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Tularemia in Germany: the tip of the iceberg?

W. D. SPLETTSTOESSER[1]*, I. PIECHOTOWSKI[2], A. BUCKENDAHL[1], D. FRANGOULIDIS[1], P. KAYSSER[1], W. KRATZER[3], P. KIMMIG[4], E. SEIBOLD[1], S. O. BROCKMANN[2] for the EMIL Study Group

[1] *Bundeswehr Institute of Microbiology, Munich, Germany*
[2] *Department of Epidemiology and Health Reporting, Baden-Wuerttemberg State Health Office, District Government Stuttgart, Stuttgart, Germany*
[3] *Department of Internal Medicine I, University Hospital Ulm, Ulm, Germany*
[4] *Department of Infectious Diseases, Baden-Wuerttemberg State Health Office, District Government Stuttgart, Stuttgart, Germany*

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SUMMARY

Tularemia is a rare, notifiable zoonosis in Germany. Since November 2004, several lines of evidence including outbreaks in humans or animals and confirmed infections in indigenous hare and rodent populations have indicated a re-emergence of tularemia in different German federal states. Unfortunately, reliable basic information on the seroprevalence in different geographical regions, permitting the identification of risk factors, does not exist. Combining a sensitive screening assay with a highly specific confirmative immunoblot test, we performed a serological investigation on 2416 sera from a population-based, cross-sectional health survey of the city population of Leutkirch, Baden-Wuerttemberg. A total of 56 sera gave positive results indicating a seroprevalence of 2·32%. Thus, the seroprevalence is tenfold higher than that previously reported in a nationwide study in 2004. *Francisella tularensis* can cause a wide variety of clinical syndromes including severe, sometimes fatal disease. Missing epidemiological data on its spatial and temporal distribution in an endemic country complicate an appropriate risk assessment necessary for public health authorities to be prepared for an adequate outbreak management. This is of special concern regarding the extraordinary potential of *F. tularensis* as an agent of bioterrorism. Our investigation performed in a presumed low-risk area demonstrated that tularemia might be seriously underestimated in Germany and probably in other central European countries as well.

Key words: Emerging zoonoses, *Francisella tularensis*, seroprevalence in Europe.

INTRODUCTION

The anthropo-zoonosis tularemia is caused by the facultative intracellular bacterium *Francisella tularensis* which can cause a wide variety of clinical syndromes ranging from asymptomatic infection to severe, sometimes fatal disease [1, 2]. The microbiological diagnosis is difficult due to the unusual growth requirements of *F. tularensis* and the limited availability of serological assays or new molecular tools [3]. For this reason and also due to the non-specific clinical symptoms, the diagnosis is often delayed or missed

* Author for correspondence: Dr W. D. Splettstoesser, German National Reference Laboratory for *Francisella tularensis*, Bundeswehr Institute of Microbiology, Department of Immunology, Neuherbergstrasse 11, 80937 Munich, Germany.
(Email: wolfsplettstoesser@bundeswehr.org)

and the number of human cases might be severely underestimated. In Europe, the distribution of endemic foci varies significantly [4]. While high numbers of cases are reported annually in Finland and Sweden, less numerous cases in France, Hungary, Turkey, Austria and Czech Republic as well as only sporadic cases are noticed from countries like Germany, Switzerland, Italy, Spain or Greece [2, 5, 6]. In Germany, the incidence in the last four decades was extremely low with only 1–5 reported human cases per 80 million inhabitants per year [5].

In 2004 and 2005 tularemia re-emerged in Germany. An airborne outbreak in humans affecting a group of hare hunters occurred in the county of Darmstadt-Dieburg [7]. Additionally, two epizootics were noticed in the county of Goettingen, Lower Saxony, where several non-human primates were infected resulting in a significant number of fatalities [8, 9]. Both affected counties were not regarded as typical endemic foci of *F. tularensis*. In 2007, the highest number of human tularemia cases was reported for almost 50 years.

Historically, the first cases in Germany were recognized in 1937 [10]. From 1949 to 1959 repeated outbreaks in humans involving hundreds of cases occurred in three main areas: Brandenburg/Mecklenburg-Western Pomerania (Northeastern Germany), Schleswig-Holstein (Northern Germany), and Bavaria (Southern Germany). From the late 1950s onwards, a sharp decline in human infections occurred [10].

Unfortunately, reliable information on seroprevalence in humans in different geographical regions of Germany, permitting the identification of risk factors, does not exist. Only one single study, published in 2004 and comprising 6632 sera from a cross-sectional investigation of a representative sample of the general German population gave evidence that the seroprevalence in Germany might be as high as 0·2% [11]. In addition to the occurrence of the first airborne outbreak of human tularemia in Germany in 2005 [7], there is also growing evidence of the re-emergence of tularemia in the last decade in Southern European (Portugal, Spain, Turkey) and Central European (Denmark, France, Austria, Bulgaria, Croatia, Former Republic of Yugoslavia) countries, respectively [6, 12–16].

In the present study we present the results of a population-based, cross-sectional health survey of the city population of Leutkirch, Baden-Wuerttemberg. We compared our data to previous results obtained from studies or reports from Germany and several other European countries, thereby summarizing for the first time the sparse data available on the incidence of tularemia in different countries in Europe.

METHODS

Design of the seroprevalence study

Briefly, 4000 of 12 475 inhabitants of an urban population were randomly selected from the registry of inhabitants and invited to participate in the study. Of the 4000 invited participants, 107 were ineligible because of having moved from the area without a forwarding address, resulting in a sample of 3893 subjects. A total of 2445 persons aged 10–65 years participated in the study leading to a participation rate of 62·8% [17]. For tularemia analysis, serum samples were available from 2416 participants.

Each interview was conducted by a trained interviewer. The standardized questionnaire included personal data (e.g. date of birth, gender, education, current work, diseases), health and social behaviour (e.g. sports activities, nutrition, alcohol consumption), as well as assumptive risk factors for infection with *F. tularensis* (e.g. pets, forest and garden work or leisure activities).

Serological testing

A screening ELISA was used to detect *F. tularensis*-specific anti-LPS antibodies as described recently [11]. ELISA results below the mean optical density (OD) plus 1 standard deviation (S.D.) calculated from 1149 negative sera collected in Germany were estimated as 'negative'. Results above the mean OD plus 3 S.D. were assumed as 'positive', whereas all results between these two values were taken as 'borderline'. Positive as well as borderline sera were additionally tested with a confirmative immunoblot [11]. Sera were considered to be positive if they showed a typical LPS ladder at a dilution of 1/500.

Data acquisition on tularemia in Europe and Germany

In many European countries including Germany, human tularemia is a reportable disease. Additionally, most countries notify human cases as well as the occurrence of tularemia in the animal population to the World Organization for Animal Health (Office Internationale des Epizooties; OIE). Therefore we extracted all relevant data on notified cases from the handiSTATUS II databank (http://www.oie.int/hs2/report.asp). In Germany, monthly updated data on the incidence of human tularemia can be directly obtained from the webpage of the Robert Koch Institute, Berlin (http://www3.rki.de/SurvStat). Almost complete historical data on the occurrence of *F. tularensis* infections in humans were recently reported based on the yearly reports to the German health authorities from 1949 to 2001 [5].

Statistical methods

Absolute and relative frequencies were calculated for qualitative factors in the descriptive statistical analysis, while, for quantitative factors, mean and standard deviation, as well as the median, minimum and maximum were determined. Statistical analysis was performed using SAS version 8.02 (SAS, Heidelberg, Germany).

Ethical agreement and informed consent

The study met the international agreements of the revised version (2000) of the World Medical Association Declaration of Helsinki regarding ethical principles for medical research involving human subjects and was approved by the ethics committee of the State Medical Chamber Baden-Wuerttemberg. Written consent was obtained from each study participant.

RESULTS

Employing a highly sensitive and specific combination of two standardized immunoassays [11], a total of 56 sera out of 2416 samples gave positive results indicating a seroprevalence of 2·32%. This result differs significantly ($P<0{\cdot}01$) from the 0·23% positive samples (out of 6673 sera) reported in a nationwide study performed in 1998 [11].

Stratification of the data indicated putative risk factors like 'hunting' (seroprevalence 6·25%) or 'working as a farmer' (3·94%) (Table 1). In German residents, seroprevalence was 2·30% (50/2162), whereas in participants of other nationalities seroprevalence was 3·70% (5/134). Due to the small number of positive samples in these subgroups, statistical tests were not performed. The analysis of age distribution showed no significant cumulation in any particular age group (Fig. 1).

Neither immigrants from Austria (0/12) nor from Turkey (0/107) showed positive results in tularemia serology. Markedly, seroprevalence differed clearly in terms of time of residency. In people living for more than 10 years in Leutkirch, seroprevalence was only 1·90% whereas prevalence varied between 4·30 and 7·90% in people living for only 1–3 or 3–5 years in this city.

Gender, outdoor activities, and exposure to ticks or pets did not appear to be associated with a higher risk for a positive test result.

The assessment of the re-emergence of tularemia in Germany by the analysis of recently notified cases through national surveillance introduced in 2001 revealed a total of 32 human cases from 2001 to 2006 (Fig. 2; http://www3.rki.de/SurvStat). In contrast to our results of the seroprevalence study, almost 75% of all infections affected males.

In the time period between 1974 and 2005, 93 serologically confirmed cases were notified in 15/16 federal states. The highest number of infections reported were in Baden-Wurttemberg ($n=18$), Hesse ($n=14$), North Rhine-Westphalia ($n=13$), and Bavaria ($n=10$), but in none of these cases was *F. tularensis* cultured or directly detected by PCR or immunological methods [5].

In contrast to that, the follow-up of all positive *F. tularensis* laboratory reports at the Bundeswehr Institute of Microbiology, Munich (2004–2007) showed that *F. tularensis* could be identified by culture and/or PCR in samples from at least seven different federal states (Fig. 2). This included the first direct detection of *F. tularensis holarctica* in a dead hare in the state of our seroprevalence study. Before 2004, tularemia in wildlife was last reported to the OIE in 1992 (http://www.oie.int/hs2/report.asp). A significant increase in the number of reported human tularemia cases occurred in 2007 compared to the last 50 years. In 2007, 21 confirmed cases were notified, including 11 cases in the federal state of Baden-Wurttemberg, where our study was performed.

Most European countries report cases of tularemia in humans, livestock, and wildlife to the OIE. In our comparative analysis we focused on human cases in the time period 1996–2004. Data from 2005 and 2006 are not yet available. Twelve European Union (EU) member states reported no human cases at all, although it should be noted, that Ireland as well as Portugal did not register tularemia at the national level until 2003. Additionally, data from Belgium and The Netherlands were incomplete. In the remaining

Table 1. *Distribution of positive sera in different strata or putative risk groups. In some groups, the sum of positive and negative samples differs from the total number described in the text. This is due to missing data in some of the questionnaires*

Key features	All		*Francisella* WB positive		*Francisella* WB negative	
	n	%	*n*	%	*n*	%
Gender						
Male	1169	48·1	26	46·4	1132	48·1
Female	1263	51·9	30	53·6	1218	51·9
Age (yr)						
> 10–20	373	15·3	6	10·7	359	15·2
> 20–35	562	23·0	11	19·6	547	23·2
> 35–50	1226	50·2	33	58·9	1180	50·0
> 50–65	279	11·4	6	10·7	272	11·5
Nationality						
German	2162	89·9	50	90·9	2091	90·0
Turkish	108	4·5	0	0	107	4·6
Others	134	5·6	5	9·1	126	5·3
Farmer						
Yes	127	5·6	5	9·3	121	5·5
No	2137	94·4	49	90·7	2065	94·5
Forest ranger						
Yes	64	2·8	1	1·9	63	2·9
No	2194	97·2	52	98·1	2119	97·1
Hunter						
Yes	16	0·7	1	1·9	15	0·7
No	2235	99·3	53	98·2	2159	99·3
Occupational exposure						
Yes	50	2·1	3	5·4	46	2·0
No	2392	98·0	53	94·6	2314	98·1
Dog owner						
Yes	404	16·6	11	20·0	389	15·6
No	2027	83·4	44	80·0	1961	84·5
Cat owner						
Yes	651	26·8	14	25·0	628	26·8
No	1777	73·2	42	75·0	1718	73·2
History of tick bite						
Yes	618	26·6	6	12·2	608	27·0
No	1706	73·4	43	87·8	1641	73·0

WB, Western blot.

15 EU member states the cumulative incidence differed significantly between single countries (Table 2). Most clearly, the incidence was highest in the Scandinavian states of Finland and Sweden with cumulative numbers of more than 37 and 20 cases/100 000 inhabitants within the 9-year period, respectively. Marked incidences in this time period were also reported from Bulgaria (3·04/100 000), Hungary (7·41/100 000), Slovakia (7·92/100 000) and Czech Republic (5·58/100 000). Although Germany directly borders the latter country, the reported incidence in Germany was only 0·03/100 000. Besides the United Kingdom, where only one imported case had been notified, only Poland, Italy and France showed results similar to those from Germany. In all four countries the cumulative incidence was only 0·5–10% of the values reported from the other European countries (Table 2).

From the 28 European countries which are not members of the EU, only very fragmentary data

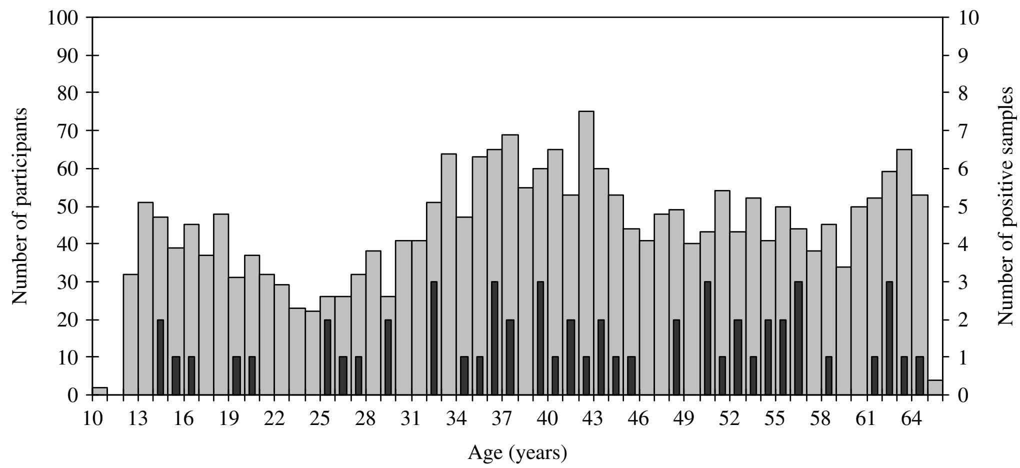

Fig. 1. Age distribution of the Leutkirch study group (■) and of seropositive samples (■).

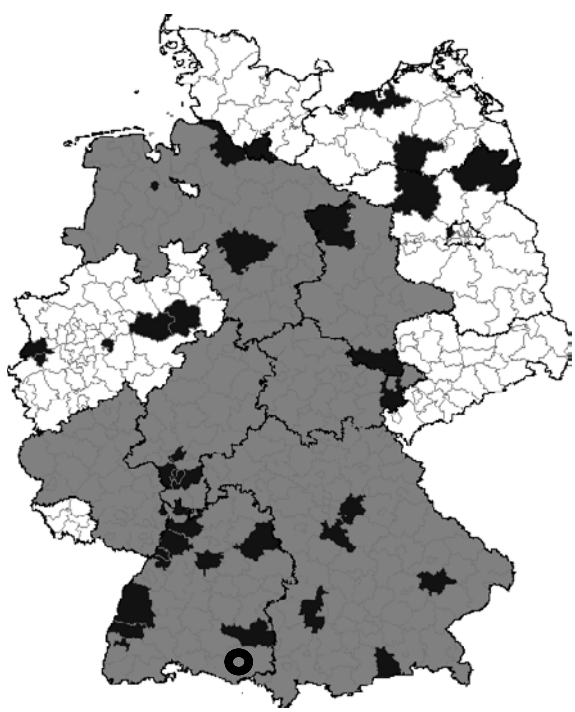

Fig. 2. Spatial distribution of reported *Francisella tularensis* infections in humans since 2001 (counties, black areas) and recovery of *F. tularensis* from rodents, hares or monkeys by PCR or culture (federal states, grey areas). Black circle (◉) denotes the study area for the seroprevalence study (city of Leutkirch). (Source modified from: SurvStat, http://www3.rki.de/SurvStat, 02.03.2008.)

were available. Here, Norway (1·85/100 000), Croatia (1·74/100 000) and the former states of Yugoslavia, Serbia and Montenegro (1·58/100 000) reported considerably high incidences of human cases. These reports are consistent with data scientifically published from these states.

DISCUSSION

The epidemiology of tularemia in Germany is characterized by three main enigmatic features: its sharp decline in the late 1950s, its irregular cycle and the irregular geographic appearance or persistence [5, 10]. Whereas between 1949 and 1959 several outbreaks involving more than 500 patients had been described from the peninsula of Eiderstadt, the Baltic Sea region of Mecklenburg-Western Pomerania and the upper Main region near Wuerzburg, the number of human cases dropped to less than 10 patients in the following years [8]. Until 1989, no significant difference in tularemia incidence between the Federal Republic of Germany and the German Democratic Republic was apparent. After the reunification of Germany, which allowed free crossing over of deer, boars and other wildlife, less than five human cases occurred annually. Even from these cases, more than 40% were assumed to be imported.

Up to this point, no methodical investigation in the German population had been described. To address this deficit, sera from a comprehensive cross-sectional study comprising a representative sample of the German population were obtained and serologically tested. Because seroprevalence studies of rare infectious diseases are often hindered by the lack of highly specific diagnostic tools, we developed a combination of a screening ELISA and an immunoblot which proved to be suited to the performance of epidemiological studies in human tularemia. From a total of 6632 serum samples from individuals between the ages of 18 and 79 years, specimens from 15 (0·23%) individuals tested positive for *F. tularensis*-specific antibodies by ELISA and confirmatory Western blot

Table 2. *Incidence of human tularemia cases in the EU member states from 1996 to 2004* (*no data are available for 2005 and 2006*)

EU member states	Population (million), 1996	Cumulative number of cases 1996–2004	Cumulative incidence (cases/100 000) 1996–2004
Austria	7·986	42	0·53
Bulgaria	8·775	267	3·04
Czech Republic	10·433	582	5·58*
Denmark	5·199	7	0·13
Finland	5·085	1912	37·61
France	58·109	37	0·06†
Germany	81·338	24	0·03
Hungary	10·319	765	7·41
Italy	58·262	29	0·05
Poland	38·792	12	0·03
Slovakia	5·432	430	7·92
Slovenia	2·052	9	0·44
Spain	39·404	640	1·62
Sweden	8·822	1825	20·69
United Kingdom	58·295	1	0·002
EU member states with no reported cases (incidence = 0): Belgium, Cyprus, Estonia, Greece, Ireland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Netherlands, Portugal			

Data were accumulated from the annual reports of each country to the World Organization for Animal Health (http://www.oie.int/hs2/report.asp).
* No data 1999–2000.
† No data 1996–2002.

[11]. These data provided some evidence that tularemia might be more abundant than is presumed from officially reported cases. Shortly after these data were published, two epizootics were recognized in the vicinity of Goettingen in Central Germany. The retrospective analysis of both outbreaks which occurred in non-human primate facilities and involved more than 20 animals, showed that the infection was passed to the monkeys by the indigenous rodent population in a region where tularemia had never been previously reported [8, 9]. Within the next 12 months, the first airborne outbreak of human tularemia in Germany was notified in Darmstadt, federal state of Hesse. Here, at least 11 hunters were affected, of which one died [7]. In 2006 and 2007, *F. tularensis* was definitely confirmed in hares from Thuringia, Rhineland-Palatinate, Hesse, Bavaria and Baden-Wurttemberg (W. D. Splettstoesser, unpublished data).

From this latter federal state the highest number of human cases was reported during the last three decades [5]. For this reason, we took the opportunity to analyse the sera derived from the Leutkirch study which was originally performed to estimate the frequency of *Echinococcus multilocularis* infections in this population [17]. Leutkirch is located in the Southwest of Germany (47° 47′ latitude, 10° 01′ longitude). Data on elevation (655 m), regional mean annual air temperature (<9 °C), and mean annual precipitation (1278 mm) (1961–2004) were obtained from the Federal Meteorological Service, Germany, but revealed no ecological risk factors associated with the endemic occurrence of *F. tularensis* [18]. No data were available on hare or rodent populations or the presence of vectors like ticks. Because the study region in the vicinity of Leutkirch shared no geographical features associated with a higher risk of long-term persistence of *F. tularensis* in the environment [8, 18], we expected an equivalent seroprevalence as in the national survey (0·23 %), or even less. To our surprise the frequency of positive samples exceeded this value by more than tenfold. The reason for this result is currently unknown, but as all results were generated in the same laboratory with the same test procedures, methodological reasons can be excluded.

Differences in stratified subpopulations gave further evidence that the seroprevalence of 2·32 % was not due to a lack of specificity of the assay combination. Markedly, the seropositive rate seemed to be dependent on the duration of residency. A lower percentage in residents living for more than 10 years in the Leutkirch area would be well in line with the assumption that this area is a low-risk region. However, on the other hand, that would mean that the seroprevalence of tularemia might even be significantly higher than 0·23% or even 2·32% in other vicinities of Germany.

In Norway, tularemia is also a common disease in small rodent and hare populations, in which large outbreaks can be observed. In humans, the yearly number of cases is low, usually less than 10. Nevertheless, serological investigations on hunters and healthy schoolchildren indicate, with up to 4·70% positive results in the latter group, that *F. tularensis* low-grade infection is widespread [19]. Similar data were reported from Austria and Poland, when either hunters or healthy forest workers were investigated [20, 21]. Seropositivity was 3·0% in 149 hunters from the provinces of Styria and Burgenland [20]. In the second study, the prevalence of antibodies to *F. tularensis* was evaluated in 480 serum samples obtained from healthy forest workers from different regions of Poland. IgA antibodies were detected in 4·60%, IgG antibodies in 3·80% and IgM antibodies in 2·70% of the serum samples [21].

All studies summarized here, coincide with our results and are in accord with the assumption that tularemia may be misdiagnosed and underestimated, especially in areas where the incidence is historically assumed to be very low. This hypothesis is additionally supported by the analysis of the cumulative incidence in all European countries according to the OIE. It is unlikely that in Germany, which shares several geographical as well as ecological features with its neighbouring countries, e.g. France, Austria, or Czech Republic, the presence of *F. tularensis* in wildlife is really significantly lower than in those states.

In Germany as well as in most other European states, modes of transmission, location of endemic foci or even the definite animal host of *F. tularensis* are either not well understood or unknown. To gain more insights into the genuine distribution of this highly virulent pathogen, clinical awareness of the disease entity, molecular characterization and typing of recovered strains and the application of sophisticated geographical information systems have to be increased. This is of great importance due to the first indication that climate change might be associated with an extension of endemic foci and an increase of human and animal tularemia [22].

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DECLARATION OF INTEREST

None.

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