Resurgence of Field Fever in a Temperate Country: An Epidemic of Leptospirosis among Seasonal Strawberry Harvesters in Germany in 2007

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Background. Although leptospirosis is a reemerging zoonosis of global importance, outbreaks related to agricultural exposures are primarily situated in tropical countries. In July 2007, a suspected leptospirosis outbreak was recognized among strawberry harvesters from Eastern Europe who were working in Germany. An investigation was initiated to identify the outbreak source and the risk factors for infection.

Methods. We conducted a retrospective cohort study with use of a questionnaire administered to harvesters by health authorities in Romania, Slovakia, and Poland. Collected serum samples were tested by microscopic agglutination test and immunoglobulin M enzyme-linked immunosorbent assay. A case patient was defined as a person who worked in the strawberry field during the period 5 June–8 September 2007 and had leptospirosis-compatible symptoms and either an antibody titer $1:800$ and a positive immunoglobulin M enzyme-linked immunosorbent assay result (for a confirmed case) or no serological confirmation (for a suspected case). Local rodents were examined for leptospirosis.

Results. Among 153 strawberry harvesters, we detected 13 confirmed case patients who had test results positive for antibodies against Leptospira species serogroup Grippotyphosa and 11 suspected case patients (attack rate, 16%). Risk of disease increased with each day that an individual worked in the rain with hand wounds (odds ratio, 1.1; 95% confidence interval, 1.04–1.14) and accidental rodent contact (odds ratio, 4.8; 95% confidence interval, 1.5–15.9). Leptospires of the serogroup Grippotyphosa were isolated from the kidneys of 7 (64%) of 11 voles.

Conclusions. This is, to our knowledge, the largest leptospirosis epidemic to occur in Germany since the 1960s. Contact between hand lesions and contaminated water or soil and infected voles was the most likely outbreak source. The unusually warm winter of 2006–2007 supported vole population growth and contributed to this resurgence of leptospirosis in Germany. Because of ongoing climate change, heightened awareness of leptospirosis in temperate regions is warranted.

Leptospirosis is a zoonotic disease caused by spirochaetes belonging to the genus Leptospira. Human infection occurs through direct contact with the urine of animal reservoirs or through contact with contaminated soil or water [1]. Clinical manifestations range from mild symptoms, including fever, chills, and headache, to life-threatening conditions (e.g., Weil disease), characterized by jaundice, renal impairment, and hemorrhage [2]. Field fever is a presentation of leptospirosis that is typically related to infections due to Leptospira species serovar Grippotyphosa [3].

Historically, outbreaks of leptospirosis in Europe were associated with agricultural exposure risks, which were mainly restricted to rural environments [4–7]. However, with the mechanization of agriculture, im-
provements in sanitation, and the reduction of animal reservoirs, epidemic leptospirosis disappeared in the early 1960s [6, 7]. Although some occupational exposures continue to exist in Germany, exposures related to recreational activities have recently been more frequently reported [8]. Recently, leptospirosis has been identified as a reemerging zoonotic disease affected by global climate change [9, 10].

In July 2007, an outbreak with unknown etiology was reported to the local health unit in Düren, Germany. Initial investigation showed that all of the individuals involved in the outbreak were seasonal strawberry harvesters from Romania, Slovakia, and Poland who had gastrointestinal symptoms and worked on a nearby farm. Thirteen persons were hospitalized, and a wide spectrum of pathogens was investigated without positive results. Simultaneously, food samples from the farm were tested, and all had negative results. Although the outbreak abated, serum samples obtained from 5 patients were eventually tested for leptospirosis; 3 samples had positive results, and leptospirosis was suspected as the possible outbreak cause. An outbreak investigation was initiated to identify the source of the outbreak and the possible risk factors for leptospirosis infection.

PATIENTS AND METHODS

Participants and study design. In September 2007, we conducted a retrospective cohort study in collaboration with the National Public Health Institutes of Romania, Slovakia, and Poland. Harvesters who were present on the strawberry farm during the outbreak month (from the middle of June through the middle of July) were identified by a list provided by the farm. Because these individuals had returned home, the corresponding national health institute contacted them and asked them to participate in the study. Participants were interviewed by health care workers using a standardized questionnaire, which was translated into the local language. The questionnaire included information on demographic characteristics, work and travel history, clinical information, and possible exposures during the outbreak month (including rodent sightings and contact, the presence of wounds, consumption of unwashed strawberries, and water activities). Verbal consent was obtained before 2 mL of blood was collected for serological testing on a voluntary basis.

A confirmed case patient was defined as an individual who was present on the strawberry farm during the period from 5 June through 8 September 2007 and who had fever and at least 1 other symptom and/or had received a diagnosis suggestive of leptospirosis (including renal impairment, meningitis, headache, flu-like symptoms, and vomiting), as well as serological test results positive (≥1:800) for antibodies against serogroup Grippotyphosa and a positive IgM antibody ELISA result. A suspected case patient was an individual who was epidemiologically and clinically associated with the outbreak for whom there was no serological confirmation of infection.

Laboratory investigation. Collected serum samples were stored at −20°C to −70°C until delivery to the Consiliary Laboratory for Leptospirosis at the Federal Institute for Risk Assessment in Berlin, Germany. The microagglutination test was used to detect anti-Leptospira antibodies in the serum samples. Seventeen reference strains comprising 14 serogroups and 17 serovars (serovars Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Pomona, Saxkoebing, Sejroe, Tarassovi, Ballum, Icterohaemorrhagiae, Pyrogenes, Hebdomadis, and Javanica) were used for microagglutination testing. Antibody titers ≥1:100 were considered to be positive for leptospirosis, whereas titers ≥1:800 were used to ascertain more-recent infections. A leptospiral antigen was considered to represent the agent of infection when cross-reactions were absent or when microagglutination test titers against this antigen (≥1:800) were at least 2 serial dilutions higher than titers against other leptospiral antigens. All samples identified as positive by microagglutination testing were further tested for IgM antibodies with use of a commercial ELISA kit (Virion/Serion).

Statistical analyses. Attack rates stratified by age, sex, and nationality were calculated. Geometric mean titers were calculated by taking the exponential of the mean of the log titer transformations. The association between exposures and leptospirosis outcome were examined by univariate analysis. Risk ratios or ORs and their 95% CIs and P values were calculated. Variables with a P < 0.1 on univariate analysis were included in multivariate logistic regression models, and stepwise backward elimination was used to identify the final model with factors significantly associated with leptospirosis infection. The final model was checked for interaction and collinearity. P < .05 was considered to be statistically significant. Analyses were performed with Stata software, version 10 (StataCorp), and SPSS software, version 15 (SPSS).

Environmental investigation. In September 2007, we investigated the strawberry field that was worked in during the outbreak over a 2-day period to identify the animal reservoir of infection. Live-catch mousetraps were laid every 10 m along 2 sides of the field and across the field. Captured rodents were removed and kept in containers until transportation to the Veterinary Faculty at the Free University of Berlin. Animals were deeply anesthetized by isoflurane inhalation anesthesia so that blood samples for microagglutination testing could be obtained. They were then euthanized, and the kidneys, bladder, and liver were removed for culture and histological examination.

The avidin-biotin-peroxidase technique was used to immunohistochemically detect leptospires in paraffin-embedded tissue specimens of mouse kidneys and livers by light micros-
copy. In brief, slides were incubated with a polyvalent rabbit antiserum against *Leptospira Grippotyphosa* (Federal Institute for Risk Assessment) at a dilution of 1:1600 for 1 h at 37°C, with anti-rabbit IgG from goat (Sigma-Aldrich) at a dilution of 1:17 for 30 min at 37°C, and finally, with streptavidin-biotin-peroxidase complex from rabbit (Sigma-Aldrich) at a dilution of 1:17 for 30 min at 37°C. 3-Amino-9 ethylcarbazol (Sigma-Aldrich) was used as the substrate-chromogen system. The slides were counterstained with hematoxylin-eosin.

Approximately 1 g of kidney sample was homogenized with a stomacher (Colworth 400) for 1 min in 10 mL of bovine serum albumin dilution. Subsequently, samples were cultured in Ellinghausen, McCollough, Johnsen, and Harris medium containing 0.15% agar (BBL 211853), 0.4% rabbit serum, and 3 inhibitory substances (rifampicin, 10 μg/mL; 5-fluorouracil, 100 μg/mL; amphotericin B, 2 μg/mL). For this purpose, 200 μL of 10⁻²- and 10⁻³-diluted suspension were inoculated as triplicates into culture tubes that were cultivated at 29°C and checked once per week for 3 months. In the case of bacterial growths, subcultures were prepared from Ellinghausen, McCollough, Johnsen, and Harris medium, as described above.

Isolates were specified for the *Leptospira* serogroup and serovar using monoclonal antibodies at the Royal Tropical Institute (World Health Organization/Food and Agriculture Organization of the United Nations Collaborating Centre for Reference and Research on Leptospirosis) in Amsterdam, The Netherlands. Rainfall and temperature records were obtained from the German meteorological office.

**RESULTS**

**Outbreak description.** Of the 185 people included in the list, 184 were contacted, and 153 (83%) agreed to participate in the study. Of the 153 individuals who agreed to participate, 77 (50%) were Polish, 71 (46%) were Romanian, and the remaining 5 (4%) were Slovakian. Fifty-four percent of the study participants were female, and the mean age of study participants was 33 years (range, 18–61 years).

In total, 13 confirmed and 11 suspected cases occurred from 19 June through 25 August 2007 among harvesters working in the largest strawberry field (19 hectares) belonging to a strawberry-producing farm near Düren, a town in North Rhine-Westphalia, Germany. The number of cases peaked in the first week of July (figure 1). Fifty percent of all cases occurred in women, the median age of participants with suspected or confirmed cases was 33 years (range 23–49), and 54% of participants with suspected or confirmed cases were of Romany origin. The overall attack rate was 16% and did not differ between male and female study participants. Although individuals of all ages from 20 years through 49 years were affected, the attack rate was highest amongst individuals aged 25–29 years (attack rate, 30%).

The distribution of self-reported symptoms and diagnoses is shown in table 1. Clinical disease was mild in most cases, with headache being the most frequently reported symptom (96% of cases), followed by gastrointestinal and respiratory symptoms. A small proportion of individuals developed complications of meningitis (21% of cases) and jaundice (13%), and 45% of the cases required hospitalization. Among hospitalized patients, leptospirosis was misdiagnosed as viral meningitis or gastrointestinal infections. No deaths were reported.

**Laboratory results.** Blood samples were collected from Romanian and Slovakian harvesters (75 of 153 of the study participants). Of the serum samples tested, 23 (31%) were positive for antibodies against serogroup Grippotyphosa (microagglutination test titer, ≥1:100), and 17 (23%) had titers ≥1:800. The mean antibody titer for serogroup Grippotyphosa was 1:902 (range, 1:100 to 1:6400). Four persons (17%) with antibodies against serogroup Grippotyphosa (titers of 1:400, 1:800, 1:3200, and 1:6400) also had antibody titers for serogroup Pomona (titers of 1:400 in the second and fourth individuals and 1:1600 in the first) or serogroup Bratislava (titer of 1:800)

**Table 1. Distribution of symptoms and diagnoses among patients with confirmed or suspected cases of leptospirosis.**

<table>
<thead>
<tr>
<th>Symptom or diagnosis</th>
<th>No. (%) of patients (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>23 (96)</td>
</tr>
<tr>
<td>Body paina</td>
<td>19 (79)</td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>17 (71)</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

| a Includes muscle, joint, calf, or abdominal pain. |
in the third individual). In 7 (30%) of the persons seropositive for serogroup Grippotyphosa, no clinical symptoms that were consistent with leptospirosis were reported (microagglutination test titers, 1:200 in 3 persons, 1:800 in 3 persons, and 1:6400 in 1 person). An additional 2 individuals had test results that were positive for other serogroups (serogroup Copenhageni, 1:200; serogroup Pomona, 1:100). All samples that tested positive with titers \( \geq 1:800 \) also had ELISA results positive for anti-leptospiral IgM antibodies.

**Analytical findings of the cohort study.** Thirteen confirmed cases (all of which occurred in Romanian individuals) and 11 suspected cases (all of which occurred in Polish individuals) were included in the analysis. Eating unwashed strawberries (relative risk, 7.7; 95% CI, 1.1–55.4; \( p = .01 \)), harvesting with uncovered hand lesions (relative risk, 3.5; 95% CI, 1.6–7.4; \( p = .001 \)), and accidental rodent contact on the field (relative risk, 3.2; 95% CI, 1.5–6.7; \( p = .01 \)) were risk factors for leptospirosis infection in univariate analysis (table 2). In univariate regression analysis, there was no statistically significant association between the number of days harvesting (OR, 1.003; 95% CI, 0.996–1.01; \( p = .99 \)) or the number of days harvesting in the rain (as continuous variables) (OR, 1.05; 95% CI, 0.99–1.1; \( p = .001 \)) and the chance of leptospirosis. However, a statistically significant association was found between the chance of developing infection and the number of days harvesting with hand wounds (OR, 1.05; 95% CI, 1.02–1.1; \( p = .004 \)) and the number of days harvesting in the rain with hand wounds (OR, 1.1; 95% CI, 1.04–1.13; \( p < .001 \)). In the final multivariable model, only harvesting in the rain with hand lesions remained a statistically significant risk factor for infection, so that with each working day in the rain with hand wounds, the odds of acquiring infection were 1.1 (95% CI, 1.04–1.14; \( p < .001 \)). The odds of infection with each day worked in the rain without hand lesions were not statistically significant (OR, 1.01; 95% CI, 0.9–1.1; \( p = .8 \)). Accidental rodent contact was also independently associated with infection (OR, 4.8; 95% CI, 1.5–15.9; \( p = .01 \)) in multivariate analysis. Exclusion of suspected cases did not affect the outcome of multivariate analysis (each working day in the rain with hand wounds: OR, 1.1; 95% CI, 1.05–1.17; \( p < .001 \); accidental rodent contact: OR, 4.7; 95% CI, 1.04–20.9; \( p = .04 \)).

**Environmental investigation.** Eleven common voles (*Microtus arvalis*) were caught in the strawberry field and surrounding areas. Leptospires were isolated from the kidneys of 7 (64%) of the mice and were identified by DNA sequencing and by monoclonal antibodies as *Leptospira kirschneri* serovar Grippotyphosa and serovar Vanderhoedeni, respectively. Through histopathological examination, a massive infestation of leptospires was confirmed in the kidneys of mice with positive culture results. Seven blood samples were tested for leptospirosis infection; of these, 6 (86%) had test results positive for the same serogroup, with microagglutination test titers ranging from 1:800 to 1:3600.

The area where the outbreak took place recorded mean monthly temperatures of 15.3–18.3°C from May through August 2007. According to meteorological records beginning in 1991, spring 2007 was the wettest spring to date, and temperatures over the winter of 2006–2007 were the warmest to date (temperatures never dropped below 5°C). From 8 through 11 June (~10 days before outbreak onset), the mean daily temperature ranged from 18.4°C through 23.1°C. During this period, a mean of 10.5 mm (range, 4.0–16.1 mm) of rain fell per day, which corresponds to 10 L of rainwater per square meter. A second period of precipitation was recorded from 18 through 21 June, during which period the mean rainfall was 8.3 mm per day and the mean temperature near the farm was 19.6°C. Inspection of the outbreak site in September 2007 revealed an unusually high density of mouse holes in the field and the surrounding areas (>10 holes/m²). After heavy precipitation, large puddles accumulated between the strawberry rows (observed on a field adjacent to the outbreak field) (figure 2). During the outbreak period, the same conditions were present

| Table 2. Univariate and multivariate analysis of the relationship between risk factors and leptospirosis infection. |
|---|---|---|---|---|---|
| Exposure | Unexposed individuals | Exposed individuals | Univariate risk ratio or OR* | Multivariate risk ratio or OR (95% CI) |
| | Case patients | All | Attack rate, % | Case patients | All | Attack rate, % | |
| Accidental contact with rodents | 7 | 18 | 39 | 16 | 131 | 12 | 3.2 (1.5–6.7) | 4.8 (1.5–15.9) |
| Harvesting with open hand wounds | 15 | 49 | 31 | 9 | 102 | 9 | 3.5 (1.6–7.4) | ... b |
| Eating unwashed strawberries | 23 | 113 | 20 | 1 | 38 | 3 | 7.7 (1.1–55.4) | ... b |
| Harvesting in the rain with open hand wounds/working day | ... | ... | ... | ... | ... | ... | 1.1 (1.04–1.1) a | 1.1 (1.04–1.1) a |
| No. of days harvesting in the rain | ... | ... | ... | ... | ... | ... | 1.05 (0.996–1.1) b | ... b |
| Harvesting with open hand wounds/working day | ... | ... | ... | ... | ... | ... | 1.05 (1.02–1.1) a | ... b |

* For continuous variables, ORs were calculated by univariate regression analysis.
  b Variables were excluded from the final multivariate regression model by stepwise backward elimination.
on the outbreak field, which had been ploughed at the time of the site visit.

DISCUSSION

There are 3 known epidemiologic patterns of human leptospirosis as defined by Faine in 1999 [11]. The first occurs in temperate climates by direct contact with infected animals through farming of cattle and pigs. The second occurs in wet tropical areas, where exposure results more often from environmental contamination, and where rodent populations represent the principal reservoir of infection. The third pattern covers rodent-borne infections in urban environments. Because it fits the tropical pattern of leptospirosis epidemiology, the present outbreak—which is, to our knowledge, the largest field fever epidemic reported in Europe since the 1960s—allows these patterns to be subjected to critical scrutiny.

Consistent with previous data, this outbreak took place in the summer months and underlines the seasonality of leptospirosis, which is most likely related to climatic factors influencing leptospiral survival in the environment and to seasonal variations in human behavior. In comparison with leptospirosis activity in Germany in previous years, however, the outbreak occurred relatively early in 2007 [8]. The shift of seasonal trends because of climatic changes has been observed for other infectious diseases [12] and might also be relevant for leptospirosis.

The clinical symptomatology is consistent with reported serovar Grippotyphosa infections, because the majority of patients experienced relatively mild symptoms, such as headache, nausea, and body pains. More-severe forms of the disease, associated with meningitis or renal impairment, however, were misdiagnosed in patients who were hospitalized during the outbreak. This bears implications for disease progression [13] and emphasizes the importance of improving knowledge among general practitioners and hospital staff to facilitate early recognition and treatment.

Days worked in the rain with hand lesions was the strongest predictor for acquisition of infection in our study. The probable mode of transmission was contact between hand wounds and water-logged soil contaminated by vole urine or direct contact with voles. The strawberry pickers rarely wore gloves while working; therefore, scratches and wounds were common and may have also been present among those case patients who did not recall having them. Skin wounds have previously been associated with leptospirosis in tropical climates [14, 15]. Despite the strong association between strawberry consumption and leptospirosis in univariate analysis, the association did not remain in the final multivariate model, which may be explained by confounding. Wounds are more common when individuals work longer in the field, and this may also increase the likelihood of strawberry consumption.

Detection of the serogroup Grippotyphosa in both patients and captured common voles strongly supports the role of the vole as the maintenance reservoir and infection source in this outbreak. Anecdotal evidence has suggested that rodent populations increased substantially in 2007 [16], which is supported by the high density of vole holes observed on the field. The epidemic potential of infections that, like leptospirosis, have a basic reproduction number close to 0 (i.e., are minimally transmissible within human populations), is largely determined by the number of introductions from animal hosts [17]. Thus, among other contributing factors (e.g., prevalence of leptospirosis in the vole population and the excretor rate), an increase in rodent populations might lead to a future increase in infection pressure from leptospirosis on the human population.

Furthermore, fluctuations in rodent populations are partly related to climatic factors. In 2006, countries across Europe recorded the warmest autumn since recordings began [18], and the winter of 2006–2007 was unseasonably warm. In the outbreak area, the mean temperature was 4.1°C warmer than the previous 4-year mean temperature of 0.2°C. May 2007 was recorded as warm, and May and June 2007 were the wettest months since 1901, when the recording of rainfall in Germany began [19]. Warmer winters with reduced snowfall support vole populations by failing to reduce food availability and failing to prevent access to food resources [20], whereas increased rainfall increases food availability. In addition, vole populations undergo fluctuations every 3–5 years, with densities of up to 5000 voles/hectare possible. These environmental conditions were

Figure 2. Strawberry field located near the outbreak field, shown after heavy precipitation in September 2007. The same conditions were present during the outbreak period.
also ideal for leptospires, which can survive in rainwater-flooded soil for at least 3 weeks [21] and find favorable conditions at temperatures ≥18°C [3]. When the ideal growth conditions for voles and Leptospira coincide, enhanced rodent-human contact, which provides greater opportunities for disease transmission, may occur.

Endemic and epidemic leptospirosis (i.e., field fever) frequently occurred in Germany during the first part of the 20th century. During the last large field fever outbreak in Germany, which occurred in 1955, 320 cases were recorded among pea harvesters after an unusual period of heavy rain, and the European hamster (Cricetus cricetus) was identified as the reservoir animal [4]. Although there are many predictors that contribute to changing disease dynamics, the climatic factors described likely contributed to the reemergence of field fever in Germany, despite the continuation of measures that successfully reduced the incidence of disease in the past several decades.

Current research predicts that temperatures will continue to increase in the future, resulting in fewer periods of very cold weather and more periods of very warm weather [22]. Although it is difficult to predict future changes in rainfall, periods of sudden heavy rain are likely to become more common in Europe [23]. Flooding is an aspect of climate change that has an important impact on health, and for rodent-borne diseases, such as leptospirosis, flooding can result in increased disease incidence [24–27]. Although previous flood-related outbreaks have occurred in tropical climates, a greater number of leptospirosis outbreaks will likely occur in temperate regions as Europe experiences warmer winters and more tropical summers.

There are some limitations to our findings. Although a highly specific case definition was used to identify recent infections in patients who provided a serum sample, we acknowledge the possibility of misclassification among individuals who met the definition for suspected case patients (i.e., those without laboratory evidence of infection). On the other hand, a more sensitive case definition more accurately reflects the actual number of cases that occur during an outbreak and has therefore also been used in other leptospirosis outbreak studies [28, 29]. In addition, the exclusion of suspected case patients did not affect the outcome of multivariable analysis. Finally, recall bias may exist for exposure history for those individuals who were ill.

In conclusion, we propose that leptospirosis may serve as a model for an endemic disease that is affected by climate change. With the impact of global warming on health still unpredictable, heightened surveillance for both human and animal populations is essential for timely interventions. For occupations associated with enhanced risk of rodent contact, rodent control may be of some benefit, and lesions should be covered with waterproof dressings. Gloves should be worn, especially by those with professions affected by heavy rain. In situations of accidental contact with rodents in the presence of open wounds, individuals may be advised to receive doxycyclin as postexposure prophylactic treatment [30].

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