,14,9). Collaboration between public health authorities in France and Spain enabled us to eliminate the association of patient 2 with the Zaragoza outbreak and establish an association of both patients with the same site in France. Control measures were taken at the hotel, but we could not obtain environmental cultures for comparison with those of the patients. Lack of environmental data prevented investigation of the relationship with other accommodation sites visited.

The SBT method provides robust genotyping with high discriminatory power (index of discrimination >0.94) (3). This method is less effective at discriminating between strains than pulsed-field gel electrophoresis (4), but it shows excellent reproducibility and may be useful in epidemiologic investigation of outbreaks caused by *L. pneumophila*. The availability of an online database with accessible information is key for sharing results and determining the geographic distribution of isolates that cause Legionnaires’ disease (4,5).

This study demonstrates the critical role of sharing results between countries that participate in a network. Agreement is essential on a standardized questionnaire that includes more information on the patient’s exposure to a disease. Moreover, despite the performance of the urine antigen test, cultures of clinical samples should be encouraged by clinicians and microbiologists. This step would permit use of techniques, such as SBT, in reference laboratories and sharing of results. Our investigation would have been more difficult without this technique in identifying the site where the infection potentially originated.

**Rosa Cano,* Sophie Jarraud,† Joana Pardos,‡ Christine Campese,§ and Carmen Pelaz* 

*Instituto de Salud Carlos III, Madrid, Spain;†Institut National de la Santé et de la Recherche Médicale E0230, Lyon, France;‡Public Health Laboratory of Girona, Catalonia, Spain; and §Institut de Veille Sanitaire, Saint-Maurice, France

**References**

1. Ricketts K, Joseph C. Travel associated Legionnaires’ disease in Europe: 2003. Euro Surveill. 2004;9:40–3.
2. European Working Group for Legionella Infections. European guidelines for control and prevention of travel associated Legionnaires’ disease. 2002 [cited 2006 Jul 28]. Available from http://www.ewgli.org
3. Gaia V, Fry NK, Afszar B, Lück PC, Meugnir H, Etienne J, et al. A consensus sequence-based epidemiological typing scheme for clinical and environmental isolates of *Legionella pneumophila*. J Clin Microbiol. 2005;43:2047–52.
4. Aurell H, Farge P, Meugnir H, Gouy M, Forey F, Lina G, et al. Clinical and environmental isolates of *Legionella pneumophila* serogroup 1 cannot be distinguished by sequence analysis of two surface protein genes and three housekeeping genes. Appl Environ Microbiol. 2005;71:282–9.
5. Scaturro M, Losardo M, de Ponte G, Ricci ML. Comparison of three molecular methods used for subtyping of *Legionella pneumophila* strains isolated during an epidemic of legionellosis in Rome. J Clin Microbiol. 2005;43:5348–50.

**Address for correspondence:** Carmen Pelaz, Laboratorio de Legionella, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220 Madrid, Spain; email: cpelaz@isciii.es

---

**Influenza A Virus PB1-F2 Gene**

**To the Editor:** Recently, Chen and co-workers described the expression of an 11th influenza A virus protein, designated PB1-F2 because this protein is encoded in the +1 open reading frame of the segment-2 RNA (1). Later, Chen et al. presented a preliminary analysis of 336 PB1 sequences from GenBank (2). We have extended the work on PB1-F2 and analyzed 1,864 partial and complete segment-2 sequences deposited in GenBank; these sequences belong to 79 influenza A virus subtypes. In summary, the following 8 observations should receive attention:

First, the size of PB1-F2 polypeptides ranges from 79 to 101 amino acids (aa); most isolates encode versions of either 87 or 90 aa. Because polypeptides of 79 aa are located within mitochondria, their truncation has no effect on the protein function. The frequency of the 79-aa PB1-F2 is ≈5%.

Second, a functional PB1-F2 is expressed by 92% of all segment-2 sequences, i.e., a polypeptide >78 aa. The proportion of intact PB1-F2 varies according to host (humans 90%, swine 76%, other mammals 100%, birds 95%).

Third, the H1N1 subtype comprises 3 genetic lineages. One clade has 2 branches: 1 branch includes the human viruses, with the pandemic 1918 virus at its root; the other branch includes the classic swine viruses. The third clade represents the European porcine isolates. Although all classic swine sequences have a truncated PB1-F2 (in-frame stop codons after 11, 24, and 35 codons), the early human isolates (H1N1 sequences from 1918 through 1947) have an intact PB1-F2. After 1956, however, a mutation became prevalent such that the recent sequences starting from A/Beijing/1/56 terminate after 57 codons. An exception to