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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 epidemiologic typing markers of *Legionella pneumophila* among reference laboratories in collaborating 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 disease and a specific tourist accommodation 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-associated Legionnaires' disease, in which strains from patients and environmental 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 symptoms 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 EWGLINET, 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 cluster 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 independently 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 (*fla*A, *pil*E, *asd*, *mip*, *momp*S, and *pro*A) 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 the 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.

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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