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Source: Journal of Wildlife Diseases, 31(4) : 541-544

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-31.4.541

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Journal of Wildlife Diseases, 31(4), 1995, pp. 541–544
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Antibody Response to Rabbit Viral Hemorrhagic Disease Virus in Red Foxes (*Vulpes vulpes*) Consuming Livers of Infected Rabbits (*Oryctolagus cuniculus*)

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ABSTRACT: Six red foxes (*Vulpes vulpes*) were given oral doses of homogenized liver from rabbits (*Oryctolagus cuniculus*) that died from rabbit viral hemorrhagic disease (RVHD) and four control foxes were given liver from uninfected rabbits. Antibodies to RVHD virus were monitored over 6 months. There was a pronounced antibody response 7 days after exposure which persisted to 14 days and then diminished. Low titers still were evident in three foxes at the end of the experiment. Based on these results, fox serum may be useful as an index of the prevalence of RVHD in sympatric rabbit populations.

Key words: Red fox, *Vulpes vulpes*, rabbit viral hemorrhagic disease, calicivirus, antibody, immune response.

Rabbit viral hemorrhagic disease (RVHD) is an acutely lethal, highly contagious disease caused by a calicivirus (Ohlinger and Thiel, 1991). It first became evident in western Europe in 1986 and now appears to affect wild rabbit (*Oryctolagus cuniculus*) populations over their entire range in Europe. While the pathology and etiology of the disease are well understood, the epizootiology and effect on wild rabbit populations is less well known (Marcato et al., 1991; Gavier-Widén, 1992). Prevalence in rabbit populations is difficult to estimate because of the high case-fatality rate of the disease and difficulty in finding sick or dead rabbits in the field (Villafuerte et al., 1994). Serological data are difficult to interpret because of the possibility that non-pathogenic but closely-related caliciviruses may be present in rabbit populations in Europe and may cross-react with RVHD virus in currently-available tests (Morisse et al., 1991; Rodak et al., 1990). Our objective was to determine whether the red fox (*Vulpes vulpes*) would produce antibodies against the virus of RVHD after consuming an infected rabbit. This was done as a preliminary investigation of the possibility of using antibodies in serum of foxes or other predators as an index of the prevalence of RVHD among rabbits sharing their habitat.

Ten male red foxes, 3 to 4-mo-old, were received from a commercial fox breeder (M. Borget, Ramasse, Ceyzeriat, France) and housed in 110 × 77 × 19 cm fox cages (Scanstar France, Villemandeur, France) for several weeks at the research station of the Centre National d'Etudes Vétérinaires et Alimentaires near Nancy, France (CNEVA-Nancy). On 1 December 1993, six foxes were selected at random and moved to cages in an isolation building. They were anesthetized with 1.5 ml ketamine-chlorobutanol (Imalgene-500®, Rhone-Merieux, Lyon, France) and 5 mg acepromazine (Vetranquil®, Sanofi Santé Nutrition Animale, Libourne, France) administered intramuscularly, and were given by stomach tube 50 ml of a homogenate of pooled infected rabbit livers equivalent to 40 g, or approximately one half of an adult rabbit liver. Homogenate also was painted on all surfaces of the oral cavity and pharynx with a brush. The four control foxes remained in the outdoor facility. Each was treated in a manner identical to the infected foxes except that liver from rabbits not infected with RVHD virus was

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used. One month after exposure, the six foxes were removed from the isolation facility and thereafter were maintained in the same outdoor facility as the control foxes. All 10 foxes received the same commercial food (Playdog® Sprint, from SARC, Crevecoeur-sur-Escaut, France) before and during the experiment. All feed was free of rabbit tissues.

The infected livers used in the experiment were from a stock of frozen livers held at the rabbit disease laboratory of CNEVA-Ploufragan and originating from experimentally-infected rabbits that died of RVHD. The homogenate was prepared at Ploufragan and was transported directly to Nancy on ice without further freezing. The presence of virus in the liver homogenate was confirmed by an ELISA assay developed at CNEVA-Ploufragan. Anti-RVHD virus sera raised in chickens and rabbits served, respectively, to capture and to identify RVHD virus antigen. Anti-rabbit IgG, conjugated with alkaline phosphatase (Sigma Chemicals, St. Louis, Missouri, USA) was used to identify specific antibody bound to virus antigen. The lower limit of detection of virus antigen in this assay was a difference in optical density (OD) of 0.1 between assays run with positive and negative rabbit sera. By this ELISA, the homogenate of livers from infected rabbits produced an OD of 1.2 at a dilution of 1/40. Frozen livers of rabbits from a RVHD-free colony at CNEVA-Ploufragan also were transported to CNEVA-Nancy where they were thawed and homogenized on the day of experimental exposure of the foxes.

Jugular blood samples were taken from the 10 foxes 11 days before and 8, 15, 35, 57, 85, 113, and 176 days after exposure to rabbit liver. Hemagglutination-inhibition antibody titers to RVHD virus were measured at the Division of Wildlife, National Veterinary Institute, Uppsala, Sweden by the method of Gavier-Widén and Mörner (1991). Competition ELISA titers for antibodies to both RVHD virus and European brown hare syndrome (EBHS) virus, a closely-related calicivirus, were measured at the Instituto Zooprofilattico Sperimentale in Brescia, Italy, the reference laboratory of the International Office of Epizootics (Paris) for viral hemorrhagic diseases of rabbits and hares, by the method of Capucci et al. (1991).

Prior to exposure to RVHD virus on 1 December, all foxes were without antibodies to RVHD or EBHS viruses. Throughout the experiment, all control foxes remained negative by hemagglutination-inhibition or ELISA. There was a marked antibody response 7 days after exposure to the infected rabbit tissues (Table 1). In general, titers remained high during the second week after exposure and then slowly diminished. However, titers to RVHD virus were maintained for the entire 6-mo-period of the experiment in three of the six foxes. During the month when both were measured, hemagglutination inhibition titers always were higher than were titers of anti-RVHD virus antibodies measured by ELISA. In five of six foxes there was an inversion of the titers of anti-RVHD virus versus anti-EBHS virus antibodies such that the latter rose as the former declined. Throughout the experiment, all foxes remained clinically healthy.

In this experiment, red foxes responded immunologically to the virus of RVHD after a single oral exposure and the response was readily detectable 7 days after exposure. Titers declined but persisted for up to 6 mo in half of the exposed foxes. These results provide support for the future use of fox serum as an index of the prevalence of RVHD in sympatric rabbit populations. Further studies are needed, however, before antibody titers to RVHD virus in foxes can be interpreted with precision in this context. The magnitude and duration of the antibody response after both single and multiple exposures to the virus require further experimental study. Serological procedures that can distinguish between antibodies to the viruses of RVHD and EBHS also may be needed since these diseases occur together over much of Europe (Mo-

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TABLE 1. Antibody titers in the serum of red foxes each given a single oral dose of a homogenate of livers from rabbits that died of acute rabbit viral hemorrhagic disease. The foxes were given infected liver on 1 December 1993.

Fox	Test	Time of blood collection (days after exposure)						
		8	15	35	57	85	113	176
A	HI[a]	1:160	1:40	1:10	ND[b]	ND	ND	ND
	RVHD	1:30	1:30	Neg.[c]	Neg.	Neg.	Neg.	Neg.
	EBHS	Neg.	1:10	Neg.	Neg.	Neg.	Neg.	Neg.
B	HI	1:320	1:160	1:80	ND	ND	ND	ND
	RVHD	1:40	1:40	1:40	1:40	1:20	1:20	1:20
	EBHS	Neg.	1:10	1:40	1:160	1:40	1:40	1:40
C	HI	1:640	>1:640	1:160	ND	ND	ND	ND
	RVHD	1:60	1:20	1:20	1:10	1:10	<1:10[d]	<1:10
	EBHS	1:10	<1:10	1:20	1:60	1:80	1:80	1:80
D	HI	>1:640	>1:640	1:40	ND	ND	ND	ND
	RVHD	1:80	1:40	Neg.	Neg.	Neg.	Neg.	Neg.
	EBHS	1:10	1:10	Neg.	1:10	1:10	<1:10	Neg.
E	HI	1:320	1:80	1:40	ND	ND	ND	ND
	RVHD	1:20	1:40	1:20	1:20	1:20	1:20	1:20
	EBHS	<1:10	1:20	1:20	1:60	1:60	1:40	1:20
F	HI	1:1,280	1:160	1:80	ND	ND	ND	ND
	RVHD	1:160	1:40	1:10	1:10	1:10	1:10	1.20
	EBHS	1:40	1:30	1:20	1:40	1:80	1:80	1.80

[a] HI, antibody titer by hemagglutination inhibition; RVHD, ELISA for anti-rabbit viral hemorrhagic disease virus antibodies; EBHS, ELISA for anti-European brown hare syndrome virus antibodies.
[b] ND, not done.
[c] Neg., negative.
[d] <1:10 are sera for which the presence of antibodies was uncertain.

risse et al., 1991). It also will be necessary to determine whether wild foxes in areas of interest are exposed to other caliciviruses. Neutralizing antibodies to San Miguel sea lion virus, for example, have been detected in grey foxes (*Urocyon littoralis*) on one island off the Pacific coast of the United States (Prato et al., 1977).

We did not determine whether RVHD virus infected, and replicated in, the exposed foxes. None of the exposed foxes became ill or died; similar results have been reported for dogs exposed to RVHD virus (Simón et al., 1994). Although the dose was not quantified precisely, it is likely that the exposed foxes received large amounts of virus antigen (Nowotny et al., 1990). While there is no *a priori* reason to suppose that actual infection of the foxes took place, the question could be of interest with respect to the epizootiology of the disease and the maintenance of the virus in nature. The inversion of titers against the viruses of RVHD and EBHS was interpreted as a qualitative change over time in the antibody response of the foxes, with production of increasingly cross-reactive antibodies. In the ELISA assay used, cross-reactive epitopes on the EBHS antigens are more reactive than those on the RVHD antigens because of differences in their structures after purification (Capucci et al., 1991). This may account for the apparent inversion of titers.

This study was supported by the Ministries of Agriculture of France and Sweden, by CNR-Italy (BTBS, 9201280-PF70) and by the University of Saskatchewan, Canada through a sabbatical leave accorded to the senior author. We thank Jacqueline Bailly, François LeDuc, Olivier Guillaumont and Evelyne Sagné (CNEVA-Nancy), Ghislaine Le Gall and Eric Boilletot (CNEVA-Ploufragan), Esteban Ri-

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vera and Helena Reineck (Sweden) and Giuliana Botti (Italy) for their assistance.

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Received for publication 7 July 1994.

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