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## Diverse *Francisella tularensis* Strains and Oropharyngeal Tularemia, Turkey

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**To the Editor:** Tularemia is a zoonosis caused by the bacterium *Francisella tularensis*; the main forms of disease that occur in humans are ulceroglandular/glandular, oculoglandular, oropharyngeal, and respiratory. In Turkey, tularemia outbreaks were described as early as 1936–1938 (1), but tularemia was not reportable until 2004. Recently, multiple tularemia outbreaks in Turkey have been described, including in regions where the disease has not been previously reported; it is now considered a reemerging zoonotic disease in Turkey (1).

The only *F. tularensis* subspecies found in most of Eurasia, including Turkey, is *holarctica*. Genetic diversity is low, probably because emergence is recent (2). However, discovery of whole-genome single-nucleotide polymorphisms (SNPs), coupled with subsequent canonical SNP (canSNP) analyses, have identified numerous phylogenetic groups within this subspecies. The distinct phylogeographic patterns provide insight into its evolutionary history (3–7).

From December 2009 through January 2011, tularemia outbreaks increased in Turkey, primarily in the central region (8). Oropharyngeal tularemia was diagnosed for

**Table.** *Francisella tularensis*–positive clinical samples from 14 patients with oropharyngeal tularemia, Turkey, December 2009–January 2011\*

Patient no. (sample no.)	City	CanSNP subgroup†	MLVA genotype‡
1 (F0737)	Corum	B.20/21/33	i
2 (F0738)	Cankır	B.28/29	b
3 (F0739)	Yozgat	B.28/29	b
4 (F0740)	Zonguldak	B.7/8	a
5 (F0741)	Corum	B.20/21/33	e
6 (F0742)	Corum	B.20/21/33	e
7 (F0743)	Corum	B.20/21/33§	ND
8 (F0744)¶	Bala/Ankara	B.20/21/33	e
9 (F0745)	Ankara	B.20/21/33	d
10 (F0746)	Corum	B.20/21/33	j
11 (F0747)	Bala/Ankara	B.20/21/33	g
12 (F0748)	Corum	B.20/21/33	f
13 (F0749)	Ankara	B.20/21/33	c
14 (F0750)	Emirdağ/Afyon	B.20/21/33	h

\*Clinical samples collected in 2011 from patients with cervical lymphadenitis at Hacettepe University Medical Faculty, Pediatric Infectious Disease unit, Ankara, Turkey. CanSNP, canonical single-nucleotide polymorphism; MLVA, multilocus variable-number tandem-repeat analysis; ND, not determined.

†Subgroups published in (3,4,6,7). CanSNP branches tested on samples in this study: B.3, B.4, B.5, B.6, B.7, B.8, B.9, B.10, B.11, B.13, B.20, B.21, B.22, B.26, B.27, B.28, B.29, B.30, B.31, B.32, and B.33.

‡MLVA markers (M03, M05, M06, M20) (2).

§Subgroup classification based on approximation because genotype for B.33 remains unresolved.

¶This patient was an adult (all others were children).

<sup>1</sup>These authors contributed equally to this article.

14 patients (13 children, 1 adult), and fine-needle lymph node aspiration was performed at the Pediatric Infectious Diseases Unit at Hacettepe University, Ankara. DNA was extracted from these 14 samples (QIAamp DNA Mini Kit; QIAGEN, Hilden, Germany) and screened by using a PCR selective for the *tul4* gene region specific to *F. tularensis* (9); all 14 samples were positive for *F. tularensis* (Table). Residual, de-identified portions of these 14 DNA extracts were used for this study.

Genetic characterization led to assignment of these 14 samples to multiple phylogenetic groups within *F. tularensis* subsp. *holarctica*. Analysis with 18 previously described (3,4,6,7) canSNP assays (Table) led to assignment of the 14 samples to 3 previously described phylogenetic groups within this subspecies: B.20/21/33 (n = 11), B.28/29 (n = 2), and B.7/8 (n = 1) (Table; online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/21/1/14-1087-Techapp1.pdf>). To identify additional genetic diversity, we used 4 previously described (2) variable-number tandem-repeat markers (M03, M05, M06, and M20) and identified 10 genotypes among the 14 samples, 8 of which were identified in the 11 B.20/21/33 samples (Table).

The genetic diversity among these samples and their widespread geographic origins from 6 provinces in central Turkey (Table; online Technical Appendix) suggest that the patients contracted tularemia from multiple independent sources. These sources might have been contaminated drinking water, which has been implicated as the source of human tularemia in previous outbreaks in Turkey (1) and could account for oropharyngeal tularemia in the 14 patients reported here.

The finding of these 3 phylogenetic groups within Turkey expands the known geographic range of these phylogenetic groups within *F. tularensis* subsp. *holarctica*. The presence of group B.28/29 *F. tularensis* in Turkey is not surprising; isolates belonging to this group were previously identified in bordering Georgia (3). Likewise, the presence of group B.20/21/33 *F. tularensis* is not unexpected, given the wide geographic distribution (Sweden, Finland, Russia, and Hungary [4]) of organisms belonging to this group (online Technical Appendix). Isolating group B.7/8 *F. tularensis*, previously thought to occur only in Scandinavia, in Turkey is of particular interest, given the relatively basal position of this group in the *F. tularensis* subsp. *holarctica* phylogeny (online Technical Appendix). Indeed, descendants of this group have, to date, been identified from North America only, suggesting a transfer from the Old World to the New World within this lineage (7). The circumstances of this transfer are unknown but might be discerned through additional knowledge of the geographic extent and genetic diversity of organisms in the B.7/8 group.

It has been suggested that Scandinavia might be the source of the historical spread of tularemia to the rest of

Europe and might be the origin of the ancestor to the B.13 clade (5). This suggestion was previously argued because *F. tularensis* subsp. *holarctica* isolates from Sweden have yielded more phylogenetic diversity than isolates from any other country. Indeed, except for the B.27 clade, much of the known phylogenetic diversity of this organism within Europe is present in Sweden (5). Some of the largest sets of analyzed samples originated in Sweden (5–7), whereas eastern Europe and much of Asia remain mostly undersampled. The high genetic diversity identified in our very limited sample set from Turkey is notable and includes 2 major lineages (B.7 and B.13; online Technical Appendix). These findings, together with the recent discovery that organisms of multiple *F. tularensis* subsp. *holarctica* phylogenetic groups exist in China (10), suggest that much additional phylogenetic diversity within this subspecies remains to be discovered in Eurasia, which will provide better information about the evolutionary history and historical spread of *F. tularensis* subsp. *holarctica*.

We have demonstrated that high-resolution genetic characterization of *F. tularensis* DNA extracted from biopsy samples is possible, and we conclude that oropharyngeal human tularemia in Turkey is caused by organisms of multiple distinct phylogenetic groups within this subspecies. This pattern, together with the wide geographic distribution of the 14 patients within Turkey (online Technical Appendix), suggests that the persons infected by *F. tularensis* during the 2009–2011 outbreaks in Turkey obtained their infections from multiple environmental sources.

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## Dengue Virus Serotype 3 Infection in Traveler Returning from West Africa to Germany

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**To the Editor:** Dengue virus (DENV) is a member of the family *Flaviviridae*, genus *Flavivirus*, and comprises 4 serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). DENV is transmitted by *Aedes* spp. mosquitoes in subtropical and tropical countries; an estimated 390 million dengue infections occurred worldwide in 2010 (1). In Africa, locally acquired dengue cases have been reported from 22 countries during 1960–2010, and there is evidence of transmission in 34 countries (2). The burden of dengue in Africa was recently estimated to be in the same range as that in Latin America (1).

In 2013, a 71-year-old man came to the Tropical Medicine Clinic at Heidelberg University Hospital (Heidelberg,

Germany) with suspected dengue fever 3 days after returning from West Africa to Germany. The patient had traveled for ≈6 weeks from mid-September through mid-October 2013 to Togo (Lomé, first 3 weeks), Benin (Ouidah, 1 week), back to Togo (Lomé, 1 week), and Burkina Faso (Ouagadougou, 3 days).

Tests results for DENV nonstructural protein 1 and IgM against DENV were positive. The result of a real-time reverse transcription PCR for DENV 1–4 was also positive. A serotype-specific real-time reverse transcription PCR identified DENV-3 (3,4).

To obtain the sequence of a 1,479-nt fragment of the complete gene of the envelope glycoprotein gene, we designed generic primers specific for all complete DENV-3 genomes available from GenBank (alignment was performed by using Geneious version 6.1; <http://www.geneious.com>), which were then sequentially adapted to sequences obtained (primers and protocol available upon request). Sequencing of the complete envelope glycoprotein gene of the virus isolated from the patient identified DENV-3 genotype III (GenBank accession no. KJ922394).

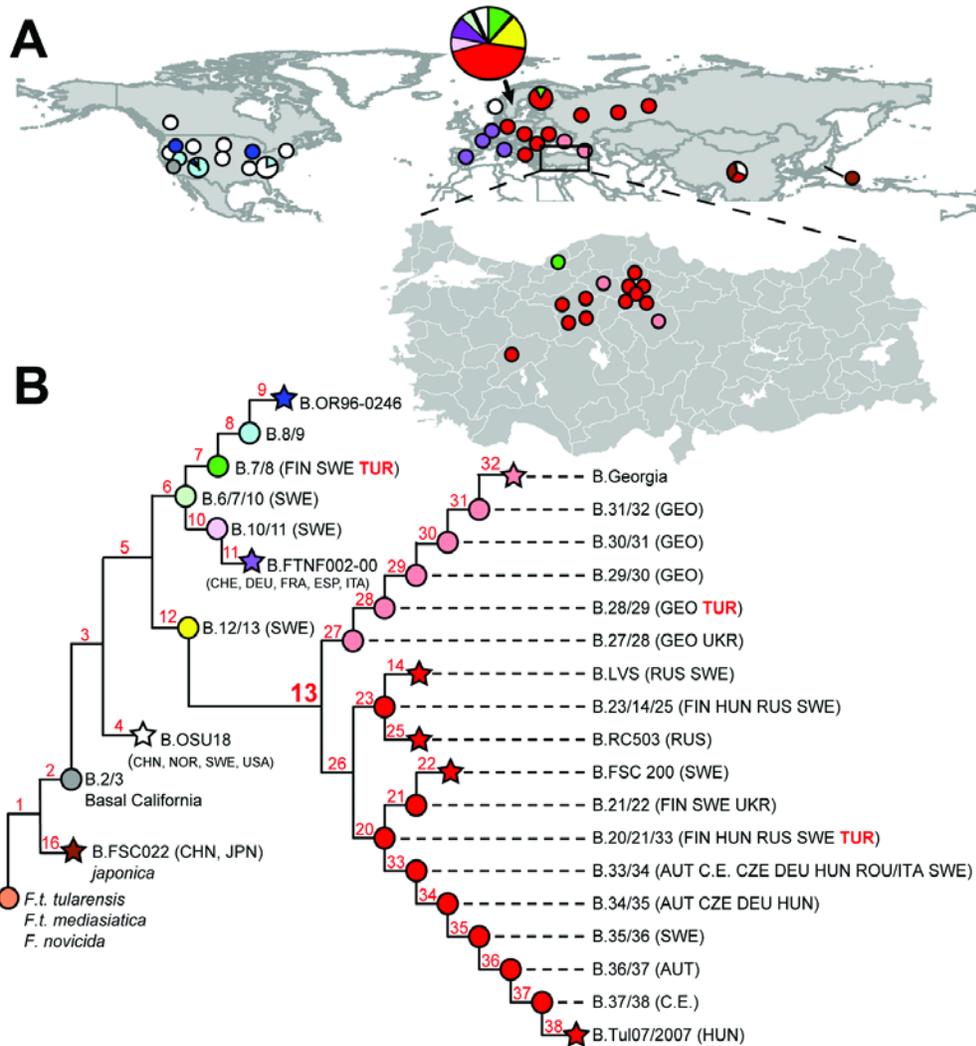
For phylogenetic comparison, we chose all DENV-3 sequences available from Africa and neighboring regions and a set of global sequences that represented different genotypes; DENV-1 was used as an outgroup. Sequences were aligned on the basis of translated nucleotide sequences, and a neighbor-joining tree with p-distance was inferred with 1,000 bootstrap replicates in MEGA version 5.2.1 (<http://www.megasoftware.net/>) for a 1,479-nt fragment spanning the complete gene of the envelope glycoprotein and a smaller fragment of 220 nt (Figure). We observed clustering of the virus sequence with those of strains from Côte d'Ivoire and Benin. Genetic identity was 99% with strains from Côte d'Ivoire (AB447989) (1,472/1,479 nt) and Benin (AB690858) (1,469/1,479nt).

Because of limited availability of only 4 complete envelope glycoprotein gene sequences from Africa, an additional phylogenetic analysis was performed with a smaller fragment of 220 nt to include more sequences of African origin. A total of 7 sequences, including additional sequences from Senegal and Cameroon, were available. Clustering of sequences of African origin was confirmed in this analysis; highest sequence identity of virus isolated from the patient was with viruses isolated in Côte d'Ivoire, Benin, and Senegal.

Nearly all sequence data for DENV-3 from Africa originate from returning travelers, such as reports of imported cases in 2006 from Cameroon to Spain (5), from Senegal to Spain in 2007 and to Italy in 2010 (5,6), from Côte d'Ivoire to France and Japan in 2008 (7,8), from Benin to Japan and France in 2010 (8,9), and from Eritrea to Finland in 2010 (5). Phylogenetic analysis of virus strains

Technical Appendix

# Diverse *Francisella tularensis* Strains and Oropharyngeal Tularemia, Turkey



Appendix Figure. Global phylogeography of *Francisella tularensis* subsp. *holarctica* and location of 14 clinical samples from Turkey. A) Global distribution of known phylogenetic groups (3,4,6,7) and expanded map of Turkey indicating provinces where the 14 patients lived (colored circles). Circle colors indicate phylogenetic groups to which the 14 samples were assigned. B) Current single-nucleotide polymorphism–based phylogenetic tree for *F. tularensis*. Red numbers indicate canonical single-nucleotide

polymorphisms (3,4,6,7). Countries of origin for strains assigned to relevant phylogenetic groups are indicated as follows: AUT, Austria; C.E., central Europe, unknown country; CZE, Czech Republic; DEU, Germany; FIN, Finland; GEO, Georgia; HUN, Hungary; ITA, Italy; ROU, Romania; RUS, Russia; SWE, Sweden; TUR, Turkey; UKR, Ukraine). Bold red text indicates phylogenetic placement of the 14 clinical samples examined in this study.