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A SURVEY OF TULAREMIA IN WILD MAMMALS FROM FENNOSCANDIA

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Abstract: A total of 2696 wild mammals from Fennoscandia were surveyed for tularemia. *Francisella tularensis* was not detected in livers/spleens or kidneys from any of the 1992 small rodents captured in Norway and Denmark as judged by one or more of the following methods: cultivation, immunofluorescence microscopy and inoculation in laboratory mice. Serologic examination of 704 wild mammals from Norway, Finland and Sweden demonstrated 11 cases of antibody titers. Agglutinating antibodies were demonstrated in 2 of 565 small rodents (titer 1:160), 2 of 26 wild rabbits (titer 1:80) and in 7 of 60 red deer (titer 1:20-1:40). The titers in red deer were low and could be due to cross reactions. No agglutinating antibodies were demonstrated in the sera of 53 domestic reindeer.

INTRODUCTION

Many cases of tularemia in man can be traced to *Francisella tularensis* in the native fauna.¹⁶ However, the maintenance of the pathogen in nature and its reappearance from time to time in the form of epidemics and epizootics is poorly understood. Kartman *et al*¹⁰ stated that epidemics in man originating in small rodents have occurred with sufficient frequency to illustrate the potential hazard of tularemia outbreaks in these animals. In Norway, reports on human tularemia are sparse.^{6,7,11,16} The general impression is that a positive relationship exists between the frequency of reported human cases and the population density of small rodents. Recently, it was reported that six of 11 patients from northern Norway with otolaryngological manifestations of tularemia had contact with rodents during a period of great numbers of Norwegian lemmings (*Lemmus lemmus*).¹¹

Prior to this century a febrile disease of humans associated with high population density of Norwegian lemmings has been described from Norway.¹⁵ As early as 1653, Worm¹⁸ described a "Leemands Soet" (lemming fever). Some of these descriptions are sufficiently thorough to leave little doubt that the causative agent was *F. tularensis*. The first definite diagnoses of tularemia were reported by Thjötta¹⁶ in 1930, who stated that most of the patients had been in contact with hares. Thus, Norway was one of the first countries in Europe where tularemia was diagnosed with certainty. Antibodies to *F. tularensis* have been reported among hunters and school children in certain parts of Norway.⁷ During the past decade a number of zoologists in Scandinavia, working with small rodents, have contracted tularemia, two being infected through rodent bites (Professor Arne Semb-Johansson and Dr. Jussi Viitala Pers. comm.).

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With this as a background we decided to investigate the presence of *F. tularensis* in a number of free-ranging mammal species, and to try to ascertain the role of tularemia in the population dynamics of small rodents.

MATERIALS AND METHODS

We used three approaches: 1) small rodents were collected from a single study area (Kviteseid) during a six-year period, including two peak phases of the small rodent cycle, 2) during September, 1973, when peak populations of rodents occurred in many parts of Norway, samples were collected throughout the country to determine if *F. tularensis* was present in any of the populations prior to the decline phase of the small rodent cycle, 3) a survey was made of red deer, reindeer and rabbits. These species were considered better indicators of the presence of *F. tularensis* in nature than small rodents since small rodents suffering from acute tularemia may be rapidly eliminated from the trappable population.

Eleven species of wild mammals from Norway, Sweden, Finland and Denmark

were included in the present study: bank vole (*Clethrionomys glareolus*), grey-sided vole (*C. rufocanus*), red vole (*C. rutilus*), field vole (*Microtus agrestis*), root vole (*M. oeconomus*), Norwegian lemming (*Lemmus lemmus*), wood mouse (*Apodemus sylvaticus*), yellow-necked field mouse (*A. flavicollis*), wild rabbit (*Oryctolagus cuniculus*), red deer (*Cervus elaphus*) and domestic reindeer (*Rangifer tarandus*).

Bacteriology

Livers, spleens or kidneys from a total of 1992 small rodents belonging to eight species captured in Norway and Denmark, were examined for *F. tularensis* using one or more of the three methods described below. The organs had been stored at -20°C for periods ranging from one week to approximately two years. Both pools and single samples were examined. Pools contained the livers/spleens or kidneys from 4 to 37 individuals. A more detailed description of the material and the methodology employed for the different samples, are presented in Tables 1, 2 and 3.

TABLE 1. A survey of tularemia in small rodents captured from 14 counties in Norway during September, 1973. Examination of pooled frozen livers and spleens by cultivation, IFAT and inoculation into laboratory mice.

County	No. of individuals	No. of pools			No. positive
		IFAT	Cultivation	Inoculation	
OEstfold	38	2	2	1	
Akershus	81	3	3	2	
Oppland	116	5	5	3	
Buskerud	27	1	1	1	
Vestfold	27	1	1	1	
Telemark	140	6	6	3	
Aust-Agder	81	3	3	2	
Hordaland	279	11	11	6	
Sogn & Fjordane	21	1	1	1	
Møre & Romsdal	54	2	2	2	
Sör-Trøndelag	24	1	1	1	
Nord-Trøndelag	399	16	16	9	
Nordland	24	1	1	1	
Troms	110	5	5	4	
Total	1421	58	58	37	0

TABLE 2. A survey of tularemia in small rodents from Norway and Denmark, captured during a six year period, by bacteriologic examination of livers and spleens.

Trapping period	Locality	No. examined	Methods of examination**	No. positive
Sept. 1969	Finse	2	B	
Aug. 1970	Kviteseid	139	BX	
Sept. 1970	Harran	17	B	
Oct. 1972	Kviteseid	2	B	
Dec. 1972	Kviteseid	56	B	
Apr. 1973	Kviteseid	2	B	
May 1973	Kviteseid	12	B	
June 1973	Kviteseid	11	B	
Aug. 1973	Kviteseid	4	B	
Sept. 1973	Kviteseid	8	B	
Oct. 1973	Kviteseid	6	B	
Dec. 1973	Kviteseid	1	B	
June 1974	Kviteseid	15	ABC	
Aug. 1974	Kviteseid	15	ABC	
Oct. 1974	Kviteseid	17	ABC	
Nov. 1974	Kviteseid	26	ABC	
Dec. 1974	Mols *	50	A C	
Jan. 1975	Mösstrand	12	A C	
Jan. 1975	Kviteseid	25	A C	
Oct. 1975	Kviteseid	20	ABC	
Total		441		0

* Denmark.

** A—Cultivation of pooled tissue homogenates.

B—Immunofluorescence microscopy of pooled tissue homogenates.

BX—Immunofluorescence microscopy of single impression smears.

C—Inoculation of pooled tissue homogenates in white mice.

TABLE 3. Attempts to detect *F. tularensis* in pooled kidney homogenates from small rodents captured in Kviteseid, Norway, using immunofluorescence microscopy.

Trapping period	No. of individuals	No. of pools	No. positive
Oct. 1972	6	1	
Jan. 1973	1	1	
Apr. 1973	5	1	
May 1973	7	1	
June 1973	6	1	
Aug. 1973	5	1	
Oct. 1973	23	4	
Aug. 1974	24	4	
Oct. 1974	17	3	
Nov. 1974	8	2	
Jan. 1975	16	3	
Oct. 1975	12	2	
Total	130	24	0

TABLE 4. Serologic survey of wild mammals from Norway, Finland and Sweden for the presence of agglutinating antibodies against two strains of *F. tularensis*.

Trapping period	Locality*	Species	No. of individuals	No. positive	Titer	
Aug. 1970	Kviteseid	A	Small rodents	91	0	—
Aug. 1972	Kviteseid	A	Small rodents	33	0	—
Dec. 1972	Kviteseid	A	Small rodents	56	0	—
Jan. 1973	Kviteseid	A	Small rodents	29	2	160**
June 1973	Kviteseid	A	Small rodents	28	0	—
Aug. 1973	Kviteseid	A	Small rodents	63	0	—
Nov. 1974	Kviteseid	A	Small rodents	36	0	—
Jan. 1975	Kviteseid	A	Small rodents	8	0	—
Sept. 1969	Finse	B	Small rodents	2	0	—
Sept. 1970	Finse	B	Small rodents	6	0	—
Sept. 1970	Harran	C	Small rodents	53	0	—
Aug. 1973	Vikedal	D	Small rodents	33	0	—
July 1974	Karigasniemi	E	Small rodents	127	0	—
Dec. 1973	Revinge	F	Wild rabbits	26	2	80***
Sept. 1973	Jotunheimen	G	Domestic reindeer	53	0	—
1971/72	Hitra	H	Red deer	37	3	20 - 40
1971/72/73	Sogndal	I	Red deer	10	0	—
1972/73	Aure	J	Red deer	13	4	20 - 40
Total			704	11		

* A, B, C, D, H, I, J—Norway, E—Finland, F—Sweden.

** *Apodemus sylvaticus* and *Clethrionomys glareolus*. The serum from the latter reacted with the "Jap Down" antigen only.

*** Agglutination obtained only against one of the antigens (HN63 (hare)).

The following methods were used to detect *F. tularensis*:

A. Cultivation of tissue homogenates on blood glucose cystein agar (BGC agar). Plates were incubated at 37 C for four days.

B. Microscopic examination of tissue homogenates and impression smears using a *F. tularensis* specific immunofluorescence technique (IFAT) as described by Karlsson *et al.*⁹

C. Inoculation of tissue homogenates in laboratory mice (NMRI (BOM) f spf). Three mice each were inoculated intraperitoneally with 0.3 ml. Necropsies were performed after about 30 days, and the mice were examined grossly for any evidence of infection. Pooled spleens and livers from all three mice were homogenized and cultivated on BGC agar.

Serology

The sera of 704 wild mammals from Norway, Finland and Sweden were tested by tube agglutination (Widal) using two antigens (Table 4). The antigens were prepared against two different strains of *F. tularensis*, "Jap Down" and "HN63 (hare)", as described by Holth Haug and Pearson.⁷

RESULTS

Results are presented in Tables 1 through 4.

F. tularensis was not detected in livers/spleens or kidneys from any of the 1992 small rodents captured in Norway and Denmark (Tables 1-3).

Serologic examination of 704 wild mammals from Norway, Finland and Sweden demonstrated 11 cases of low and medium antibody titers against *F. tularensis* (Table 4).

DISCUSSION

There is evidence that infectious disease may be a decimating factor in populations of small rodents.⁴ However, detection of the responsible microbial agent is difficult, especially when the pathogen causes acute, fatal disease. *F. tularensis*

kills microtine rodents within a few days after infection.^{2,14} This is especially pertinent to North America inasmuch as *F. tularensis tularensis* is generally considered to have a greater virulence than *F. tularensis palearctica*.⁵ However, both variants probably are fatal for small rodents before any immunological reaction is measurable. Also, animals with acute infections may remain in their nests and burrows and therefore are not a part of the trappable population. Most surveys of small rodents during interepizootic periods have demonstrated few infected animals.^{1,17} On the other hand, a very high prevalence has been reported during tularemias epizootics.^{10,12} For example, *F. tularensis* was isolated from 18 of 49 voles during an outbreak of tularemia in Sweden in 1966/67.¹² The epizootics, however, seemed to be confined to relatively small areas and were probably of short duration,¹⁰ thus making it difficult to find the epizootic foci.

Less sensitive animal species are better indicators of the occurrence of tularemia, since in such species infection with the pathogen frequently leads to non-fatal, immunizing infections rather than progressive fatal infections. For example, serologic surveys have shown the presence of antibodies to *F. tularensis* in rabbits and in several deer species.^{3,5,17} In the present study antibodies were demonstrated in 7 of 60 red deer and in 2 of 26 wild rabbits (Table 4). These red deer come from areas where *Ixodes ricinus* is abundant and thus the infections might have been tick-borne. However, the titers in red deer were low and could be due to cross reactions. The possibility of cross reactions with *Brucella abortus* can be excluded since brucellosis was considered virtually eradicated in Norway in 1951.¹³

Highly susceptible animals would not function as effective reservoirs for *F. tularensis*. Nevertheless, not all cases of tularemia in microtine rodents are fatal infections. The present demonstration of antibodies to *F. tularensis* in two small rodents support this view. Bell and Stewart² demonstrated tularemic nephritis associated with chronic bacteriuria in voles infected orally with *F. tularensis palearctica*. Such a chronic process would

seem to fulfill the requirements for more long term maintenance of *F. tularensis* in these populations. Their results prompted us to study the kidneys of small rodents (Table 3).

The number of small rodents examined was small and for practical reasons specimens were not collected at optimal intervals (for example twice monthly). Thus, the present results do not permit evaluation of the role of *F. tularensis* in the population dynamics of small rodents in Norway. The main problem seemed to be obtaining representative samples from the populations, especially during the winter months when the decline phase of

the small rodent cycle often occurs under thick layers of snow. Even in known foci, a large number of samples collected over long periods are evidently needed for successful detection of *F. tularensis*.¹

In future studies of *F. tularensis* in wildlife, we feel that a serologic survey of indicator species, such as carnivores or cervids, would be a logical first step. In this way, the area with the highest percentage of positive animals could be ascertained. Further studies in this particular area would then, in turn, increase the chances of detecting *F. tularensis* in the environment, small mammals and biting arthropods.

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