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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

kit (Seeplex PneumoBacter ACE Detection Multiplex PCR Seegene, Inc., Korea).

2.4. Diagnosis of the other patients

After the diagnosis of the index case and his spouse, we were able to contact only four of the people in their tourist group because they all lived in Eastern Turkey far from Istanbul where our study was conducted. We telephoned the individuals to explain the situation and invite them to our medical center. We were unable to reach the other 44 people, despite our efforts.

3. Results

3.1. Serogroup analyses

The dilutions were evaluated as: borderline: (+), weak positive: +, positive: ++, or strong positive: +++. Using this serogrouping technique, the *L. pneumophila* of the index case K.Y. (M/54) was found to be sg 12 and that of F.G. (F/28) was found to be sg 11. The *L. pneumophila* of Z.Y. (M/50) was sg 14, that of B.T. (M/45) was sg 4 and sg 6 and that of A.K. (M/50) was sg 4. However, the sg of *L. pneumophila* could not be identified in Z.C. (F/27). The strength of the reaction was 1/320 for sg 4 and sg 6, 1/100 for sg 11, sg12 and sg14.

3.2. Index case

On 10 January 2014, a 54-year-old man (K.Y.) without any prior disease was admitted to Internal Medicine Department of Cerrahpasa Medical Faculty with complaints of fever, chills, weight loss and joint pain that lasted for two weeks (Table 1). No significant findings were observed upon physical examination, except splinter hemorrhage. Microbiological analyses were negative for any microorganisms known to be pathogenic (Table 2). Electrolyte imbalances and other biochemical signs were absent. Echocardiography revealed no thrombus, vegetation or myxoma. Urine analysis, fundoscopic examination and immunological parameters revealed normal vital parameters. Thoraco-abdomino-pelvic computed tomography (CT) revealed ground-glass consolidation and the tree-in-bud sign in the medial segment of the right lung (Fig. 1). In addition, a thick, irregular-walled cavitary lesion was observed in the upper segment of the right lung (Fig. 2). In the light of the radiological images, the patient was pre-diagnosed with cavitary pneumonia. Due to the CT images and the clinical signs, three consecutive sputum samples were examined and bronchoscopy was performed with suspect of tuberculosis. Bacterial or fungal pathogens were not detected (Table 2). The result of trans-bronchial biopsy was not specific. Moreover, the clinical progression was too rapid for pulmonary tuberculosis. The patient was also evaluated for granulomatous angiitis. Rhinoscopic examination revealed no mucosal ulcers. The patient denied hearing loss. The presence of arthralgia, polyneuropathy and cavitary pulmonary lesions prompted us to analyze the patients.

The patient reported that he had been on a religious trip to Iraq with a large group 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 also experienced the same symptoms.

One week after the admission (on 17 January 2014), his serum samples arrived to our ELISA unit for analyses of *L. pneumophila* IgM and IgG levels. The result was positive with a titer of 1/32 titer for IgM. The serum IgG level was negative with a 1/100 titer (Table 2). The diagnosis was Legionnaires' disease (Table 1). The patient was treated with 200 mg/day of doxycycline. The antibiotic therapy lasted 14 days and after one month, the control chest CT revealed that the pulmonary consolidation was resolved completely and the cavitary lesion had disappeared. A tractional bronchiectasis was observed in the latter region (Fig. 3); this lesion was accepted as a residual lesion. The patient's complaints disappeared completely, and he was fully recovered.

To confirm the presence of Legionnaires' disease with serologic assays, a 4-fold increase in serum titers was required. The second serum sample obtained after a 3-week of admission to the hospital (on 09 February 2014) was positive for *L. pneumophila* IgM with a titer of 1/256 and with a titer of 1/100 IgG. The index case's 5th month (i.e., May) control tests for *L. pneumophila* IgM and IgG were also positive with titers of 1/32 and 1/100, respectively.

The patient's 50-year-old spouse (Z.Y.) who attended the same trip was admitted with similar but less severe complaints of intermittent fever, shivering, headache and mild myalgia. Upon her laboratory test findings, she was not

Table 1 Demographic characteristics of the patients and important dates during their infections.

Demographic characteristics of patients	K.Y.	Z.Y.	F.G.	B.T.	Z.C.
Age	54	50	28	45	27
Gender	Male	Female	Female	Male	Female
IMPORTANT DATES					
Hospitalization date in CMF	10.01.2014	(−)	20.01.2014[a]	(−)	(−)
Admission date as outpatients in CMF	(−)	18.01.2014	(−)	27.01.2014	27.01.2014
Diagnosis of infection date	17.01.2014	20.01.2014	24.01.2014	28.01.2014	28.01.2014
Confirmation date of infection	09.02.2014 (Lp)	09.02.2014 (PPf)	15.02.2014 (Lp)	19.02.2014 (PPf)	19.02.2014 (PPf)

Lp: The causative agent of infection was confirmed as *Legionella pneumophila*.
PPf: The infection was confirmed as possible Pontiac fever.
CMF: Cerrahpasa Medical Faculty.
[a] FG visited first Erzurum Governmental Hospital (Eastern Turkey) (on 12 January 2014).

Table 2 Laboratory and CT findings of patients.

	K.Y.	Z.Y.	F.G.	A.K.	B.T.	Z.C.
Laboratory findings						
White blood cells	13,000	5700	8400	10,400	7500	5300
Neutrophils	75%	53%	64%	56%	55%	49%
Hemoglobin	13.4	13.9	12.3	14.6	14.1	12.9
Hematocrit	42%	41%	37%	41%	41%	38%
Plateletes	245×10^3	163×10^3	233×10^3	309×10^3	290×10^3	213×10^3
C reactive protein	3.4	3.2	3.7	2.1	1.80	1.5
ESR	20*35**	5*11**	7*18**	12*28**	6*15**	8*23**
Aerobic blood culture	(−)	(−)	(−)	(−)	(−)	(−)
Anaerobic blood culture	(−)	(−)	(−)	(−)	(−)	(−)
Brucella serology	(−)	(−)	(−)	(−)	(−)	(−)
Salmonella serology	(−)	(−)	(−)	(−)	(−)	(−)
Hidatic cyst serology	(−)	ND	(−)	ND	ND	ND
Lp IgM^1 titers	1/32	1/32	1/64	1/64	1/128	1/32
Lp IgM^2 titers	1/256	1/128	1/256	1/256	1/512	1/256
Lp IgG^1 (+) titers	Negative	1/100	1/100	1/100	1/100	1/100
Lp IgG^2 (+) titers	1/100	1/100	1/100	1/100	1/100	1/100
L. pneumophila DNA (S, NS, WB)	(−)	(−)	(−)	(−)	(−)	(−)
Lp serogroup	12	14	11	4	4 & 6	ND
PPD (mm)	4	4	5	8	7	4
Tuberculosis cultures (blood, S, BAL)	(−)	ND	(−)	ND	ND	ND
Non-specific culture (sputum, BAL)	(−)	ND	(−)	ND	ND	ND
Gram/ARB (sputum, BAL)	NP/-	ND	NP/−	ND	ND	ND
CT findings						
Tree-in-bud in CT	(+)	ND	(+)	ND	ND	ND

ESR: erythrocyte sedimentation rate *: 1. hour ESR, **: 2. hour ESR, Lp: *Legionella pneumophila*, CT: computed tomography, IgM^1, IgG^1: result from the first serum sample, IgM^2, IgG^2: result from the second serum sample, ND: not done, B.A.L: bronco alveolar lavage, S: sputum, NS: nasopharyngeal swabs, WB: whool blood, NP: not pathological.

hospitalized (Tables 1 and 2). On 20 January 2014 (i.e., the 10th day of hospitalization of K.Y.) the IgM and IgG results of patient were: IgM was positive with a titer of 1/32 and IgG was positive with a titer of 1/100. In contrast to the primary case, a thoracic CT failed to reveal any specific pathology related to Legionnaires' disease (i.e., ground-glass opacification, etc.). In the light of these findings, the patient was clinically diagnosed with possible Pontiac fever caused by *L. pneumophila* and no other supportive or specific treatment was administered. *L. pneumophila* DNA was investigated in nasopharyngeal swabs, sputum and whole blood samples of K.Y. and his spouse, Z.Y. using a multiplex PCR kit (Seeplex PneumoBacter ACE Detection Multiplex PCR Seegene, Inc., Korea). The results were

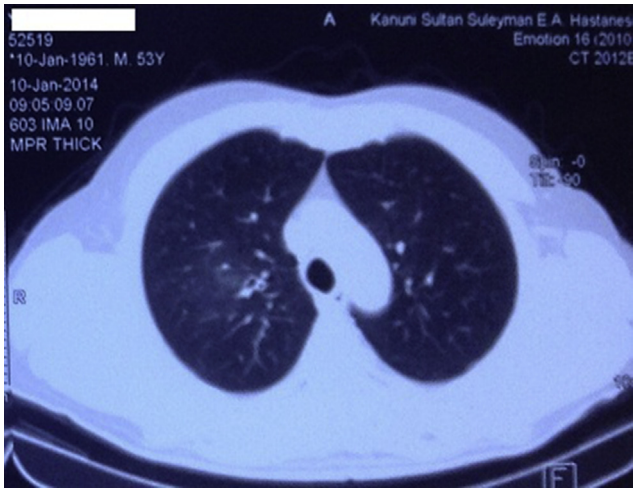

Figure 1 The image of ground glass consolidation in thoraco-abdomino-pelvic CT during the primary diagnosis of the index case.

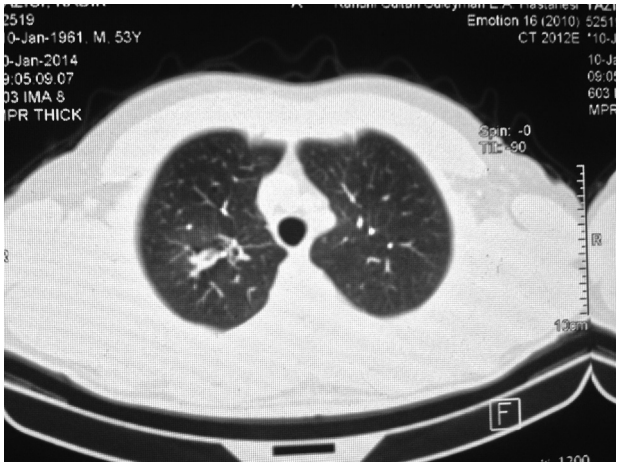

Figure 2 The image of cavitary lesions in thoraco-abdomino-pelvic CT during the primary diagnosis of the index case.

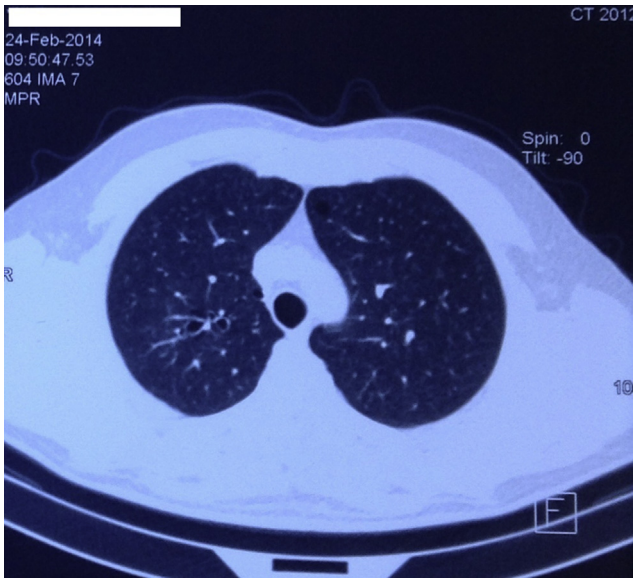

Figure 3 The control CT of the index case after treatment (disappeared pulmonary consolidations and cavitary lesions, presence of residual lesions of tractional bronchiectasis).

negative whereas the second blood sample of Z.Y. tested for *L. pneumophila* IgM and IgG levels (on 09.02.2014) revealed an IgM positivity with a titer of 1/128 and an IgG positivity with a titer of 1/100.

3.3. Other cases

After the diagnosis of the index case (K.Y.) and his spouse (Z.Y.), we tried to reach the other four people (F.G., A.K., B.T., Z.C.), who were in contact with the index case and had clinical findings and medical history related with this infection. We phoned the individuals to explain their situation and invite them to our medical center because they were not living in Istanbul but in an Eastern Anatolian region of Turkey.

These four cases were described below. F.G. was a 28-year-old female who visited Erzurum Governmental Hospital (Eastern Turkey) on 12 January 2014, near her home village with clinical symptoms identical to those of the index case. Thoracic CT revealed similar pathological findings. *L. pneumophila* sg 1 antigen was not found in the urine sample. IgM and IgG tests could not be evaluated due to the lack of resources in Erzurum Governmental Hospital; therefore, the diagnosis was not completed. An oral empirical treatment was started with 2 × 500 mg of clarithromycin and 2 × 1000 mg of amoxicillin–clavulanic acid. The patient accepted our invitation and visited our center, because her complaints did not cease and continued at a moderate level. During the follow-up period of the patient in Chest Disease Department, laboratory tests were performed, her serum samples were tested for *L. pneumophila* IgM and IgG. The IgM test was positive with a titer of 1/64, and the IgG test was positive with a titer of 1/100 on 24 January 2014 (Table 2). On the thoracic CT scan, tree-in-bud images were observed in the lateral region of the middle lobe of the right lung, in the basal region of the inferior lobe, and in the lingula of the upper lobe of the left lung. Based on these results, the patient's treatment was reorganized to include 10 days of 2 × 500 mg of clarithromycin. On the day that the primary case was discharged (after 4 weeks of hospitalization), *L. pneumophila* serum IgM and IgG levels of FG were reactive with titers of 1/256 and 1/100, respectively (on 15 February 2014) (Tables 1 and 2). The patient's serum sample was stored at −80 °C for serogrouping analyses. In contrast to the index case (K.Y.) and F.G., the other invited cases (i.e., A.K., a 50-year-old male, B.T., a 45-year-old male, and Z.C., a 27-year-old female) exhibited no typical legionellosis symptoms after their travel in Iraq. These cases only had some complaints of fever, headaches and intermittent myalgia and never reported any respiratory distress. Consequently, no CT scans were performed and the patients were sent to their home addresses from their regional medical center with pain relief medications and oral antibiotics (2 × 1000 mg of amoxicillin–clavulanic acid). After the admission of these patients to CMF, laboratory tests were performed and the serum samples of these patients were analyzed (on 28 January 2014) for *L. pneumophila* IgM, and the results were positive, with titers of 1/64 for A.K, 1/128 for B.T., and 1/32 for Z.C. *L. pneumophila* IgG was also positive for all of these patients, with identical titers of 1/100 (Table 2). After three weeks the second serum samples of these patients were tested for *L. pneumophila* IgM and IgG levels (on 19.02.2014). The results revealed an IgM positivity with titers of 1/256 for A.K, 1/512 for B.T., and 1/256 for Z.C. and an IgG positivity was with a titer of 1/100. These four cases (Z.Y., A.K., B.T. and Z.C.) were diagnosed with possible Pontiac fever. The serum samples of these six cases were stored at −80 °C for serogrouping.

4. Discussion

In our study, we aim to present six cases of legionellosis, four of which (Z.Y., A.K., B.T. and Z.C.) were diagnosed as possible Pontiac fever based on the absence of laboratory confirmation and a tenuous link between the trip and the clinical symptoms/laboratory diagnostics available at the time; reliance on a single positive IgM test in convalescence, poorly characterized illnesses post-Iraq trip, and the fact that seropositivity was not per se temporally-linked to the Iraq trip. The other two cases (K.Y. and F.G.) were presented as Legionnaires' disease.

The involved patients had visited the Iraqi city Najaf, which is an important city for Shiites, where civil war and political, religious and sectional conflicts exist. They (50 shia pilgrims from Kars city of Turkey) visited the tomb of Ali. Ali was the cousin and son-in-law of the Islamic prophet Muhammad. The Shias regard Ali as the most important figure after Muhammad. Shia pilgrims usually go to Mosque of Ali in Najaf for visiting and pray there [6]. During their 10 day trip in Iraq, these cases stayed in different hotels. All complained about poor hygiene and sanitation in their hotels (e.g., showers were outdated with limited water flow, rooms were cleaned only every other day, clean sheets and towels were moist, and the drinking water served in the hotels was not from closed bottles). Due to the geographical location and hot climate in Iraq in

December 2013, water consumption was high. During that period in the Iraqi city Necef, which is an important city for Shiites, suffered from civil war with frequent occurrence of shootings, and political, religious and sectional conflicts. For these reasons, the city had unhealthy living conditions and a deficient infrastructure. Different sgs might have been acquired by these people during their stay in different hotels. Source of these various sgs might have been drinking water or water from the outdated showers. In a study reported from France, three different Lp (*L. pneumophila*) sgs (Lp1, Lp10 and Lp12) isolated from the same natural biofilms of a sulfur-rich warm spring of a French thermal spa, were reported as the source of infection [7]. In 2013, in drinking water of different regions of Iraq (i.e., Basra), the frequencies of Lp sgs 2—15 were reported as 22.9% [8]. With these six cases of legionellosis, we hope to draw attention to epidemics due to the movement of people in the context of mutual visits between countries or neighboring countries for tourism-related or immigration-related purposes.

Reingold et al. [9] indicated in their retrospective review that two-thirds of legionellosis cases were caused by sg 1 and sg 6. In 2002, Yu et al. [10] reported sg 1 had a frequency of 84.2% (in 428 cases), sg 6 a frequency of 1.7% (in 9 cases). Faris et al. [11] reported 4 cases caused by sg 13 in 2005. In a study (2013) performed in Basra region of Iraq, the most frequent sg was demonstrated to be sg 1, with a rate of 77.1%. In contrast, sg 2—15 were found at a rate of 22.9% [7].

In our study, the index case K.Y. and F.G. were diagnosed with Legionnaires' disease caused by *L. pneumophila* sg 12 (K.Y.) and sg 11 (F.G.). The patient's history of staying in a hygiene-deficient hotel and patient's clinical findings and radiological information (particularly patient's CT images before and after treatment) were important for his diagnosis. In addition, although the sg 1 antigen test was negative twice, the *L. pneumophila* IgM test reactiveness, the four-fold titer increases in the serum samples and the good response to anti-legionellosis treatment were all considered as important parameters. The Centers for Disease Control (CDC) in Atlanta, USA reported that paired sera that show a four-fold increase in antibody levels when drawn shortly after illness and several weeks following recovery are acceptable for confirming the diagnosis [12]. Similarly, a four-fold increase in antibody levels was observed in all of our cases. The *Legionella* culture could not be performed due to technical difficulties, such as the need for special culture media and technically skilled personnel [13].

In two (Z.Y.: F/50 and A.K.: M/50) of our four possible Pontiac fever cases, *L. pneumophila* sg 14 and 4 were found, respectively, and in the third possible Pontiac fever case (B.T.: M/45), sg 4 and 6 were presented together. These patients had similar clinical symptoms and patient histories, but they stayed in different hotels and none of them had typical thoracic CT findings.

The primary case prompted us to perform a surveillance study, and *L. pneumophila* IgM and IgG tests were completed for each patient. Similar to the study of Yu et al. [10], which reported that in three of 508 cases, the sg of the involved *L. pneumophila* could not be identified, in our final case (Z.C.: F/27), the sg was not identified.

A major limitation of this work is the absence of genotyping and the serogroup difference as 12 and 14 between index case, K.Y and his wife, Z.Y, respectively who shared the same hotel. We suggest that this serogroup difference may be caused by (for men and women) sitting separately in Islamic rules.

4.1. Conclusions

In conclusion, although the diagnosis of legionellosis was supported by genotyping, it is emphasized that in infected patients for whom the diagnosis of legionellosis is ruled out due to negative test results for the routine *L. pneumophila* sg 1 urinary antigen test, rare sgs may play a role for legionellosis. These rare sgs might represent a serious public health problem. Additionally, 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. Turkey that neighbors other Middle Eastern countries, may also be affected by this situation. 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.

Consent

Written informed consent was obtained from each patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Authors' contributions

Prof. Dr. Bekir KOCAZEYBEK: made substantial contributions to conception and design, analysis and interpretation of data, have been involved in drafting the manuscript or revising it critically for important intellectual content.

Dr. Pelin YÜKSEL: acquisition of data, or analysis and interpretation of data.

Dr. Reyhan ÇALIŞKAN: acquisition of data, or analysis and interpretation of data.

Dr. Mert KUŞKUCU: acquisition of data, or analysis and interpretation of data.

Dr. Zafer HABİP: acquisition of data, or analysis and interpretation of data.

MSc. Student Harika Öykü DİNÇ: acquisition of data, or analysis and interpretation of data.

Prof. Dr. Hrisi Bahar Tokman: made substantial contributions to conception and design analysis and interpretation of data.

Assoc. Prof. Dr. Sevgi Ergin: made substantial contributions to conception and design.

Md. Yağız Meriç ALTUN: acquisition of data, or analysis and interpretation of data.

Dr. Dilek Keskin: acquisition of data, or analysis and interpretation of data.

Dr. Serap Şahin Yavuzer: acquisition of data, or analysis and interpretation of data.

Dr. Mahir Cengiz: acquisition of data, or analysis and interpretation of data.

Dr. Suhail Sheikh: acquisition of data, or analysis and interpretation of data.

Prof. Dr. Nail YILMAZ: acquisition of data, or analysis and interpretation of data.

Assoc. Prof. Dr. Suat Saribas: made substantial contributions to conception and design have been involved in drafting the manuscript or revising it critically for important intellectual content.

PhD student: Asiye Karakullukcu: acquisition of data, or analysis and interpretation of data.

Authors' information

B.S.K: still works in Istanbul University, Cerrahpasa School of Medicine, Department of Internal Medicine, Istanbul Turkey, as the head of the serology/ELISA laboratory. His research interest includes antimicrobial resistance, pathogenesis (especially *Helicobacter pylori*, *Chlamydophila pneumoniae*, Bifidobacteria), *Toxoplasma gondii* isolation, treatment and epidemiology. He is the coordinator of a continuing project conducted in conjunction with Stanley Medical Research Institute, USA (Project title: Effect of antipsychotic drugs on *T. gondii* IgG serology). Additionally he is collaborating with a group from Johns Hopkins School of Medicine in a multicentric research study based on serotyping *T. gondii*. Furthermore he is working on the value of new serological and molecular methods and recently on some acute phase reactants for the diagnosis of endocarditis or other clinics. He has published 66 research articles and 5 case reports in international journals. He was the advisor for 3 doctorate thesis, 7 Master of Science thesis and 4 medical doctors specialization thesis.

Conflict of interest

There is no conflict of interest related with this study.

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