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Travel-associated infections caused by unusual serogroups of *Legionella pneumophila* identified using Legionella BIOCHIP slides in Turkey and Iraq

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Summary *Background:* Although *Legionella pneumophila* serogroup 1 is the common disease causing serogroup, rare serogroups can also may cause legionellosis. A 54-year-old male patient (index case) reported that he had been on a religious trip (for visiting, tomb of Ali, which is important for Shias) to Iraq with a large group (50 shia pilgrims from Kars city of Turkey) two weeks prior to admission. Due to civil war, the hotel where the patient stayed in Iraq lacked proper hygiene. A large number of people in the travel group were experiencing the same

List of abbreviations: sg, serogroup; sgs, serogroups; Lp, *Legionella pneumophilia*; CT, computed tomography; CMF, Cerrahpasa Medical Faculty; WHO, World Health Organization; DFA, direct fluorescent antibody; CDC, The Centers for Disease Control; PPf, possible Pontiac fever (in Table 1); ESR, erythrocyte sedimentation rate (in Table 2).

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Legionellosis

symptoms. Other five cases were 2 males (ages; 50, 45) and 3 females including the wife of the index case (ages; 50, 28, 27).
Method: The detection of *L. pneumophila* IgG and IgM was performed by anti-*L. pneumophila* Indirect Immunofluorescent IgM, IgG kit. *Legionella* 1 biochip/verification BIOCHIP slides were used for serogrouping in Euroimmun AG, Leubeck, Germany.
Results: In index case, *L. pneumophila* IgM was positive with a titer of 1/32 titer. IgG was negative with a 1/100 titer. Another case (28 year old female), had clinical symptoms identical to the index case. *L. pneumophila* IgM and IgG were positive with titers of 1/64 and 1/100, respectively. These two cases were diagnosed with Legionnaires' disease caused by *L. pneumophila* serogroup 12 (index case) and female (28-year-old) by serogroup 11. The other 4 cases were diagnosed with possible Pontiac fever caused by *L. pneumophila* serogroups 14 (wife of the index case), 4, and 6 whereas the serogroup of *L. pneumophila* detected in 27 years old female case could not be identified.
Conclusion: A major limitation of this work is the absence of genotyping and the serogroup difference between index case and his wife who shared the same hotel. We suggest that this serogroup difference may be caused by (for men and women) sitting separately in Islamic rules.

On the other hand, the movement of people in the context of mutual visits between countries or neighboring countries for tourism-related (i.e., for religious events or visits to holy sites) or immigration-related reasons, may cause some epidemic diseases. This study reemphasized that not only *L. pneumophila* serogroup 1, but other rare serogroups might cause also legionellosis which may increase in frequency and cause regional epidemics. We propose that increased financial resources for improving the hygiene conditions and performing routine legionella surveillance studies in touristic hotels would be useful measures for legionellosis prevention and control.

1. Background

Legionnaires' disease is a severe bacterial infection that can lead to morbidity and mortality, and threatens human health and holds an important place among community-acquired sporadic atypical pneumonias. In contrast, Pontiac fever, which is a mild acute upper respiratory infection, is non-fatal and resolves spontaneously. *Legionella pneumophila* responsible for primarily pulmonary involvement rarely causes extra-pulmonary involvement colonizes water and it is transmitted to humans via inhalation and aspiration. Among *Legionella* species, *L. pneumophila* has 16 (serogroups) sgs; among these sgs, sg 1 is the most prevalent disease-causing sg [1—3].

Legionellosis and Legionnaires' disease may appear as sporadic cases or clusters or outbreaks of large numbers of cases. The most critical factor that determines the number of cases is the route of exposure to contaminated water sources, especially in tourism-related locations, such as hotels and holiday camp villages [4,5].

In this study, we reviewed six cases including an index case and five others from a group of Turkish tourists, who stayed in different hotels in Iraq (between 15 and 25 December, 2013). These individuals returned to Turkey after a religious visit and were treated for legionellosis at Cerrahpasa Medical Faculty (CMF) of Istanbul University. In the context of these six cases of legionellosis caused by rarely encountered sgs of *L. pneumophila*, this study aimed to emphasize the regional epidemic threats of infectious diseases occurring by the movement of people in the context of mutual visits between countries or neighboring countries for tourism-related or immigration-related purposes.

2. Methods

2.1. The detection of *L. pneumophila* IgG and IgM

The analyses of IgG and IgM were performed in our unit using the anti-*L. pneumophila* indirect immunofluorescent IgM, IgG kit (Euroimmun AG, Leubeck, Germany). The serum IgM level was evaluated with titers from 1/32 to 1/256.

2.2. Serogrouping analysis

All these 6 serum samples, including the index case's sample, were sent under special transport precautions to the Euroimmun AG Clinical Immunology Laboratory in Lubeck, Germany for serogrouping. In that laboratory, *Legionella* 1 Biochip/Verification BIOCHIP slides were prepared using the TiterPlane technique. Serogrouping of *L. pneumophila* 1—14 was subsequently performed. In the study, the primary dilution was 1/100 and the subsequent dilutions were 1/320 and 1/1000.

2.3. PCR studies

L. pneumophila DNA was investigated in nasopharyngeal swabs, sputum and whole blood samples obtained from the index case, K.Y. and his spouse, Z.Y. using a multiplex PCR

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