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REVIEW

Re-emergence of tularemia in Turkey

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Summary Four tularemia epidemics were reported from three different regions of Turkey between 1936 and 1953. After a long interval, a new tularemia epidemic was reported from the area around Bursa in the northwestern part of Turkey in 1988. Following this first epidemic in Bursa, small epidemics occurred in areas around Bursa between 1988 and 2002. Other tularemia epidemics in different regions of Turkey were reported between 1988 and 2005. Almost all of the cases involved the oropharyngeal form of the disease. However, ulceroglandular and oculoglandular forms were detected in the Bursa epidemics; all of the ulceroglandular cases had dermatitis on their hands. To date, 1300 cases have been serologically confirmed. We reviewed one of the biggest tularemia epidemics in Europe.

Introduction

Tularemia is a zoonotic disease caused by the Gram-negative coccobacillus *Francisella tularensis*, which was first isolated from rodents in 1911 in Tulare County, California, USA. Tularemia has been found in more than 250 animal species. *F. tularensis* strains have been identified as belonging to several subspecies including *tularensis* (also known as type A or subspecies *nearctica*), *palaearctica* (also known as type B or subspecies *holarctica*), *mediasiatica*, and *novicida*. This classification has been done principally on the basis of virulence, citrulline ureidase activity, and acid production from glycerol. Subspecies *holarctica* is now the more widely used terminology in place of subspecies *palaearctica*. Type A is reported to have a terrestrial cycle with the main reservoirs being cottontail rabbits (*Sylvilagus spp*) and ticks. Type B is reported to have a mainly water-borne cycle with aquatic rodents as reservoirs. These rodents include muskrats (*Ondatra zibethicus*) and beavers (*Castor canadensis*) in North America, and ground voles (*Arvicola terrestris*) in the former Soviet Union. However, it is not clear whether these animal species are the true reservoir of the bacterium in the environment. Type A is considered more virulent than type B for humans.[1—3]

F. tularensis is transmitted to humans through various modes, including direct handling of infected animals, ingestion of contaminated food or water, arthropod bites, or inhalation of infectious dusts or aerosols. There is evidence that the bacteria can persist in watercourses, possibly in association with amoebae. Type A is usually transmitted to humans by tick bites or contact with rabbits; type B is associated with water and animals living near water.[4—7]

Tularemia has several clinical forms in humans, including ulceroglandular, glandular, pneumonic, oropharyngeal, oculoglandular, and systemic (typhoidal). The clinical forms mostly depend on the port of entry into humans. Ingestion typically results in the oropharyngeal form and is associated with symptoms such as fever, pharyngitis, cervical lymphadenitis, and suppuration.[8]

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A wide range of arthropod vectors have been implicated in the transmission of tularemia between mammalian hosts. In the USA, biting flies are the most common vectors in Utah, Nevada, and California, while ticks are the most important vectors east of the Rocky Mountains. In the former Soviet Union, *F. tularensis* is transmitted by both mosquitoes (*Aedes*, *Culex*, and *Anopheles* species) and the *Ixodes* species of tick.[1]

Ohara et al. reported an increase in arthropod-borne tularemia between 1972 and 1998 in Japan, although no arthropod-borne cases were reported before 1951. They also reported that the occurrence of disease caused by contact with diseased hares was biphasic, with the higher peak occurring during the winter, whereas the occurrence of arthropod-borne tularemia was found to be common from spring to autumn.[9]

In central Europe, *Dermacentor reticulatus* and *Ixodes ricinus* ticks are important vectors for *F. tularensis*.[10,11]

Two epidemics, which included mostly ulceroglandular forms, were reported from Spain between 1997 and 1998. The main transmission route in the epidemics was found to be crayfish fishing in a contaminated freshwater stream and direct contact with infected hares. *F. tularensis* type B was the responsible bacterium.[12,13]

A widespread outbreak of tularemia in Sweden was reported in 2000. The outbreak was investigated in a case–control study, and also clinical comparisons were made between endemic areas and outbreak areas. Mosquito bites were found to be the main risk factor, and swollen lymph nodes and wound infections were found to be more common in the outbreak area, while pneumonia was more common in the disease-endemic area.[7]

Forty-nine cases with oropharyngeal tularemia associated with the consumption of water from an unchlorinated water system were reported from Italy in 1982.[14]

Three hundred twenty-seven patients with tularemia confirmed by serology in Kosovo in 2000 were investigated, and the results of a case–control study were found to be consistent with the idea that the tularemia outbreak was food-borne. This was based on associations of illness with large numbers of rodents in the peridomestic environment, rodent contamination of food storage and preparation areas, and the eating of some uncooked foods.[15]

A total of 262 laboratory-confirmed tularemia cases were reported from Bulgaria between 1998 and 2003. The majority of the patients had the oropharyngeal form.[16]

In the present study, the Turkish and English literature regarding the epidemiology of tularemia in Turkey were retrospectively reviewed. In addition, results of the Turkish Tularemia Reference Laboratory were analyzed.

Tularemia in Turkey

Four tularemia epidemics were reported from three different regions of Turkey between 1936 and 1953.[17–20] After a long interval, a new tularemia epidemic was reported from the area around Bursa (Bursa epidemic), which is in the north-western part of Turkey.[21–23] Following this first Bursa epidemic, small epidemics occurred in different parts of the area surrounding Bursa between 1988 and 2002; during the same period (1988–2005), further tularemia epidemics were reported from different regions of Turkey.[24–30]

The characteristics of the tularemia epidemics reported from Turkey are shown in Table 1.[17–25,28–34] In addition, cases from serologically confirmed but unpublished epidemics from different regions were provided by Professor Suna Gedikoğlu for this retrospective analysis.[35] To date, 1300 cases have been serologically confirmed. The microagglutination method was used for serological diagnosis. The antigen used in serological tests was prepared with intraperitoneal inoculation of the first *F. tularensis* strain that was isolated from a lymph node aspirate, into guinea pig and rats. The test antigen was compared with a standard antigen (Difco code No. 2251–2256), which has been found to be suitable for testing sera for antibodies by microagglutination.[24] Antibody titers $\geq$1:160 or a four-fold or greater titer change were accepted as positive for serological diagnosis. All of the regions that had a tularemic focus according to serological confirmation or reported epidemics are shown in Figure 1.

Table 1 Characteristics of the tularemia epidemics reported in Turkey

Year	Region	No. of cases	Transmission	Clinical form[a]	Mortality	Ref.
1936	Lüleburgaz Kırklareli Tekirdağ	150	Water-borne	Oropharyngeal	1	17
1938	Van	6	Food	Oropharyngeal	0	18
1945	Lüleburgaz	18	Water-borne	Oropharyngeal	0	19
1953	Antalya	200	Water-borne	Oropharyngeal	0	20
1988–2002	Bursa	205	Water-borne	Oropharyngeal	0	21
2000	Ayaş, Ankara	16	Water-borne	Oropharyngeal	0	25
2004–2005	Zonguldak Bartın Kastamonu	61	Water-borne	Oropharyngeal	0	29
2004–2005	Kocaeli	145	Water-borne	Oropharyngeal	0	30
2004	Samsun	43	Water-borne	Oropharyngeal–glandular	0	31
2005	Edirne	10	Water-borne	Oropharyngeal	0	32
2004–2005	Kars	56	Water-borne	Oropharyngeal	0	33
2005	Gölcük, Kocaeli	5	Water-borne	Oropharyngeal	0	34

[a] Most of the cases or all of the cases.

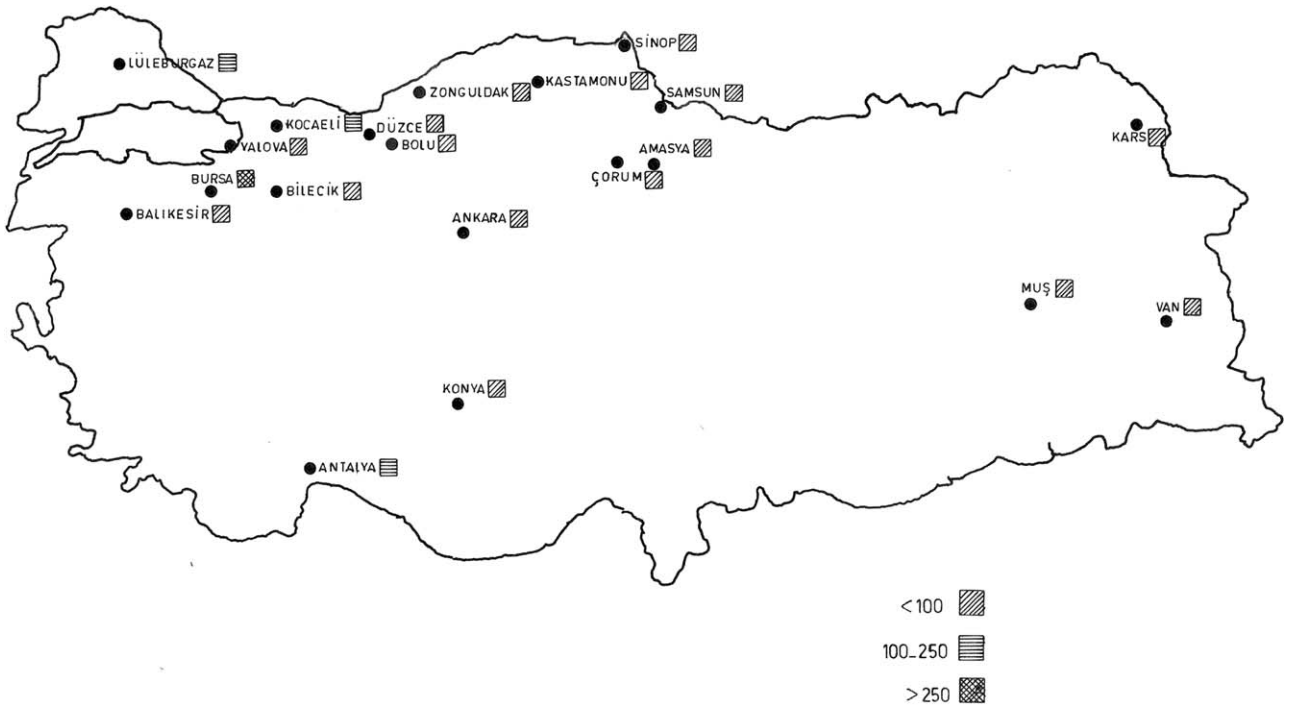

Figure 1 Regions having a tularemic focus according to serological confirmation or reported epidemics from Turkey (number of patients).

There was no isolation of *F. tularensis* from the suspected water in these epidemics despite intensive efforts. All age groups, males and females were equally affected during these epidemics. All of the cases appeared in the autumn or winter.[24,25,28]

Almost all of the reported cases involved the oropharyngeal form. However, ulceroglandular and oculoglandular forms were also detected in the Bursa epidemics; all of these ulceroglandular cases had dermatitis on their hands.

We believe that all of the tularemia epidemics that occurred in Turkey were water-borne. During the epidemic, three water samples from different epidemic areas were positive by PCR assay.[31,32,36] The findings that suggest water-borne epidemics are shown in Table 2. On the other hand, *F. tularensis* was not isolated from the water. This might have been due to delays in taking water samples for cultures (assuming the bacteria can persist in water over a prolonged period) because of the late presentation of symptoms in affected patients.[6,37]

Table 2 Findings suggestive of water-borne epidemics

Only one mortality caused by tularemia (indirectly suggests type B and water-borne epidemics)
Almost all of the cases are of the oropharyngeal form; presence of oculoglandular form
The lack of the ulceroglandular form (except in those patients who had dermatitis previously)
Limitation of the epidemics depends on the use of certain aqueducts in the same area
The lack of chlorination of the water system in some epidemic areas
PCR positivity for *F. tularensis* in water from epidemic areas

In our opinion, the water was contaminated by infected rodents and hares. Unfortunately, we did not test this hypothesis until recently. However, there is some evidence supporting this hypothesis. Gürcan et al. reported that Turkish isolates contained the *holarctica* subspecies.[38] In Europe, tularemia is most frequently found in hares (*Lepus spp*), although hares probably do not constitute a reservoir for the disease.[2] In the Kosovo epidemic, the lipopolysaccharide (LPS) antigen of *F. tularensis* was found to be positive in the liver tissue of *Apodemus agrarius* (field mice) recovered from a well in the village where the index case of the epidemic was reported. In addition, authors reported that fecal specimens collected from *A. agrarius* and *Rattus rattus* (black rats) were positive for the *F. tularensis* antigen.[15] The presence of lagomorphs such as *Lepus europaeus* Palas and rodents such as *Arvicola terrestris*, *Microtus arvalis*, and *A. agrarius* in Turkey, support our hypothesis.[39] An increase of voles before the epidemic occurred in the area around the Bursa epidemic during 1988 (reported by rural villagers), also supporting our hypothesis.

The tularemia epidemics in Turkey do not seem to have been tick-borne, although there are several reports that suggest the presence of *Ixodes ricinus* in Turkey. The presence of a few patients with the ulceroglandular form (most of them had dermatitis before the epidemics) and geographical differences between the epidemics and the regional distribution of *Ixodes ricinus* ticks (according to published reports) indirectly support the same hypothesis.[40—43]

Tularemia epidemics occurred in different regions of Turkey between 1988 and 2005. It is not clear whether these epidemics were due to the spreading of bacteria or to exacerbation of old tularemic foci. The widespread geographic distribution of *F. tularensis* might be explained by an occasional transport of infectious immature ixodid ticks parasitizing migratory birds, although the evidence (i.e.,

direct detection of the agents in the ticks attached to migrating birds) is still lacking. Surprisingly, fleas can also be transported over long distances on migrating birds. In water-borne infections, the agent can be shed by infectious migrating birds, resulting in contamination of water with feces, nasal discharges, and respiratory exudates.[44]

Two questions remain to be answered about the epidemiology of tularemia in Turkey. The first question is ''What is the epidemiology of *F. tularensis* in rodents, hares, ticks, and fleas in Turkey?'' We need environmental studies and a focus on the ecology of *F. tularensis* in epidemic areas. The second question is ''Why were there no tularemia cases between 1953 and 1988, and why did the disease re-emerge in 1988?'' Re-emergence of tularemia might have been due to ecological changes.[45–47] We know that climate conditions have changed significantly over the last 50 years in Turkey.[48] We believe that these questions should be answered by further studies.

Tularemia should be considered in the differential diagnosis of patients with fever, pharyngitis or tonsillitis and/or cervical lymphadenopathy, and who have not responded to beta-lactam antibiotics. We believe that passive surveillance is sufficient to detect new case clusters and outbreaks. For prevention of new outbreaks, national and local health authorities should be aware that tularemia may be disseminated to other regions of the country and they should check sources of all drinking water for adequate sanitation.

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