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## **$\beta$ HEMOLYTIC STREPTOCOCCAL INFECTION IN RED FOXES (*VULPES VULPES* L.) IN FRANCE: THE NATURAL DISEASE AND EXPERIMENTAL STUDIES**

J. Barrat,<sup>1</sup> J. Blancou,<sup>1</sup> C. Demantke,<sup>2</sup> and Y. Gerard<sup>1</sup>

**ABSTRACT:**  $\beta$  hemolytic streptococcal infections, usually of group G and C, were identified in red foxes in France. In a study of 31 animals, septicemia and jaundice were found to be the main signs of the disease. Gross and microscopic lesions consisted of generalized inflammation of viscera and joints, jaundice, cellulitis and abscesses of spleen, liver, lungs and kidneys. The disease was reproduced in foxes by intramuscular inoculation of less than the minimal quantity of bacteria lethal to mice. When challenged, recovered animals were resistant to infection that proved to be lethal to control animals.

### **INTRODUCTION**

In Europe, streptococcal infections in red foxes have been reported by Grini (1945), Ross and Fairley (1969), Blancou et al. (1982) and Blackmore (1964). The latter author reported that 13% of natural deaths of 60 wild foxes in Great Britain were caused by streptococci.

The results of our observations on the disease in foxes in France indicate that the natural disease is widespread, that certain types of streptococci are pathogenic for foxes, and that prior streptococcal infection can result in immunity.

### **MATERIALS AND METHODS**

In France, our study centre has kept, for experimental purposes, an average of 50 wild foxes for the past 12 yr. The animals were captured in April–May in three regions of France (Massif Central, Bretagne and Normandie). Over 10% of the animals have died each year of primary or secondary bacterial infections. Twenty-one of these foxes died of streptococcal infection during the 6-mo mandatory quarantine period.

Of the 21 strains of  $\beta$  hemolytic streptococci isolated from foxes dying of the natural disease, two strains grouped by the Lancefield system (one G and one C) were used for experimental

infection. Inocula were prepared from Bio-Streptosef broth (Biomérieux-69260 Charbonnières-les-Bains, France) incubated 48 hr at 37 C. One ml of broth was inoculated by either the intramuscular (in the thigh) or the intravenous route. The inoculum contained various quantities of live streptococci (see Tables 1 and 2 for details), as determined following bacterial counts on nutrient agar.

Thirty-one red foxes (including 13 males and 16 females) 14 mo old, kept in captivity since they were captured, were used for pathogenicity experiments. Those which survived were re-infected 1 mo later for assessing possible post-infection immunity.

### **RESULTS**

#### **Natural disease**

During the mandatory quarantine period of 6 mo prior to placement of the foxes in the experimental area (Kazandjian, 1977), nine males and 12 females were found to be infected with streptococci and eventually died. Irrespective of the streptococcal group causing the disease, this natural disease was characterized by septicemia (anorexia, fever, depression), usually with generalized hemolytic jaundice (yellowish-orange mucosa) and incoordination. In most cases, the extremely brief course of the disease (24 to 48 hr) precluded specific antibiotic therapy, although the strains were usually susceptible in vitro to penicillin and erythromycin. Microscopic examination of the primary focal lesion in muscle revealed suppura-

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TABLE 1. Death rates and intervals between inoculation and death in red foxes inoculated by intramuscular or intravenous route with various quantities of group G or group C streptococci.

Streptococcus group	Route of inoculation	Quantity inoculated	Foxes dying	Interval between inoculation and death (days)
			(No. dead/no. inoculated)	
C	Intravenous	$8 \times 10^8$	1/2	12
		$8 \times 10^7$	0/2	—
		$8 \times 10^6$	0/2	—
	Intramuscular	$8 \times 10^8$	0/2	—
		$4 \times 10^8$	0/2	—
		$2 \times 10^8$	0/2	—
G	Intravenous	$9 \times 10^8$	2/2	3-3
		$9 \times 10^7$	2/2	4-5
		$9 \times 10^6$	2/2	5-6
		$1.4 \times 10^6$	1/1*	8
		$1.4 \times 10^5$	0/1*	—
		$1.4 \times 10^4$	1/1*	30
		$1.4 \times 10^3$	0/1*	—
	$1.4 \times 10^2$	0/1*	—	
	Intramuscular	$8 \times 10^8$	2/2	3-5
		$4 \times 10^8$	0/2	—
$2 \times 10^8$		1/2	7	
		$1.4 \times 10^8$	2/2*	5-9

\* Red foxes used as controls during the challenge of those which survived this first inoculation (Table 2).

tive cellulitis. Small abscesses, which were not walled off and which contained masses of streptococci, were seen in spleen, liver, lungs and kidneys. In 50% of the cases, there was localized inflammation of connective tissue and muscles adjacent to a skin injury which was the probable portal of entry of the infection.

Streptococci were usually isolated in pure culture from the blood of sick or dead foxes, but were sometimes contaminated with *Escherichia coli* in other organs. The strains showed classical characteristics of streptococcal colonies. Their biochemical reactions were also characteristic of the genus (hydrolysis of starch, arginin, seculin, solatin) with some differences as far as trehalose is concerned (positive reaction with group C, negative with G).

TABLE 2. Post-infection immunity as indicated by death rates and intervals between inoculation and death in red foxes challenged\* after having survived a previous inoculation of group C or G streptococci.

Streptococcus group	Route	Quantity	Previous inoculation (Table 1)	Foxes dying	Interval between inoculation and death (days)
			(No. dead/no. inoculated)		
C	Intravenous	$8 \times 10^8$	1/1	16	
		$8 \times 10^7$	0/2	—	
		$8 \times 10^6$	1/2	8	
	Intramuscular	$8 \times 10^8$	2/2	8-12	
		$4 \times 10^8$	2/2	5-8	
		$2 \times 10^8$	1/2	16	
G	Intramuscular	$4 \times 10^8$	1/2	13	
		$2 \times 10^8$	1/1	16	

\* An inoculum of  $1.4 \times 10^8$  group G streptococci was used by intramuscular route. Different control foxes were used; see Table 1.

#### Experimental studies

**Group G Streptococcus:** Inoculation by the intravenous route resulted in the death of all six foxes inoculated, after various intervals of time depending on the quantity inoculated. However, when the intramuscular route was used, only three of the six foxes inoculated died (Table 1).

**Group C Streptococcus:** Inoculation by the intravenous route resulted in the death of only one out of six foxes. Intramuscular inoculation was not lethal to any of the six foxes inoculated (Table 1).

The dead foxes presented the same signs and lesions as the animals that had died of the natural disease.

At necropsy macroscopic findings included generalized inflammation of viscera in all cases. Hemolytic jaundice and lesions of joints, liver, spleen and kidneys were observed. Arthritis was frequent with an hyperemic and edematous synovial membrane and the joint cavity was sometimes filled with a fibrinous material. The liver was enlarged with degeneration zones. Histologically centrolobular fatty degeneration and interstitial hepatitis with

microabscesses were often noted. Most of the time, spleen and kidneys were observed with multiple small abscesses and congestive areas. Histologically these abscesses were surrounding bacterial colonies, the spleen showing also hypoplasia of lymphoid follicles, foci of non-encapsulated purulent necrosis along with purulent and edematous areas with intra- and extravascular bacterial colonies.

Streptococci were re-isolated (for grouping) in all cases, in pure culture, from heart blood and from all lesions observed. Those animals which survived, after transitory signs of illness such as anorexia and slight hyperthermia, developed scars at the sites of intramuscular inoculation.

The three foxes that survived inoculation with group G streptococci and the 11 animals that survived the group C inoculation were challenged with an intramuscular injection of a quantity of group G streptococci that killed two other unexposed control foxes.

One out of the three foxes in the first group and four out of the 11 foxes in the second group resisted the challenge (Tables 1 and 2).

#### DISCUSSION

Streptococcal infection appears to be a common disease in wild foxes. This confirms data presented by the authors cited above. These infections are probably a major cause of death during the first year of the fox's life. Seventy to 75% of young foxes do not reach adulthood (Artois, 1979).

This infection appears to be relatively

specific to the vulpine species, in view of the fact that streptococci were not frequently found in other wild carnivores submitted for analysis in our study centre during the same period.

Non-lethal group C or G streptococcal infections in adult foxes appear, on the other hand, to prevent reinfection by a *Streptococcus* of the G group. This could limit the spread of epizootics in the wild, as it does in captive foxes. A carrier state of streptococcal infection might also be possible in foxes, as suggested by other observations at our field station.

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