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INFECTIONS WITH *FRANCISELLA TULARENSIS* BIOVAR *PALAEARCTICA* IN HARES (*LEPUS TUMIDUS, LEPUS EUROPAEUS*) FROM SWEDEN

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ABSTRACT: The occurrence of tularemia was studied in 1,500 hares submitted to the National Veterinary Institute, Uppsala, Sweden for postmortem examination during 1973 through 1985. A total of 109 tularemia cases was recorded based on the fluorescent antibody (FA) test for *Francisella tularensis* and on the gross and microscopic pathology. Tularemia was diagnosed only in the varying hare (*Lepus timidus*) and not in the European brown hare (*Lepus europaeus*). The geographical distribution of the 109 cases indicates that tularemia has not spread in Sweden during the last 45 yr, with the exception of an endemic occurrence of the disease on the island of Stora Karlsö in the Baltic sea. The disease was most frequent in the autumn and only a few cases were recorded during winter. Cases were not seen in the spring. The annual prevalence varied, with several cases in 1974 and 1981, but there were no cases in 1976 and 1980. The postmortem findings in hares dying of tularemia in the autumn were characterized by focal coagulative necrosis in liver, spleen and bone marrow, with high numbers of bacteria FA-positive for *F. tularensis*. In hares dying during winter months, the most characteristic findings were hemorrhagic enteritis and typhlitis, although necrotic lesions could occur in liver, spleen and bone marrow. Diseased hares on the island of Stora Karlsö were demonstrated to be infected with ticks, while hares on the mainland of Sweden generally were fed upon by mosquitoes. Twenty-six of the 109 hares with tularemia were examined bacteriologically and *F. tularensis* biovar *palaearctica* was isolated from eight. The lung extract antibody test for *F. tularensis* was performed in 18 of the 109 hares. All were negative. In addition to the field study, an experimental study with *F. tularensis* biovar *palaearctica* was performed. Four varying hares and three European brown hares were inoculated. None of the hares died from tularemia, and generalized infection was not demonstrated.

Key words: Tularemia, *Francisella tularensis* biovar *palaearctica*, varying hare, European brown hare, *Lepus timidus*, *Lepus europaeus*, pathology, experimental infection, LEAT, FA-test, ELISA.

INTRODUCTION

As a zoonosis, tularemia is primarily a disease of wild lagomorphs and rodents. In North America, two subspecies of *Francisella tularensis* are seen: type A, or *F. tularensis* biovar *tularensis* (synonym *nearctica*) occurs most frequently in lagomorphs such as the cottontail rabbit (*Sylvilagus* spp.) (Jellison, 1974); and type B, *F. tularensis* biovar *palaearctica* (synonym *holarctica*), predominates in aquatic rodents such as the beaver (*Castor canadensis*) or the muskrat (*Ondatra zibethicus*) (Jellison, 1974).

In Europe and Asia only type B occurs (Bell and Riley, 1981) where it is reported from several species of lagomorphs and rodents (Jusatz, 1961a). Olsufjev (1974) reported that the water vole (*Arvicola terrestris*) was the main reservoir of the disease in the Union of Soviet Socialistic Republics (USSR).

In Sweden, tularemia is commonly named "hare plague" because of its appearance as an epizootic with high mortality in the varying hare (*Lepus timidus*) (Borg et al., 1969). However, it has been reported from several other species such as lemmings (*Lemmus lemmus*), squirrels (*Sciurus vulgaris*), small rodents and birds

(Olin, 1942; Borg and Nyström, 1960; Berglund, 1965; Rehbinder and Karlsson, 1979; Mörner and Mattsson, 1983). The disease is reported to occur mainly as an epizootic among varying hares in late summer and early autumn (Borg et al., 1969), and occasionally at other times of the year (Mörner, 1986). The disease seems to appear only in the northern and middle part of Sweden, with the exception of two islands off the Swedish coast (Mörner, 1986).

The postmortem picture in hares in Sweden dying from tularemia during epizootics has been described as rather characteristic with acute focal necrosis in the liver, spleen and bone marrow (Borg et al., 1969). The role of the varying hare in the epizootiology of tularemia in Sweden is unclear. Serological surveys of varying hares for antibodies against *F. tularensis* (Borg et al., 1969; Mörner and Sandstedt, 1983) have been negative. This may be due to the high susceptibility of varying hares to tularemia with few surviving infection, and consequently they do not act as the reservoir of the disease in Sweden.

This study was initiated to further study the occurrence of tularemia in Sweden, the postmortem picture and the susceptibility of hares to tularemia in Sweden. The study was based on postmortem examination of dead hares submitted to the National Veterinary Institute (NVI; Uppsala Sweden) during the period 1973 through 1985. In addition, an experimental study with *F. tularensis* biovar *palaearctica* in varying hares and European brown hares (*Lepus europaeus*) was conducted.

MATERIALS AND METHODS

Animals

During the period 1973 through 1985 approximately 1,500 wild hares were sent in to the NVI for postmortem examination. Approximately 50% of the hares were varying hares and 50% were European brown hares. The hares were sent in from all parts of Sweden. One hundred and nine cases of tularemia were recorded in hares during this period. The diagnosis was based on gross and histopathological examination and the fluorescent antibody (FA) test for bacteria either on fresh tissue imprints (Karlsson et al., 1970) or on formalin fixed tissue sections (Mörner, 1981).

Experimental infection

Four varying hares and three European brown hares were experimentally infected with *F. tularensis* biovar *palaearctica*. One varying hare and the three European brown hares were pen raised, while three varying hares were wild caught in the central part of Sweden (Västmanland, 59°45'N, 15°30'E), an area where tularemia was not active. The hares were caged separately in an isolated room at the NVI during the entire study period. They were fed hay, rabbit pellets (Mälardalens Lantmän, Uppsala, Sweden) and whole oats, and provided water ad libidum.

The *F. tularensis* used in this study was isolated at NVI from a varying hare which died of tularemia in the northern part of Sweden in the autumn of 1985. The organism was morphologically and biochemically characterized as *F. tularensis* biovar *palaearctica* (Eigelsbach and McGann, 1984). The bacteria were stored at −20 C and recultured on cysteine-agar medium (tryptone broth with thiamine 2.0%, cysteine-HCl 0.5%, sodium thioglucolate 0.2%, glucose 1%, agar 1% and 5% rabbit blood) prior to the experiment. The bacterial growth was suspended in physiological saline solution and inoculated intraperitoneally into white mice (NMRI strain, NVI, P.O. Box 7073, S-750 07 Uppsala, Sweden). After the mice died, 2 to 4 days after inoculation, livers and spleens were excised and cultured on cysteine agar. The reisolated growth was suspended in physiological saline solution and spectrophotometrically adjusted to densities of 1×10^3, 1×10^7 and 1×10^9 bacteria/ml.

The pathogenicity of the bacterial isolate was tested by inoculating 0.5 ml of the suspension into white mice (NRMI strain). Dead mice were necropsied and the FA test for *F. tularensis* was performed on the liver and spleen. An organ-suspension was also performed for experimental inoculation in two hares by homogenizing in saline the liver from a white mouse dead of experimental tularemia.

Route of infection, number of bacteria inoculated and the day of death/euthanasia are listed in Table 1. The two brown hares were sedated with 0.7 ml Hypnorm® (LEO, 252 42 Helsingborg, Sweden) before reinfection. The hares that did not die were killed with an overdose of pentobarbital natricum (Apoteksbolaget, Stockholm, Sweden).

Blood samples were taken from all hares before the experiment; from the two brown hares when they were reinfected and from the ani-

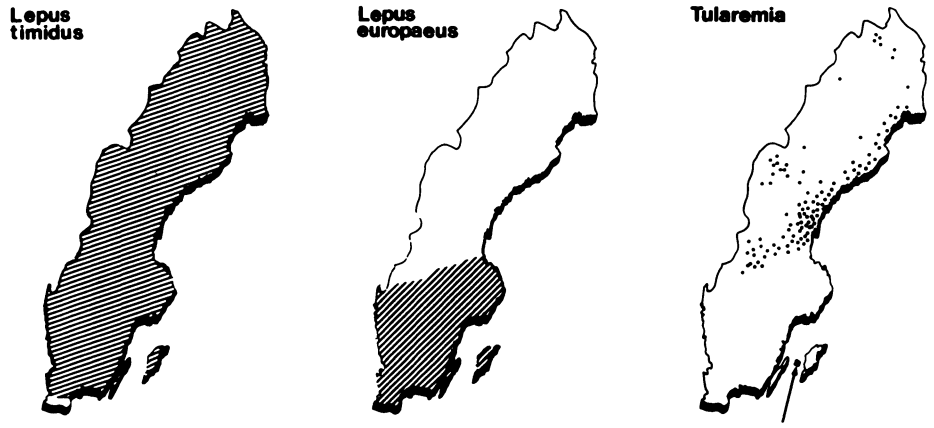

FIGURE 1. Distribution of varying hares (*Lepus timidus*) and European brown hares (*L. europaeus*) in Sweden, and of 109 recorded cases of tularemia in varying hares in the years 1973 to 1985 (each dot represents one hare that died of tularemia; the location of the island of Stora Karlsö is indicated by the arrow).

mals when they were killed. Urine and fecal samples from experimentally infected hares were tested for the presence of *F. tularensis* with a sandwich-ELISA test (Sandström et al., 1986).

Postmortem examination

The hares found dead in the field were necropsied; specimens from liver, spleen and bone marrow were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned, stained and examined histologically. If macroscopic lesions were not typical for tularemia, specimens from other organs, such as kidneys, heart, lungs, intestines, lymph nodes, muscles or brain also were examined histologically. The FA test for *F. tularensis* (Mörner, 1981) was performed on liver, spleen and bone marrow and on other organs from some animals. Specimens from lungs were taken from 18 hares for the lung extract antibody test (LEAT) (Mörner et al., 1987) for *F. tularensis*.

Experimentally infected hares (Table 1) were necropsied <6 hr after death. Specimens from liver, spleen, bone marrow, kidneys, heart, lungs, skin, muscles, regional lymph nodes, brain, thymus, intestines and intestinal lymph nodes were fixed in 10% neutral buffered formalin, paraffin embedded and examined histologically and with the FA test for *F. tularensis* (Mörner, 1981). Specimens from liver, spleen, bone marrow and lungs were examined bacteriologically. Lung tissue was taken for the LEAT for *F. tularensis*.

Bacteriology

Specimens from liver, spleen and bone marrow from 26 hares diagnosed as cases of tularemia, based on the FA test for *F. tularensis*, were cultivated on blood agar base number 2 (Difco Manual, Difco Laboratories, Detroit, Michigan 48232, USA) with 5% horse blood, and on cysteine agar (Mörner and Mattsson, 1987). Suspensions of liver, spleen and bone marrow from five of these hares were inoculated into white mice. Dead mice, or mice killed 5 days after inoculation, were necropsied and specimens were cultivated bacteriologically and FA-tested for *F. tularensis*.

Serological investigation

Sera and lung extracts were tested for the presence of antibodies against *F. tularensis* with the ELISA method as described by Sandström et al. (1986), with the exception that dilute samples of serum and lung extract were coated on the micro-plates. The antigen used was prepared from a live vaccine strain of *F. tularensis* (Sandström et al., 1984).

RESULTS

One hundred nine cases of tularemia were recorded during the study period (1973 to 1985). All the recorded cases were found in the varying hare and there were no cases observed in the European brown hare. The geographical distribution of the field cases is shown in Figure 1. All cases originated from the northern and middle part of Sweden with the exception of four cases of tularemia from the island of Stora Karlsö in the Baltic Sea.

The seasonal prevalence of tularemia is shown in Figure 2. Cases were most frequent in the autumn with 52 cases in August, 19 in September, 14 in October and

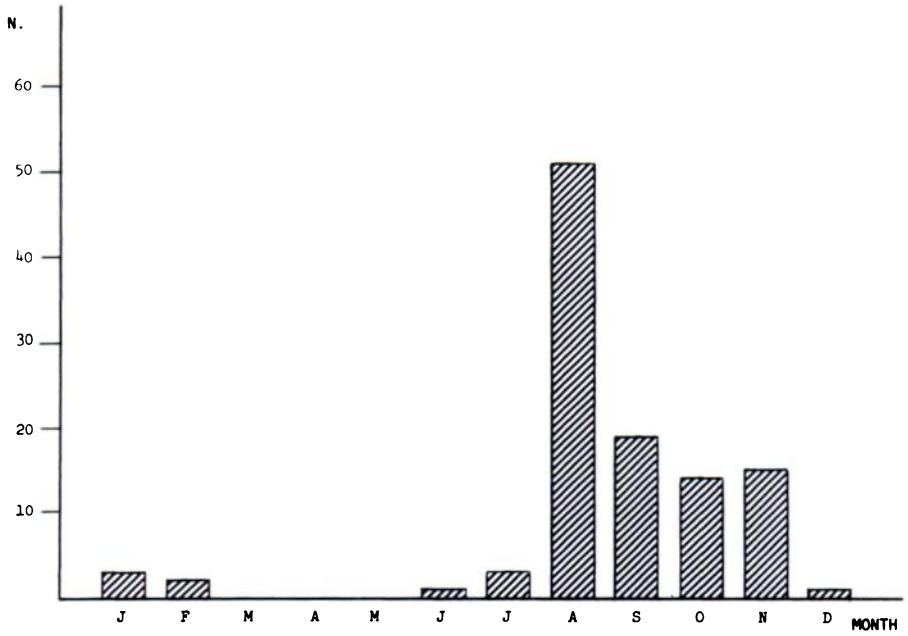

FIGURE 2. Seasonal distribution of varying hares (*Lepus timidus*) with tularemia diagnosed at the National Veterinary Institute (Sweden) during 1973 to 1985 (*n*, number of hares).

15 in November. There were no cases found in March, April and May. The cases found in December (1), January (3) and February (2) were all found in the winter of 1984/1985.

The yearly prevalence of recorded cases of tularemia is shown in Figure 3. The highest numbers of cases were seen in 1974 (28) and 1981 (37). The sex ratio in wild hares with tularemia was 49 males (45%)

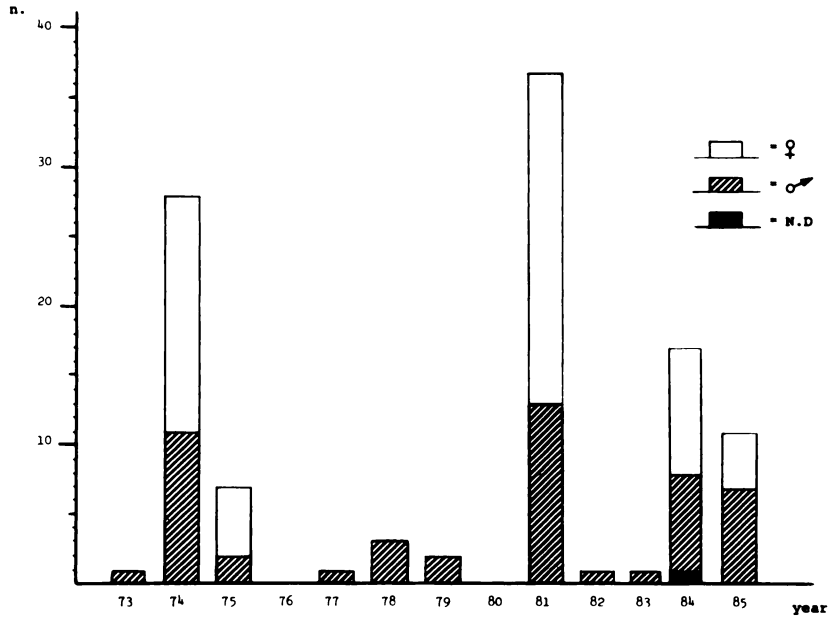

FIGURE 3. Yearly distribution and sex of varying hares (*Lepus timidus*) with tularemia diagnosed at the National Veterinary Institute (Sweden) during 1973 to 1985 (*n*, number of hares; N.D., sex not determined).

TABLE 1. Route of infection, number of bacteria inoculated, day of reinfection, and day of death/killed of varying hares (*Lepus timidus*) and European brown hares (*L. europaeus*) experimentally infected with *Francisella tularensis* biovar *palaearctica*.

Species[a]	Route of infection[b]	Number of bacteria inoculated	Day of reinfection	Number of bacteria reinoculated	Postinoculation day of death
V.H.	IM	1×10^3	—	—	5 (K)[d]
V.H.	IM	1×10^7	—	—	5 (K)
B.H.	IM	1×10^7	—	—	5 (K)
V.H.	PO	1×10^7	—	—	5 (K)
B.H.	IM	1×10^9	29	10^9	42 (K)
B.H.	IP	1×10^9	29	0.5 ml[c]	42 (K)
V.H.	IM	0.5 ml[c]	—	—	12 (D)[e]

[a] V.H. = varying hare, B.H. = European brown hare.
[b] IM = intramuscularly, PO = per os, IP = intraperitoneally.
[c] Organ suspension of in saline homogenized liver from mice dead in experimental tularemia.
[d] K = killed.
[e] D = died.

and 59 females (54%) (Fig. 3, Table 2). The sex was not determined in one hare. The sex ratio differed between different years with the highest proportion of females in the years 1974 (61%) and 1981 (65%). In the years with few recorded cases of tularemia (<4), the disease was observed only in males. The Chi-square test on the relative prevalance of tularemia in females and males submitted to NVI in the 2 yr with epizootic outbreaks (1974 and 1981) showed no statistically significant difference between the sexes ($P > 0.05$). However, if the figures from the 2 yr were tested together, using the Cochran-Mantel-Haenszel test (Cochran, 1954; Mantel and Haenszel, 1959), a statistically significant difference was demonstrated ($0.01 < P < 0.05$).

Generally, the postmortem findings in hares with tularemia were characterized by acute focal necrosis without cellular reaction in liver, spleen and bone marrow. However, the postmortem and histologic findings in the hares was not as consistent as described by Borg et al. (1969) in that hares dying during the winter months (December, January and February; $n = 6$), and hares from the island of Stora Karlsö ($n = 4$) deviated from the general pattern. Necropsy findings in hares dying from tularemia during the period July to November were similar to those described by Borg et al. (1969); a good state of nutrition and a very acute course. The most pronounced macroscopic finding was moderate enlargement of the spleen and pinpoint white necrotic foci in the liver, spleen and bone marrow. Gross lesions in other organs were generally lacking. The microscopic findings were similar also to those described by Borg et al. (1969); coagulative necrosis in the liver, red pulp of the spleen and in the bone marrow. Necrosis was occasionally seen in lymph nodes, but not in other organs such as kidneys, brain or lungs.

The FA test for *F. tularensis* on histological sections (Mörner, 1981) revealed a high concentration of bacteria in the necrotic foci in the liver, spleen and bone marrow (Fig. 4). Bacteria were demonstrated also in other organs such as lymph nodes, lungs, brain and kidneys, but in these organs bacteria were most frequently restricted to the intravascular spaces.

The six hares dying during the period from December to March were all in a poor nutritional condition. The most characteristic finding was a pronounced hemorrhagic enteritis and typhlitis. The mu-

TABLE 2. Number of females and males of varying hares (*Lepus timidus*) submitted to the National Veterinary Institute (Sweden) diagnosed with tularemia or other causes in 1974 and 1981.

	1974			1981		
	Tularemia	Other causes	Total	Tularemia	Other causes	Total
Females (F)	17	70	87	24	34	58
Males (M)	11	70	81	13	40	53
Total number of hares	28	140	168	37	74	111

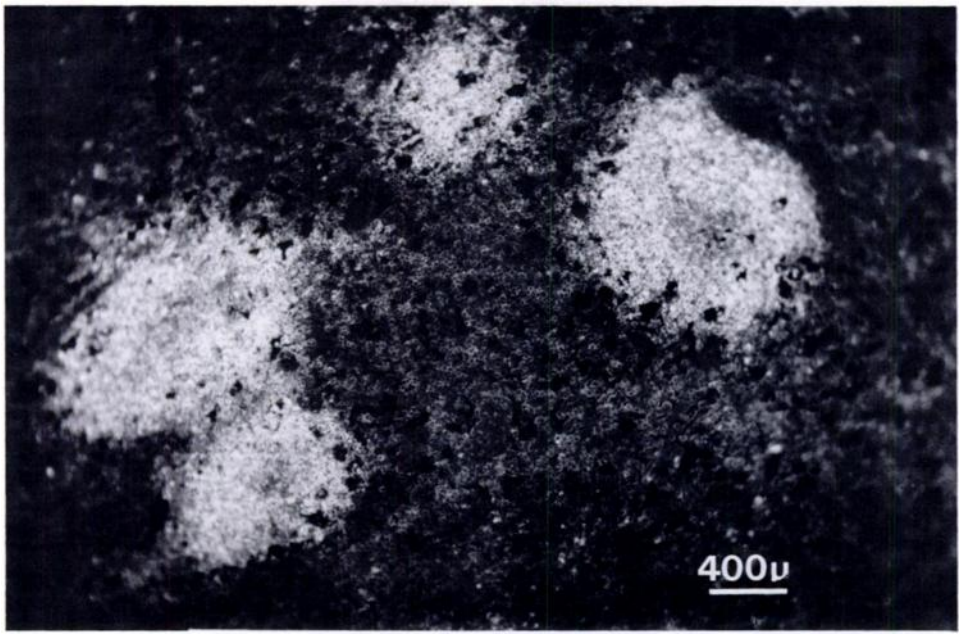

FIGURE 4. Necrotic foci in the formalin-fixed bone marrow of a varying hare (*Lepus timidus*) with positive fluorescent antibody reaction to *Francisella tularensis*.

cosa in the jejunum and caecum was congested and necrotic in various places. Acute coagulative necrosis was found generally in the liver, spleen, intestinal lymph nodes and bone marrow, but was not present in all hares. In the FA test on histological sections, *F. tularensis* was found in all organs and not concentrated in areas of

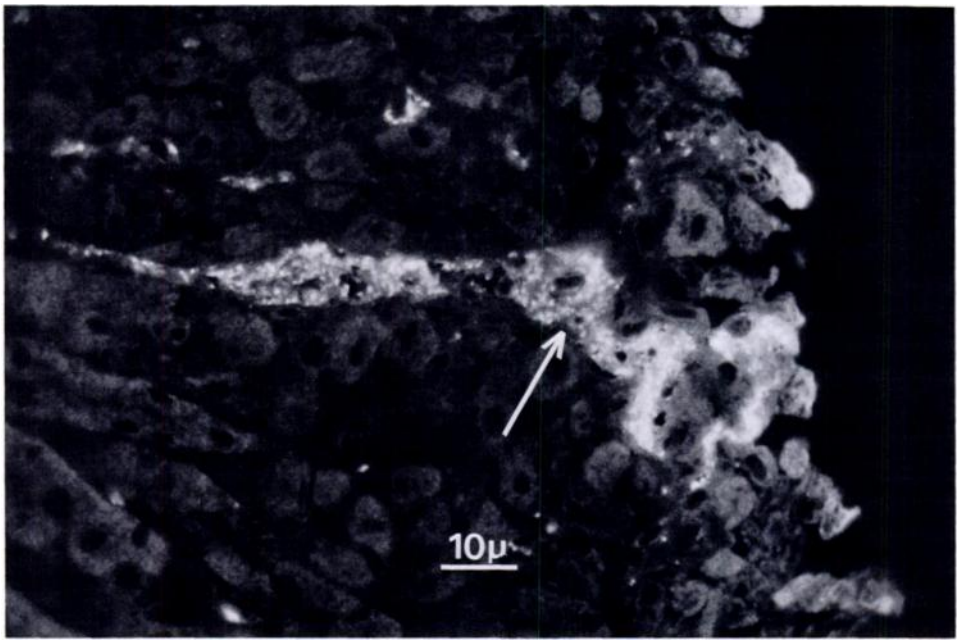

FIGURE 5. Intestines from a varying hare (*Lepus timidus*) dying during the winter months with fluorescent antibody positive *F. tularensis* in the intestinal mucosa and crypts (arrow).

necrosis. *Francisella tularensis* was demonstrated in both the epithelium and interstitial tissue of the kidneys, and also in the mucosa, crypts and lumen of the intestines (Fig. 5).

Four hares from the island of Stora Karlsö were submitted to NVI during September and October 1983 and 1984. The lesions of tularemia in hares from this island were different than those in hares from the mainland of Sweden. The nutritional status was poor. The intensity of intestinal parasites was high, and pneumonia caused by lung nematodes was observed in all four animals. The hares were found also to be heavily infested with ticks (*Haemaphysalis punctata*, *Ixodes ricinus* and/or *Ixodes trianguliceps*) around the neck. Subcutaneously to this infestation, a local phlegmon was seen around one or two ticks on each hare. Microscopically, this phlegmon was characterized by diffuse necrosis in the epidermis and in underlying tissues, with a large number of macrophages and plasma cells, and a moderate number of neutrophils and eosinophils. The axillary lymph nodes were congested and edematous and somewhat necrotic. A catarrhal enteritis was seen in all four hares. Acute coagulative necrosis was found in the liver and spleen of all four hares and in the bone marrow of two. The FA test for *F. tularensis* indicated high concentrations of bacteria in the necrotic foci in liver, spleen, bone marrow and lymph nodes. Bacteria were demonstrated also in kidneys, lungs, intestines, brain and in the vessels. The FA test on skin from the neck demonstrated high concentrations of bacteria in the phlegmons, but not in the tissue in areas around ticks where no inflammatory reaction was seen. The FA positive bacteria could be demonstrated in the blood and mouth parts of a tick attached to the middle of a phlegmon.

From three of the 26 hares examined bacteriologically *F. tularensis* biovar *palaearctica* was isolated on cysteine agar plates. *Escherichia coli* was isolated from 16 hares and in seven cases pathogenic bacteria were not found. Organ suspensions from five hares which were negative for *F. tularensis* upon primary bacteriological investigation were inoculated into white mice; *F. tularensis* biovar *palaearctica* was subsequently isolated. In the four hares in our material from the island of Stora Karlsö we isolated *F. tularensis* from two hares by mouse inoculation. The bacteria were characterized as *F. tularensis* biovar *palaearctica*, and differed in no way from the strains isolated from the mainland of Sweden. The 18 lung extracts investigated with the ELISA test for *F. tularensis* were all negative.

One of seven experimentally infected varying hares injected with 0.5 ml of the organ suspension died 12 days after infection. The postmortem findings in this hare were dominated by hemorrhagic enteritis. Necrosis was not found in liver, spleen or bone marrow. Necrosis and suppuration with a large number of neutrophils, basophils, macrophages, plasma cells and lymphocytes, but no giant cells, were found in the subcutaneous tissues at the site of inoculation. The FA test on liver, spleen, bone marrow, heart, lung, kidney, intestines, lymph nodes, brain and thymus was negative for *F. tularensis* in this hare. The FA test on muscles and skin from the site of injection was positive in the necrotic debris, but not in adjacent tissues.

Three varying hares and one European brown hare were killed 5 days postinoculation and two European brown hares were killed 42 days postinoculation. The hares were slightly depressed several days following inoculation. Other clinical signs were not observed during the experimental study.

In the varying hare infected per os, lymphocytes, monocytes and plasma cells were increased in numbers in the lamina propria of the intestines. Lymphoid hyperplasia was found in the intestinal lymph nodes. The FA test for *F. tularensis* was negative in intestines, liver, spleen, bone marrow, kidneys, heart, lungs, brain, thymus and lymph nodes.

In the five remaining hares infected parenterally, profound purulent dermatitis and myositis with a large number of neutrophils, basophils, macrophages, lymphocytes and plasma cells, but no giant cells, were found at the inoculation site. Lymphoid hyperplasia was seen generally in the regional lymph nodes and the spleen, but necrosis was not observed. The FA test for *F. tularensis* were only positive in the necrotic debris at the site of injection. Specimens taken from liver, spleen, bone marrow and lungs were negative on bacteriological examination. The ELISA test on serum was negative for antibodies against *F. tularensis* in samples collected before the experiment started and in serum samples and lung extracts collected when hares were killed. All fecal and urine samples investigated with a sandwich-ELISA test for the presence of *F. tularensis* were negative.

DISCUSSION

In Europe, tularemia is reported from both the varying and the European brown hare (Jusatz, 1961a; Olsufjev, 1974; Kemenes, 1976; Sterba and Krul, 1985). However, there are several additional reports on tularemia that mention "hares" but do not specify the species studied. The pathology of tularemia in the European brown hare is described as chronic; granulomas with central necrosis, particularly in the lungs and kidneys (Kemenes, 1976; Sterba and Krul, 1986) and occasionally in the liver, spleen, bone marrow and lymph nodes. The granulomas contain neutrophils, mononuclear leucocytes and giant cells, as well as calcified areas. This is completely different from our findings and those of Borg et al. (1969) in varying hares in Sweden; only acute necrosis in liver, spleen, bone marrow and lymph nodes was seen in these hares. We did not find a case with chronic necrosis, or necrosis or other inflammatory reactions in the lungs. Neither did we detect a case of tularemia in the European brown hare despite careful microscopic examination or using the FA test for *F. tularensis*.

To our knowledge, there are no reports from central Europe describing postmortem lesions in varying hares with tularemia, or describing the comparative pathology of tularemia in the varying hare and the European brown hare. In Finland, tularemia is found in both the varying hare and the European brown hare (B. Westerling, pers. comm.). The postmortem lesions are similar in both species, and also similar to those observed in varying hares in Sweden. Chronic cases have not been reported in either of the two species, neither in Finland, or in Sweden. The apparent difference in pathology observed in hares dead of tularemia in Sweden and Finland versus central Europe is unexplained. This could result from differences in the pathogenicity of the different strains, and/or varying susceptibility of hares in the Nordic countries compared to those in central Europe. This aspect of tularemia in Europe needs to be further examined.

The reason why tularemia is not found in European brown hares in Sweden is unknown. However, tularemia seems to occur only in areas in the middle and northern part of Sweden where brown hares are not endemic (Fig. 1). The geographical distribution of tularemia in our study is similar to that reported by Olin (1942) and Borg et al. (1969). It could be assumed that tularemia should spread southward in Sweden, as has been reported to occur in Europe (Jusatz, 1961b) and in Finland (B. Westerling, pers. comm.), but this has not happened. Jellison (1974) states that the occurrence of tularemia in humans in North America is closely related to the distribution of the cottontail rabbit. This species obviously acts as reservoir for tularemia in the United States. In Sweden animal tularemia is found predominately in the varying hare in the middle and northern part of the country (Fig. 1), and the distribution of animal tularemia also coincides with the distribution of human cases. Alternatively, the distribution of tu-

laremia and varying hares does not coincide in that varying hares commonly are found in all parts of Sweden (Fig. 1). These observations indicate that the varying hare is not the reservoir of the disease in Sweden and is only an accidental host. This opinion is supported also by results in serological surveys in varying hares in Sweden (Borg et al., 1969; Mörner and Sandstedt, 1983) where all the examined hares were negative for antibodies against *F. tularensis*.

Mörner (1986) proposed that further spread of tularemia in Sweden is limited by the absence of a suspected beaver (*Castor fiber*) reservoir in the southern part of the country. *Francisella tularensis* biovar *palaearctica* is reported to mainly have an aquatic cycle (Jellison, 1974; Morton, 1981). Based on this, it could be hypothesized that some aquatic rodent may be involved primarily in the epizootiology of the disease in Sweden. The beaver in Sweden is found in the middle and northern part of the country, and sporadically in some isolated areas in the south (Statens Naturvårdsverk, 1975). The geographic distribution of the beaver and tularemia in Sweden are very similar. Although cases of tularemia have not been found in the beaver in Sweden, a serological survey for antibodies against *F. tularensis* (Mörner and Sandstedt, 1983) indicated that 50% of these animals had positive serological titers to *F. tularensis*.

There are other aquatic rodents in Sweden that could be reservoirs for tularemia. The water vole, reported to be the reservoir of tularemia in the USSR (Pollitzer, 1963; Olsufjev, 1974) is found in most parts of Sweden. However, in contrast to the occurrence of tularemia, this rodent is more common in the southern than in the northern region of the country (Bjärvall and Ullström, 1985). Pollitzer (1963) reported that water vole related tularemia in the USSR occurs in the spring. However, our findings that the disease occurs mainly in the autumn further indicates that water voles are not important in the epidemiology of tularemia in Sweden. Another aquatic rodent, the muskrat, is only found in a limited area in the far northern part of Sweden. Cases of tularemia have not been recorded in either the muskrat or water vole in Sweden. However, in Finland the muskrat is found in most parts of the country, both in areas with and without tularemia. Tularemia has been found occasionally in muskrats in Finland (B. Westerling, pers. comm.). This may explain why tularemia is spreading in Finland; there is an abundant and suitable reservoir host. Why this does not happen with the hare populations could probably be explained by the high susceptibility to tularemia in these species. Serological surveys in varying hares for antibodies against *F. tularensis* in Sweden (Borg et al., 1969; Mörner and Sandstedt, 1983) and in Finland (B. Waltonen, pers. comm.) has always been negative, suggesting that infections in this species are often fatal. Two other species of rodents in Sweden, *Myopus schistocolor* and *Clethrionomys rufocanus*, have a geographic distribution (Bjärvall and Ullström, 1985) similar to that of tularemia. However, tularemia in these two species or in other small rodents has not been studied. Likewise, the occurrence of *F. tularensis* in vectors such as mosquitoes or ticks, or the occurrence of *F. tularensis* in water in different parts of Sweden has not been studied. This, as well as climatic factors, has to be further examined before there is an understanding of the distribution of tularemia in Sweden.

The sex ratio in populations of wild hares in Sweden is reported to be almost 1:1 (Frylestam, 1979). Thus, it could be assumed that the sex ratio in hares with tularemia would be 1:1 also. In our material we found only male hares with tularemia; there were no infected females in the years with <5 cases. However, there was an overrepresentation of females in the 2 yr with epizootic outbreaks of tularemia (Fig. 1). The explanation for the difference in sex ratio in hares with tularemia between the different years is unknown. Borg et al. (1969) observed an overrepresentation of females in the epizootic of 1967. They sug-

gested that this difference was due to a higher risk of females being exposed to infected mosquitoes. Olin (1942) reported a higher prevalence of tularemia in women than in men and that this was caused by different levels of exposure to the mosquitoes. Another explanation could be that female hares are more susceptible to tularemia at this time of the year since they normally are pregnant or just have given birth to the second litter. Sex related difference in the occurrence of tularemia in both humans and animals needs further study.

Hörnfeldt et al. (1986) stated that tularemia follows short-term cycles in small rodent populations with peaks approximately every fourth year. This is supported by the present study and data show that the yearly distribution of tularemia follows a regular cyclic pattern with high peaks in 1974 and 1981. These data plus the reported epizootic of 1967 (Borg et al., 1969) indicate a trend with high numbers of cases every seventh year, followed by years with few or no cases.

Tularemia in humans from Sweden is normally spread by mosquitoes and it can always be traced to the occurrence of tularemia in animals (Olin, 1942). The seasonal distribution of tularemia in humans (Olin, 1942) and hares (Fig. 2) is similar; the majority of cases occur in early autumn. Based on this, we believe that tularemia in hares in Sweden is normally transmitted by mosquitoes. The occurrence of tularemia in the winter indicates that other routes of infection might exist, since there are no arthropods active during the cold northern winters. The pathological findings in hares dead from tularemia during the winter with mainly gastrointestinal lesions, support this opinion. As reported from North America (Jellison, 1974) and the USSR (Pollitzer, 1963; Olsufjev, 1974) *F. tularensis* is normally found in the water in infected areas and there are reports of infections acquired from contaminated water (Jellison, 1974). It is unlikely that the hares in our study which died of tularemia during the winter got the disease from contaminated water. Hares normally do not drink water from the streams during wintertime; moreover, most streams are frozen during this time of the year. Therefore, there remains the question of whether or not infection results from direct transmission from diseased animals or alimentary infection from some other source. However, it is unlikely that hares carry tularemia subclinically after exposure to the disease earlier in the autumn considering the demonstrated high susceptibility of the varying hare to tularemia. Furthermore, unknown sources and reservoirs of the infection should not be overlooked.

The discovery of tularemia on the island of Stora Karlsö (Mörner and Krogh, 1984) was surprising. An endemic outbreak of tularemia occurred on this island in 1983 with high mortality among the hares during September and October. Stora Karlsö is located far out of the area in Sweden were tularemia is frequently found, and is a unique island in that its only terrestrial mammal is the varying hare. Among arthropods no species of mosquitoes that normally serve as vectors for tularemia are found on the island. Alternatively, the island is heavily populated with ticks (*Ixodes ricinus, I. trianguliceps, Haemaphysalis punctata*). Hares frequently are infested by these ticks which attach in a ring around the neck. Normally these ticks do not cause any inflammatory reaction. However, one of the hares we examined that had died of tularemia had a phlegmon with large number of *F. tularensis* and the FA test on one of the ticks demonstrated the bacteria. These results strongly suggest that ticks may be the vector for tularemia on the island of Stora Karlsö.

Our study is the first record in Sweden of transmission of tularemia by ticks. How tularemia was spread to the island is unknown, but it could have been introduced to the endemic population of hares by ticks carried on birds. There are two reports of tularemia from birds in Sweden; one on

birds of prey (Mörner and Mattsson, 1983) and one of ravens (*Corvus corax*) (Rehbinder and Karlsson, 1979). The island of Stora Karlsö is known as a resting place for birds migrant from the north in the autumn. Ticks of the same species as those found on hares have been found on dead birds from the island. There are no reported human cases of tularemia from the island, nor from any person visiting the island. Domestic animals are not allowed on the island.

Of interest is the more chronic nature of tick transmitted tularemia in hares from Stora Karlsö, in contrast to the acute nature of the disease in hares dying from tularemia transmitted by mosquitoes on the mainland of Sweden. Bell et al. (1979) reported that tick infestation can cause resistance to infection with *F. tularensis*. This reported resistance to tularemia may explain the difference in the nature of tularemia in these animals, rather than a possible strain difference in the virulence of the bacteria.

Our experimental infections were performed to obtain animals with tularemia from a known route of infection. The result of the experimental study was unexpected because no hares died from tularemia. The hare that died with a hemorrhagic enteritis showed no signs of a generalized infection with *F. tularensis*, nor could *F. tularensis* be demonstrated in tissues other than at the site of inoculation. The results of our experimental study are in contrast to those of Borg et al. (1969) because in their study no hare survived longer than 72 hr. The bacteria used in the study of Borg et al. (1969) was isolated from a hare which died during an epizootic and it was not reported how long the bacteria were treated or stored prior to the experiment. The survival of the hares in our study was probably due to lower virulence of the bacteria. Hood (1977) reported that solutions of sodium chloride decapsulate *F. tularensis* and make the organism less virulent. The virulence of sodium chloride-decapsulated bacteria was tested in different animals and they were less virulent when inoculated into guinea pigs, but there was no loss of virulence when inoculated into white mice. It was suggested that, when treated with saline solution, the majority of bacteria were decapsulated. The remaining capsulated bacteria were insufficient to kill guinea pigs, but were enough to be fatal for mice. This could explain why the hares in our study did not die, although the mice died during the pathogenicity tests. Another explanation of the lower virulence could be that deep freezing and reculturing of *F. tularensis* during the time the bacteria was stored at NVI had reduced the virulence of the culture. The reason for the lower virulence of the bacteria used in our study was not studied further.

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