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A General Overview of *Francisella tularensis* and the Epidemiology of Tularemia in Turkey

Francisella tularensis ve Türkiye'de Tularemi Epidemiyolojisine Genel Bir Bakış

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SUMMARY

Tularemia, caused by the facultative intracellular gram-negative bacterium Francisella tularensis, is a multisystemic disease in humans and some animals. Tularemia occurs predominantly between 30° and 70° latitude in the northern hemisphere, but has rarely been found in the southern hemisphere. F. tularensis can infect a wide range of animals (more than 250 animal species). Small rodents are the main natural hosts, and blood-sucking ectoparasites are the most important vectors. Humans can acquire the infection through bites from infected arthropods (usually ticks), contact with infected animal tissues or fluids, ingestion of contaminated water or food, or inhalation of aerosolized bacteria. The clinical picture and severity of the disease in humans vary depending on the route of transmission, the infecting dose, the Francisella subspecies involved, and the immune status of the host. Clinical presentations include the six classic forms of tularemia: ulceroglandular, glandular, oculoglandular, oropharyngeal, typhoidal, and pneumonic. Tularemia was first recognized in Turkey in 1936, when an outbreak occurred in a military garrison and rural community in the Luleburgaz region. Since then, tularemia epidemics have been reported from different regions of Turkey, but the majority of outbreaks have occurred in the Marmara and western Black Sea region. To date, more than 2000 cases have been serologically confirmed. Recently, tularemia outbreaks emerged in several provinces, mainly located in the central parts of the country. In this review, general characteristics of F. tularensis and the epidemiology of tularemia in Turkey are summarized.

Key Words: *Tularemia, Francisella, Epidemiology, Disease outbreaks, Turkey*

ÖZET

Francisella tularensis ve Türkiye'de Tularemi Epidemiyolojisine Genel Bir Bakış

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Fakültatif hücre içi gram-negatif bakteri olan Francisella tularensis tarafından oluşturulan tularemi, insan ve bazı hayvanların multisistemik bir hastalığıdır. Tularemi esas olarak kuzey yarım kürede 30° ve 70° enlemler arasında görülürken, güney yarım kürede nadiren tanımlanmıştır. F. tularensis çok geniş bir yelpazede yer alan hayvan türlerini infekte edebilir (250'den fazla sayıdaki hayvan türü). Tu-

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laremi için, küçük kemiriciler ana doğal konakçı ve kan-emen ektoparazitler ise en önemli vektörlerdir. İnsanlar, infekte eklembacaklılar (çoğunlukla keneler) tarafından ısırılma, infekte hayvan dokuları veya sıvıları ile temas, kontamine su veya gıdaların alınması veya bakterinin inhale edilmesiyle infeksiyona yakalanabilirler. Hastalığın klinik formları ve şiddeti bulaşma yoluna, bakterinin infektif dozuna ve alttürü ile konağın bağışıklık durumuna göre değişim göstermektedir. Tularemide ülseroglandüler, glandüler, okületandüler, orofarengeal, tifoidal ve akciğer tularemisi olmak üzere altı klasik klinik formda açığa çıkabilir. Ülkemizde tularemi, ilk olarak Lüleburgaz bölgesinde askeri garnizon ve yakınındaki köylerde açığa çıkan salgın ile 1936 yılında tanımlanmıştır. O tarihten sonra, çoğunluğu Marmara ve Batı Karadeniz bölgesinde olmak üzere farklı bölgelerde tularemi salgınları bildirilmiştir. Bugüne kadar, serolojik olarak 2000'den fazla olgu tanımlanmıştır. Son zamanlarda özellikle ülkemizin iç bölgelerinde yer alan birçok ilde tularemi salgınları ortaya çıkmıştır. Bu derlemede, F. tularensis'in genel karakteristikleri ve Türkiye tulareminin epidemiyolojisi özetlenmiştir.

Anahtar Kelimeler: *Tularemi, Francisella, Epidemiyoloji, Salgın hastalıklar, Türkiye*

"I know of no other infection of animals communicable to man that can be acquired from sources so numerous and so diverse.

In short, one can but feel that the status of tularemia, both as a disease in nature and of man, is one of potentiality".

R. R. Parker, 1934

BACKGROUND

Tularemia is a potentially fatal multisystemic disease in humans and other animals caused by the facultative intracellular gram-negative bacterium *Francisella tularensis*[1]. The disease was first defined by George McCoy and Chapin in the face of a plague-like epizootic in ground squirrels in Tulare County, California in 1911. However, tularemia was first described as a disease in humans prior to the discovery of the causative agent, in Japan as early as 1818 and in Norway since at least 1890 and perhaps as early as 1653[2,3].

Edward Francis, a United States (US) Public Health Service surgeon, dedicated much of his scientific career to research on the disease, including classification of the various clinical manifestations of the disease, its diagnosis (cultivating the organism and developing a serum agglutination test), determination of its histopathology, and elucidation of its various mechanisms of transmission. Up to 1928, he evaluated the clinical and epidemiological features of more than 600 human cases[4]. Until 1925, tularemia was believed to occur only in the US; subsequent reports from around the world documented cases in Japan, Sweden, Norway, Canada, Austria, and the former Soviet Union. Although known as a zoonotic and human pathogen for almost a century, knowledge on its intracellular lifestyle, its virulence mechanisms and its ecology is still fairly limited[5-7].

The family *Francisellaceae* contains a sole genus, *Francisella*, and only two recognized species, mainly on the basis of 16S rDNA sequencing and fatty acid composition: *F. tularensis* and *Francisella philomiragia*. According to the geographical distribution, epizootiology, biochemical characteristics, and virulence assay in rabbits, four subspecies (subsp.) of *F. tularensis* have been distinguished to date: *F. tularensis* subsp. *tularensis*, subsp. *holarctica*, subsp. *mediasiatica* and subsp. *novicida*. Although all have been associated with human disease, subsp. *tularensis* and *holarctica* are of particular clinical and epidemiological relevance[6-9].

The highly virulent subsp. *tularensis*, also known as type A biovar, is restricted almost exclusively to North America (except for a single report from arthropods in Europe), and is associated with wild rabbits, ticks and tabanid flies. The infective dose in humans is extremely low, 10 bacteria when injected subcutaneously and 25 when given as an aerosol, making this one of the most highly infectious bacterial pathogens known[2,6,8,9]. *F. tularensis* subsp. *holarctica* (type B) exists throughout the northern hemisphere, representing the most important subspecies involved in human and animal infection. Subsp. *holarctica* is less virulent than type A and usually requires an inoculum of 10.000 organisms or more to cause illness in humans. Type B strains cause milder infection with lower mortality rates in humans, but high mortality in wildlife[6-10].

F. tularensis is a fastidious organism that requires enriched medium supplemented with sulfhydryl compounds (cysteine, cystine, thiosulfate, isovitalex) for

growth. However, some strains of *F. tularensis* lack an overt requirement for cysteine or enriched medium for growth. Cysteine-enriched media enhance the growth of *F. tularensis*, but are not required for growth of isolates of *F. tularensis* subsp. *novicida* and *F. philomiragia*[1,5,6,8,9].

Eco-Epidemiology and Host Range

The ecology and epidemiology of *F. tularensis* are complex and incompletely understood. *F. tularensis* seems to occur in circumscribed areas; moreover, the organism may be present in the environment without associated outbreaks of tularemia[6,9-11]. *F. tularensis* is able to infect a wide range of animal species - warm-blooded and cold-blooded vertebrates, invertebrates, and numerous arthropods (more than 250 animal species). However, several mammalian hosts are important to its ecology in any geographic region and most commonly associated with human risk[9,11-13]. Small- and medium-sized mammals including lagomorphs (hares, cottontail and jack rabbits), semi-aquatic animals (e.g. water voles, beavers, muskrats), a variety of rodents including voles, field mice, hamsters, vole rats, squirrels, and lemmings, have long been assumed as the principal natural foci of tularemia (reservoir). Nonetheless, hares and rabbits are globally important as sources for infec-tion in humans (Figure 1)[13,14].

Two ecologic cycles of tularemia, terrestrial and aquatic, have been described[13]. In the terrestrial cycle, lagomorphs (cottontail rabbits-Sylvilagus spp. and hares) typically serve as amplifying hosts, and ticks or biting flies are arthropod vectors in comparatively dry environments. In the aquatic cycle, beaver, muskrat and voles serve as important mammalian hosts and appear to shed live organisms into their environments. *F. tularensis* subsp. *tularensis* is reported to have a terrestrial cycle, whereas subsp. *holarctica* has a mainly water-borne cycle with aquatic rodents as reservoirs. *F. tularensis* subsp. *holarctica* is linked to rodents including muskrats (*Ondatra zibethicus*), American beavers (*Castor canadensis*) and voles (*Microtus* spp.) in North America, and to varying hares (*Lepus* spp.), European brown hare, and Japanese hare in Eurasia along with voles (especially water vole) and muskrat. However, the rodents in North America (muskrats and beavers) and in the former Soviet Union (ground voles- *Arvicola terrestris*) as well as hares and rabbits have long been assumed as the principal natural foci of tularemia (reservoir)[2,6,9,10-13]. This consideration of the reservoirs of *F. tularensis* has recently been questioned by some authors, owing to the high susceptibility of all these species to the agent and mostly fatal disease, particularly in rodents and lagomorphs[9,15]. The true reservoir(s) of *F. tularensis* remain to be elucidated, and currently, there "…is no proof that mammals constitute the reservoir of *F. tularensis* in the environment"[5,11,16].

The diversity of both biological and mechanical vectors is also multitudinous. A number of blood-sucking ectoparasites such as ticks, mosquitoes (> 10 mosquitoes species, e.g. Aedes, Culex and Anopheles spp.) and biting flies (the deerfly, e.g. hrysops spp.), mites, and fleas might be capable of transmitting *F. tularensis*[1,5,12]. Ticks are biological vectors, some species of which serve as both hosts and reservoirs for *F. tularensis*. At least 15 species of ticks, such as the dog tick (*Dermacentor variabilis*), wood tick (*D. andersoni*), Lone Star tick (*A. americanum*), and *Ixodes* spp. commonly involved in North America have been found to be naturally infected with *F. tularensis*. In central Europe, *D. reticulatus* and *Ixodes ricinus* ticks are considered as important vectors for *F. tularensis*[11,12,15,17]. The possibility of transovarial passage in the tick remains dubious because of a high mortality rate in *F. tularensis*-infected ticks. Ticks may harbor *F. tularensis* in their saliva/gut for as long as two decades and may be inoculated either directly or indirectly into the bite wound[2,12-14]. Although tick transmission traditionally has been associated with subsp. *tularensis* (primarily a tick-borne disease), several outbreaks of tick-borne tularemia caused by subsp. *holarctica* (type B), which is more often linked to water, rodents, and aquatic animals, have been reported[3,11,18].

Biting flies including mosquitoes and tabanids serve as mechanical vectors. Mosquitoes may acquire infection from other components of the aquatic cycle shown to carry the organism or more likely due to their development in the larval stage in water. Mosquitoes can maintain *F. tularensis* up to 43-50 days and transmit it for up to 22-35 days. Mosquitoes of certain species have been strongly implicated as mechanical vectors of tularemia in Russia, Sweden and

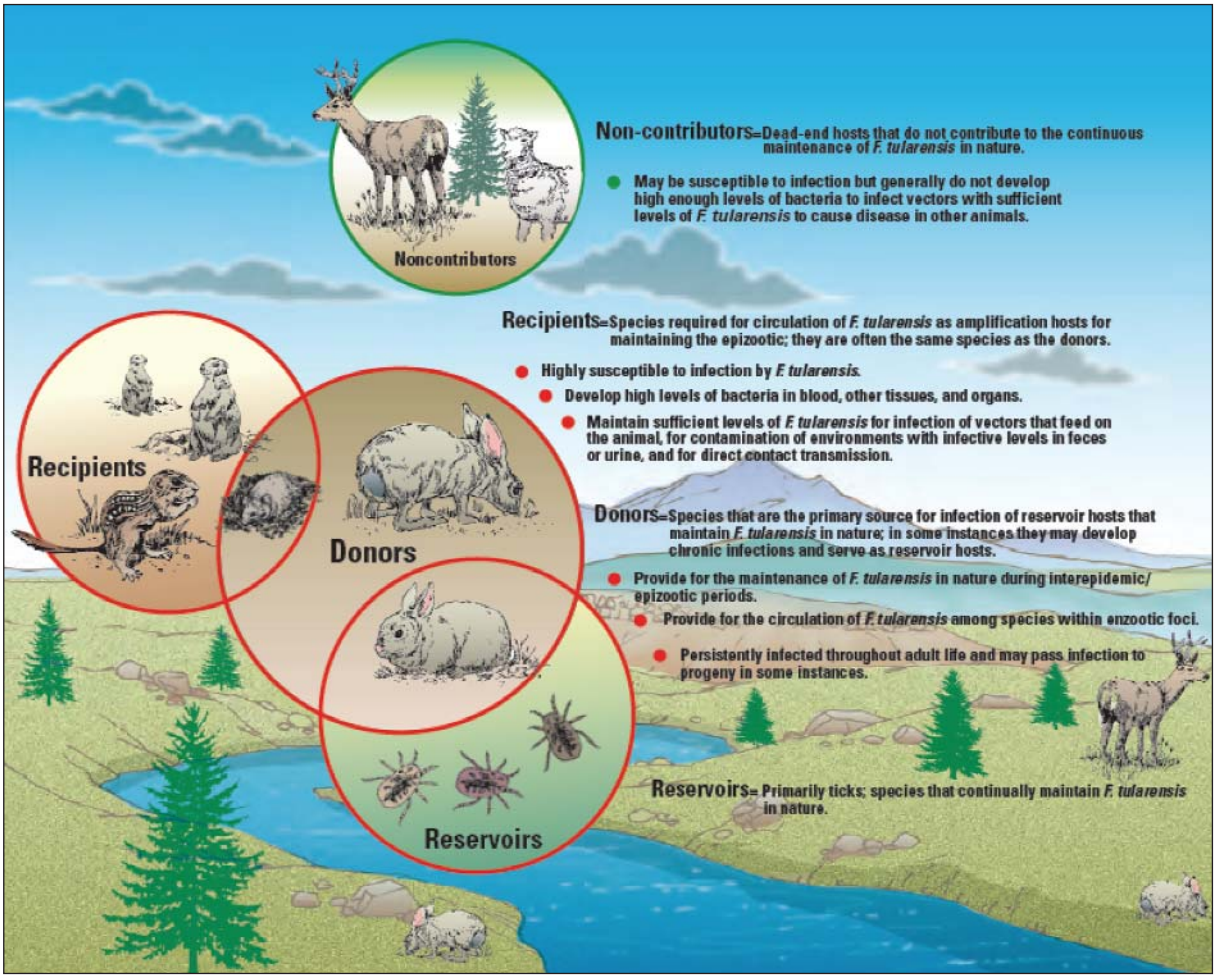

Figure 1. Non-contributor, reservoir, donor and recipient species in the ecology of tularemia[2].

Finland[2,11,12]. Another vector, tabanids, is a cosmopolitan family of usually medium-to-large-sized Diptera, commonly known as horse or deer flies. Deer (Chrysops spp.), stable and horse flies (Tabanus spp. and Chrysozona spp.) are of importance in specific areas such as Utah and the former Soviet Union; transmission occurs mainly between June and September in the western US[2,3,11-14]. Consequently, *F. tularensis* is maintained in nature for several years through an enzootic cycle involving lagomorphs (rabbits, hares, and pikas), rodents, and ticks, mosquitoes and other blood-sucking arthropods. Invertebrates, primarily ticks, have substantial roles both in the maintenance of *F. tularensis* in nature and in disease transmission to humans, especially in type A infection (Figure 1)[1-3,7,13,14].

Humans and other mammalian species, e.g. domestic farm animals (sheep, cattle, goat, swine), dogs, cats, horses, wild animals (bears, foxes, coyotes), and several species of birds, fish and amphibians are considered to be incidental hosts[19-21]. Sheep are the domestic mammal most commonly associated with tularemia, and transmission to humans via shearing, skinning, and slaughtering of infected sheep and from infected ticks previously attached to sheep has been reported from the US, Canada and Russia. However, other livestock species may have serologic evidence of exposure to *F. tularensis*, but clinical disease is rare. Infection in nonhuman primates has been reported in a pet monkey and animals housed in zoos and laboratory facilities as well[2,9,12]. Of domestic species, cats and dogs can acquire infection, and dogs may serve as reservoirs for the orga-

nism or maintenance hosts for the tick vector. In contrast to dogs, clinical illness is more common in cats, and recently, domestic cats have become an increasing source for human cases of this disease[1,8,11,22,23].

Environmental Persistence

F. tularensis is quite stable in the environment under humid and cold conditions and may survive for weeks at low temperatures in surface water, mud in stream bottoms and ponds, decaying or frozen animal carcasses, hides, and hay or straw[11,15]. Survival of *F. tularensis* in nature is dependent upon a variety of factors such as temperature, direct exposure to sunlight, and other physical factors that generally affect the survival of microbes (Figure 2). While the bacterium is quite stable in the environment, it is sensitive to heat, and cooking at 56°C for 10 minutes renders meat of animals (e.g., rabbits, hares, and game birds) safe for eating. The organism also does not

Environmental substrate	Survival time	Comments
Water 	14 weeks	Field samples stored at 7°C[180]
	90 days	?[27]
	4 months	At 4-6°C[19]
	3 months	Tap water
	3 weeks	At 20-21°C in presence of carcass of a water vole dead from tularemia[142]
Soil 	14 weeks	Mud samples stored at 7°C[25]
	30 days	Humid soil[27,181]
	62 days	Mud[232]
Fodder 	4 months	Grain, straw at 4-6°C[25]
	6 months	Dry straw litter[19]
	133 days	Wheat grain[27]
Live ticks 	764 days	Virulence maintained within body of *Ornithodoros turicata*[27]
	701 days	*Ornithodoros parkeri*[19]
	700 days	*Dermacentor marginatus*[25]
Animal carcass 	3 to less than 4 weeks	General survival in carcass tissues[180]
	At least 20 days	Hides of water rats that died of tularemia (15-20°C)[181]
	4 + 31 days	Urine in bladder of beaver dead of tularemia and storage of that urine at 15-28°C[181]
Laboratory culture 	22 years	At 10°C[27] (culture media not reported)
DESTRUCTIVE FACTORS		
Heat 	10 minutes	At 56-58°C[27]
Direct sunlight 	3 hours	At 29°C[19,27]

Figure 2. Examples of environmental persistence of *Francisella tularensis*[2].

survive in chlorinated water sources. Recently, free-living amoebae such as *Acanthamoeba* species, commonly found in natural aquatic systems, have been shown to harbor *F. tularensis* and may play an important role as a reservoir in aquatic cycles[24].

Contamination of surface waters has been suggested to occur primarily through shedding of bacteria via urine and feces from infected animals (primarily voles) and the carcasses of animals dying from tularemia[2,11,13,14]. Nevertheless, either process would explain contamination of water only during epizootics, but each fails to explain persistence between outbreaks[24]. *F. tularensis* circulates in populations of small rodent-endemic regions, and outbreaks in human populations frequently mirror outbreaks of disease occurring in wild animals[25].

Geographic Distribution

Tularemia occurs worldwide in the northern hemisphere (Holarctic region) between latitudes 30° and 71° north, with great variation in geographic and temporal occurrence. Tularemia has a patchy distribution in the northern hemisphere, which may be attributable to specific environmental conditions and the presence of appropriate hosts and vectors[2,7,11,13].

Cases are reported from North America, throughout continental Europe, throughout the states of the former Soviet Union, and in the Far East, in northern China, Korea, and Japan (Figure 3). Tularemia has not been described in sub-Saharan Africa, South America, or Southern Asia. To date, a single isolate

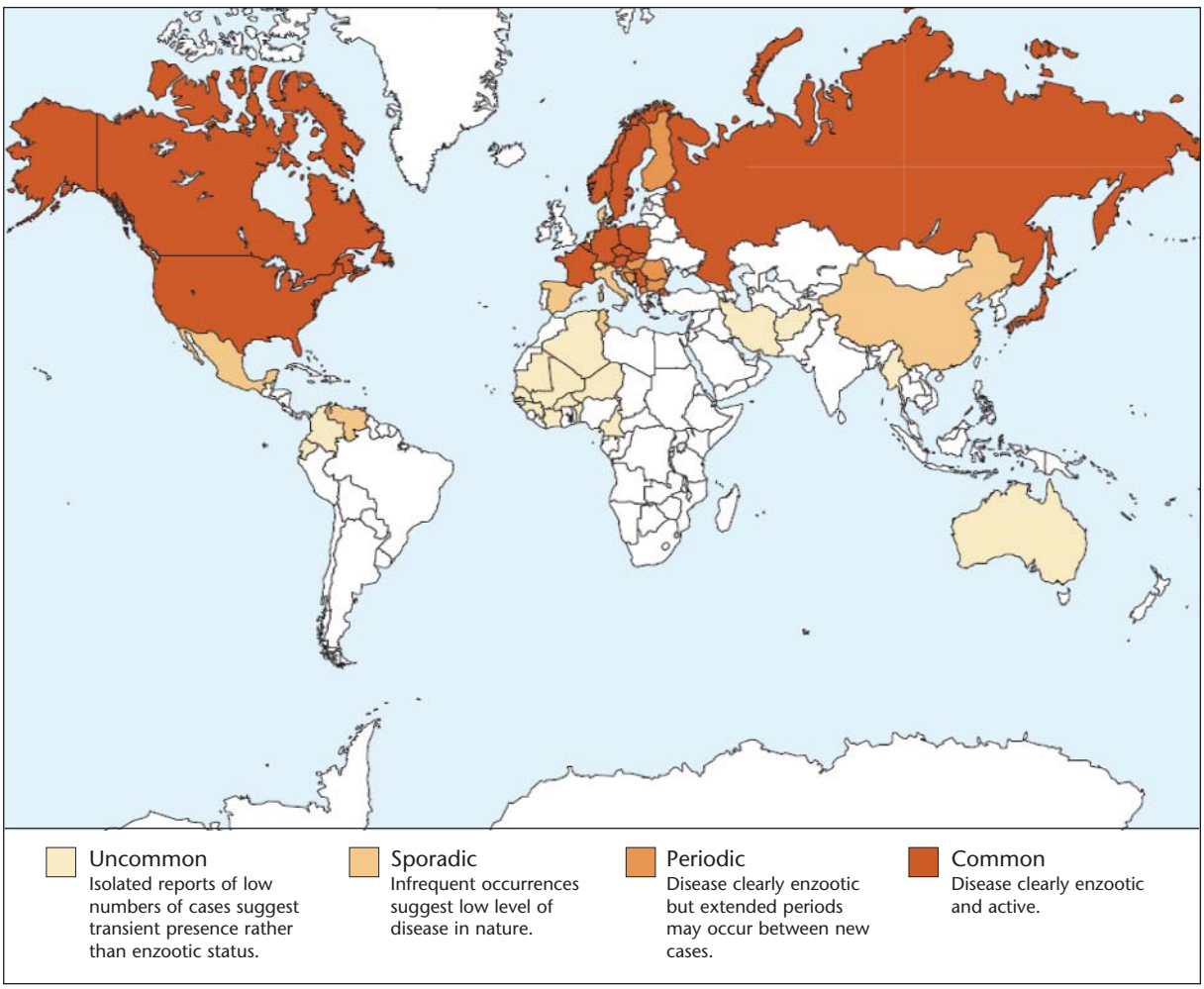

Figure 3. Worldwide occurrence of tularemia.

Table 1. Examples of reported human cases of tularemia by country

Country	Year of first report	Reporting period	Number of cases	Average
USA	1911	1924-1960	30.851	857.0
		1990-2000	1368	136.8
Japan	1924	1924-1994	1372	19.6
Former Soviet Union	1926	1926-1942	73.300	4581.3
Canada	1929	1930-1950	79	4.0
		1946-1958	73	6.1
Sweden	1931	1931-1996	5963	91.7
France	1938	1946-1947	404	36.7
Kosova	1999-2000		327	
Bulgaria	1962	1995-2005	285	

of *F. tularensis* subsp. *novicida* has been reported from the southern hemisphere, in Australia in 2003. Although tularemia is widely distributed over the Eurasian continent, it is typically a disease of Central and Northern Europe; Scandinavia and countries of the former Soviet Union have reported the most human cases. However, no case has been reported from the British Islands thus far[1,6,8,10,11] (Table 1).

In Norway, infection in a considerable proportion of cases occurred through contaminated water supplies, whereas in Sweden, most patients are infected via mosquito bites, but transmission by ticks and rain flies (*Hematopota pluvialis*) occurs as well. It should be emphasized that the highest incidence rates in the world have been reported from certain areas in Sweden and Finland, but with a remarkable variation in the number of human cases among years. A close relationship with some outbreaks and an increase in rodent tularemia has been reported[25,26]. In Sweden and Finland, respiratory tularemia occasionally occurs among farmers during the summer[26]. In comparison with the Nordic countries, in Central and Southern Europe, infection is uncommon, but has been reported from Italy and France[27]. In Spain, tularemia in humans was first reported in 1997, when 585 cases occurred associated with direct contact with infected hares (hare hunting). A year later, a second outbreak of ulceroglandular tularemia associated with crayfish (*Procambarus clarkii*) fishing in a contaminated freshwater stream was reported. In Spain, *F. tularensis* subsp. *holarctica* was identified as a causative agent in both epidemics[21].

In Bulgaria, a total of 262 laboratory-confirmed tularemia cases, the majority of which were oropharyngeal form, were reported between 1998 and 2003 [18]. In Asia, tularemia is most prevalent in Japan. Although no arthropod-borne cases were reported before 1951, the increase in the number of arthropod-borne tularemia cases was conspicuous between 1972 and 1998[2,11].

Transmission

F. tularensis is transmitted to humans through various modes, including direct contact with infected animals, from animal bites, via arthropod vectors, by ingestion of water or food contaminated by the excrement or carcasses of infected animals, arthropod bites, or inhalation of infectious dusts or aerosols (Figure 4)[6,8,9,11,15]. Vector-borne infection generally leads to the ulceroglandular form of tularemia. Mosquitoes constitute the dominant mode of transmission in Sweden, where transmission by ticks or deerflies is seen only occasionally. While human cases related to arthropods are frequently seen in the summer and fall, cases or outbreaks during the fall and winter are generally associated with exposure to infected animals or contaminated water[11].

While water from contaminated wells constitutes the dominant mode of transmission in Turkey, and to a lesser extent in Russia and the Balkan countries, it

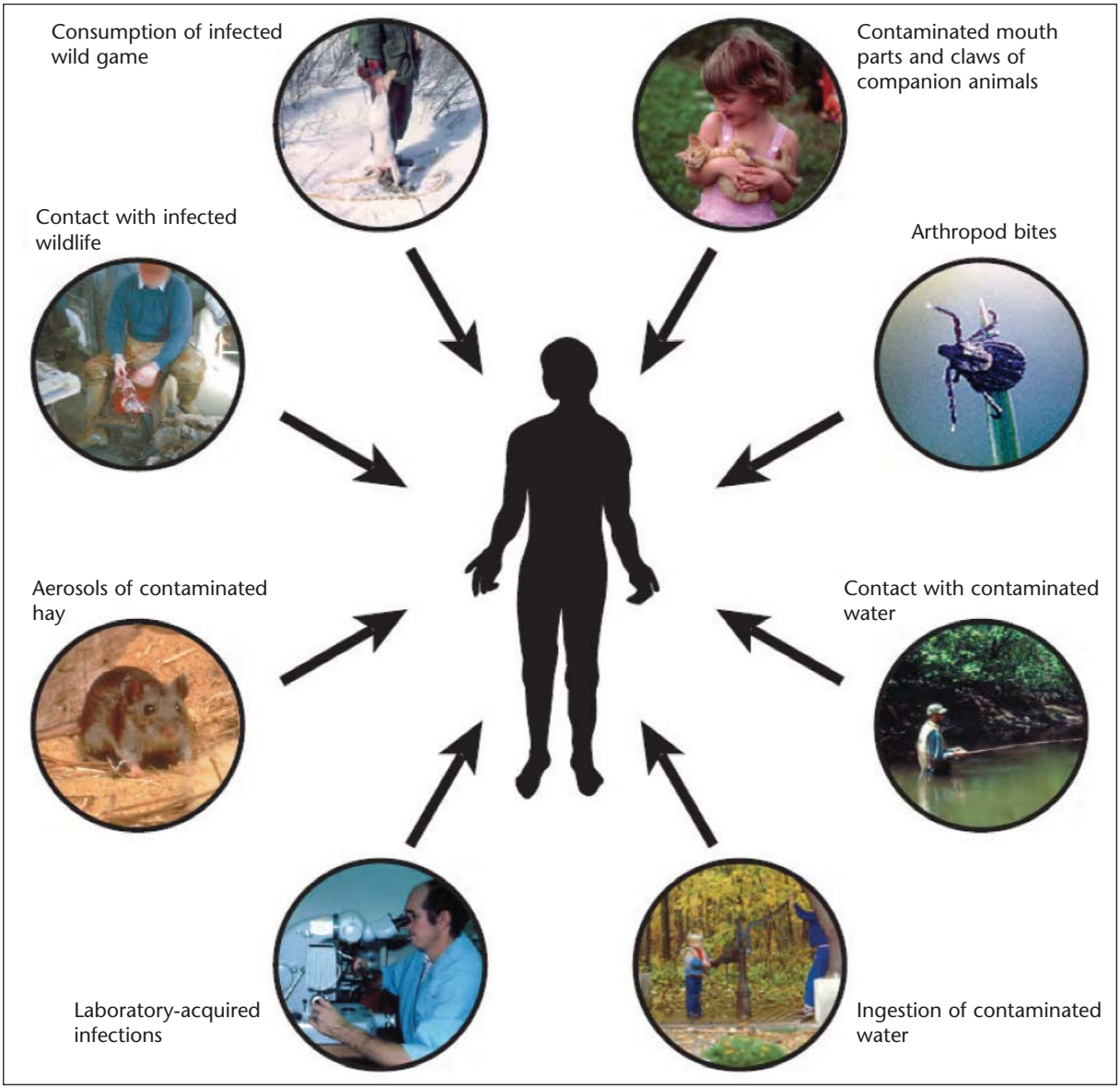

Figure 4. Routes of human exposure to *Francisella tularensis*[2].

is rarely described in the US and northern Europe. 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[1,8,12-14]. Infective dusts from contaminated hay, litter and other substrates associated with agriculture are occasional sources for infection in humans. Laboratory personnel are at great risk of infection via the respiratory route while handling diagnostic samples. Infection through inhalation generally leads to respiratory or the typhoidal form of tularemia[28].

Clinical Manifestations

The clinical picture and severity of the disease in humans vary depending on the route of transmission, the infecting dose, the *Francisella* subsp. involved, and the immune status of the host. The disease can take a variety of clinical forms, from the severe generalized form, through glandular variants, to subclinical cases fortuitously diagnosed by serology. Clinical presentations include the six classic forms of tularemia: ulceroglandular, glandular, ocu-

loglandular, oropharyngeal, typhoidal, and pneumonic (Table 2)[1,3,6,9,10].

Although the ulceroglandular form is the most common throughout the endemic areas of the world, oropharyngeal tularemia with oral ulcers, tonsillopharyngitis and enlargement of cervical lymph nodes is the most commonly occurring syndrome in Turkey[29].

A GENERAL OVERVIEW of THE EPIDEMIOLOGY of TULAREMIA in TURKEY

In Turkey, tularemia has been known since the 1930's. The history of tularemia in our country dates back to 1913 when a case was diagnosed on the basis of serology and clinical signs[2]. Tularemia in Turkey (1936-2009) can be evaluated in three periods for the convenience of the analysis: the first 18 years between the first epidemic in 1936 and 1953, the middle 16 years (1988-2004), and the last (third period) 5 years (2005-2009). Surprisingly, no tularemia cases were reported between 1953 and 1988, and the reasons for this variation are still not known.

Prior to 2004, tularemia was not a reportable disease, but was included in the official list of reportable infectious diseases in 2004 due to the increased case numbers in other parts of Turkey with jeopardization to human health. Hence, there were no official statistics concerning tularemia in Turkey during the period 1936-2004, and all data rely mainly on published studies that evaluated tularemia outbreaks or cases.

Characteristics of the First Period

The first period covers the years from the first epidemic in 1936 to the largest epidemic of oropharyngeal forms of tularemia in 1953. A total of 374

Table 2. Clinical presentation and route of acquisition of *Francisella tularensis* infection

Clinical form	Route of acquisition	Clinical signs and symptoms
Ulceroglandular	Direct contact by skinning and dressing infected animals (rabbits, squirrels, muskrats) Animal bites, scratches Bites of ticks, deerflies, mosquitoes	Cutaneous ulcers: predominantly on hands and painful LAP Fever, myalgia, headache Auto-inoculation to other sites possible Ulcers: head, neck, back
Glandular		Absence of primary lesion at site of inoculation Regional LAP
Oculoglandular	Rubbing of eyes after handling infected animals **or** contaminated water	Conjunctivitis with discrete ulcers Periauricular, parotid, submaxillary LAP Erythema and edema of eyelids
Oropharyngeal	Ingestion of contaminated food or water **or** Inhalation of large infected droplets	Exudative pharyngitis, stomatitis or painful mucosal ulceration Anterior-posterior cervical LAP complications: suppuration of lymph node and retropharyngeal abscess Enteritis, peritonitis, and appendicitis rare Primary and secondary cutaneous lesions
Typhoidal	Unclear	Local signs possibly absent Fever, vomiting, diarrhea, myalgia, shaking chills Hematogenous spread, sepsis, DIC, ARDS and meningitis (rare)
Pneumonic	Inhalation of contaminated aerosols, dust (primary) Hematogenous spread (secondary) Pulmonary involvement possible regardless of route of transmission	Bronchopneumonia Pleural effusion Peribronchial infiltration Hilar lymphadenopathy

LAP: Lymphadenopathy, DIC: Disseminated intravascular coagulation, ARDS: Acute respiratory distress syndrome.

human cases were reported from three different regions in four epidemics. The first tularemia outbreak in humans was reported among military personnel from the Luleburgaz garrison in the Thrace region in the summer of 1936[30]. The outbreak subsequently spread to civilians in the villages (Turgut Bey) in the region and affected a total of 150 cases: 133 soldiers and 17 civilians. The clinical presentation of the cases was reported as 59% glandular (oropharyngeal), 32% oculoglandular and 9% ulceroglandular forms. While the portal of entry for glandular cases was not determined, portals of entry were eyes for oculoglandular cases and cutis for ulceroglandular cases. Only one death (0.67%) was recorded in this outbreak. After the first widespread epidemic, studies of the epidemiology and ecology of tularemia were initiated in this region. Despite studies on the presence of *F. tularensis* in various animals (such as rabbits, rats, moles, sheep, quails, ticks and barn flies), the source of the outbreak could not be determined[30].

The causative agent was not recovered from laboratory animals to which clinical samples obtained from cases were inoculated. On the other hand, while handling the strains (namely "Corlu and Gulhane"), laboratory-acquired tularemia infection occurred when laboratory staff inadvertently splashed their eyes, and bacterium was isolated from conjunctival samples[30]. One year later, isolation of *F. tularensis* from the Kaynarca stream led to the consideration of water as the source of the outbreak. This outbreak was the first water-source epidemic in the world literature, together with that reported in Russia by Antonov and Karpov. After this epidemic, clinical histories of cases compatible with tularemia were recorded and some of these were confirmed by positive agglutination titers in this region[31]. Thereafter, intrafamilial tularemia consisting of six cases (five were siblings) resulting from consumption of rabbit flesh were determined in Tatvan in 1937[32].

During this first period, sporadic cases were also determined in various parts of the country, in Konya and Haymana. After the first epidemic in the Thrace region, tularemia was seen sporadically in Luleburgaz at various times, accompanied by another outbreak comprising 18 cases, occurring in September 1945. The source was again determined to be the Kaynarca stream[33].

The largest reported epidemic in Turkey involved more than 200 cases in the village of Bademagaci in Antalya in August-September 1953. Humans acquired the oropharyngeal and typhoidal forms of the disease by consuming contaminated water. Though the agent could not be isolated from water samples, it was suggested that fountain water was the source of the outbreak. It should be emphasized that 8% of the cases were of the typhoidal form, which had not previously been reported in Turkey. A study conducted in the village involved the application of the tularemia agglutination test. Seventy-one serum samples were available for testing by tube agglutination, and 69 (97.2%) were confirmed to be positive for the diagnosis of tularemia by the serological method[34]. In general, tularemia was observed in six provinces in our country between 1936 and 1953.

Characteristics of the Second Period

Interestingly, from 1953 to 1988, no tularemia case was recorded in Turkey. After a long interval, for unknown reasons, the disease emerged in northwestern Turkey in 1988, and thereafter, several small epidemics occurred in different parts of the area surrounding Bursa. Approximately 1000 cases of tularemia were registered with the Turkish Ministry of Health in the period 1989-2004.

The first outbreak of the second period consisting of 64 cases occurred in villages in the Karacabey and Mustafa Kemal Pasa districts of Bursa in 1988. The Department of Infectious Diseases at the University Hospital of Uludag evaluated small epidemics and sporadic cases seen around Bursa during the 10-year period since 1988 and assumed the duty as the primary referral center for tularemia in Turkey. In one epidemic, 205 tularemia cases were observed, the majority of cases (83%) were of the oropharyngeal form, and the epidemics were thought to be water-borne. A notable finding is the isolation of *F. tularensis* in 10 patients, five of whom were serologically negative[35]. During the period 1988-2005, further tularemia epidemics and sporadic cases continued to occur in different regions of Turkey, 372 of which were determined by microagglutination test (MAT)[36].

In November-December 1997, an outbreak consisting of 16 cases was reported in the village of Yagmurdere in the town of Ayas near Ankara. This was

the first outbreak recorded in the Central Anatolia region. It was suggested that all the cases were of the oropharyngeal form and the epidemic was again thought to be water-borne[37]. In 1998, an outbreak occurred in the village of Ahmetler in the town of Pazarcik in Bilecik. The ulceroglandular form prevailed notably in this outbreak consisting of 35 cases[36]. According to the data of the Department of Infectious Diseases and Microbiology at the University of Uludag, cases of tularemia were also reported from Marmara and Western and Central Black Sea regions between 1999 and 2001. Thirty-seven cases in Zonguldak in 2001, 40 cases in Sinop and 22 cases in Yalova in 2000, and 34 and 15 cases in Samsun in 1999 and 2001, respectively, were determined[36].

A breakdown of infrastructure caused by natural disaster, as in 1999, may lead to the emergence of tularemia in Turkey. Cases of tularemia were observed in villages in the district of Akcakoca in Duzce where no previous epidemic had been reported. Between March and June 2000, 22 patients from two neighboring villages situated in a forest region of Duzce-Akcakoca were diagnosed by MAT. In this outbreak, the principal form of tularemia was reported to be the oropharyngeal form (19 cases). Residents also reported increased rodent activity around poultry houses and villages prior to the outbreak. Additionally, the areas where the patients lived were supplied with water that was not treated with any disinfection or chlorination and the spring water was contaminated with Rattus carcass and excrement as well as that of other wild rodents. On the grounds of these observations, the authors concluded that the outbreak was water-associated[38].

A year later in September, there was an outbreak with 21 cases in the village of Yazikara in the Gerede district of Bolu. Since the majority of the cases (62%) were of the oropharyngeal form, the outbreak was reported as water-associated. The authors stated that the existence of rodents such as voles and rats in the peridomestic environment and contamination of spring water as well as cellars by these reservoirs could contribute to the rising probability of being infected among rural residents. It has also been emphasized that earlier cases might have been overlooked due to the previous absence of this illness in the region[39].

Another tularemia outbreak was recorded in the urban center of Balikesir province and its districts and villages between January and March 2002. One hundred twenty-six tularemia cases (81 females, 45 males) with the oropharyngeal form were determined in the outbreak. Of the cases, 97 were from Balikesir urban provincial center, 14 from the township of Manyas and 15 from various villages in Balikesir. Though the causative agent was not recovered from water samples, as in the case of previous tularemia epidemics, based on clinical presentation of cases as oropharyngeal tularemia, the most probable vector for spread was water[40].

Up to 2004, the majority of epidemics in Turkey have taken place in the two adjacent regions, Marmara and the western Black Sea, which borders the Marmara region. These regions have similar climatic and ecologic characteristics, such as the heavily forested nature of the area and abundant rodents with contact to the water.

In contrast to previous tularemia epidemics, there was an outbreak broke in the town of Suluova in Amasya in 2004. Suluova is situated on high ground inland within the central Black Sea region and near the Yesilirmak. Although the disease had previously been reported from surrounding cities, there was no record of tularemia in Suluova. During the epidemic, 86 cases were recorded by the health authorities and 43 cases (28 suspected and 15 probable cases) were evaluated with a matched case-control study to identify the potential factors associated with infection sources and modes of transmission. A significant feature of this outbreak was the detection of the causative agent from a water sample obtained from the rivulet and from lymph node aspiration fluid samples of two patients by polymerase chain reaction (PCR). Therefore, this was the first water-source epidemic in which the source of the outbreak was properly determined[41].

The authors emphasized that contamination of the rivulet water that passes through Suluova may have an impact on the occurrence of epidemics. Stock farming [cattle, sheep, and poultry (hens, ducks)] in the areas where the patients lived may cause contamination of the water. Irrigation of raw vegetables with the rivulet water without proper washing and/or exposure to contaminated water during

plucking and cleaning of animals may contribute to humans being exposed to infection[41]. In addition to these factors, in the multivariate logistic regression model, keeping a domestic animal in the garden was associated with an increased risk of contracting the disease. The demonstration of *F. tularensis* by PCR in the water sample partly supports the hypothesis that small domestic animals might play a role in the transmission of the disease. However, this suggestion seems doubtful because contamination of the surrounding area and the rivulet by dead rodents cannot be ruled out.

Three outbreaks of tularemia occurring from January to March and September in 2004 (first and second) and January to March in 2005 (third) were reported from the Sarikamis area in Kars city located in northeastern Turkey. A total of 56 tularemia-suspected cases were detected, and serological investigation revealed that 39 had antibody titers of ≥ 1/160 against *F. tularensis* by the microagglutination assay[42]. Although isolation and/or detection of the etiological agent was not achieved, it seems most likely that the source of the epidemics was contaminated water. Many people living in the same area contracted the disease in the same time period, and the clinical nature of the cases (oropharyngeal tularemia) may also implicate a common source such as water for transmission to humans.

In general, reported human tularemia cases have substantially increased during the past two decades within endemic areas of Turkey. Cases have been reported in 15 provinces during epidemics, mainly restricted to Marmara and the western Black Sea region, whereas cases were recorded in only six areas in the first period. Nevertheless, years in which high numbers of cases have been recorded followed periods in which tularemia was virtually absent.

Characteristics of the Third Period

At the beginning of 2005, a new and completely revised communicable disease notification was launched nationwide in Turkey, and tularemia was instated to notifiable status because of the emergence in our country. It is obvious that reported human cases can provide important information for evaluating patterns and trends for this disease.

In our country, more than 1000 human cases of tularemia with one death were recorded prior to 2005, and a total of 1091 human cases were reported between 2005 and the end of 2009[43]. The characteristics of tularemia epidemics recorded from Turkey are shown in Table 3.

In addition to increased case numbers, geographic prevalence of tularemia within Turkey has markedly changed. In contrast to the more northwestern Turkey and western Black Sea occurrence of tularemia cases during the 20th and first five years of the 21st century, most human cases currently occur in the areas adjacent to Central Anatolia regions[43, unpublished laboratory data]. Changes in the pattern of disease may also reflect local and regional changes in wildlife and vector populations due to changes in the landscape and in human activities. The regions with a tularemic focus according to reported epidemics or microbiological confirmation are shown in Figure 5.

In Turkey, tularemia is endemic and 1091 cases were reported from 2005 through 2009 (Figure 6). The general pattern of tularemia within Turkey is small clusters of human cases. The average annual number of cases from 2005 to 2009 was 218.2, and based on this figure, the reported incidence is 3.1 cases per 1 million inhabitants[43].

The incidence gradually decreased after 2005 (from 0.54/100.000 population in 2005 to 0.06/100.000 in 2008), with the average number being 96.3 during 2006-2008. However, an abrupt increase occurred in 2009 with an annual number of 428 (annual incidence rate 0.6/100.000) (Figure 7). The incidence in endemic areas varies greatly from year to year, and regional outbreaks are seen every two to three years. Additionally, prevalence varies widely from region to region throughout the country due to several factors like eco-epidemiology of *F. tularensis* and living under circumstances of substandard housing, hygiene, and sanitation.

The outbreak clusters typically comprise a group of family members and acquaintances who contracted tularemia from a common point source. Tularemia is a disease of rural areas and presents itself as small clusters or an epidemic due to a common source of infection. The presence of tularemia in households seems to be a risk factor, and epidemics have allowed us to calculate the attack rate. The presence

Table 3. Characteristics of the tularemia epidemics reported in Turkey (1936-2005)

					Clinical form (%)				
Year	Region	Cases (n)	Season	Transmission	Oropharyngeal	Oculoglandular	Ulceroglandular/ glandular	Typhoidal	Mortality
1936[30]	Luleburgaz	150	Summer	Water-borne	59	32	9		1
1937[32]	Tatvan	6		Food	100				0
1945[33]	Luleburgaz	18	Spring	Water-borne	82	18			0
1953[34]	Antalya	200	Autumn	Water-borne	45	2	45	**8**	0
1988-1998[35]	Bursa	205	Winter	Water-borne	83	8	2		0
1997[37]	Ankara	16	Winter	Water-borne	100				0
2000[38]	Duzce	21	Autumn	Water-borne	62	38			
2001[39]	Bolu	14	Autumn	Water-borne	100	38			
2002[40]	Balikesir	115	Winter	Water-borne	100				
2004[41]	Suluova	43	Autumn	Water-borne	4.6	11.6	**83.7**		0
2004-2005[45]	Zonguldak	61	Winter	Water-borne	100				0
2004-2005[30]	Kocaeli	145	Winter-Spring	Water-borne	100				
2004-2005[42]	Kars	56	Winter-Spring Autum	Water-borne	100				
2005[44]	Kocaeli	129	Winter	Water-borne	100				0
2005[46]	Tokat	8	Winter	Water-borne	88		12		
2005[47]	Edirne	10	Winter	Water-borne					
2005[48]	Duzce	11	Winter	Water-borne	73	27			0

Figure 5. Provinces in which tularemia has been recorded according to tularemia epidemics or serologically confirmed cases.

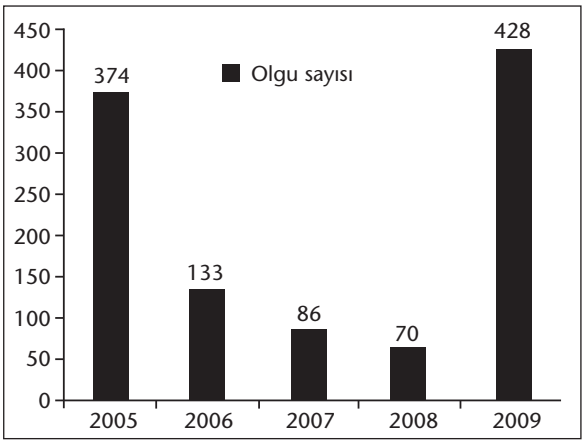

Figure 6. The number of tularemia cases, 2005-2009.

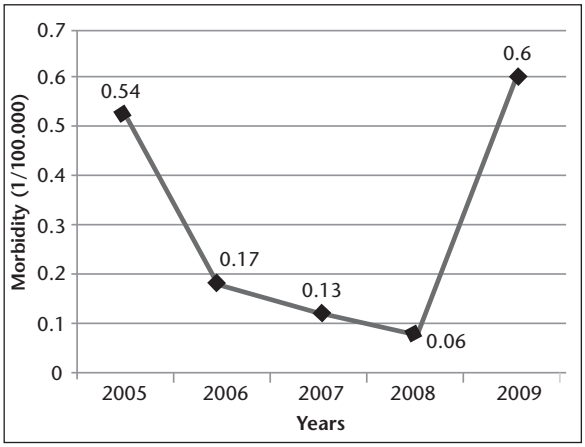

Figure 7. Morbidity rate of tularemia, 2005-2009.

of tularemia in households has ranged between 13.6% and 50% in the previous outbreaks[38,44,45]. The attack rate of tularemia has been reported to be 50% in a large single family exposed to the same risk factors[46].

Age and Sex Distributions

When the sex and age distribution of the 1091 cases were analyzed, tularemia was determined more likely to occur in females than males, and adults aged 30 or older, and may reflect activity patterns that enhance opportunities for exposure. The female to male ratio for all of the cases reported during the five-year period was calculated to be 1.18 and the ratio gradually increased during this period (Figure 8). The increase in tularemia in females and older people in recent years may be due to the fact

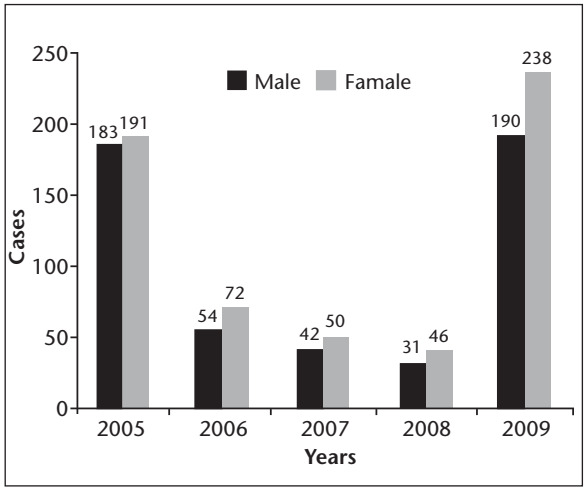

Figure 8. Gender-dependent distribution of 1091 cases of tularemia in Turkey, 2005-2009.

that they are now more involved in doing chores inside leading to a greater representation of women having contact with contaminated water and to lesser extent handling animal excrement in the food storage areas.

With respect to age distribution in Turkey, the mean age of females was slightly higher than that of the males (38.7 vs. 30.6 years, respectively). The age distribution changed during the survey period; in generations over 30 years of age, the rate gradually increased, compared to generations 10-19 years of age (Figure 9). The distribution of affected individuals according to age groups was determined as follows: 1-14 (10.4%), 15-19 (8.9%), 20-29 (16.9%), 30-44 (28.2%), 45-64 (25.6%), and above 65 (9.3%). The change in the age distribution of tularemia appears to

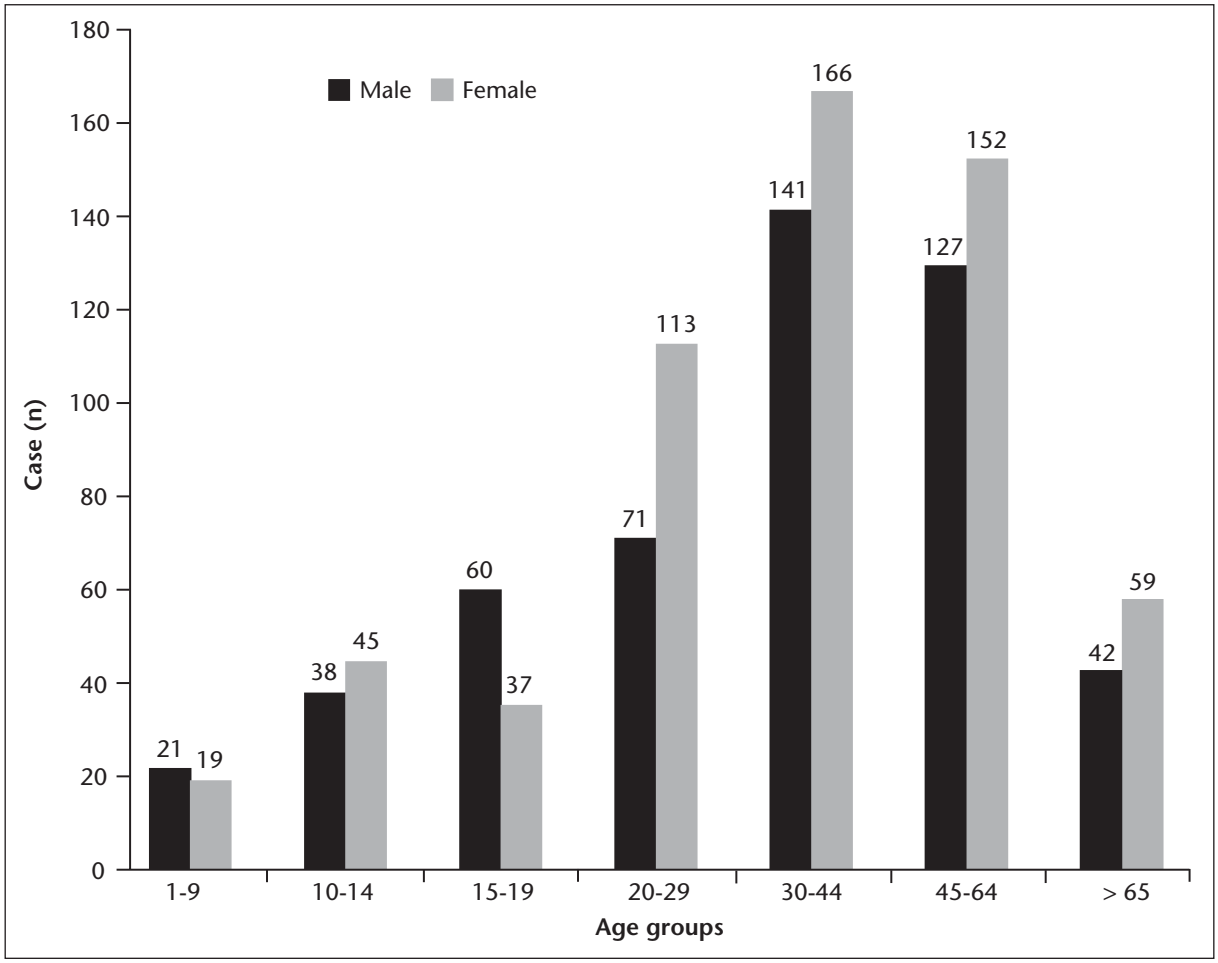

Figure 9. Distribution of 1091 tularemia cases according to age and gender, 2005-2009.

be an absolute increase in the older population owing to a decrease in the younger population staying in rural communities.

Interestingly, a slight male predominance was seen in people aged 15-19 years, probably reflecting the amount of time spent in nature and hunting, whereas gender distribution had a female predominance between 2005-2009. Although tularemia affects all age groups, tularemia is not well known in children and the pediatric cases are often misdiagnosed. In our country, tularemia is reported to be rare in childhood; however, during the last four years, the number of pediatric cases seems to be higher than previously reported. The figures in the last five years indicated that the pediatric cases constitute approximately 10% of tularemia cases in our country.

Seasonal Occurrence of Tularemia

Tularemia epidemics have seasonal characteristics, and *F. tularensis* subsp. *holarctica* causes outbreaks during autumn and winter months. Although tularemia was observed throughout the year in Turkey, seasonal distribution data demonstrated an increase during late fall (november) and winter months with a gradual decrease in spring and summer (very rarely). This tendency was observed throughout all three periods (1936-2009) (Table 3). The seasonal distribution of the disease, on the other hand, was not the same for all geographical areas.

In our country, tularemia has occurred predominantly in the rural areas (villages) where cycle of infection can readily come into existence. The existence of reservoirs (semi-aquatic rodents) in peridomestic areas may be responsible for contaminating water supplies and food storage areas (cellars) in rural areas. Further, water sources are not hygienically controlled or treated with periodic cleansing or disinfection/chlorination procedures, and are inadequately protected from the entry of small animals[38-40,44,45,47-50]. Community water supplies, including village fountains supplied by stream and spring waters, may be easily contaminated with the pathogen due to lack of monitoring and disinfective procedures, which may also lead to exposure of humans through consumption of water.

Modes of Infection

When reported tularemia cases were analyzed, in contrast to other European countries where ulceroglandular form was more prominent, the oropharyngeal form of the disease was the most common presentation of the tularemia outbreaks in Turkey, with a prevalence of 99% (Table 3). In outbreaks, almost all of the cases are of oropharyngeal form, which is related to the consumption of contaminated water or food. A rare variation of ulceroglandular disease, the oculoglandular form, is the second most common clinical presentation, while ulceroglandular and glandular form have been rarely reported. The oculoglandular form emerges as a result of the transfer of bacteria on the fingertips or from swimming in contaminated water or contaminated splashes[6,8-10,16]. From the case histories, the main mode of transmission in this rare clinical form is exposure to contaminated water by washing the face or from splashes. In light of the epidemiological features of tularemia in Turkey, most of the cases were infected with contaminated water; to a lesser extent, infection was due to ingestion of contaminated food, reflecting water-associated epidemics.

The ulceroglandular form is most commonly associated with arthropod bites or direct handling of infected animals in the US and Europe[2,6,12,15]. However, in our country, it is entirely a result of contact with contaminated water or wild rodent excrement, since most of the patients recall no history of risk factors such as tick bite or contact with dead animals. Infection presumably by insect bite was reported in three cases in Duzce[38]. Pneumonic tularemia associated with oropharyngeal disease was reported in one case in the Bursa epidemic[35]. The most severe clinical form of tularemia, typhoidal tularemia, was determined in eight cases during the Antalya outbreak (1953), and a case was recently reported from Duzce[34,51].

Asymptomatic or Subclinical Cases

Tularemia is likely to occur in persons who showed no symptoms but who had been exposed to the same risk factors. It is noteworthy that the rate of asymptomatic or subclinical cases reported in outbreaks in Turkey varies between 4-19%[35,39,47]. This significant difference may be partly related to the

magnitude of the population and to a lesser extent to the geographic area. Among individuals without symptoms, but with tularemia antibodies in ≤ 1/80 dilution, some were evaluated as "probable cases". On the other hand, asymptomatic cases with titers of agglutination test ≥ 1/160 were defined as "tularemia case" in the previous reports.

Risk Factors for Tularemia

From the epidemiological point of view, risk factors of tularemia may include independent variables such as drinking spring water, eating wild rabbit, contact with the excreta of rodents, consumption of unhygienic food, working in a poultry house, increased rodent population at home or in the surroundings, and outdoor activities. It has been reported by some authors that the risk of infection was significantly correlated with the consumption of water from natural springs in the outbreak region[38,50]. In conclusion, the most common transmission route of tularemia is exposure to infected animals or ticks in the international literature, whereas in Turkey the main mode of transmission of *F. tularensis* is considered to be drinking unchlorinated water or uncontrolled spring water[35,39,41,44,45,47,48].

Occupation-Dependent Distribution

In Turkey, tularemia occurs mainly among occupations related to a rural area due to water-borne outbreaks. Since the disease was not notifiable before 2005, we do not have adequate data for epidemiological analyses. When reported tularemia cases were arranged according to occupations, almost all of the cases were engaged in agriculture. Tularemia has been most commonly reported among farmers and their families, housewives, children, hunters or forest workers[29,38,40,45,48,50].

Seroepidemiological Studies

According to the literature, only four seroprevalence studies have been conducted to date-in Bursa, Bolu and the Thrace region. Results of these seroprevalence studies, using MAT, indicate that the prevalence of seropositivity appears to vary considerably in different geographic areas, seasons and populations studied. The prevalence of antibody to *F. tularensis* in the general population varied widely, between 0.3% and 20.9%[39,52,53].

Following the first outbreak in the Bursa region, a serologic survey was performed to trace the prevalence of the disease in humans. This first survey indicated that 82 (20.9%) of 393 sera in the Bursa region had serological evidence of previous *F. tularensis* infection[52]. This higher prevalence rate may be partly due to the time of the study (immediately after the outbreak) and to a lesser extent to a geographic location.

A study by Gurcan in Bolu-Gerede found that 16.7% of 108 sera had antibodies to *F. tularensis*[39]. After the third outbreak in the Thrace regions (Edirne-Lalapasa-Demirkoy) in 2005, a total of 390 sera obtained from villagers and pupils were investigated regarding the prevalence of antibodies to *F. tularensis*. Ten (2.6%) subjects were found to have antibodies against tularemia at titers of 1/10 and over[47].

In contrast to these previous studies performed in outbreak areas, a large scale seroepidemiological study was carried out in 90 different villages of Edirne, Kirklareli, and Tekirdag provinces in the Thrace region. The presence of *F. tularensis* antibodies was screened in 1782 subjects by MAT, and 5 (0.3%) contained antibodies between the titers of 1/20-1/160. Thus, the authors considered the presence of low-level antibodies against tularemia in the serum samples of those subjects to be cross-reactions due to the antigenic similarities in other bacteria, in particular brucellosis. Rose Bengal test was also found positive in three of the seropositive subjects, making the results a probable cross-reaction with brucellosis. With exposure to ticks, livestock animals kept in close proximity to housing and male gender are the major risk factors for acquiring *F. tularensis* infections[53]. Results of these seroprevalence studies in humans suggest that tularemia has been more widespread in the areas of endemicity in Turkey than was previously assumed.

Tularemia in Animals in Turkey

It is noteworthy that almost nothing is known about the incidence and geographical distribution of tularemia in animals in Turkey, although the disease has been known to occur since 1936.

Rodent Population in Turkey

Among animals species considered as a reservoir in the northern hemisphere, lagomorphs such as *Le-*

pus europaeus Palas (wild or Angora rabbit) and rodents such as *Arvicola terrestris* (water vole; found throughout Turkey), *Microtus arvalis* (common vole- "Doğu Anadolu tarla faresi"; only lives in eastern Turkey and altitudes over 1900 m), *Apodemus agrarius* (striped field mouse; found in the coastal region of the Thrace region), and *Rattus rattus* (black rat or ship rat; found throughout Turkey) are also present in Turkey[54].

The hypothesis that contamination of water sources by these mentioned infected rodents and hares accounts for the spread of tularemia may be supported by the presence of these animal species in Turkey. Additionally, an increase in the rodent population (voles and rats) prior to the epidemic has been reported by rural villagers in Luleburgaz (1936), the area around the Bursa epidemic (1988), Duzce (2000), and Edirne (2005), most probably supporting this hypothesis.

To our knowledge, only two studies in domesticated animals have been documented in the literature thus far, but there was no report of *F. tularensis* infection in smaller rodents. The first animal study was conducted as an academic dissertation thesis in the Kars region in 1996 in order to determine the incidence of tularemia in sheep by means of serological and cultural methods. A total of 1412 sera taken from different residential areas were analyzed with three serological methods, tube agglutination test, MAT and ELISA test, and tularemia antibodies were determined in 28 (1.98%), 50 (3.54%), and 110 (7.80%), respectively. Consequently, the incidence rate of tularemia was calculated as 0.14%[55].

Another study was carried out in Demirkoy village, Edirne during the investigation of the outbreak. Since there was a rabbit farm and villagers recounted that some of the domesticated rabbits died two months before the outbreak, the investigators started epidemiological studies not only in the rabbits but also other animals belonging to patients and ticks collected from animals. While one of the 25 rabbits and 19 of the cows tested had low-level (1/20-1/80) tularemia antibodies, none of the sheep had antibodies. The authors evaluated the seropositivities in the rabbit and cows as non-specific, making domestic animals unimportant in this outbreak. Tissue samples obtained from dead rabbits and eight *Rattus rattus* carcasses found in the homes of patients were subjected to PCR and culture; however, no successful result was obtained[4].

Microbiological Perspectives

Tularemia case definition in Turkey involves confirmation of suspected cases by culture and serologic method (Figure 10)[56].

Unequivocal diagnosis of tularemia requires isolation of the causal agent. Culture of oropharyngeal and wound swabs and/or lymph node aspirates is the method of choice, but specimens need to be obtained early, and cultures often need long periods of incubation. In addition, failed detection of the pathogen is a frequent occurrence. The diagnosis of tularemia is mainly based on serological analysis, because isolation of the causative agent is time-consuming, extremely hazardous and requires a biosafety level-3 containment in order to avoid risks of laboratory infection[5-9]. Recently, detection of *F. tularensis* in clinical specimens using PCR-based laboratory tests can be considered a routine diagnostic method. It should be stressed that the PCR method should not be used alone; samples should also be analyzed by culturing. Molecular sub-typing methods are used to differentiate subspecies of *F. tularensis* and can be directly applied in clinical samples (no requirement of isolation). Additionally, PCR has been shown to work best as a final confirmation with culture-positive samples. The PCR method is reliable, reproducible, less time-consuming, and more practical than culture[57]. Nevertheless, there are drawbacks to PCR, the most obvious being the need for an equipped laboratory with qualified technicians, which is not feasible in remote tularemia endemic areas that lack sufficient infrastructure. These limitations make serology the most useful tool for laboratory diagnosis of tularemia infection[5,8,9,58].

The MAT remains the most common method used to detect antibodies against *F. tularensis*. MAT is considered as the current reference method for the serodiagnosis of tularemia[1,5,8,58]. The ELISA, on the other hand, is purported to be more sensitive than agglutination for the diagnosis of tularemia. It has been recommended that negative results in suspected patients should be confirmed by ELISA and Western blotting[5,8,58,59].

Tularemia: Standard Case Definition in Turkey	
Clinical definition:	Clinical illness: An illness characterized by several distinct forms, including the following: • **Ulceroglandular:** Cutaneous ulcer with regional LAP • **Glandular:** Regional lymphadenopathy with no ulcer • **Oculoglandular:** Conjunctivitis with preauricular LAP • **Oropharyngeal:** Stomatitis or pharyngitis and cervical LAP • **Pneumonic:** Primary pleuropulmonary disease • **Typhoidal:** Febrile illness without early localizing signs and symptoms
Laboratory criteria for diagnosis:	(a) Isolation of *F. tularensis* from a clinical specimen (b) Specific antibodies at significantly high titers (≥ 1/160) by MAT (without documented four-fold or greater change) (c) A four-fold or greater increase in serum antibody titer in paired serum samples
Case classification	Probable Case: (a) Any person meeting the clinical criteria and with an epidemiological link without any laboratory criteria Confirmed Case: (a) At least one laboratory criteria positive in suspected case

Figure 10. Standard case definition of tularemia in Turkey[56].

In the presence of compatible symptoms, sustained high titers of 1/160 or greater in an acute specimen support a presumptive diagnosis of tularemia. However, a titer ≥ 1/160 may also reflect past infection. An equivocal titer may be due to cross-reactive antibodies (*Brucella, Yersinia*, or *Rickettsia*), past infection, or very recent infection. A four-fold rise in titer between acute and convalescent sera is required for definitive serologic diagnosis of tularemia. Serology is of limited use in acute infection because antibody levels are generally not detected until 10 days after the onset of the disease[2,5,8,9,58]. Detection of *F. tularensis* nucleic acid in a clinical specimen has been included recently in the laboratory criteria in Europe, but in the US, PCR positivity is considered as a presumptive laboratory criterion in the diagnosis of tularemia. Another diagnostic technique on the agenda is fluorescent assay, and detection of *F. tularensis* in a clinical specimen by fluorescent assay was accepted as a presumptive laboratory criterion by the Centers for Disease Control and Prevention[60].

When the laboratory diagnosis of tularemia in our country is reviewed, the first isolation of *F. tularensis* was reported by Gottslich in 1936. In the report of the Luleburgaz outbreak, two mentioned strains were named as "Gulhane and Corlu", possibly in the honor of the men, Omer Bican and Kemal Huseyin Plevnioglu, who pioneered research on the disease at Corlu and Gulhane military hospitals[30]. Unfortunately, we have only one strain and no solid confidential information about other strains at present. Another strain was isolated from the Kaynarca stream one year after the outbreak. Since then, isolation of *F. tularensis* has not been reported. During a 10-year period, *F. tularensis* was isolated from 10 of 205 patients (4.9%) by Gedikoglu[35]. However, further analyses to determine subspecies and molecular typing methods to elucidate genetic relatedness among Turkish isolates have not been attempted yet.

In 2005 and 2006, two strains were isolated from lymph node aspirates and named as "Yazikara and Nuhoren", referring to the sites of the outbreak. Gurcan et al. evaluated antimicrobial susceptibility and genetic relatedness using multiple-locus variable-number tandem repeat analyses (MLVA) with Bulgarian isolates. Results of MLVA-genotyping consisting of six selected markers displayed that the ge-

notypes of the Turkish isolates obtained from two patients in 2005 and 2006 and one of the four Bulgarian outbreak strains isolated in 2003 were identical[61]. This interesting finding was construed by authors as indicating the existence of a common Balkan genotype in Eurasia (Thrace and Anatolia) and spread of this genotype in regions over 1000 km from one another through either infected rodent or immigrant birds as well as ticks. Another possible explanation for sharing the same MLVA genotype is that the MLVA-$_6$ genotyping system is not highly discriminatory to distinguish very closely related isolates, for instance, strains within a local outbreak and to some extent phylogenetically relevant in many countries.

Despite intensive efforts, there was no isolation of *F. tularensis* from the suspected water prior to 2009, which may be partly due to delays in taking water samples for cultures and low-sensitivity of culture. On the other hand, recovery of the causative agent in water was achieved in the last epidemic in the Central Anatolia region (pending result). Molecular techniques have been applied in the diagnosis of tularemia since 2004. The first successful result was obtained in the Suluova outbreak, Amasya in 2004, and *F. tularensis* was detected not only in the water sample obtained from the rivulet but in samples obtained from the ulcerated lesions of two patients as well[41]. The isolation and demonstration of bacteria from water sources in the affected area (Suluova 2004, Duzce 2005, Karamursel 2005, Edirne 2006) has proven water as the route of infection. This finding is further supported by the observation that most of the cases represent the oropharyngeal form[41,47,48,59]. Successive application of PCR yielded 47 PCR positivities in clinical specimens before 2009[41,44,45,47,50,61].

Current Status

The number of provinces in which tularemia cases are reported increases yearly. In late 2009 and early 2010, 400 individuals were diagnosed with tularemia particularly in areas of Central Anatolia and the Aegean region border with Central Anatolia, where cases of tularemia had been rarely recorded in only a few provinces thus far. Tularemia has emerged in nine provinces in Central Anatolia, Eastern Anatolia, Eastern Black Sea, Aegean, and Mediterranean regions. Currently, tularemia has been observed in 40 provinces throughout the country (Figure 5).

Since October 2009, ongoing tularemia epidemics, mainly in the Central Anatolia region, have led to isolation of numerous bacteria and detection of *F. tularensis* by PCR from clinical samples as well as water. Results of molecular studies have confirmed that *F. tularensis* subsp. *holarctica* is the causative agent of tularemia in our country (unpublished laboratory data). Considering that we have a sufficient number of isolates and genetic material of *F. tularensis*, this presents us with a good opportunity to determine the antimicrobial susceptibility of Turkish isolates and to apply molecular typing methods for discriminating isolates originating from restricted geographic sources, as well as for the trace-back analyses. Additionally, in these circumstances, there is an opportunity to create the link between tularemia outbreaks, water sources and reservoir.

CONCLUSIONS

The majority of epidemics in Turkey are seen in the Marmara region. The outbreaks are usually water-borne and the oropharyngeal form generally predominates, and the disease follows a mild course. One remarkable observation is that early cases are principally overlooked in outbreaks, and the diagnosis was initially missed. This is somewhat surprising, since tularemia has been known as an infectious disease and as having caused outbreaks in that region since 1936. It is unclear how often this diagnosis is missed in either epidemic or non-epidemic regions. The suggestion of the link between tularemia outbreaks and water sources and *F. tularensis* subsp. *holarctica* as a causative agent has been proven by recent studies (pending results). Another notable finding is the observation of asymptomatic cases in the outbreaks in about 4 to 19% of the patients, which highlights the need and importance of active surveillance to detect new case clusters in the outbreak area. Another remarkable observation is that a group of patients with tularemia were misdiagnosed and treated as tuberculosis in some centers[45, unpublished laboratory data].

Unanswered Questions About the Epidemiology of Tularemia in Turkey

Two questions remain to be answered about the epidemiology of tularemia in Turkey: "What is the

epidemiology of *F. tularensis* in reservoirs and vectors in Turkey?" and "Why was there an apparently disease-free period (no tularemia cases) between 1953 and 1988 and a subsequent re-emergence in 1988?".

It must also be considered that climate conditions have changed significantly over the last 50 years in Turkey, and the re-emergence of tularemia might have been due to these ecological and climate changes. Thus, there is an obvious need for further studies, in both wild and domestic animals, as well as environmental studies and a focus on the ecology of *F. tularensis* in epidemic areas in an effort to elucidate the eco-epidemiology of tularemia in Turkey.

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