# Tularaemia Transmitted by Ticks (*Dermacentor andersoni*) in Saskatchewan

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## ABSTRACT

**Common wood ticks (*Dermacentor andersoni*) collected from Saskatchewan Landing Provincial Park, Saskatchewan in the spring of 1982 transmitted a lethal tularaemia infection to four of six rabbits. *Francisella tularensis* organisms were isolated from tissues taken from the dead rabbits and identified from subcultures using an indirect immunofluorescent antibody assay. One human associated with the animals developed symptoms of tularaemia and, after successful therapy, had a significant increase in titre of specific antibodies to *F. tularensis*. This is the first time tick-transmitted tularaemia has been reported in Saskatchewan in more than 25 years.**


**Key words:** Ticks, tularaemia, Saskatchewan.


## RÉSUMÉ

**Les auteurs rapportent la transmission d'une tularémie fatale à quatre lapins expérimentaux, sur un total de six, par des tiques communes des bois, *Dermacentor andersoni*, récoltées dans le Parc provincial Landing de la Saskatchewan, au printemps de 1982. Ils isolèrent *Francisella tularensis* des tissus prélevés sur les lapins morts et l'identifièrent à partir de subcultures qu'ils soumirent à une épreuve d'immunofluorescence indirecte. La technicienne qui s'occupait des lapins manifesta des symptômes de tularémie et, après un traitement efficace, elle afficha une élévation appréciable de son titre d'anticorps à l'endroit de *F. tularensis*. Depuis 1956, c'est le premier cas de tularémie imputable à des tiques qu'on rapporte en Saskatchewan.**


**Mots clés:** tiques, tularémie, Saskatchewan.

## INTRODUCTION

*Francisella tularensis*, the cause of tularaemia, infects a wide range of organisms, from arthropods to humans. It is prevalent throughout the northern hemisphere (1) and there have been several endemic foci in Canada (2). Greenberg and Blake (2) reported that 7% of approximately 800 Canadian natives tested in 1957 had significant titres of *F. tularensis* agglutinins, with the greatest prevalence from Ontario.

*Francisella tularensis* is an aerobic, Gram-negative coccobacillary organism that forms transparent colonies on cysteine-glucose agar (3). Two varieties of the organism have been described: *F. tularensis* var. *tularensis* (or type A) ferments glycerol, may be highly pathogenic for rabbits, produces a moderate mortality rate in untreated humans (5-7%) and may be associated with ticks and their dryland habitat hosts (1). *Francisella tularensis* var. *palearctica* (or type B) does not ferment glycerol, is less virulent for rabbits, produces a very low mortality rate in untreated humans (1%) and may be associated with wetland habitats and their fauna (1).

In general, tularaemia is pathogenic for approximately 100 species of wild and domestic animals including lagomorphs, rodents, canids, felids, ungulates and bears. Twenty-five species of birds (including waterfowl, gallinaceous, predatory and scavenger species) have been reported to be infected with tularaemia, as have several species of fish, frogs and toads (1, 4, 5).

Tularaemia may be transmitted by arthropods, including fleas, deer flies, stable flies, lice, bedbugs, mosquitoes and ticks (1, 5). Ticks transmit this bacterium vertically by both transstadial and transovarial mechanisms, and horizontally by bite-site contamination with faeces, coxal fluid or by salivary injection (5). The principal tick vectors include species in the genera *Amblyomma, Dermacentor, Haemaphysalis, Ixodes* (1) and *Ornithodoros* (6). In Canada, the wood tick (*Dermacentor andersoni*) (6, 7), the dog tick (*Dermacentor variabilis*) (8), the rabbit tick (*Haemaphysalis leporis-palustris*) (9, 10) and the common bird tick (*Haemaphysalis chordeilis*) (11) have all been implicated as tularaemia carriers.

The purpose of this paper is to report, for the first time since

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1956, the occurrence of tick-borne tularaemia in Saskatchewan.

## MATERIALS AND METHODS

Between mid-April and mid-May 1982, approximately 4000 adult *Dermacentor andersoni* ticks were collected for other research purposes using flannel flags to sweep vegetation along game trails in the prairie watershed ravines of Saskatchewan Landing Provincial Park (approximately 108° west longitude, 51° north latitude). The ticks were brought into the laboratory and, prior to further experiments, some ticks were allowed to feed on a number of six to ten week old New Zealand White rabbits. These host animals were housed with rabbits from other experiments, but caged individually. *Dermacentor andersoni* ticks were confined in plastic capsules cemented to the shaved flanks of the rabbits and, unless otherwise stated, were removed after five days. Rabbits number 1 and 2 each received 50 female and 25 male ticks, whereas rabbits number 3, 4 and 5 were each infested with 100 female and 50 male ticks. Rabbits number 3 and 4 were submitted for necropsy shortly following death. Rabbit number 5 was exsanguinated when moribund, then frozen and stored for subsequent necropsy. Rabbit number 6 was infested with 100 female and 50 male ticks. Ten female ticks were removed from it each day until death occurred.

Routine necropsies were performed on rabbits 3-6: heart, lung, liver, spleen, thymus, lymph node and other tissue samples were fixed in buffered 10% formalin and processed to 6 $\mu$ paraffin sections. Sections were stained with haematoxylin-eosin and selected sections were also stained with Gram, Periodic acid-Schiff, Giemsa, Warthin-Faulkner, Ziehl-Nielsen or Grocott stains.

Selected tissues were processed for various microbiological tests. Viral agents were sought by injecting supernatants of saline-homogenized lung, spleen and thymus into bovine and feline kidney cell cultures and monitoring cytopathic effects. Attempts to demonstrate rickettsial agents were made by the injection of similar supernatants of spleen, liver, lung and thymus into the yolk-sac of eight day old chick embryos. In addition, heat or acetone-fixed impression smears of spleen tissues were tested for the presence of Spotted Fever Group rickettsiae (as well as *F. tularensis*) in the laboratory of Dr. W. Burgdorfer (Rocky Mountain Laboratory, NIAID, Hamilton, Montana) using an indirect immunofluorescent antibody (IFA) assay.

Fresh or frozen liver and cervical lymph node sections were ethanol dipped, flamed, saline-homogenized and used to inoculate MacConkey agar, blood agar and cysteine heart agar (CHA) (Difco Laboratories, P.O. Box 1058a, Detroit, Michigan) plates. After aerobic incubation at 37°C, bacterial colonies were used as the substrate for both agglutination and indirect IFA *F. tularensis* diagnostic assays (Difco Laboratories). Commercial *F. tularensis* organisms (Difco) and *Pasteurella multocida* organisms were used as the positive and negative controls, respectively.

A serum sample collected within 12 h of onset of illness, from a 23 year old laboratory technician (WG) was tested for anti-*F. tularensis* agglutinating antibodies. This person was subsequently treated with tetracycline for one week. Another serum sample, collected three weeks later, was also tested for antibodies to *F. tularensis*. Both of these tests were performed at the Province of Sask. Health Lab. in Regina, Saskatchewan.

## RESULTS

Rabbits number 1 and 2 remained asymptomatic throughout the course of this study, and did not have anti-*F. tularensis* antibodies six weeks postinfestation. Rabbits 3, 4 and 5 appeared healthy on the evening of the fifth day of the tick infestation. Rabbits 3 and 4 were found dead in their cages on the morning of day 6; rabbit number 5 was weak, dehydrated and had diarrhea the same morning. Rabbit number 6 was found dead in its cage on the morning of day 6. It still had 40 female and 50 male ticks attached. No other rabbit housed in the same room with the tick-infested rabbits developed any signs of disease.

Ulcers with encrusted blood, and numerous subcutaneous hemorrhages were evident at the sites of tick attachments. Lymph nodes of the head, neck and the cranial mediastinum were congested and swollen, with numerous large hemorrhages in the subcapsular and cortical regions. The lungs were moderately congested and edematous and had areas of atelectasis. Numerous small hemorrhages were scattered over the epicardium and the serosal surfaces of the pleural and peritoneal cavities.

The livers had numerous diffuse pale, round to slightly irregular, sharply demarcated necrotic foci. The spleens were swollen and contained multiple pale foci approximately 1-2 mm in diameter.

In histological sections, polymorphonuclear leukocytes were scattered lightly throughout the lymph nodes, lymphoid cells were depleted in general and many of those that remained were karyorrhectic. In the lungs, alveolar macrophages were often present in excess. The alveolar septa were thickened and contained mononuclear cells. Fibrin strands partially occluded many small arterioles. The ventricular myocardium had small focal hemorrhages accompanied by increased fibre eosinophilia and nuclear pyknosis.

The necrotic hepatic foci varied from small areas involving only a few hepatocytes to macroscopically visible foci involving most of a lobule. The affected hepatocytes were completely destroyed, leaving only cellular debris and remnants of nuclei interspersed with low numbers of small Gram-negative coccobacilli. These bacteria were occasionally seen in adja-

cent intact hepatocytes. Occasional mononuclear cells were also found at the periphery of the necrotic foci. In the spleen, red pulp cellular necrosis was widespread and severe, while periarteriolar lymph follicles were either destroyed, or activated and composed of blast-like cells. Small Gram-negative coccobacilli were found in low numbers in the areas of cellular debris.

No evidence of rickettsial agents was found. Impression smears of splenic tissue from rabbits number 3 through 6 contained *F. tularensis*. Furthermore, this bacterium was isolated on CHA plates from liver and lymph node tissue, but could not be cultured on blood or MacConkey agar plates.

The human patient reported here had symptoms characteristic of the very early phase of tularaemia (1): a fever of 40°C, headache, chills and general malaise. Although she had several minor abrasions on her hands at the time of handling the affected ticks and rabbits, no ulcerous lesions typical of ulcer-glandular tularaemia (4) developed. Her serum titre of specific antibodies was negligible at initiation of antibiotic treatment and her symptoms disappeared within 24 hours. Her three week (i.e. convalescent phase) serum titre against *F. tularensis* was 10,240.

## DISCUSSION

Tularaemia in Canada has been associated with both wetland and dryland habitats. In general, endemics in Ontario, Manitoba (10, 12) and N. Saskatchewan (4) have occurred in wetland habitats, while those in S. Saskatchewan (8), Alberta (6, 7) and British Columbia (9) have occurred on the prairie (i.e. dryland or tick habitats). Martin and coworkers (4) reported in 1982 that most recent human tularaemia cases in Saskatchewan have been confined to trappers and hunters of wetland habitat animals in central and northern parts of the province.

In 1956, Gregson (11) reported that *F. tularensis* was carried by various ticks in Saskatchewan, including *Dermacentor variabilis* in the Carlyle Lake area and *D. andersoni* in the southwestern areas. The studies of Greenberg and Blake (2) indicated that in 1957, approximately 10% of the natives from southern Saskatchewan had significant *F. tularensis* antibody titres, despite the fact that few of these people had reported illness.

To our knowledge, this is the first time that tick-borne tularaemia has been reported in Saskatchewan since 1956. In 1971, and then again each year from 1974 to 1981, between 1000-4000 apparently disease-free *D. andersoni* were collected from Saskatchewan Landing Provincial Park. In 1982, we found for the first time that a second tick species, *Haemaphysalis chordeilis* (the common bird tick), was coincidentally collected in low numbers. This tick is capable of harbouring *F. tularensis* in Canada (6, 10). Although there is no direct evidence, it is possible that the new appearance of both the disease and this tick at Saskatchewan Landing are related.

It is significant that those rabbits that were infested with 150 ticks contracted tularaemia while the two that received only 75 ticks remained disease-free and did not develop anti-*F. tularensis* antibodies. Therefore, it seems feasible that a low proportion (ie $< 0.5\%$) of the tick population carried a highly virulent strain of *F. tularensis*.

In addition to the many other species that *D. andersoni* parasitizes, man is also capable of being a host for this tick (11). Bow and Brown (7) and Black and Thomas (9) both report instances in Western Canada where patients developed tularaemia following the bite of *D. andersoni*. The human infection in this report was not caused by the bite of a tick. It seems likely that either the bacteria entered through the skin during handling of the infected ticks or rabbits, or that they entered through the respiratory mucosa after inhalation (1). Therapy was initiated before symptoms characteristic of either the ulceroglandular or the pneumonic form of tularaemia set in, so there is no basis for judging the actual portal of entry.

We conclude that a potential zoonotic problem exists in the Saskatchewan Landing Provincial Park area and that it will require monitoring to establish its true extent.

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