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## EXPERIMENTAL INFECTION OF FIVE SPECIES OF RAPTORS AND OF HOODED CROWS WITH *FRANCISELLA TULARENSIS* BIOVAR *PALAEARCTICA*

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**ABSTRACT:** Sixteen raptors and three hooded crows were infected experimentally with *Francisella tularensis* biovar *palaearctica*. The birds were infected parenterally or per os. One goshawk, one sparrow hawk and one hooded crow died during the experimental period, and the remaining 16 birds were killed 14–77 days after the first infection. *Francisella tularensis* was not isolated from any bird. Antibody levels against *F. tularensis* measured in nine birds varied from 0 to 1:1,280. In one goshawk with a titer of 1:1,280, positive fluorescent antibody reactions against *F. tularensis* were seen in the liver and spleen. These results are similar to those found by other authors indicating that raptors and corvids are normally resistant to infections with *F. tularensis*.

**Key words:** Tularemia, *Francisella tularensis*, raptors, hawks, buzzards, owls, crows, *Accipiter* spp., *Buteo* spp., *Strix aluco*, *Corvus corone cornix*, fluorescent antibody test, tube-agglutination test, serology, experimental infection.

### INTRODUCTION

Tularemia is a highly infectious disease ubiquitous in the Northern Hemisphere and most commonly seen in mammals of the orders Rodentia and Lagomorpha (Jellison, 1979). *Francisella tularensis* has been found also in several other mammalian species, in birds, reptiles, fish and arthropods (Hopla, 1974; Jellison, 1974).

The occurrence of tularemia in birds has attracted particular interest because birds might serve as disseminators of the disease (Hopla, 1974; Reh binder and Karlsson, 1979; Mörner and Krogh, 1984). Tularemia in birds has been reported by Cabelli et al. (1964) and Hopla (1974) and reviewed by Jellison (1974). Naturally acquired infections seem to occur in gallinaceous birds (Green and Wade, 1929; Green and Shillinger, 1932; Kursban and Forshay, 1946; Karlovic and Aleraj, 1973) and Parker et al. (1932) reported an outbreak in sage grouse (*Centrocercus urophasianus*) in Montana, USA. Tularemia has also been found in waterfowl and Frankling gulls (*Larus pipicans*) (Stahl et al., 1969), in ravens (*Corvus corax*) (Reh binder and Karlsson, 1979) and in several species of raptors (Nakamura, 1950; Mörner and Mattsson, 1983). Dobrokhotov and Mescheryakova (1969) demonstrated *F.*

*tularensis* in faeces from raptors in an area where there was an outbreak of tularemia among small rodents.

Birds probably are exposed via bites of infected ticks, mainly of the genus *Hae-maphysalis* (Jellison, 1974). This tick is often found on rodents and lagomorphs as well as on birds.

The susceptibility of raptors and corvids to tularemia is of particular interest since these groups normally feed on sick and dead animals during tularemia outbreaks. Several studies of experimental tularemia in birds have been performed (Green and Wade, 1928; Green et al., 1928; Cabelli et al., 1964). Alonso et al. (1975) infected five common buzzards (*Buteo buteo*) per os and parenterally; no deaths occurred, although elevated titers of antibodies against *F. tularensis* were found in some birds.

While the postmortem picture in rodents and lagomorphs is well known and fairly characteristic (Borg et al., 1969; Moe et al., 1975; Bell and Riley, 1981) with focal necrosis in the liver, spleen, lymph nodes and bone marrow and moderate enlargement of the spleen it is non-specific in birds. Because of this, many cases of tularemia in birds are probably overlooked unless a specific test or cultivation on special medium is performed. The sponta-

neous cases of tularemia in birds (Green and Wade, 1929; Green and Shillinger, 1932; Nakamura, 1950; Mörner and Mattsson, 1983), seem to have been discovered by chance. Despite the few published reports, little is known about the susceptibility of birds to this disease and the post-mortem picture in these hosts. The aim of the present study was to determine the susceptibility of five species of raptors and of crows to tularemia and to describe the associated gross and microscopic lesions.

## MATERIALS AND METHODS

### Animals

Nineteen birds including 11 goshawks (*Accipiter gentilis*), one sparrow-hawk (*A. nisus*), one common buzzard (*Buteo buteo*), one rough-legged buzzard (*B. lagopus*), two tawny owls (*Strix aluco*), and three hooded crows (*Corvus corone cornix*) were used in the study. The selection of species was based on the availability of wild birds, and not on earlier findings of tularemia in these species.

The birds were caught near Uppsala, Sweden (60°00'N, 17°59'E) between early October and the end of January during 1980 through 1982. Tularemia has never been found in the area where the birds were captured, and the closest recorded case is from approximately 100 km away. Due to quarantine regulations the experimental studies had to be performed in a room designed for highly infectious diseases. Due to limited space in the experimental room only a limited number of birds could be infected at the same time, and the study was therefore performed on five different occasions, giving five groups of birds (Groups I–V). The birds were maintained 2 days prior to the experiment to allow for adjustment to captivity. They were held individually in cages during the entire study period. The raptors were fed daily with three to six freshly-killed white mice, and the crows with dead mice and moistened commercial dog-food (Triumph, AB Calcium, Häbolund, 197 00 Bro, Sweden). The birds had free access to water.

### Experimental infection with *F. tularensis*

The bacterial strain used in the study was isolated from a Ural owl (*Strix uralensis*) (Mörner and Mattsson, 1983). Upon bacteriological examination small *F. tularensis*-like colonies grew on the cysteine-agar plates. The isolates were morphologically and biochemically characterized as *Francisella tularensis* biovar *palearctica*. This was further verified by Profes-

sor N. G. Olsufjev (Tularemia Laboratory, Moscow, Union of Soviet Socialist Republics). To prepare bacterial suspensions for the experimental study the deep frozen (–20) bacterial strain was recultured on cysteine-agar medium. The growth was suspended in physiological saline solution and inoculated intraperitoneally into white mice (NMRI strain, National Veterinary Institute, P.O. Box 7073, Uppsala, Sweden). After the mice had died, which happened 3–5 days after inoculation, livers and spleens were excised at necropsy and cultured on cysteine-agar. The reisolated colonies were suspended in physiological saline solution and spectrophotometrically adjusted to a density of  $1 \times 10^7$  bacteria/ml. The pathogenicity of the bacterial suspension was checked by intraperitoneally inoculating the suspension into white mice. The mice died 2–5 days after inoculation. At necropsy a fluorescent antibody (FA) test for *F. tularensis* (Mörner, 1981) was made on liver and spleen.

The bacterial suspension was injected intramuscularly, intraperitoneally or subcutaneously into 14 of the birds. Five birds were fed with white mice which had died from tularemia (Table 1).

Group I consisted of three birds (one goshawk, one common buzzard and one rough-legged buzzard), Group II of seven birds (four goshawks, one sparrow hawk and two tawny owls), Groups III and IV each of three birds (goshawks) and Group V consisted of three hooded crows.

The birds in Group I and two crows in Group V were infected per os with mice which had died from tularemia (Table 1). The rest of the birds were infected parenterally (Table 1). Route of infection, number of bacteria inoculated, day of reinfection and day of death/euthanization are listed in Table 1. Birds that did not die were killed with an overdose of pentothal sodium. Birds in Groups III, IV and V were blood sampled prior to the experiment.

### Postmortem examination

All birds were necropsied and specimens from liver, spleen, bone marrow, heart, lung, and kidneys were fixed in 10% neutral buffered formalin and examined microscopically. Intestines were inspected macroscopically for parasites. However, due to the risk of human infection a closer examination for parasites was not conducted.

### Bacteriology

Specimens from liver and spleen (and lung from the goshawk that died at day 4) were cultivated on blood agar base No. 2 (Difco Manual, Difco Laboratories, Detroit, Michigan 48232, USA) with 5% horse blood and on cysteine-agar (tryptose broth with thiamine 2.0%, cysteine-HCl

TABLE 1. Route of infection, number of bacteria inoculated, day of reinfection, and day of death/killed of five species of raptors and of crows experimentally infected with *Francisella tularensis* biovar *palaearctica*.

Species <sup>a</sup>	Route of infection <sup>b</sup>	Number of bacteria inoculated <sup>c</sup>	Day of reinfection	Number of bacteria reinoculated	Day of death <sup>d</sup>
GH	p.o.	one mouse	14	four mice	26 (K) <sup>d</sup>
C.B.	p.o.	one mouse	14	four mice	26 (K)
R.B.	p.o.	one mouse	14	four mice	26 (K)
GH	i.m.	$0.5 \times 10^7$	—	—	4 (D)
T.O.	i.m.	$0.5 \times 10^7$	—	—	32 (K)
GH	i.m.	$0.5 \times 10^7$	—	—	32 (K)
GH	i.m.	$1.0 \times 10^7$	—	—	21 (K)
GH	i.p.	$0.5 \times 10^7$	—	—	21 (K)
T.O.	i.m.	$0.5 \times 10^7$	—	—	21 (K)
S.H.	i.m.	$0.5 \times 10^7$	—	—	21 (D)
GH	i.p.	$1.0 \times 10^7$	—	—	14 (K)
GH	i.m.	$1.0 \times 10^7$	—	—	14 (K)
GH	s.c.	$1.0 \times 10^7$	—	—	14 (K)
GH	i.m.	$1.0 \times 10^7$	—	—	14 (K)
GH	i.m.	$1.0 \times 10^7$	—	—	53 (K)
GH	i.m.	$1.0 \times 10^7$	53	$1.0 \times 10^7$	77 (K)
C	i.m.	$1.0 \times 10^7$	—	—	14 (K)
C	p.o.	three mice	—	—	24 (K)
C	p.o.	three mice	—	—	24 (K)

<sup>a</sup> GH, Goshawk; C.B., Common buzzard; R.B., Rough-legged buzzard; T.O., Tawny owl; S.H., Sparrow hawk; C, Hooded crow.

<sup>b</sup> p.o., per os; i.m., intramuscularly; s.c., subcutaneously; i.p., intraperitoneally.

<sup>c</sup> ml of a bacterial suspension with  $10^7$  *F. tularensis* per ml; the number of *F. tularensis* in the mice were not calculated.

<sup>d</sup> K, killed; D, death.

0.5%, sodium thioglycolate 0.2%, glucose 1%, agar 1.0% and 5% rabbit blood).

#### Fluorescent antibody (FA) test

Formalin-fixed liver, spleen, bone marrow and lung were examined for *F. tularensis* by the FA technique described by Mörner (1981). Also, fresh imprints from liver and spleen were examined with the FA test as described by Karlsson et al. (1970).

#### Serological investigation

Serum was tested by the tube-agglutination (TA) test (Mörner and Sandstedt, 1983) and the indirect fluorescent antibody (IFA) test. The antigen for the TA test consisted of heat-destroyed whole cells of *F. tularensis* (National Bacteriological Laboratory, Stockholm, Sweden). Titrations were made with dilutions 1:20, 1:40, 1:60, 1:80 and then in two-fold dilutions to 1:2,560. The titer was recorded as the reciprocal of the highest serum dilution that gave visible agglutination.

The IFA test was performed as follows: One drop of a solution of  $1 \times 10^7$  bacteria/ml of formalin-treated *F. tularensis* was placed on a glass slide, fixed in methanol, air dried, rinsed with water and air dried again. One drop of the test serum, undiluted or diluted to 1:10, 1:20,

1:40, 1:60, 1:80 and then in two-fold dilutions was added and the slide was then transferred to a moist chamber at 20 C for 30 min. After the slide had been washed in phosphate-buffered saline solution (PBS), pH 8.6, for 5 min and air dried, a drop of rabbit hyper-immune-anti-species (anti-goshawk- and anti-crow-serum prepared at the National Veterinary Institute, Uppsala, Sweden) serum diluted 1/20 was put on the glass, incubated at 20 C for 30 min and then washed in PBS for 5 min and air dried. One drop of FITC (fluorescein isothiocyanate)-conjugated sheep anti-rabbit serum (National Bacteriological Laboratory, S-105 21 Stockholm, Sweden) diluted 1/5 (as recommended by the manufacturer) was then added on the slide, and the slide was incubated in a moist chamber for 30 min and then washed in PBS for 5 min. One drop of phosphate-buffered glycerol, pH 8.6, was placed on the slide before it was covered with a coverslip. Serum from a specific pathogen free (SPF) rabbit was used as negative control instead of the rabbit-anti goshawk/crow serum.

Slides were examined in a Leitz fluorescent microscope with fluorescent attachment incident light, excitation 490 nm, emission 530 nm and magnification 400 $\times$ . The titer was recorded as the reciprocal of the highest serum dilution

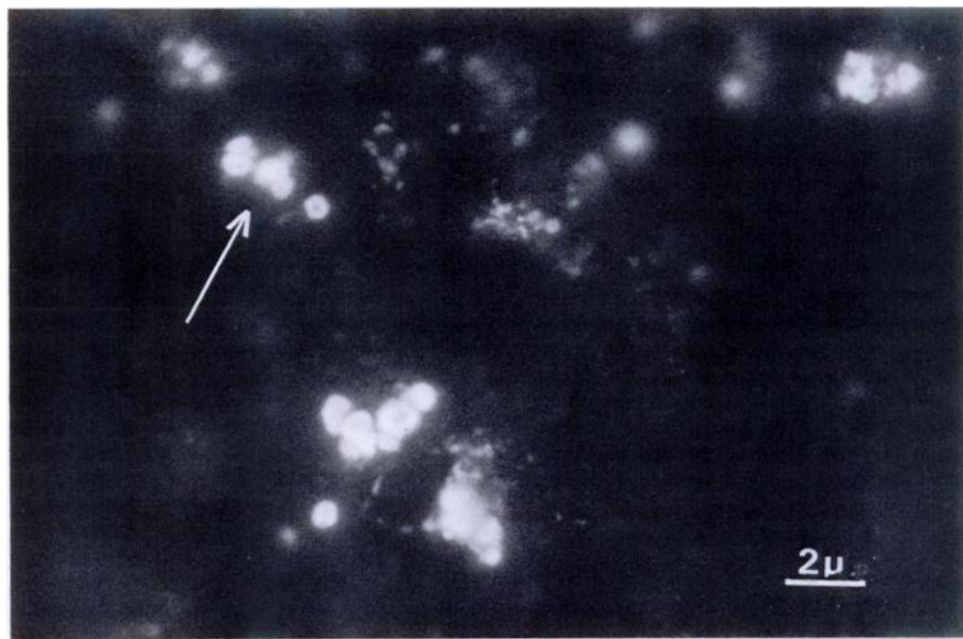


FIGURE 1. Positive fluorescent antibody reaction to *Francisella tularensis* in the spleen of a goshawk. Bacteria (arrow) occur within a germinal center.

that gave a strongly positive FA reaction from the bacteria followed by a weakly positive reaction and secondly by a negative FA reaction.

### RESULTS

Of the 19 birds used in this study, three died during the experimental period: one goshawk, one sparrow hawk, and one crow. The goshawk died on day 4 (Table 1). On postmortem examination purulent pneumonia and splenitis were seen. *Proteus vulgaris* was isolated from liver and lung, but *F. tularensis* was not isolated. The FA test on liver, lung, heart, spleen, bone marrow, intestines and kidney was negative also for *F. tularensis*. The sparrow hawk and the crow died 21 and 14 days, respectively, after infection (Table 1). Both birds were emaciated, but specific gross or microscopic changes were not seen in any organ. The FA tests on liver, spleen, kidney, lung, and bone marrow and cultures were negative for *F. tularensis*.

Gross lesions were not observed in the 16 birds that were killed. Histologically, necrosis were seen in the liver of one gos-

hawk and one tawny owl. An increased number of lymphocytes and macrophages was observed in the spleen and liver of two goshawks, one common buzzard and the rough-legged buzzard. Nephritis characterized by infiltration of lymphocytes and histiocytes was observed in one goshawk. Other microscopic changes were not found in any of the killed birds. The FA tests on formalin-fixed specimens were positive in liver and spleen of one goshawk (Fig. 1). In this bird several FA-positive bacteria were seen in leucocytes, especially in the germinal center of the spleen. All other birds were negative in the FA test on fixed specimens, and the FA tests on fresh imprints were negative in all birds. *Francisella tularensis* was not cultured from any bird.

The results of serologic examinations by tube agglutination and indirect fluorescent antibody tests in nine of the birds are listed in Table 2. The titers against *F. tularensis* were similar in the two tests on 15 occasions. On four occasions the difference was one dilution step and on two occasions a

TABLE 2. Serum antibody titers against *Francisella tularensis* in experimentally infected goshawks and hooded crows.

Animal		Day postinoculation					
		10	14	24	53	64	77
Goshawk	TA <sup>a</sup>	1:20	<1:2	— <sup>b</sup>	—	—	—
	IFA <sup>c</sup>	<1:2	<1:2	—	—	—	—
Goshawk	TA	1:1,280	1:1,280	—	—	—	—
	IFA	1:320	1:1,280	—	—	—	—
Goshawk	TA	1:20	1:160	—	—	—	—
	IFA	1:40	1:160	—	—	—	—
Goshawk	TA	—	1:60	—	—	—	—
	IFA	—	1:60	—	—	—	—
Goshawk	TA	—	1:60	1:80	1:20	—	—
	IFA	—	1:320	1:80	1:80	—	—
Goshawk	TA	—	1:320	1:160	1:20	1:320 <sup>d</sup>	1:640
	IFA	—	1:320	1:160	1:20	1:640	1:640
Hooded crow	TA	1:320	<1:2	—	—	—	—
	IFA	1:320	<1:2	—	—	—	—
Hooded crow	TA	<1:2	<1:2	—	—	—	—
	IFA	<1:2	<1:2	—	—	—	—
Hooded crow	TA	<1:2	<1:2	—	—	—	—
	IFA	<1:2	<1:2	—	—	—	—

<sup>a</sup> Tube-agglutination test.<sup>b</sup> Not done.<sup>c</sup> Indirect fluorescent antibody test.<sup>d</sup> Bird was reinjected with *F. tularensis* on postinoculation day 53.

two-fold difference was observed. The titers found in the goshawks varied between 0 and 1:1,280 and in the crows between 0 and 1:320. The highest titer was found in the goshawk that was positive in the FA test on liver and spleen tissue (Table 2).

### DISCUSSION

None of the birds in this study died from tularemia, nor did any of the birds show the pathological changes including acute necrosis in liver, spleen and bone marrow that is reported as typical of tularemia in other species (Borg et al., 1969; Bell and Riley, 1981). The necrosis found in the liver of two birds were characterized by infiltration of mononuclear leucocytes, and were negative with the FA test for *F. tularensis*.

One goshawk died of pneumonia. The only bacteria isolated from this bird was *P. vulgaris*. To our knowledge there are no previous reports of *P. vulgaris* causing

pneumonia in birds, although there are a few reports of isolation of this bacteria from birds of prey (Cooper, 1978; Halliwell and Graham, 1978). *Proteus vulgaris* is an unusual pathogen, but known to overgrow other bacteria, which could have happened in this case. The microscopic picture with a purulent pneumonia indicates a bacterial infection, but the FA test for *F. tularensis* on the lung was negative.

An active infection with *F. tularensis* was present in the goshawk with the highest antibody titers. There were no signs of parasitic infection, poor state of nutrition or any other predisposing factor to explain why this bird acquired an active infection and the others did not.

With the exception of this goshawk, antibody titers did not exceed 1:640 in any bird. In two birds, antibody titers declined by 53 days postinoculation. This is in agreement with a study of experimental *F. tularensis* infection of common buz-

zards (Alonso et al., 1975). They found titers of up to 1:100 by serological test (method not specified) 58 days after infection and all titers had declined to 0 by 69 days postinfection.

These results indicate that the species of raptors and crows studied are resistant to infections with *F. tularensis*. Reviews of tularemia in birds (Hopla, 1974; Jellison, 1974) support our findings that these birds seldom become ill from tularemia.

In Sweden, tularemia has been observed in ravens (*Corvus corax*) that appeared to be healthy (Rehbinder and Karlsson, 1979). The bacteria have also been demonstrated in two birds of prey (Mörner and Mattsson, 1983), but it was not clear in these cases whether the infection was the cause of death.

Most reports of avian tularemia in North America describe the isolation of the bacteria either from birds living in an area where an epizootic was occurring or had occurred among rodents or lagomorphs, or from human patients who have acquired tularemia from birds. There are no reports indicating that tularemia occurs as an epizootic disease among birds.

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## BOOK REVIEW . . .

**Arthropod-Borne and Rodent-Borne Viral Diseases**, WHO Technical Report Series No. 719. World Health Organization, 1211 Geneva 27, Switzerland. 1985. 116 pp. \$4.50 U.S.

This pocket-sized book is the written report of the 1983 meeting of the World Health Organization Scientific Group on Arthropod-Borne and Rodent-Borne Viral Diseases. The last published report from this WHO Scientific Group was in 1967. An updated report was needed because the number of identified viruses has more than doubled, the knowledge about the diseases and the viruses has greatly expanded, and the techniques for diagnosing viral infections and identifying viruses have changed dramatically since 1967.

This report succinctly summarizes the pertinent information on the arthropod- and rodent-borne viruses, including geographic distribution, vectors and vertebrate hosts, clinical disease and pathogenesis in humans, laboratory diagnosis, biological hazards, surveillance and control, and preparedness and emergency operations. It is a very concise report that contains a textbook-amount of information in 116 pages. The report is written such that the reader needs to have a background in arbovirology to comprehend some sections. There are no descriptions or diagrams of the natural transmission cycles of the viruses, although there are tables listing the viruses, the types of vectors involved

and the clinical symptoms of diseases these viruses cause. The report contains much useful information on the viral diseases that are of the most public health concern in the various regions of the world.

The report emphasizes the viruses, diagnoses, clinical disease in humans, and vectors and vector control. There is very little information on the involvement or role of vertebrates in these viral diseases, but information on rodent control is provided. There are good sections on emergency and long-term vector control and the research needs for vector control. The sections on pathogenesis and the diseases and their management are also quite informative. Although the list of references is short and seemingly incomplete for the broad range and depth of the subjects discussed, the members of the scientific group writing the report were able to provide information from their own fields of expertise.

It is an inexpensive book that contains valuable information on many important viral diseases in the world. It is a handy reference and an excellent summary of the current knowledge of and research needs for these viral diseases.

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