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TULAREMIA IN DEER MICE (*PEROMYSCUS MANICULATUS*) DURING A POPULATION IRRUPTION IN SASKATCHEWAN, CANADA

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ABSTRACT: Type B tularemia caused by *Francisella tularensis* subsp. *holarctica* was diagnosed in deer mice (*Peromyscus maniculatus*) found dead at four sites in west-central Saskatchewan during April and May 2005. The occurrence of tularemia coincided with a decline in the number of deer mice in part of a large area (>22000 km^2) in which deer mice had been extremely abundant during the autumn of 2004 and spring of 2005, and in which mice caused damage to crops in the autumn of 2004. This is apparently the first report of tularemia as a cause of death of wild deer mice. The bacterium isolated from deer mice was atypical in that cysteine was not required in the media used for isolation. Three isolates tested were genotypes not previously identified in Canada. There were no reports of human disease in the area.

Key words: Crop damage, deer mouse, epizootic, *Francisella tularensis*, *Peromyscus maniculatus*, tularemia, zoonosis.

INTRODUCTION

Tularemia, caused by the bacterium *Francisella tularensis*, is a zoonotic disease that occurs over much of the northern hemisphere. It has a broad host range and has been reported from at least 250 species (Farlow et al., 2001). Four subspecies of *F. tularensis* are recognized, of which three occur in North America (Johansson et al., 2004). *Francisella tularensis* subsp. *tularensis* (Type A) is found predominantly in North America. *Francisella tularensis* subsp. *holarctica* (Type B) is found throughout much of the northern hemisphere. *Francisella tularensis* subsp. *novicida* is rarely isolated (Johansson et al., 2004).

Some rodent species undergo sporadic irruption ("a sudden increase in an animal population" [Nichols, 2001]) during which they may damage crops and may constitute an increased risk to humans because of disease agents that they carry. Examples include "plagues" of house mice (*Mus musculus*) in Australia (Pech et al., 2003) and irruptions of voles (*Microtus* spp.) in the western United States (Beck, 1958), Sweden (Dahlstrand et al., 1971), and Mongolia (Pech et al., 2003). Although little is known about the role of infectious disease in population changes among rodents (Begon, 2003), tularemia was diagnosed in voles found dead during irruptions of *Microtus californicus* in California in 1927 (Perry, 1928) and *Microtus montanus* in Oregon and California in 1957–1958 (Jellison et al., 1958). A sudden population decline, during which many voles (*Microtus agrestis*, *Microtus raticeps*) were found dead in hay storage barns, coincided with an outbreak of respiratory tularemia among farmers in Sweden (Dahlstrand et al., 1971). Tularemia was suspected to have caused the decline of a *Microtus canicaudus* population in Oregon (Wolff and Edge, 2003).

We describe the occurrence of tularemia during an irruption of deer mice (*Peromyscus maniculatus*) in west-central Saskatchewan.

MATERIALS AND METHODS

Description of the outbreak

During March 2005, as winter snow was melting, reports appeared in local media of massive numbers of dead deer mice on some

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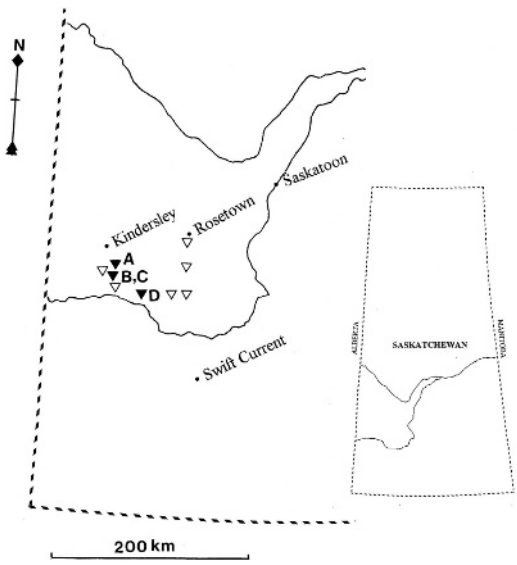


FIGURE 1. Map of west-central Saskatchewan showing sites at which dead deer mice were collected (filled triangles, A–D) and other sites at which dead mice were not found (open triangles).

highways in west-central Saskatchewan. On 18 April 2005, an adult male deer mouse was submitted for necropsy to the Canadian Cooperative Wildlife Health Centre, Saskatoon, Saskatchewan by a municipal Pest Control Officer (PCO) who had observed hundreds of dead deer mice on 13 April while checking farm buildings (N 51°20′, W 109°00′) near Madison, Saskatchewan. The mouse was seen to be panting heavily and died while being observed. It was held frozen until submitted. On 24 April, the senior author inspected the site where the mouse was found (Site A) and two other sites (B, C) where the PCO had seen dead deer mice (Fig. 1). Site A consisted of granaries in a field distant from any occupied farm buildings. There was barley (*Hordeum* spp.) and dry peas (*Pisium sativum*) on the ground about the granaries. Many desiccated but intact (mummified) deer mouse carcasses were evident about the granaries and in the surrounding field. One live and many dead deer mice were found under pieces of wood on the ground about the granaries. Sites B and C were groups of granaries in fields located 12 km from site A and about 1 km apart. Mummified deer mice were evident in the grass about the buildings at site B and >50 dead deer mice and a live house mouse were found under pieces of wood lying on the ground. Three recently dead deer mice were collected. There were many mummified deer mice surrounding the granaries at site C; three recently dead deer mice found under wood lying on the ground were collected.

On 3 May, the senior author examined 15 unoccupied farm building sites located east and south of Sites A–C that were similar to those where dead deer mice had been found previously. These were chosen opportunistically along a line that extended south from near the town of Rosetown (51°32′N, 107°59′W) for approximately 60 km and then west for approximately 80 km (Fig. 1). No dead deer mice were found except at eight sites within a 10-km radius of the hamlet of Tyner (51°00′N, 108°25′W), at all of which mummified deer mice were evident. A farmer reported seeing many dead deer mice in this area earlier in the spring. The only specimen suitable for collection was at Site D (51°00′N, 108°37′W) located 47 km from Site A and 34 km from Sites B and C (Fig. 1). A recently dead deer mouse that was collected, a live house mouse, a live deer mouse with pups, and >20 mummified deer mice were found near granaries at this site. Precautions proposed by Mills et al. (1995) were observed when collecting dead deer mice.

Necropsy and detection of *F. tularensis*

Necropsies were performed within a biosafety cabinet. Samples of major organs were preserved in 10% buffered formalin and liver was frozen until used for bacteriology and/or testing by polymerase chain reaction (PCR). Fixed tissues were processed routinely, sectioned at 5 μm, and stained with hematoxylin and eosin for histology. Liver from the initial mouse from Site A was inoculated on Tryptic Soy Agar supplemented with 5% sheep blood and MacConkey's agar plates (Becton-Dickinson-Canada, Oakville, Ontario, Canada) that were incubated at 37 C in the presence of 5% CO_2. Liver from this mouse and from a single mouse from each of Sites B–D was tested for the presence of *F. tularensis* by PCR using primers specific for *F. tularensis* subsp. *tularensis* outer membrane protein fopA gene (Ftul-F 5′ GTGTTAGGGATTTCGAGGAGTCT-3′, Ftul-R 5′-CTGGCCAGTTCTATCTTGAGG-3′) (Sibley et al., 2005). DNA was extracted with the use of standard proteinase K and phenol/chloroform extraction. The primers amplified a 459 bp product. Reagent-only (no DNA) reactions were used as negative controls to detect potential contamination, whereas DNA from cultured bacteria (field strain) was used as a positive control. Replicate unstained histologic sections of tissues from all mice from each site (total of eight) were used for immunohistochemistry

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with avidin–biotin complex polyclonal anti–*F. tularensis* antiserum (Difco Laboratories, Detroit, Michigan, USA).

A culture of *F. tularensis* from the initial animal from Site A, as well as liver from one animal from each of Sites B and C that had tested positive for *F. tularensis* with the use of PCR were sent to the National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba (NML) for further typing. *Francisella tularensis* was cultured from the specimens from Sites B and C at NML and DNA was prepared from the three cultures of *F. tularensis* with the use of a thermolysis procedure (Keim et al., 2000). Multiple-locus variable-number tandem repeat analysis (MLVA) was performed for molecular subtyping of the isolates. MLVA targets were PCR amplified as described in Johansson et al. (2004). Amplicons were purified with the use of Microcon centrifugal filter devices (Millipore, Billerica, Massachusetts, USA) and MLVA marker alleles were sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with the use of BigDye terminator V3.1 cycle sequencing chemistry. Bionumerics software (Applied Maths, Belgium) was used to analyze sequence data. The raw genotype scores were analyzed by using an unweighted pair group method arithmetic average (UPGMA) cluster analysis with a categorical coefficient.

Information on deer mouse populations

To collect information on the deer mouse population in west-central Saskatchewan, a questionnaire was sent during May 2005 to rural municipalities in which dead mice had been found or where large numbers of mice had been reported in the media. Based on the response to these initial questionnaires, questionnaires then were sent to additional municipalities. The initial question asked was: Have unusual numbers of deer mice been reported in your municipality at any time since January 2003? If the answer was affirmative, respondents were asked to answer further questions including: When were unusual numbers of mice first observed? Was the entire municipality involved? Did the number of mice increase suddenly or gradually? Were unusual numbers of mice associated with any particular crop? Has the number of mice remained stable, increased or decreased in the spring of 2005? Have large numbers of dead mice been observed? If so, when and where?. In total 55 municipalities were polled. Questionnaires were supplemented by telephone contact with the PCO or other knowledgeable persons in some municipalities.

RESULTS

All eight mice examined were adult (five male, three female); five had full stomachs. The only gross lesion seen was splenomegaly in one mouse. All had microscopic foci of necrosis in liver and spleen, with large numbers of bacteria evident within the lesions and in vessels in other tissues. The initial animal from site A had focal suppurative pneumonia, one mouse had diffuse thickening of alveolar septa, and the remainder had pulmonary edema.

The initial mouse from Site A was submitted as an unknown diagnostic specimen. Because of the risk to laboratory personnel from Sin Nombre virus and *Bartonella* spp. carried by deer mice, bacteriological culture and PCR examination of liver from this animal were done within biosafety cabinets. On bacteriologic culture, small colonies grew after 48 hr of incubation on 5% blood agar but not on MacConkey's agar. On Gram stain, the organism appeared as gram-negative coccobacilli. There was no reaction on triple sugar iron. Additional biochemical tests including oxidase, urease, and nitrate were negative, suggesting a *Francisella* sp.–like organism; however, the isolated organism did not require cysteine for growth. To reduce the risk of infection of laboratory personnel, bacteriologic isolation from any of the other deer mice was not attempted in the Saskatoon laboratory.

Liver from the initial mouse from Site A and from a single mouse from each of Sites B–D was examined by PCR; all were positive for *F. tularensis*. Sections of fixed liver from all mice collected from Sites A–D were positive for *F. tularensis* with the use of immunohistochemistry.

To determine the extent of diversity among the three isolates sent to NML, 25 variable-number tandem repeat (VNTR) markers were analyzed for molecular

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subtyping. All three isolates were designated *F. tularensis* subsp. *holarctica*. Sequence analysis of the Ft-M19 locus indicated a 30 bp deletion in this region, which has been shown to be diagnostic for *F. t. holartica* isolates (Farlow et al., 2001; Johansson et al., 2004). Of the 25 VNTR markers analyzed, only two hypervariable markers, Ft-M3 and Ft-M4, showed any variation (Table 1). Both markers are known, historically, to have the highest diversity index (Ft-M3: $D=0.95$; Ft-M4, $D=0.65$) among those tested (Johansson et al., 2004). At the Ft-M3 locus, isolate EBD05-005 contained 15 copies of a 9 bp tandem repeat unit, EBD05-004 had 16 copies and EBD05-003 had 19 copies. At the Ft-M4 locus, EBD05-003 had 4 copies of a 5 bp tandem repeat whereas both EBD05-004 and EBD05-005 each had 5 copies. The deer mouse MLVA profiles were compared to others in the Canadian national collection of *F. tularensis* bionumerics database and all three isolates represent new genotypes.

A response was received from each municipality sent the questionnaire, but not all respondents answered all questions. Abnormally large numbers of deer mice were reported during 2004 and 2005 in 23 municipalities, with a total affected area of approximately 22,300 km^2 (Fig. 2). The actual density of mice is unknown, but comments from respondents are illustrative: "during harvesting, sieves on the combine get loaded with mice," "observed pavement with flattened mouse carcasses every foot or so," "farmers with pails of water with flip lids [homemade traps] report catching up to 75 mice a night" [in farm buildings], "people who have never had mice in their house are complaining of mice." Some other municipalities also reported that the number of deer mice may have increased, but not to an extent that they constituted a problem. The number of deer mice was reported to have increased suddenly in the summer or autumn of 2004 (6/14 respondents) or in the spring of 2005 (7/14 respondents).

TABLE 1. The number of repeats at each of 25 variable-number tandem repeat markers (M1 to M25[a]) in three isolates of *F. tularensis* from deer mice and in reference strain SCHU S4 (*F. t.* subsp. *tularensis*).

Strain	M1	M2	M3	M4*	M5	M6	M7	M8*	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24	M25
SCHU S4[a]	3	4	25	3	3	4	4	4	4	18	5	2	2	3	2	2	3	4	2	3	3	2	2	1	5
EBD05-003[b]	3	2	19	4	2	5	2	2	2	2	5	2	1	3	3	1	2	2	1	3	2	3	1	2	4
EBD05-004[c]	3	2	16	5	2	5	2	2	2	2	5	2	1	3	3	1	2	2	1	3	2	3	1	2	4
EBD05-005[d]	3	2	15	5	2	5	2	2	2	2	5	2	1	3	3	1	2	2	1	3	2	3	1	2	4

[a] From Johansson et al. (2004).
[b] From Site C.
[c] From Site B.
[d] From Site A.

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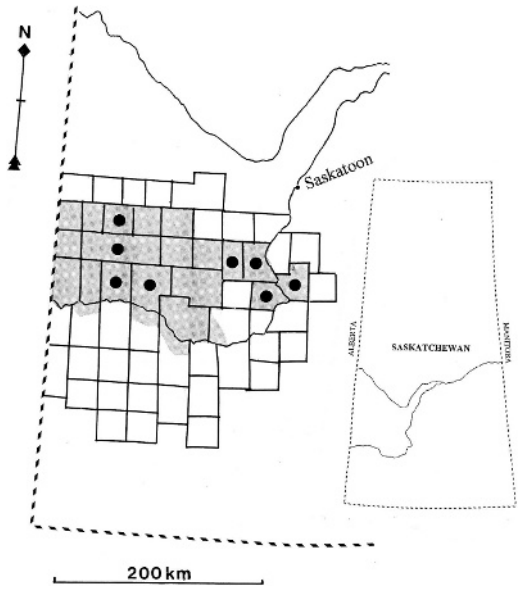


FIGURE 2. Map of west-central Saskatchewan showing the borders of the rural municipalities to which questionnaires were sent. The shaded area represents the region within which a very high density of deer mice was reported by respondents. The municipalities marked with a dot are those in which dead mice were seen by respondents in the spring of 2005.

One respondent reported that the number of mice had increased gradually from 2002 to 2005. Deer mice were reported to be particularly numerous in association with crops of canaryseed (*Phalaris canatiensis*) and lentils (*Lens culinaris*). Respondents from 10 municipalities indicated that the mouse population declined in the spring of 2005 and many dead deer mice had been observed in eight municipalities (Fig. 2). Only one respondent recalled a similar density of deer mice at any time in the past. He reported that many deer mice occurred in one year in the 1950's, during a period of several years with above-normal rainfall.

DISCUSSION

We found no report of populations of deer mice of the type that occurred in this area of Saskatchewan, or of damage to crops caused by deer mice. The actual density number of deer mice is unknown, but mice were considered to be a problem over a large area. Australian farmers generally do not perceive that a mouse problem exists until densities are >200 mice/ha (Brown et al., 2003). Severe damage to standing crops of canaryseed, and wheat (*Triticum* spp.), and less severe damage to chickpea (*Cicer arietum*), mustard (*Brassica* spp.), and flax (*Linum usiatissimum*) crops occurred near Kindersley in September 2004 (Phelps, 2005). Some crops were destroyed completely by mice clipping off the seed heads. Approximately one mouse burrow per square meter of soil was present in severely affected fields, and as many as 1,000 deer mice/ha may have been present in some fields in Saskatchewan (Phelps, 2005), a density similar to that in Australian mouse plagues. Population density of *Peromyscus* spp. has been characterized as typically being low, with less variation than in other small mammals, and with "no evidence for outbreaks of the type exhibited by *Lemmus*, *Mus musculus*, and various species of *Microtus*" (Terman,1968, p. 437). A five-fold variation between peak and low populations of deer mice was found in natural habitats in Montana (Douglass et al., 2001) and a 3–20-fold variation was detected in New Mexico (Yates et al., 2002). The granaries at Sites A–D were probably points where mice congregated and, hence, were more obvious, but dead mice also were evident in some fields in May 2005.

Factors that led to this irruption are unknown. The natural vegetation of the area was mixed grass prairie similar to grasslands in Montana, where the average number of deer mice ranged from 7.7 to 22.1/ha (Douglass et al., 2001). The area now consists of extensive grain fields with only tiny remnants of native habitat. In common with other highly modified agricultural landscapes, the area lacks biodiversity but contains plentiful energy-rich food on a seasonal basis. Feedback mechanisms that influence rodent popula-

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tions, including predator–prey, plant–herbivore, and social interactions may not occur in agricultural landscapes that are disturbed frequently (Leirs, 2003).

We believe this to be the first report of tularemia in wild deer mice, although deer mice are susceptible to experimental infection with *F. t.* subsp. *tularensis* (Stagg et al., 1956; Vest and Marchette, 1958). *Francisella tularensis* has been isolated from deer mice during surveys for plague, including from one mouse in Alberta (Ozburn, 1944), two of 58 pooled samples from 301 mice in California (Burroughs et al., 1945), and from ectoparasites from mice in Utah (Stagg et al., 1956). The subspecies of *F. tularensis* isolated in these surveys was not reported. Burroughs et al. (1945) indicated that mice in their survey were latently infected and that septicemic tularemia was not present. Vest et al. (1965) and Lane and Emmons (1977) did not isolate *F. tularensis* from any of 4404 and 64 deer mice collected in Utah and California, respectively.

The organism isolated from the initial mouse from Site A was atypical in that cysteine was not required in media for growth. Bernard et al. (1994) reported that seven isolates of *F. tularensis* from humans in Canada lacked a requirement for cysteine and speculated that strains that lack a requirement for cysteine and enriched media for growth may have reduced virulence. The organisms from mice from Sites A–C were identified as *F. t.* subsp. *holarctica* with the use of MLVA. We note that MLVA should be used with caution for assigning *F. tularensis* subspecies; however, Type A and Type B biovars were consistently distinguished by the concurrent use of six VNTR loci (Farlow et al., 2001; Johansson et al., 2004). This subspecies is considered to be less virulent than *F. t.* subsp. *tularensis* and is usually associated with voles and muskrats (*Ondatra zibethicus*) in North America and with water rats (*Arvicola terrestris*) and microtine voles in Eurasia (Bell, 1980). Most human cases of tularemia in Saskatchewan have been linked to contact with muskrats (Martin et al.,1982). Epizootics of Type B tularemia occur in varying hares (*Lepus timidus*) in Sweden and Finland, but hares are thought to be accidental rather than reservoir hosts of the disease (Mörner et al., 1988). Multiple-locus variable-number tandem repeat analysis, which uses VNTR-based polymorphisms, provides a high level of discrimination among *F. tularensis* isolates. Two of 25 VNTRs tested in this study were polymorphic among the three deer mouse isolates. Comparison of VNTR profiles with those in the Canadian *F. tularensis* database, with the use of a categorical coefficient and UPGMA cluster analysis, resulted in three new genotypes.

Tularemia only is obvious when many animals die of the disease or humans become infected. Deer mice are nocturnal and secretive, so mortality caused by tularemia is unlikely to be observed in populations at low density. The epidemiology of the disease is extremely diverse (Bell, 1980) and the reservoir for the bacterium is unclear (Tärnvik et al., 1996). *Francisella tularensis* is believed to be an obligate host-dependent bacterium (Larsson et al., 2005) and although it is associated with rodents and lagomorphs, it is unclear if these mammals are true reservoirs of the bacterium (Ellis et al., 2002). Bell and Stewart (1975) proposed that chronic nephritis in voles, resulting in water contamination, might be a method by which *F. t.* subsp. *holarctica* may persist. This seems an unlikely source of infection for deer mice living in agricultural fields in a semiarid environment. Isolation of *F. tularensis* from live-trapped deer mice living in dry fields without access to standing water (Burroughs et al., 1945) suggests that subclinically infected deer mice might be a reservoir.

The diagnosis of tularemia coincided temporally with a reported decline in the number of deer mice in 10 municipalities. The disease was diagnosed in dead mice at

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four widely separated sites located in two of these municipalities. During the survey on May 3, 15 sites were examined in five municipalities that reported large numbers of deer mice. Dead mice were found in two municipalities that reported mouse mortality; no dead mice were found at 4 sites in three municipalities in which mortality was not reported. The actual spatial extent of the disease and its impact on the deer mouse population are unknown. Based on the mummified condition of most mice found in April and May, much of the mortality probably occurred during March or earlier. The number of mummified carcasses suggests that mortality exceeded the ability of predators and scavengers to remove sick and dead mice before carcasses desiccated.

Public health officials were advised of the diagnosis of tularemia in deer mice but there were no reported cases of human tularemia in the area, although many dead mice were present in and around farm buildings and equipment. Humans have been infected with type B tularemia through contact with dead animals, contaminated food and water, ticks and biting insects, inhalation, and bite wounds (Bell, 1980; Ellis et al., 2002). Respiratory infection as a result of inhalation of dust occurred in buildings contaminated by rodent carcasses in Sweden (Dahlstrand et al., 1971; Tärnvik et al., 2004) and in association with haying and threshing in Finland (Syrjälä et al., 1985). During the investigation, several individuals volunteered that their dogs and cats caught many deer mice. Transmission of tularemia to humans by cats has been reported (Baldwin et al., 1991; Capellan and Fong, 1993), including an instance in which a cysteine-independent strain of *F. tularensis* was transmitted by a cat bite (Bernard et al., 1994). Occurrence of human tularemia is grossly underestimated, because of the relatively benign nature of human disease caused by some strains of *F. tularensis* (Ellis et al., 2002). During an irruption of *M. montanus* in Oregon, there was extensive environmental contamination with *F. tularensis*, but no overt human tularemia outbreak (Kartman et al., 1958). However, retrospective serology identified 12 human cases, none of which had been suspected to have had tularemia (Osgood et al., 1958). It was concluded that mild and atypical clinical symptoms, perhaps related to the low virulence of the organism, failed to arouse the suspicion of either patients or physicians.

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