MAJOR ARTICLE

Outbreaks of Tularemia in a Boreal Forest Region Depends on Mosquito Prevalence

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Background. We aimed to evaluate the potential association of mosquito prevalence in a boreal forest area with transmission of the bacterial disease tularemia to humans, and model the annual variation of disease using local weather data.

Methods. A prediction model for mosquito abundance was built using weather and mosquito catch data. Then a negative binomial regression model based on the predicted mosquito abundance and local weather data was built to predict annual numbers of humans contracting tularemia in Dalarna County, Sweden.

Results. Three hundred seventy humans were diagnosed with tularemia between 1981 and 2007, 94% of them during 7 summer outbreaks. Disease transmission was concentrated along rivers in the area. The predicted mosquito abundance was correlated (0.41, $P < .05$) with the annual number of human cases. The predicted mosquito peaks consistently preceded the median onset time of human tularemia (temporal correlation, 0.76; $P < .05$). Our final predictive model included 5 environmental variables and identified 6 of the 7 outbreaks.

Conclusions. This work suggests that a high prevalence of mosquitoes in late summer is a prerequisite for outbreaks of tularemia in a tularemia-endemic boreal forest area of Sweden and that environmental variables can be used as risk indicators.

Tularemia is an acute febrile illness that resembles plague but is generally less severe. Early and correct antibiotic treatment is needed to avoid progression of lymph node swelling into abscess formation and long-lasting drainage of pus [1]. It is unclear what factors determine the timing and magnitude of tularemia outbreaks among humans. The disease is a zoonosis that occurs in the northern hemisphere, and it is caused by the intracellular bacterium *Francisella tularensis*. Human disease often occurs as geographically localized summer–autumn outbreaks, presumably transmitted by blood-feeding arthropods, by inhalation of infectious dust, or by ingestion of *F. tularensis*–contaminated food or water [2]. The ecology of tularemia is complex, and no simple model for its maintenance and spread in nature exists [3]. Classically, tularemia is divided into type A and type B tularemia caused by the highly virulent *F. tularensis* spp. *tularensis* (type A) or *F. tularensis* spp. *holarctica* (type B). Type A tularemia occurs in North America and is associated with dry environments, hares, and tick or tabanid fly transmission to humans. Type B tularemia occurs throughout the Northern Hemisphere, is associated with fresh water and numerous rodent species, and seems to be transmitted to humans by ticks, tabanid flies, or mosquitoes. Hard ticks are considered the main type B reservoir and disease vector in the continental United States and continental Europe, whereas mosquitoes are believed to be the main disease vectors in the large boreal forest regions of Alaska, Sweden, Finland, and Russia [3–5]. Although this is the general view among inhabitants and physicians in these boreal forest regions, there is surprisingly little firm evidence for mosquito involvement as vectors of

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human tularemia [6, 7]. To some degree, this may be explained by the lack of recent systematic observations of the mosquito fauna, but the transmission route also remains controversial because it is, to our knowledge, the only bacterial disease proposed to be disseminated predominately through mosquitoes.

Although globally a rare disease, local outbreaks may be extensive. Large outbreaks of 100–900 cases per year have been regular in both Sweden and Finland in the 2000s [8, 9]. The geographical distribution is typically uneven, with resurgence in some counties. The large majority of patients contract the ulceroglandular form of tularemia with a primary skin ulcer at the site of *F. tularensis* inoculation, perhaps resulting from an infective mosquito taking blood and a corresponding lymph node enlargement.

In this work, we took a traditional epidemiologic approach based on geographical transmission data combined with mathematical modeling of meteorological and hydrological data to explore possible connections between environmental parameters, mosquito abundance, and type B tularemia in Sweden. Specifically, we asked: Can tularemia outbreaks among humans be explained by mosquito abundance?

MATERIALS AND METHODS

Study Setting and Tularemia Data

Dalarna County of central Sweden has a population of 278 000 and is located longitudinally from 14°30'00"E to 15°36'02.11"E and latitudinally from 61°00'00"N to 60°40'42.81"N. Lakes and watercourses cover about 8% of the total area of the county, with over 6300 lakes larger than 1 ha in size. The largest river is Dalälven, which is formed by the two forks Västerdalälven and Österdalälven, and flows southeast from the mountains along the Norwegian border into the Gulf of Bothnia (Figure 1). Since 1968, tularemia is a reportable disease, according to the Communicable Diseases Act of Sweden. The epidemiological investigation performed in this work conformed to the law-enforced standards. Mandatory report information includes date of disease onset (time), the likely geographical location of disease transmission (place), and identification of the individual (person). All reports on human tularemia in Dalarna from 1981 to 2007 were assessed for time, place, and person data at the local Department of Communicable Disease Control. Two rules in sequence were used to define the location of disease transmission: (1) the stated location of disease transmission, and (2) the residential address, except for reports on residents living in the two urban communities of the county, Borlänge (39 000 inhabitants) and Falun (36 000 inhabitants). Rule 2 was applied for minimizing bias that may result from using only the residential address as the place of transmission. Maps of tularemia transmission sites were prepared using ArcGIS version 9.3 software (ESRI, Redlands, CA).

Modeling Mosquito Abundance Using Mosquito Catch Data

The mosquito abundance in the late summer (July–September) was observed at 3 locations in the mosquito modeling area, Färnebofjärden, for the period 2001–2007 (Figure 1). Mosquitoes were sampled roughly every 14 days with light traps, as previously described [10]. Typical mosquito numbers per trap and night varied greatly over the season, ranging from a few to tens of thousands. Observations from 2 locations were used to model the standardized mosquito abundance (*SM*) as a linear function of the temperature (T) and the standardized river flow (*SR*) observed at nearby meteorological and hydrological stations in Näs and Gävle. Data from the third trap were used for evaluation.

For each time point (t), the *SM* variable was defined as

$$SM = \frac{N - \bar{N}}{S_N},$$

where $N = \log_2(M+1)$ and M is the average number of mosquitoes observed at time t. The sample mean and standard deviation ($\bar{N}$, S_N) for variable N were obtained using all observations from the period 2001–2007. The variable *SR* was defined similarly:

$$SR = \frac{W - \bar{W}}{S_W},$$

where W is the river flow observed at the hydrological station and where the variables sample mean and standard deviation were obtained using all summer observations (18 May–23 August) from 1981 to 2007.

The variable *SM* at time t was modeled using multiple linear regression [11] with the final model being

$$SM = -2.76 + 0.67Q_1 + 0.62Q_2 + 0.19T,$$

where *Q1* and *Q2* are the maximum *SR* for two periods preceding time t (36–42 days and 22–28 days, respectively, before time t) and where T is the mean temperature 1–7 days before time t.

The *SM* variable was transformed into the relative mosquito abundance (*RMA*), which can be interpreted as the number of mosquitoes relative to the median mosquito abundance in the region. The transformation can be motivated as follows. The *SM* variable can be transformed to the original M-scale (mosquito abundance in the region) by the transformation

$$M - 1 = 2^{S_N SM + \bar{N}},$$

where $\bar{N}$ is the region's average mosquito abundance (on a $\log_2$ scale) and S_N the corresponding standard deviation. Here, neither $\bar{N}$ or S_N can be observed. The problem was solved by assuming that the regions' standard deviations could be approximated by the standard deviation observed in the mosquito modeling area.

Figure 1. Dalarna County, central Sweden, and the area used for modeling mosquito abundance (hatched oval). The hydrological and meteorological stations used for collecting data are indicated.

Furthermore, it can be argued that $2^{\bar{N}}$ is a reasonably good estimator of the median of M. Hence, the RMA variable is defined as

$$RMA = 2^{S_{N(F)}SM} = \frac{2^{S_{N(F)}SM+\bar{N}}}{2^{\bar{N}}} \approx \frac{M}{median\ M}, \qquad (1)$$

where $S_{N(F)} = 3.089$ is the standard deviation observed in the mosquito modeling area. R^2 for the third trap location in the mosquito modeling area was 0.50, and the correlation between the observed and predicted RMA values was 0.61.

Prediction of Mosquito Abundance in Dalarna County

Relative mosquito abundance was predicted for the time period 10 July–15 September for 4 regions in Dalarna County (Figure 1), using the predictive mosquito model 1 together with local temperature (T) and standardized river flow (SR). The 4 regions were defined by pairs of hydrological and meteorological stations: Forshuvudforsen–Falun, Gråda–Falun, Lima–Malung and Mockfjärd–Idkerberget. Mosquito profiles, describing the seasonal variation in mosquito abundance, were predicted for each of the regions, and the county's annual RMA value was obtained by taking the average of median RMA values.

Environmental Variables

Meteorological and hydrological data were obtained from the Swedish Meteorological and Hydrological Institute (www.smhi.se). Daily observations on humidity, precipitation, snow cover, and temperature were recorded at the meteorological stations Malung, Mora, Falun, and Idkerberget (Figure 1). River flow data were recorded at the hydrological stations Forshuvudforsen, Gråda, Lima, and Mockfjärd. The daily data were reassigned to the county level by calculating averages of the stations measurements and were used to calculate a number of summary variables. The mean temperature, mean humidity, and total precipitation were calculated for the following periods: summer (June–August), spring (April–May), winter (December–March), previous fall (September–November of the preceding year), and previous summer (June–August of the preceding year). In addition, we counted the annual number of cold winter days (<-7.3°C; ie, the 10th percentile) with a minimum snow cover (<10 cm) observed during the preceding winter (December–March). The number of tularemia cases the preceding year was also included as an explanatory variable.

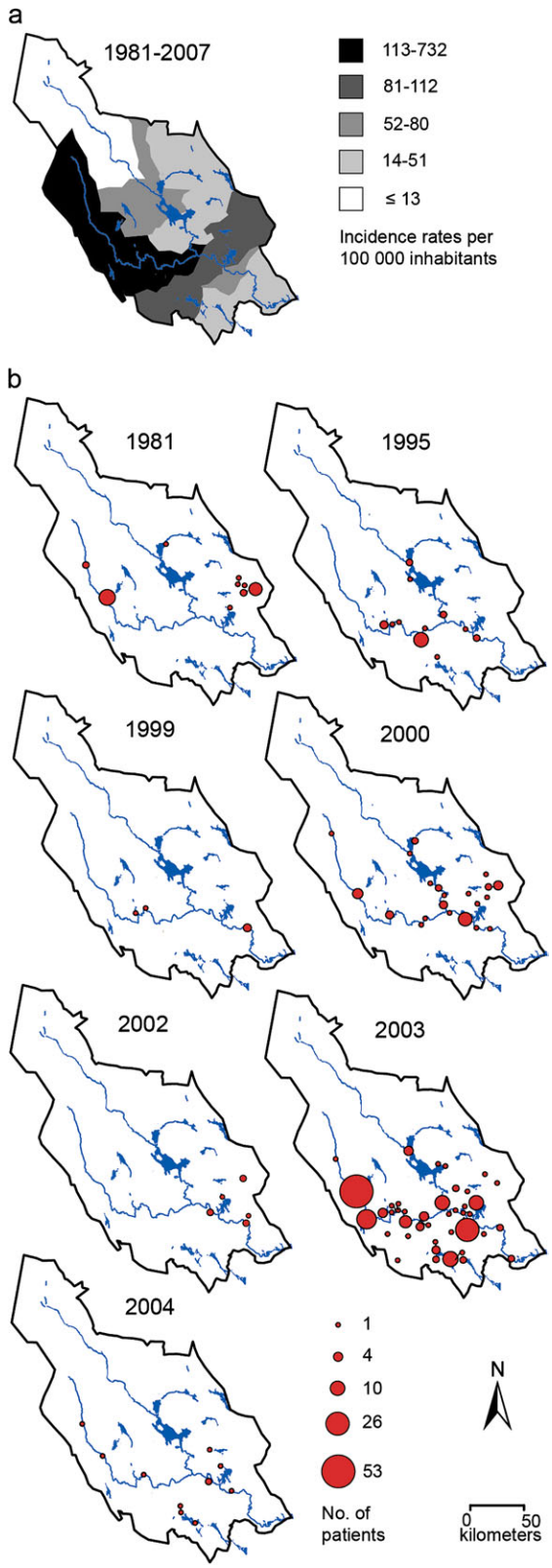

Figure 2. The total human tularemia incidence rate for the time period 1981–2007 in the municipalities of Dalarna County. White represents low and black represents high incidence rates. *B*, The geographical distribution and number of tularemia patients during the 7 outbreaks with ≥5 patients (year 1981, n = 29; 1995, n = 42; 1999, n = 5; 2000, n = 48; 2002, n = 9; 2003, n = 202; and 2004, n = 14).

Modeling Tularemia Cases

The annual number of tularemia cases was modeled in a 2-step procedure. Initially, we removed variables that were not significantly correlated with tularemia ($P > .05$). In the second step, the tularemia cases were modeled by a negative binomial model [12]. The final model was obtained using backward selection (including all the remaining variables, but no interaction terms) with the Akaike Information Criterion [13]. The model was validated by considering the model's pseudo R^2 [14]. Its predictive power was estimated by the predicted R^2 obtained by leave-one-out cross-validation [15]. Throughout this paper, we used Spearman rank correlation [16] if nothing else is stated. All analyses were done using R version 2.9.1 (R Development Core Team, 2009).

RESULTS

Descriptive Epidemiology

In total, 370 humans were diagnosed with tularemia between 1981 and 2007 in Dalarna County, and 349 (94%) of those cases occurred during 7 years; 1981, 1995, 1999, 2000, 2002, 2003, and 2004 (Figure 2). Regardless of year, the outbreaks occurred during the summer time period, with onset of disease reported between July 8 and October 12. The mean age of the patients was 45 years (range, 1–83 years; median, 48 years). Cases were reported in 221 men and 149 women. Tularemia was distributed among age groups, with highest incidences among children and the middle-aged to elderly. There were 11 patients aged 0–4 years, 47 aged 5–14 years, 16 aged 15–24 years, 33 aged 25–34 years, 46 aged 35–44 years, 74 aged 45–54 years, 73 aged 55–64 years, 49 aged 65–74 years, and 21 aged 75–84 years.

Information regarding presumed location of disease transmission was available for 332 patients and showed an evident geographical clustering of cases (Figure 2). A reported location of disease transmission was used for 303 patients and the residential address was used for 29 patients. Analysis per outbreak year revealed the presence of stable geographical foci of tularemia with little evidence for disease movement. Nearly all identified sites of tularemia transmission were located in close proximity to watersheds, in particular along a stretch of the river Västerdalälven (Figure 2).

The incidence rates were highest in areas near a major watercourse in municipalities with a low population density (Figure 2). The highest annual incidence rates per municipality were 521 per 100 000 inhabitants (year 2003 in Vansbro Municipality, which had 7291 inhabitants), 490 per 100 000 inhabitants (year 2003 in Malung Municipality, which had 10 799 inhabitants), and 208

per 100 000 inhabitants (year 1995 in Gagnef Municipality, which had 10 075 inhabitants).

Association Between Predicted Mosquito Abundance and Reported Tularemia in Humans

The annual *RMA* ranged from 0.09 to 39 units with a median of 0.72. The lowest *RMA* was predicted in 2004 and the highest in 1995 with considerable annual variation (Figure 3). Among the 7 high incidence years (≥5 cases), 6 had *RMA* values above 1.5, whereas only 2 out of the remaining 20 low-indices years had *RMA* values above 1.5. The 3 exceptions were 2004 (6 cases, $RMA = 0.09$), 1987 (no cases, $RMA = 16$), and 1985 (2 cases, $RMA = 2$). The correlation between the annual *RMA* values and the number of human tularemia cases was 0.41 ($P < .05$).

Under the assumption that mosquitoes are vectors for transmission to humans, a period with high mosquito abundance should be followed by a period of tularemia cases. Indeed, for all high-incidence years, the mosquito peaks (ie, the date at which the highest mosquito abundance was predicted during the period 10 July–15 September) occurred before the median onset times of tularemia (Figure 4). The observed delay ranged from 6 to 35 days. The temporal correlation (Pearson correlation) between the mosquito peaks and the median onsets was 0.76 ($P < .05$). These results suggest that mosquitoes are involved in the transmission of tularemia and that mosquito abundance partially explains the annual and seasonal variability of tularemia.

Modeling Human Tularemia Outbreaks Using Environmental Variables

The annual number of tularemia cases (*Tul*) was modeled by considering 20 environmental variables. Five variables were included in the final model; the logarithm of RMA ($\log_2 RMA$), the summer temperature the preceding year (ST_{lag}), summer precipitation (*SP*), cold winter and low snow coverage (*CW*), and the logarithm of the preceding year's number of tularemia cases ($\log_2 Tul_{lag}$). In the final model, *Tul* was modeled as

$$Tul = \exp\left(-11 + 0.52\log_2 Tul_{lag} + 0.54\log_2 RMA + 0.65ST_{lag} + 0.012SP - 0.15CW\right). \quad (2)$$

All explanatory variables were significant ($P < .05$) in the final model and $\log_2 RMA$ had the lowest *P* value. The average summer temperature ranged from 12.2°C to 16.8°C, with a median of 14.5°C. The correlation between ST_{lag} and *Tul* was 0.43 ($P < .05$). The mean summer precipitation ranged from 143 to 342 mL/M^2, with a median of 239 mL/M^2. The variable was not significantly correlated with *Tul*, but because the correlation was moderately high (0.22), it was included in the final modeling step. The *CW* variable ranged from 0 to 28 days with a median of 5 days and was negatively correlated (−0.40, $P < .05$) with *Tul*. The $\log_2 RMA$ variable was correlated (0.41, $P < .05$) with *Tul*. The number of tularemia cases the preceding year was not significantly correlated with *Tul* but was included in the final model because of an apparent temporal clustering of tularemia between flanking years in the raw data (see Figure 5).

Model 2 predicted 6 out of the 7 high-incidence years, but also erroneously predicted 31 cases in 1987 when no cases were reported (Figure 5). The model's pseudo R^2 was 0.92, and the correlation between the predicted and observed tularemia abundance was 0.71. The model's predicted R^2 was 0.20. Most of the unexplained variation was due to the tularemia peak in 2003, when 203 cases occurred but only 74 cases were predicted. In summary, several environmental variables are associated with tularemia, and the final model predicts the occurrence of outbreaks but not their exact extent.

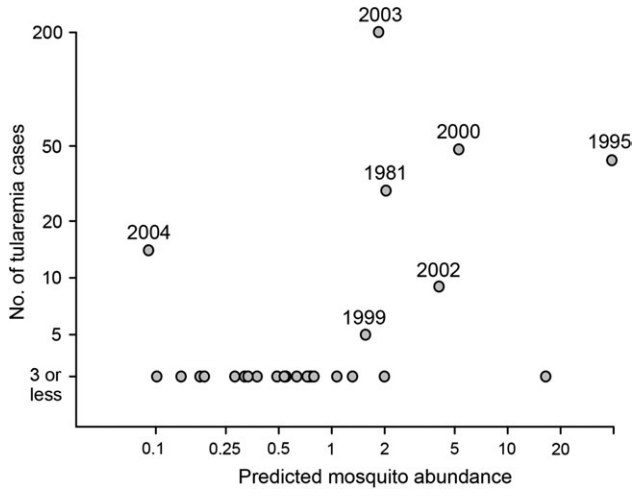

Figure 3. Predicted annual relative mosquito abundance (expressed in median units) and annual number of recorded tularemia cases in Dalarna County, central Sweden, for the period 1981–2007.

DISCUSSION

This study documents the geographical concentration of repeated human tularemia outbreaks in Dalarna County, Sweden. By using statistical modeling, we provide evidence for an important role of mosquitoes in tularemia transmission to humans. Additionally, analysis of local hydrological and meteorological data allowed for identification of several abiotic factors linked to occurrence of human outbreaks.

We found a significant correlation between predicted mosquito prevalence and the number of human tularemia cases. For 6 out of the 7 outbreaks during 1981–2007, there was also a predicted high prevalence of mosquitoes. Furthermore, predicted seasonal mosquito peaks were followed by human tularemia outbreaks in consistency with the described incubation period of tularemia of 1–21 days [2]. Among 20 environmental variables tested in the model, the most significant was the predicted mosquito abundance.

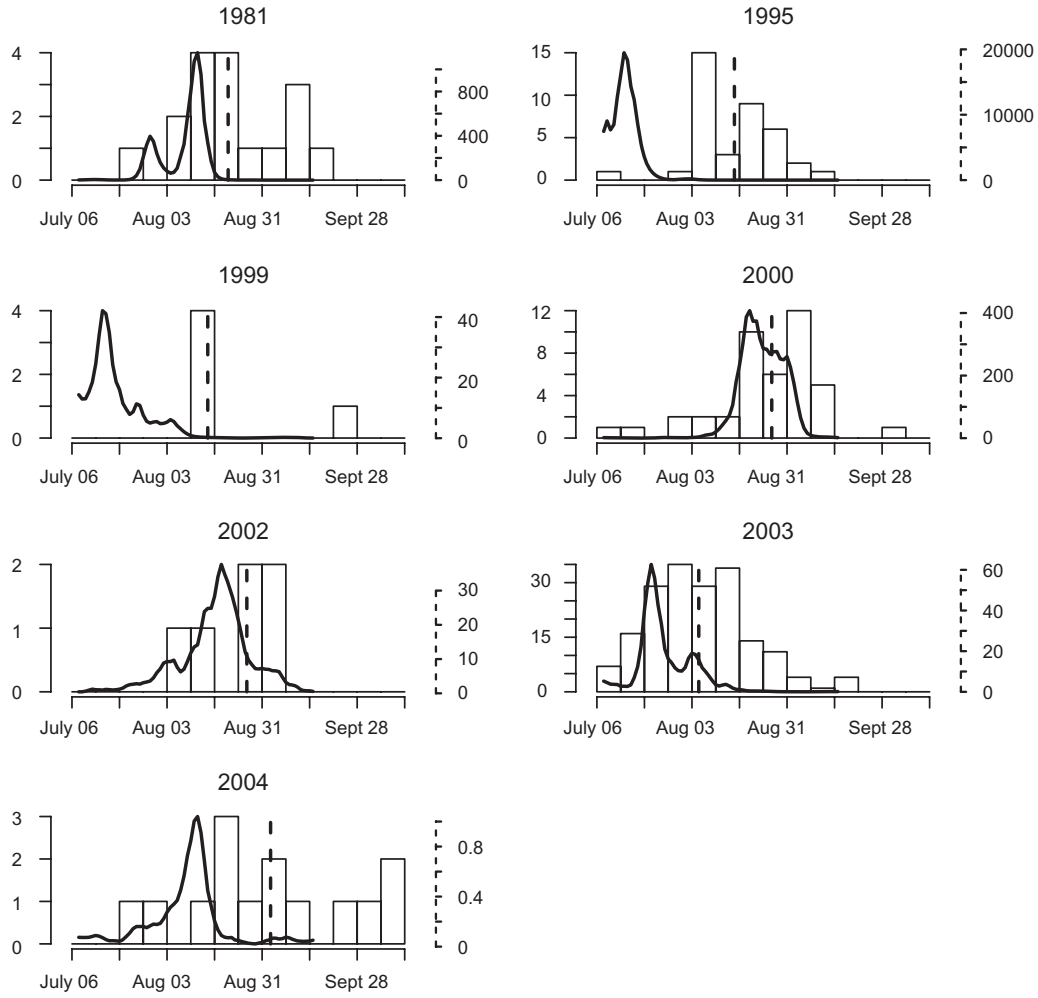

Figure 4. Temporal distribution of predicted relative mosquito abundance (black line) and date of tularemia onset for patients during 7 high-incidence years in Dalarna County, central Sweden. Bars represent the number of tularemia patients within 7-day periods. The median date of tularemia onset is indicated (hatched line).

These results provide evidence that mosquitoes are important for tularemia transmission to humans.

The time and place analysis of patient data during 26 years strongly suggests that there exists a "nest of disease" in nature (ie, that tularemia is a disease of nidality as was suggested by Pavlovsky already during the 1960s) [17]. We found that human outbreaks occurred repeatedly in a few small communities. Our data thus support the view that *F. tularensis* resides in nature at very specific locations in a geographically stable but hitherto unknown natural reservoir located in proximity to watersheds or in the water (Figure 2) [7, 18, 19].

A model predicting the number of annual human tularemia cases was built considering a selected set of environmental variables with possible impact on tularemia transmission to humans. The selection was based on previous descriptions of tularemia as a disease related to mosquito bites, natural fresh water, water flooding events, and a presumed existence of a local disease reservoir in nature [5, 17, 20–22]. The final model contained several environmental parameters that are of interest to explore in the future.

A negative association was apparent for the cold winter and low snow coverage parameter. A possible reason for this finding is that the effective population of the infectious agent, *F. tularensis*, is reduced after many days with low temperature and little snow cover. This may result from a reduction of a rodent reservoir for the disease, from decreased survival of an important arthropod or protozoan reservoir, or from a direct effect on the survival of *F. tularensis* bacteria during the winter. Another significant variable was summer temperature the preceding year. Possibly, this is again linked to the size of the *F. tularensis* population in nature. The replication of *F. tularensis* outside a mammal may depend on favorable summer temperatures. The number of tularemia patients the preceding year was a significant variable of the final model, a finding compatible with a carry-over effect between years dependent on increased bacterial or vector

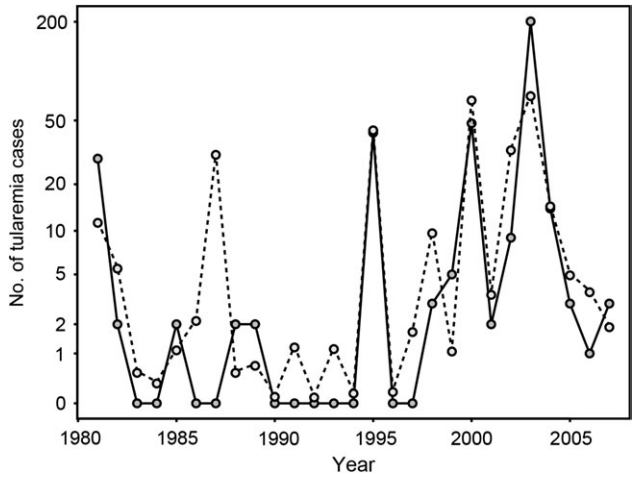

Figure 5. Observed and predicted annual human tularemia abundance for 1981–2007 in Dalarna County, central Sweden. The solid line is observed values, and the dashed line is predicted values.

population size in nature. Taken together, these findings suggest that the above variables have impact on a still unidentified disease reservoir of tularemia in nature.

For a useful prediction of disease burden from tularemia, the final model has limitations, since both summer precipitation and mosquito abundance rely on measurements performed immediately before or during an outbreak. A possible future improvement to resolve this limitation is to use early summer meteorological data to predict both mosquito abundance and summer precipitation.

There is also an apparent lack of parameters reflecting changes in human behavior. For example, the strong tradition of forest berry picking and hunting among residents in Dalarna County represents behavioral parameters that might have a strong influence on tularemia exposure. Possibly, variation in such behavioral factors might explain the incorrect prediction of a tularemia occurrence in year 1987 (Figure 5). The first alarm in Europe detecting anomalous nuclear fallout from the Chernobyl disaster in April 1986 came from Forsmark, Sweden, which is situated only 80 km east of Dalarna County (Figure 1). In 1987, governmental authorities warned the population about eating forest-picked berries and mushrooms as well as fish and game from major parts of eastern and central Sweden. The inhabitants in Dalarna County likely minimized forest berry picking and hunting during the summer of 1987, and thereby minimized exposure to tularemia, because of the Chernobyl disaster.

The nature of this study does not allow for identification of a causative relationship between environmental parameters and tularemia but adds to the evidence that mosquitoes are important for tularemia transmission to humans in boreal forest regions. It is still unclear how the mosquitoes become infected, but the very focal geographical source of disease as exemplified in this study suggests that identification of infectious sources should be possible. Given the high tularemia incidence numbers recorded for some geographical areas investigated in this study, interventions to halt disease transmission to humans, both at the level of mosquitoes and infectious sources, are warranted.

Notes

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References

1. Tärnvik A. WHO guidelines on tularaemia, 1st ed. Vol 1. Geneva: World Health Organization, **2007**:116.
2. Penn RL. *Francisella tularensis* (Tularemia). In: Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's principles and practice of infectious diseases, 7th ed. Vol 2. Philadelphia: Elsevier/Churchill Livingstone, **2010**:2927–37.
3. Keim P, Johansson A, Wagner DM. Molecular epidemiology, evolution, and ecology of *Francisella*. Ann NY Acad Sci **2007**; 1105: 30–66.
4. Eisen RJ, Mead PS, Meyer AM, Pfaff LE, Bradley KK, Eisen L. Ecoepidemiology of tularemia in the southcentral United States. Am J Trop Med Hyg **2008**; 78:586–94.
5. Eliasson H, Lindbäck J, Nuorti JP, Arneborn M, Giesecke J, Tegnell A. The 2000 tularemia outbreak: a case-control study of risk factors in disease-endemic and emergent areas, Sweden. Emerg Infect Dis **2002**; 8:956–60.
6. Hubalek Z, Halouzka J. Mosquitoes (*Diptera: Culicidae*), in contrast to ticks (*Acari: Ixodidae*), do not carry *Francisella tularensis* in a natural focus of tularemia in the Czech Republic. J Med Entomol **1997**; 34: 660–3.
7. Lundström JO, Andersson AC, Bäckman S, Schäfer ML, Forsman M, Thelaus J. Transstadial transmission of *Francisella tularensis holarctica* in mosquitoes, Sweden. Emerg Infect Dis **2011**; 17:794–9.
8. Swedish Institute for Infectious Disease Control. Data and statistics. Tularaemia. http://www.smittskyddsinstitutet.se/in-english/statistics/tularaemia. Accessed 12 March 2011.
9. The National Institute for Health and Welfare (THL) Finland. The statistical database of the Infectious Diseases Register, *Francisella tularensis*. http://www3.ktl.fi/stat/. Accessed 12 March 2011.
10. Schäfer ML, Lundström JO, Petersson E. Comparison of mosquito (*Diptera: Culicidae*) populations by wetland type and year in the lower river Dalälven region, Central Sweden. J Vector Ecol **2008**; 33: 150–7.
11. Kleinbaum DG, Kupper LL, Muller KE, Nízam A. Applied regression analysis and other multivariate methods. 3rd ed. Pacific Grove, CA: Duxbury Press, **1998.**
12. Lawless JF. Negative binomial and mixed Poisson regression. Can J Stat/La Revue Can J Stat **1987**; 15:209–25.
13. Hocking RR. The analysis of and selection of variables in linear regression. Biometrics **1976**; 32:1–49.

14. Nagelkerke NJD. A note on a general definition of the coefficient of determination. Biometrika **1991**; 78:691–2.
15. Stone M. Cross-validatory choice and assessment of statistical prediction. J Roy Stat Soc **1974**; 36:111–47.
16. Wackerly DD, Mendenhall W III, Scheaffer RL. Mathematical statistics with applications. 6th ed. Pacific Grove, CA: Duxbury Press, **2002.**
17. Pavlovsky E. Natural nidality of transmissible diseases. Urbana: University of Illinois Press, **1966.**
18. Svensson K, Bäck E, Eliasson H, et al. Landscape epidemiology of tularemia outbreaks in Sweden. Emerg Infect Dis **2009**; 15:1937–47.
19. Broman T, Thelaus J, Andersson AC, et al. Molecular detection of persistent *Francisella tularensis* subspecies holarctica in natural waters. Int J Microbiol **2011**; doi:10.1155/2011/851946.
20. Anda P, Segura del Pozo J, Diaz Garcia JM, et al. Waterborne outbreak of tularemia associated with crayfish fishing. Emerg Infect Dis **2001**; 7:575–82.
21. Jellison WL. Tularemia in North America, 1930–1974. Missoula: University of Montana Foundation, **1974.**
22. Hopla CE, Hopla AK. Tularemia. In: Beran GW, ed. Handbook of zoonoses. 2nd ed. Boca Raton, FL: CRC Press, **1994**: 113–26.