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MAJOR ARTICLE

Seroepidemiology of human tularemia – systematic review and metaanalysis of seroprevalence studies

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Background: Seroepidemiologic studies of human tularemia have been conducted throughout the northern hemisphere. The purposes of this study are (1) to provide an overview of *F. tularensis* seroprevalence data, and (2) to generate an estimate of the proportion of study participants whose infection remained subclinical.

Methods: We conducted a systematic review of *Francisella tularensis* seroprevalence studies according to the PRISMA guidelines. We searched Pubmed®, Embase® and Web of Science™covering the period from 1951 to 2023.

Results: The weighted pooled seroprevalence among 44'486 participants recruited in 52 studies was 3.7% (95% confidence interval (CI) 2.7-5.1). Reported seroprevalences ranged between 0.2%

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and 31.3%. Occupational activities associated with an increased likelihood of exposure (risk ratio (RR) 3.51 (3.2-3.86)) and studies from North America vs. Europe and Asia (4.53 (4.15-4.94)) were associated with significantly increased seropositive rates. Twenty-eight datasets (47%) reported clinical information on a total of 965 seropositive participants. The weighted pooled estimate for subclinical seropositivity was 84.4% (95% CI 72.9-991.7). Studies from *F. tularensis* type A areas (RR 0.37, 95% CI 0.27-0.51) and studies from sites where pulmonary tularemia prevailed (RR 0.38, 95% CI 0.28-0.51) reported lower subclinical seropositivity rates than studies from type B areas and from areas of predominance of (ulcero-) glandular or oropharyngeal tularemia, respectively.

Conclusions: Throughout the northern hemisphere, only a small proportion of study participants showed serologic evidence of exposure to F. *tularensis.* Eight outof 10 seropositive participants had no historical evidence of past clinical tularemia.

Keywords: tularemia – seroepidemiology – seroprevalence – subclinical – systematic review

Key points: The weighted seroprevalence of human antibodies against *Francisella tularensis* in endemic areas of the northern hemisphere was 3.7%. Eight out of 10 seropositive participants had no historical evidence of past clinical tularemia.

INTRODUCTION

Human tularemia is a bacterial zoonosis caused by *Francisella tularensis*, a small, gram-negative coccobacillus with the capacity to infect a wide range of mammals, arachnids, insects and other animals. There are two main subspecies. Type A (*F. tularensis subsp. tularensis*) is mainly restricted to North America, although a few strains have been isolated in Europe [1]. Type B (*F. tularensis subsp. holarctica*) is distributed throughout the Northern hemisphere and has also been isolated in Australia [2]. Modes of acquisition in humans are diverse and include arthropod bites (ticks, mosquitos), ingestion of contaminated freshwater or soil, direct contact with infected live or dead animals, and inhalation of contaminated aerosols. Accordingly, clinical manifestations vary and include (ulcero-) glandular, oropharyngeal, typhoidal, and pulmonary manifestations [3].

While both the molecular pathogenesis of tularemia [4] and the clinical manifestations in humans have been studied in detail [3, 5], important questions remain unanswered. An issue of particular interest to clinicians is the likelihood of subclinical infection among exposed individuals (i.e., a- or oligosymptomatic, medically unattended infection). Some authors have postulated that the majority of infections remain undetected [6], while others believe that most cases cause a distinct clinical syndrome [7, 8]. A comprehensive review of the available data is lacking. Such information, however, is important, as it may elucidate whether subclinical infection as opposed to clinically overt disease is the typical human response to *F. tularensis* exposure. It may assist in

the clinical interpretation of diagnostic test results and contribute to what is known on how effectively the human immune system deals with *F. tularensis.*

One means of addressing this question is to examine seroprevalence studies. Seropositive individuals without a history of clinical disease compatible with tularemia can be considered to have experienced subclinical infection. If they represent the majority of seropositive individuals, it follows that clinical disease cannot usually be explained by pathogen virulence alone, but requires a particular set of additional conditions for it to occur. This may be particularly relevant in geographic areas, where the less virulent type B circulates [9]. Clinical disease could then be considered as evidence of some sort of immune compromise around the time of infection. Evidently, alternative explanations are possible and include the mode of acquisition, the infectious dose, strain-specific virulence determinants and genetic predisposition, which may impact on the extent of clinical disease.

Thus, the purposes of this study were (1) to generate an overview of *F. tularensis* seroprevalence rates reported from endemic areas worldwide, and (2) to generate an estimate of the proportion of human tularemia cases identified by detectable serum antibodies that had no history of past clinical manifestations suggestive or confirmed to be clinical tularemia (subclinical cases). Because each study usually identifies only a handful of seropositive individuals, we conducted a systematic review of tularemia seroprevalence studies between the 1940s and 2023 in accordance with the PRISMA guidelines for systematic reviews [10].

METHODS

Data source and search strategy

Pubmed® (1946 to present), Embase® (1947 to present) and Web of Science™ (1921 to present) were searched using the following search term combinations: (Tularemia OR Francisella) AND (seroprevalence OR seroepidemiolog*); (Tularemia OR Francisella) AND antibody AND prevalence; antibod* prevalence tulare* human. Refinements were added as needed. The list of references of each retrieved article was searched for additional suitable articles. No language limitations applied. Studies written in languages other than English, German or French were full-text translated using Deeple Pro® (www.deepl.com). The generated list of articles was screened by title and abstract independently by two authors (CM and CA) who applied the predefined inclusion/exclusion criteria (s. below). Discrepancies were resolved by consensus.

Inclusion and exclusion criteria

We included studies, research letters and abstracts that reported original data on the prevalence of serum antibodies against *F. tularensis* in humans. No restriction applied regarding the population studied (e.g., geographic location, general vs. risk populations, age, gender, ethnicity) and outbreak vs. non-outbreak time periods of serum sampling. Publications were included if published before

30 April 2023. We excluded papers without original data or those duplicating previously published data, papers focusing on clinical cases, and papers without methodologic description of antibody detection tests used. Studies reporting a seroprevalence of 0 were recorded, but excluded from analyses.

Quality assessment

We next assessed all retrieved studies using a critical appraisal checklist adapted from the Joanna Briggs Institute checklist for prevalence studies [11, 12] (supplementary file 1, table S1). We included studies, which scored ≥5 of 7 points (>70%) in the 7-question checklist. In addition, we devised a list of criteria identifying high-specificity serologic testing for use in subanalyses (supplementary file 1, table S2).

Data retrieved from selected articles

The following data were extracted from all articles included in the analysis: study year and location, population characteristics (age, sex, risk factors for *F. tularensis* exposure), use of a study questionnaire for participants, serologic tests used, tests for cross-reactivity used, cut-offs values defining seropositivity, number and proportion of participants testing positive, number and proportion of "subclinical" participants testing positive, narrative clinical description of participants testing positive.

Definition of subclinical infection

We defined, for the purpose of this study, a "subclinical case" as an individual who was seropositive for *F. tularensis* and explicitly asymptomatic at the time of serum sampling and had a past medical history lacking episodes of known tularemia or tularemia-like illness.

Statistical analysis and software used

Pooled counts and weighted proportions of participants seropositive for *F. tularensis* were calculated for the entire set of studies and subgroups of interest. Weighted proportions were calculated using both a fixed effect model and a random effect model using restricted maximum likelihood estimation (REML) of the between-study variance [13], because substantial heterogeneity between the studies (e.g., geographic region, risk of exposure to *F. tularensis*) other than sampling errors were expected to occur. No limit for heterogeneity as expressed by the I^2 statistic applied. Forest plots are presented with a 95% confidence interval (95% CI) and group size. Weighted seroprevalences rates for subgroup analyses and risk ratios (RR) were calculated with the same model when the comparative datasets were complete. When incomplete, the RR were calculated from the weighted seroprevalences directly. For linear correlation analysis, the Pearson correlation coefficient was calculated. Funnel plots were constructed by plotting the log odds against the study size as recommended by Hunter et al [14] for metaanalyses of proportion studies (figure S3). "R" software package, version 4.0.3 (Vienna, Austria; https://www.R-

project.org/) and VassarStats (www.vassarstats.net) were used for analysis. The map (figure S2) was created using ArcGIS®, intellectual property of Esri (www.esri.com).

RESULTS

Bibliographic data and study settings

We identified 52 articles fulfilling the predefined selection criteria. A flowchart detailing the selection process adapted from PRISMA [15] is provided in figure 1. Six additional seroprevalence studies extracted during the search process were excluded, because no seropositive individuals were identified [16-21]. Seven selected articles provided 2 datasets [6, 22-27], resulting in a total of 59 datasets (tables 1 and 2). The distribution of the publication years over time is shown in figure S1 (supplementary data file 1). The geographic distribution of study countries is displayed in figure S2 (supplementary data file 1). Key study settings are summarized in table 1. Details of each study are provided in the supplementary data file 2.

Pooled seroprevalence data

A total of 44'486 participants were included. Seroprevalence rates as reported in each study ranged from 0.2 to 31.3 percent (table 2). Sample size (n) and seroprevalence rate were not significantly correlated (Pearson r -0.203, 95% CI -0.436 - 0.056, p=0.123). The weighted seroprevalence rate of 59 pooled datasets calculated with the random effects model was 3.7% (95% CI 2.7-5.1) (figure 2). The I^2 statistic was 96% (96-97; p<0.01) indicating major heterogeneity. This was expected because of real between-study differences in the participant cohorts' risk of exposure to *F. tularensis*. A funnel plot designed for proportion studies [14] (figure S3, supplementary data file 1) confirmed the heterogeneity in that there was no convergence of prevalence rates as the sample sizes increased.

Male and female individuals accounted for 18'106 and 9'885 participants, respectively, in a total of 28 datasets. Sex was not specified for another 16'477 participants in 31 datasets. The serostatus could be attributed to sex in 10'994 male and 8'094 female participants (table 3). The respective weighted seroprevalence rates were 4.7% (95% CI 3.0-7.3) and 5.2% (95% CI 3.1-8.8), and the RR was 0.83 (95% CI 0.66-1.05). We did not calculate age-specific pooled seroprevalence rates because the reporting of age and age group in the original studies was very heterogeneous and did not allow consistent grouping across a majority of studies.

Table 3 also lists the pooled weighted seroprevalence rates and estimated risk ratios according to the geographic region of the study sites, occupational risk of exposure, serologic testing strategy and the availability of clinical information. The pooled seroprevalence of studies conducted in North America was greater than that from Europe and Asia combined (9.6% vs. 2.7%; RR 4.53, 95% CI 4.15-4.94). A combined total of 16'554 study participants (37%) reported an occupational risk of exposure to *F. tularensis*. Occupational risk factors included hunting and trapping (16

studies), military service (5), animal husbandry, farming and ranching (5), butchering and slaughterhouse work (5), forestry work (4), veterinary medicine (3) and landscaping (1). Included are North American native participants whose lifestyle placed them at an increased risk of exposure. The pooled weighted seroprevalence of these at-risk populations was significantly greater than in studies of populations without such risk factors (5.5% vs. 2.4%, RR 3.51 (3.20-3.86); table 3). However, only 5 studies provided a complete comparative dataset of individuals with vs. without an increased risk of exposure and the respective seropositivity rates [28-32]. The pooled seropositivity rates of these studies were 2.7% (95% CI 1.5-4.9) and 2.4% (95% CI 1.3-4.5), respectively, with a risk ratio of 1.20 (95% CI 0.94-1.53).

Grouping of the 52 studies according to the serologic testing strategy revealed that high-specificity testing according to our ad hoc definition (supplementary file 1, table S2) was associated with a lower weighted seropositivity rate than non-high specificity testing (3.5% vs. 4.6%, RR 0.49 (0.45-0.53), table 3).

Subclinical tularemia

Clinical detail provided on study participants testing seropositive varied widely and was predominantly limited to summary statements. A total of 28 datasets [6-8, 22-24, 31, 33-53] comprised a total of 13'807 individuals, for whom clinical information was available. Of these, 965 tested positive (weighted seroprevalence 5.5% (95 CI 3.8-7.8) vs. 2.6% (95% 1.6-4.2) in studies without clinical data) (table 3). Table S3 in the supplementary data file 1 lists pertinent quotations from the narrative clinical accounts given in each study.

A past medical history compatible with tularemia or a tularemia-like illness (with or without a physician-confirmed diagnosis) among these 965 seropositive individuals could be elicited in 143, leaving 819 considered as having had subclinical tularemia. Figure 3 provides a Forest plot with the weighted rates of subclinical seropositivity reported in these studies. Overall, the weighted subclinical seropositivity rate was 84.4% (95% CI 72.9-91.7). Additional subanalyses (table 4) revealed that this rate was lower in studies from areas where, according to the literature [9, 54, 55], *F. tularensis* type A was prevalent [6, 52] than in studies from type B areas. Similarly, studies from areas with a predominance of pulmonary tularemia around the time of serum sampling [6, 8] reported lower subclinical seropositivity rates than studies from areas where (ulcero-) glandular or oropharyngeal tularemia prevailed. Subanalyses according to occupational risk of exposure and testing strategy are also listed in table 4.

DISCUSSION

This systematic review and metaanalysis covers 52 studies from North America, Europe and Asia. Studies from North America dominated the first 50 years of observation between 1951 and 2002, while studies from Europe and Asia combined (mostly its westernmost region including Turkey

and Iran) prevailed between 2003 and 2023 (figures S1 and S2). The latter contributed more than three quarters of all study subjects (table 3), reflect the more recent regional seroepidemiology, and mainly used advanced, commercially available methods for testing. Their weighted pooled seroprevalence was greater than four-fold smaller than the corresponding rate derived from North American studies (table 3). Possible explanations include differences in virulence of circulating *F. tularensis* subtypes and clades, different transmission paths and dynamics, the predominance of indigenous participants in North American studies [22, 24, 33, 34, 38, 41, 44, 56-58], whose lifestyle may have led to frequent to exposure, and possibly less specific serologic methods used in the earlier decades of the observation period. However, the absolute difference in seroprevalence being 6.9% only, we chose to pool study data in subsequent analyses irrespective of their geographic origin.

The weighted overall seroprevalence rate of 3.5% for *F. tularensis* antibodies (figure 1) and the equally important finding that 90% of reported rates ranged between 0.3% and 18% (table 2) emphasize that only a small minority of individuals living in endemic areas provides serologic evidence of past infection. These findings were the result of pooling data that were generated by the use of different serologic assays. Our original intention to group and compare the studies according to the test type used was not feasible as the combinations of methods and cut-off values, the choices of confirmatory tests and the reporting formats varied widely (table 2 and supplementary data file 2). Thus, we devised an ad hoc definition for "high-specificity" testing (table S2), which indeed identified a group of studies that yielded a lower pooled seroprevalence rate suggesting greater specificity than non-"high-specificity" testing (table 3). The absolute difference of the pooled seroprevalences grouped accordingly of 1.1% (table 3) again was not clinically or epidemiologically relevant.

The finding of low seroprevalence rates across most of the reviewed studies is important both epidemiologicallyand clinically. It may reflect that tularemia transmission can be highly focal and even within endemic areas the risk of acquisition is extremely heterogeneous. Also, as serum antibodies persist for decades, cross-sectional serosurveys capture exposure events dating back many years, which underscores the rarity of tularemia. Clinically, the interpretation of a positive serology result in a patient is facilitated by knowing the pretest-likelihood of seropositivity in the community. Even when such data are not available at a particular location, this figure indicates that the "background" seropositivity rate in endemic areas is predictably low irrespective of the serologic test system used. A low seroprevalence was also found for study participants reporting occupational or lifestyle activities expected to increase the risk of exposure. While their likelihood of testing positive was indeed more than threefold that of non-at-risk participants (table 3), their pooled seroprevalence rate remained low at 5.5% with 90% of studies reporting a rate below 15% (table S2 and supplementary data file 2).

The second objective of this review was to quantify the proportion of seropositive individuals who had undergone the infection subclinically, i.e., who did not report a history compatible with past tularemia. The use of seroprevalence studies to address the issue of subclinical infection is well

established and has been used previously for other pathogens, e.g., *Borrelia burgdorferi* [59, 60]. Our calculation indicates that this proportion was 84.4% (figure 3) and was not affected by reported risk factors for exposure (table 4). Interestingly, subclinical infection appears to the norm for many vector-borne zoonoses, e.g., Lyme disease [59] or tick-borne encephalitis [61]. The interest in establishing the rate of subclinical tularemia is epidemiologic, clinical and scientific. In clinical epidemiology, mandatory reporting of tularemia cases is a common public health tool used to monitor the disease activity over time. Our data suggest that this tool likely catches 10-20% of infections at best as subclinical cases remain unreported. Knowledge of the rate of subclinical infection thus provides a basis to roughly estimate true infection rates. Our findings also suggest, however, that such extrapolation may not be applicable in outbreak situations and when pneumonic tularemia indicates aerosol transmission [6, 8].

In clinical practice, knowing the rate of subclinical seropositivity provides additional information for the interpretation of a positive serology, even when seroconversion around the time of an acute illness appears to strongly suggest true tularemia. Considering the high rate of subclinical tularemia indicates that the positive predictive value (PPV) of a given serologic test may be lower than commonly reported, e.g., for ELISA tests [62, 63]. Definitions for "true positive" used to calculate the PPV often rely on compatible clinical illness (reviewed by Maurin [1]) and may lead to overestimation of the PPV as compatible clinical illness, such as acute lymphadenopathy or pneumonia, has multiple causes with tularemia being an infrequent one. Thus, when tularemia is suspected, care should be taken to identify the organism by culture or PCR whenever possible.

In science, an estimate of the rate of subclinical seropositivity may add a puzzle piece to what is known about host susceptibility to *F. tularensis*. Our finding suggests that the immune defenses of most individuals, who mount a detectable humoral immune response, control and eliminate *F. tularensis* in the absence of substantial clinical manifestations. The question arises why then a 10-20% minority of becomes clinically ill at all. Only isolated cases of patients with defined immunodeficiencies and severe tularemia have been described (reviewed by Bahuaud and co-workers [64]). The vast majority of patients appear immunologically healthy before and after the disease. Future research could be directed towards the identification of subtle deviations within the framework of virulence factors and immunoprotective events associated with tularemia that could explain a temporal susceptibility to symptomatic disease in otherwise immunocompetent individuals. This could entail a comprehensive "systems-level" approach [65] comparing immune functions in previously healthy individuals with severe tularemia with individuals with subclinical infection.

The results of this systematic review need to be taken with caution. In the former Sovjet Union, tularemia was extensively studied, but publications were inaccessible using the search strategy we used. Limitations also include the large time window of study dates and widely scattered locations, virulence differences of and within the two main subspecies, partly incomplete description of participant cohorts, diverse serologic test systems, and the often sketchy descriptions of how the clinical history of seropositive participants were obtained. It also needs to be kept in mind that the

fundamental problem of serosurveys for estimating subclinical infection rates is the often low disease prevalence in these settings. Consequently, the PPV of serology is low with a tendency to overestimate subclinical infection because of contamination by false-positive results. In addition, as the majority of clinical information obtained was historical in nature and reflected the participants' recollections, it can be assumed that the data are incomplete and likely overestimate the rate of subclinical infections.In conclusion, we find that in temperate and arctic zones of the northern hemispheres where human tularemia occurs, only a small proportion of the population has ever been exposed to *F. tularensis*. Eighty to 90% of exposed persons are not aware of ever having had overt tularemia or a clinical illness compatible with it.

Author contributions:CA conceived the study and wrote the study protocol. CM and CA independently conducted the literature search, study screening and critical study appraisal, agreed on the final list of studies for analysis, and aggregated the data. PKAA and SA conducted statistical analyses. NS, MB and AD commented on analysis and drafting of the manuscript, which was prepared by CM and CA. All authors critically reviewed the various versions of the manuscript and approved the content and format of its final version.

Patient consent statement: All patient data used in this systematic reviewed had previously been published. Informed consent was not required for systematic reviews according to the competent local ethics committee.

Conflict of interest statement: The authors declare no conflict of interest.

Funding sources: No external funding.

Figure 1 Bibliographic search and selection flow diagram adapted from the PRISMA statement [46].

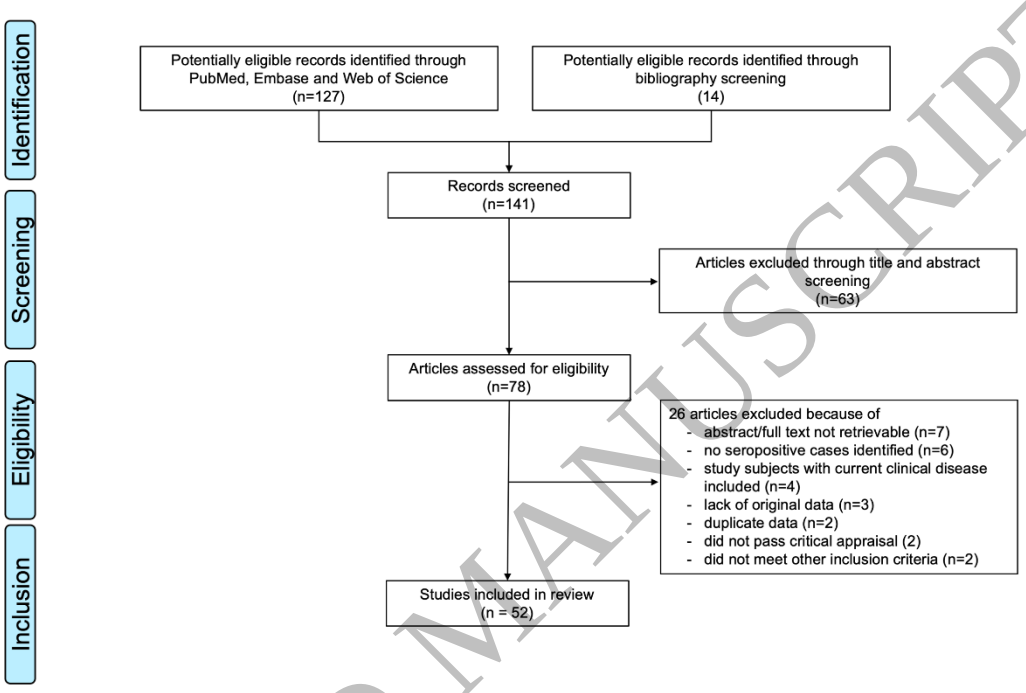

Figure 2 Forest plot of the pooled seroprevalence rates for antibodies against *F. tularensis*.

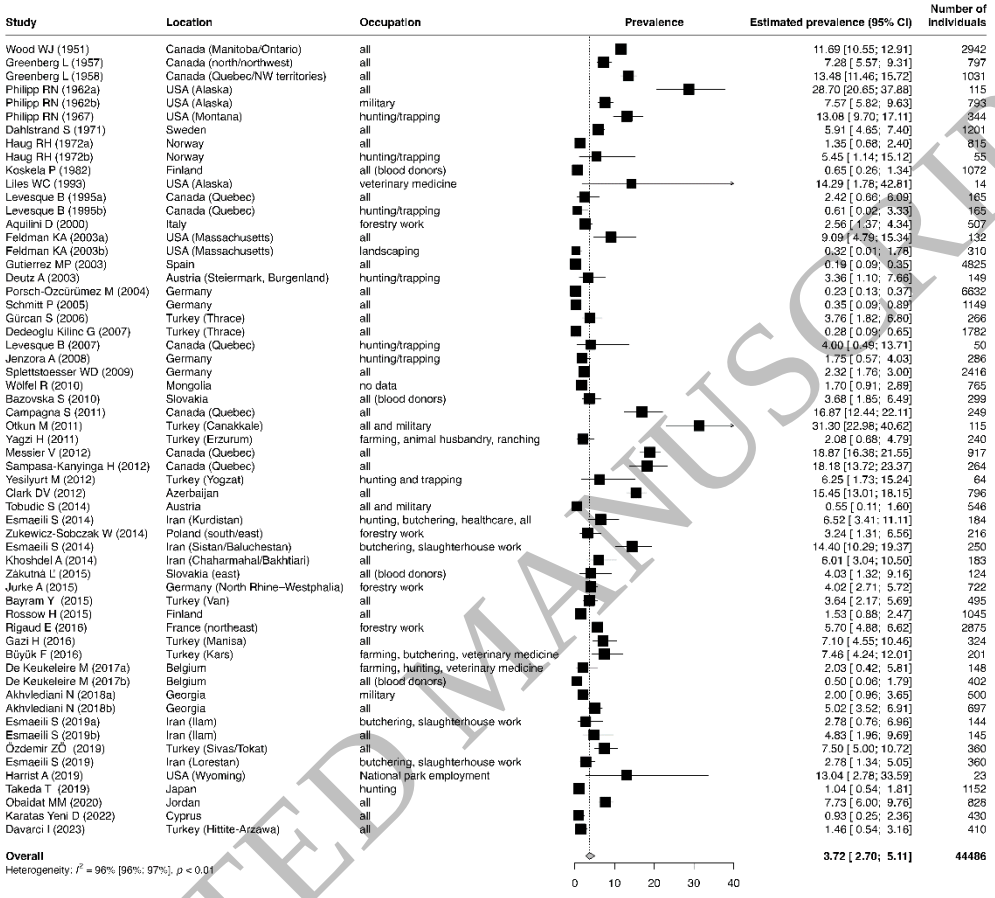

Figure 3 Forest plot of the pooled rates of subclinical seropositivity for antibodies against *F. tularensis*.

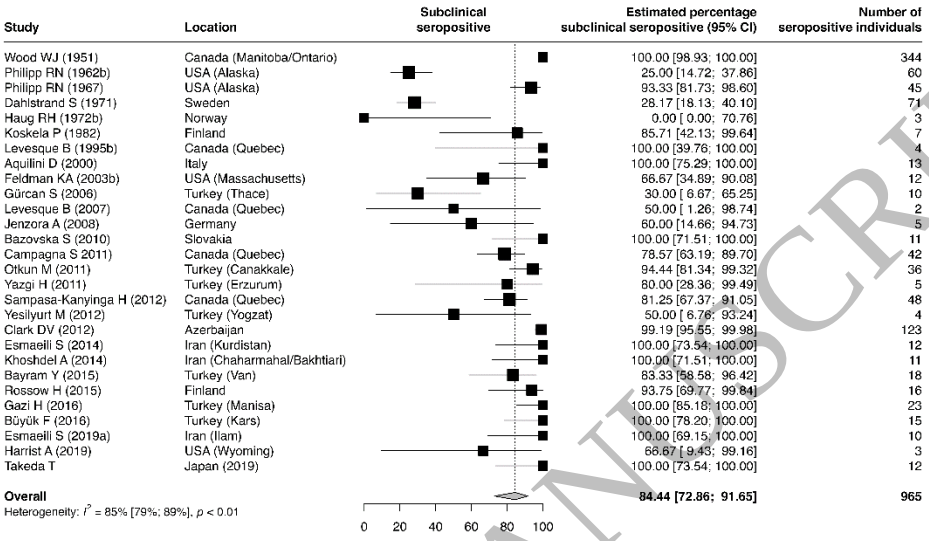

Table 1 Main settings of human *F. tularensis* seroprevalence studies included in this systematic review

Characteristic	n (%)
No. of studies	52 (100)
No. of studies with 2 datasets	7 (13)
No. of datasets	59
Geography	
Study site (world region)	
North America	13 (21)
Eastern Europe	4 (7)
Northern Europe	4 (7)
Western Europe	11 (21)
Middle East	18 (34)
East Asia	2 (4)
Study site in *F. tularensis* type B region	50 (96)
Study site in *F. tularensis* type A region	2 (4)
Participant characteristics	
Age span specified	52 (100)
Participants below 15 years of age included	10 (19)

Participants exclusively below 18 years of age	1 (2)
Sex distribution of seropositive individuals specified	28 (54)
Risk factors for *F. tularensis* exposure identified	33 (64)
Clinical information on seropositive participants provided	28 (56)
Participant questionnaire used	39 (75)
Serology for F. tularensis	
Primary antibody test used	
Tube agglutination test (TAT)	16 (36)
Microagglutination test (MAT)	12 (23)
Enzyme Linked Immuno Sorbent Assay (ELISA)	19 (36)
Other	5 (8)
Confirmatory secondary test(s) used	12 (23)
Cross-reactivity against *Brucella sp.* tested	26 (50)
High-specificity test strategy used	39 (75)
Non high-specificity test strategy used	13 (25)

Table 2 Human *F. tularensis* seroprevalence studies included in this systematic review

		All participants			**Seropositive participants**				
Author (year)	**Country**	**Age (y)**	**n**	**Male/female (n)**	**n**	**%**	**Clinically evaluated (n)**	**Subclinical (n)**	**Antibody test(s)**
Wood (1951) [33]	Canada	2-88	2'942	1'623/1'319	344	11.7	344	344	TAT[1]
Greenberg et al (1957) [56]	Canada	3-93	797		58	7.3			TAT[1]
Greenberg et al (1958) [57]	Canada	0-78	1'031		139	13.5			TAT[1]
Philip et al (1962) [34]	USA	all	115		33	28.7			TAT
	USA	18-65	793	793/0	60	7.6	20	15	TAT
Philip et al (1967) [22]	USA	15-84	344	332/12	45	13.1	45	42	TAT
Dahlstrand et al (1971) [8]	Sweden	all	1'201		71	5.9	71	20	TAT[1]
Haug et al (1972) [23]	Norway	13-17	815		11	1.3			TAT (Widal)
	Norway	adult	55		3	5.5	3	0	TAT (Widal)
Koskela et al (1982) [35]	Finland	adult	1'072		7	0.7	7	6	TAT[1]
Liles et al (1993) [66]	USA	adult	14		2	14.3			TAT[1]
Lévesque et al (1995) [24]	Canada	40±12	165	157/8	4	2.4	4	4	LAT

	Canada	adult	165		1	0.6			LAT
Aquilini et al (2000) [36]	Italy	21-65	507	507/0	13	2.6	13	13	IF
Feldman et al (2003) [6]	USA	adult	132	104/28	12	9.1	12	8	MAT
	USA	adult	310	154/156	1	0.3			MAT
Gutiérrez et al (2003) [67]	Spain	14-92	4'825	2'324/2'486	9	0.2			MAT
Deutz et al (2003) [68]	Austria	adult	149	146/3	5	3.4			MAT
Porsch-Ozcürümez et al (2004) [63]	Germany	18-79	6'632		15	0.2			ELISA+WB
Schmitt et al (2005) [69]	Germany	adult	1'149		4	0.3			ELISA+WB
Gürcan et al (2006) [37]	Turkey	all	266		10	3.8	10	3	MAT
Dedeoglu Kilinc et al (2007) [28]	Turkey	6-92	1'782	1'213/569	5	0.3			MAT
Campagna et al (2011) [41]	Canada	>15	249	105/146	42	16.9	42	33	TAT
Lévesque et al (2007) [38]	Canada	50	50	22/28	2	4.0	2	1	TAT
Jenzora et al (2008) [39]	Germany	adult	286		5	1.7	5	3	ELISA+WB+IFA
Splettstoesser (2009) [70]	Germany	10-65	2'416	1'169/1'263	56	2.3			ELISA+WB
Bazovska et al (2010) [71]	Slovakia	adult	299		11	3.7	11	11	TAT[1]
Wölfel et al (2010) [72]	Mongolia	adult	765	670/95	13	1.7			ELISA and/or IF
Otkun et al (2011) [42]	Turkey	0.5-76	115	60/55	36	3	36	34	TAT
Yagzi et al (2011) [43]	Turkey	16-77	240	134/106	5	2.1	5	4	ELISA+MAT
Sampasa-Kanyinga et al (2012) [44]	Canada	>18	264	110/157	48	18.2	48	39	TAT
Messier et al (2012) [73]	Canada	18-74	917		173	18.9			TAT
Yesilyurt et al (2012) [45]	Turkey	18-67	64	64/0	4	6.3	4	2	MAT+ELISA
Clark et al (2012) [74]	Azerbaijan	>18	796	347/449	123	15.5	123	122	ELISA
Tobudic et al (2014) [75]	Austria	18-60	546	534/12	3	0.5			ELISA
Esmaeili et al (2014) [47]	Iran	>18	184	184/0	12	6.5	12		ELISA
Esmaeili et al (2014) [29]	Iran	>18	250	206/44	36	14.4			ELISA
Khoshdel et al (2014) [48]	Iran	2-18	183	89/94	11	6.0	11	11	ELISA
Zukiewicz-Sobczak et al (2014) [30]	Poland	35-55	216	176/40	7	3.2	0		ELISA

Baryam et al (2015) [49]	Turkey	18-93	495	152/343	18	3.6	18	15	MAT
Zákutná et al (2015) [76]	Slovakia	adult	124	77/47	5	4.0			ELPAGA+WB
Rossow et al (2015) [7]	Finland	30-92	1’045	481/564	16	1.5	16	15	ELISA+WB
Jurke et al (2015) [32]	Germany	18-66	722	569/153	29	4.0			ELISA+WB
Gazi et al (2016) [50]	Turkey	49±17	324	156/168	23	7.1	23	23	ELISA
Büyük et al (2016) [51]	Turkey	>15	201	178/23	15	7.5	15	15	MAT+ELISA
Rigaud et al (2016) [77]	France	17-81	2’875	2916/59	164	5.7			TAT+ELISA
De Keukeleire et al (2017) [25]	Belgium	25-72	148	128/20	3	2.0			ELISA+ICT
	Belgium	18-68	402	118/90	2	0.5			ELISA+ICT
Akhvlediani et al (2018) [26]	Georgia	18-59	500	476/13	10	2.0			MAT
	Georgia	18-65	697	310/387	35	5.0			MAT
Esmaeili S et al (2019) [27]	Iran	30-50	144	144/0	4	2.8			ELISA
	Iran	27-53	145	145/0	7	4.8			ELISA
Esmaeili S et al (2019) [31]	Iran	18-78	360	275/85	10	2.8	10	10	ELISA
Harrist et al (2019) [52]	USA		23	13/10	3	13.0	3	2	MAT
Takeda et al (2019) [53]	Japan	18-90	1’152		12	1.0	12	12	RSA
Özdemir et al (2019) [78]	Turkey	20-80	360	180/180	27	7.5			ELISA
Obaidat et al (2020) [79]	Jordan	all	828	339/489	64	7.7			ELISA
Karatas Yeni et al (2022) [80]	Cyprus	>18	430		4	0.9			MAT
Davarci et al (2023) [81]	Turkey	2-89	410	226/184	6	1.5			MAT

Abbreviations: TAT, Tube Agglutination Test; LAT, Latex Agglutination Test; IF, Immunofluorescence Assay; MAT, MicroAgglutination Test; ELISA, Enzyme Linked ImmunoSorbent Assay; WB, Western Blot; ICT, ImmunoChromatography Test; RSA, Rapid Slide Agglutination

[1]The term «agglutination reaction» is used in the original publication.

Table 3 Pooled weighted seroprevalence of *F. tularensis* antibodies according to sex, geographic region, risk of exposure, testing strategy and clinical data availability in study participants of 52 studies included in this review

	Characteristic present					Characteristic absent							
			Proportions (95% CI)					Proportions (95% CI)			Risk ratio (95% CI)		
Characteristic	n	Seropositive	Raw	Fixed effect	Random effect	n	Seropositive	Raw	Fixed effect	Random effect	Raw	Fixed effect	Random effect
Male sex*	10994	566	5.1	8.8 (8.1-9.5)	4.7 (3.0-7.3)	8054	394	4.9	9.1 (8.3-10)	5.2 (3.1-8.8)	1.05 (0.93-1.19)	0.99 (0.92-1.05)	0.83 (0.66-1.05)
North America (vs. Europe or Asia)	8311	967	11.6	12.9 (12.1-13.7)	9.6 (6.3-14.3)	36175	929	2.6	5.0 (4.7-5.3)	2.7 (1.9-3.9)	4.53 (4.15-4.94)	4.53 (4.33-4.74)	4.53 (4.15-4.94)
Occupational risk of exposure	16554	1281	7.7	10.0 (9.5-10.6)	5.5 (3.9-7.8)	27932	615	2.2	5.1 (4.7-5.5)	2.4 (1.5-3.8)	3.51 (3.19-3.86)	3.51 (3.35-3.69)	3.51 (3.20-3.86)
High-specificity serologic testing used	34486	1189	3.4	7.5 (7.1-8)	3.5 (2.4-5.1)	10000	707	7.1	8.9 (8.3-9.6)	4.6 (2.6-8.1)	0.49 (0.45-0.54)	0.49 (0.47-0.51)	0.49 (0.45-0.53)
Clinicial information available	13807	965	7.0	9.4 (8.9-10)	5.5 (3.8-7.8)	30679	931	3.0	6.8 (6.4-7.2)	2.6 (1.6-4.2)	2.30 (2.11-2.51)	2.30 (2.20-2.41)	2.30 (2.11-2.51)

* information on sex was available in a subset of studies only

Table 4 Pooled weighted subclinical seropositivity rate for *F. tularensis* according to study location, geographic predominance of type A vs. type B, predominance of different clinical syndromes in the study area, presence or absence of occupational risk of exposure, and serologic testing strategy

	Characteristic present					Characteristic absent						
				Proportion (95% CI)			Proportion (95% CI)			Risk ratio (95% CI)		
Characteristic				Fixed Raweffect	Random effect			Fixed Raweffect	Random effect	Raw	Fixed effect	Random effect
North America (vs. Europe or Asia)	560	488	87.1	65.6 (57.6-72.9)	80.7 (54.5-93.5)	405331	81.7	63.0 (54.5-70.8)	86.3 (72.5-93.8)	1.07 (1.01-1.13)	1.07 (1.04-1.10)	1.07 (1.01-1.13)
F. tularensis type A area (vs. type B)	75	25	33.3	33.4 (23.1-45.6)	47.1 (18.7-77.6)	890794	89.2	72.5 (66.6-77.7)	87.3 (76.7-93.5)	0.37 (0.27-0.51)	0.37 (0.32-0.44)	0.37 (0.27-0.51)
Predominance of pulmonary tularemia (vs. (ulcero-) glandular or oropharyngeal tularemia)	83	28	33.7	33.6 (23.9-44.9)	44.6 (14.2-79.8)	882791	89.7	74.1 (68.3-79.2)	86.7 (76.1-93.1)	0.38 (0.28-0.51)	0.38 (0.32-0.44)	0.38 (0.28-0.51)
Occupational risk of exposure	639	559	87.5	68.3 (61.2-74.7)	82 (66.2-91.3)	326260	79.8	57.8 (48.1-66.9)	88.1 (67.6-96.4)	1.10 (1.03-1.17)	1.10 (1.06-1.13)	1.10 (1.03-1.17)
High-specificity serologic testing	446	321	72.0	51.0 (43.7-58.2)	78.3 (61.1-89.3)	519498	96.0	86.4 (80.3-90.8)	93.0 (82.3-97.4)	0.75 (0.71-0.80)	0.75 (0.73-0.77)	0.75 (0.71-0.80)

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