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ANTIBODIES AGAINST RABBIT HEMORRHAGIC DISEASE VIRUS IN FREE-RANGING RED FOXES FROM GERMANY

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ABSTRACT: Antibodies against rabbit hemorrhagic disease virus (RHDV) from 352 red fox (*Vulpes vulpes*) sera collected in Germany (Mecklenburg-Vorpommern) in 1993 were tested by a blocking enzyme-linked immunosorbent assay (ELISA) test kit. Ninety samples with positive or suspected results also were analyzed by the hemagglutination inhibition test (HIT). Eighteen serum samples (5%) were positive with the blocking ELISA and eight of these also were positive with HIT. The 18 positive sera also were tested by blocking ELISA for antibodies against European brown hare syndrome virus (EBHSV) and by sandwich ELISA to detect fox antibodies against RHDV and EBHSV antigen. Antibodies were not found against EBHSV using the blocking ELISA. With the sandwich ELISA, six samples were positive against RHDV and also against EBHSV, indicating cross-reactivity between determinants of both viruses. However, antibody titers against RHDV were higher than against EBHSV in five samples, and in one animal titers were similar. In addition, two positive samples were investigated by Western blot immunoassay which showed clear positive reactions with the two main peptide bands of EBHSV and RHDV. Comparison of the areas below the peaks of the bands after immunoblotting indicated that there was a stronger reaction with the two main polypeptides of RHDV than with the two main peptides of EBHSV. This is the first report of calicivirus antibodies in free-ranging red foxes. Based on the specificity of the tests, the antibodies detected against caliciviruses may be induced by RHDV. There is a potential link for RHDV between free-ranging rabbits and foxes.

Key words: Caliciviruses, comparative immunological testing, European brown hare syndrome virus, rabbit hemorrhagic disease, red foxes, serological survey, *Vulpes vulpes*.

INTRODUCTION

Carnivores come into contact with many infectious agents due to their position at the top of the food chain and are potential carriers of some infectious agents that occur in their prey species. For most of these infections, the predators do not develop clinical signs or are not susceptible to the diseases (Dedek, 1995).

If the kinetics of antibody development in top carnivores to certain pathogens of their prey are known, conclusions may be drawn about the presence of infected prey in an area by testing carnivores for evidence of exposure. The role of top carnivores in the spread and maintenance of infectious diseases is of great interest. In Germany, red foxes (*Vulpes vulpes*) are of special interest, because they are very adaptable in respect to habitat and choice of prey (Zimen, 1981). We investigated exposure of free-ranging red foxes to rabbit hemorrhagic disease virus (RHDV) and European brown hare syndrome virus (EBHSV). Foxes feeding on infected rabbits might contribute to the spread of the disease among rabbits by carring the virus to new locations via feces.

Rabbit hemorrhagic disease was first observed in China in 1984 (Liu et al., 1984). Since 1988 RHDV and EBHSV have been reported in many European countries (Morisse et al., 1991; Frölich et al., 1996). European brown hare syndrome virus and RHDV are caliciviruses (Ohlinger and Thiel, 1991) and only lagomorphs are susceptible to experimental infections with RHDV and EBHSV (Smid et al., 1991; Xu, 1991). Rabbit hemorrhagic disease virus is spread by oral and nasal transmission (Xu and Chen, 1989); the fecal-oral mode of transmission is probably the most important one in the field (Morisse et al., 1991). There is no evidence of natural exchange of caliciviruses between hares (*Lepus* spp.) and rabbits (*Oryctolagus cuniculus*) (Gavier-Widen and Mörner, 1993).

Leighton et al. (1995) gave red foxes oral doses of homogenized liver from rabbits which died of RHD. There was a pronounced antibody response 7 days after exposure. In general, these titers remained high during the second week after exposure and then slowly diminished. However, low titers were maintained for the entire 6-mo-period of the experiment in three of the six foxes. All foxes remained clinically healthy throughout the experiment. Based on these results the authors felt that monitoring of RHD antibodies in foxes could be used to provide information on the spread of this disease in sympatric rabbit populations (Leighton et al., 1995). In this study, our objective was to determine whether free-ranging red foxes in Germany have antibodies against RHDV or EBHSV.

MATERIALS AND METHODS

The study area of Mecklenburg-Vorpommern is in northern Germany (53°10′N to 54°50′N, 10°90′E to 14°10′E) and covers an area of 24,000 km^2. Rabbit hemorrhagic disease was first detected in rabbits in Mecklenburg-Vorpommern in 1988 (Schlüter, 1988); no information is available regarding occurrence of EBHS in this area. Our samples came from throughout the region and included 352 red foxes hunted in 1993. Blood was collected immediately after death. After centrifugation, serum samples were inactivated at 56 C for 30 min and stored at −20 C.

We used RHDV and EBHSV antibody-blocking enzyme linked immunosorbent assay (ELISA) test kits according manufacturer instructions (Danish Veterinary Institute for Virus Research, Kalvehave, Denmark). Sera were tested at four dilutions: 1:4, 1:10, 1:100, and 1:1,000, and analyzed as quadruplicates. Briefly, 50 µl of the diluted serum sample were added to rabbit IgG anti-RHDV or IgG anti-EBHSV precoated wells followed by 50 µl of either RHDV or EBHSV, giving an optical density (OD) value of about 1.0 with negative serum. These virus antigens were processed as follows: liver and spleen of an infected hare or rabbit 4 days post infection were suspended in phosphate-tween salt (0.5 M) buffer solution (pH 6.0), triturated and centrifuged (3,200 g, 10 min). This supernatant was used as diagnostic material and was kindly provided by L. Ronsholt, Danish Veterinary Institute for Virus Research, Kalvehave, Denmark. Dilutions of the supernatant were suitable to detected antibodies in all ELISA's used in this study, according to Frölich et al. (1996). Following an incubation period overnight at 5 C and a washing step, immunosorbent treated guinea-pig anti RHDV hyperimmune serum or guinea-pig anti EBHSV hyperimmune serum (detecting serum) was added to two of the wells, and the same concentration of normal guinea-pig serum to the other two wells per test sample. Following another incubation period of 30 min at 37 C and another washing procedure, the wells were further incubated with rabbit anti-guinea-pig peroxidase conjugate (Denmark code P141, DAKO Diagnostik GmbH, Hamburg, Germany). After washing, the fixed enzymes reacted for 10 min with *o*-phenylene-diamine (OPD) substrate including perhydrol (Sigma-Chemie GmbH, Deisenhoven, Germany). The reaction was stopped with H_2SO_4 and the results were read at 492 nm on a MTF10 spectrophotometer (Wissenschaftlicher Gerätebau, Berlin, Germany). Sera from three farmed foxes were used as negative controls. The mean OD of the negative control sera was calculated. Sera that deviated >3 standard deviations from the mean of negative control sera were considered antibody positive.

An indirect sandwich ELISA was established to verify the detection of the antibodies against RHDV or EBHSV, as well as for comparing RHDV and EBHSV cross-reactivity. All positive samples by the blocking ELISA, as well as three negative farmed fox sera, were studied. Briefly, microtiterplates (Costar, Cambridge, Massachusetts) were coated with RHDV or EBHSV solutions (50 µl 1:500 in phosphate buffered saline, PBS) overnight at 4 C. After washing (PBS, 0.05% Tween 20) and blocking with PBS containing 2% bovine serum albumin (Serva Feinbiochemica GmbH, Heidelberg, Germany), fox sera were added in doubling dilutions from 1:100 to 1:12,800 and the plates were incubated at 22 C for 2 hr. After another washing procedure, 50 µl of an affinity isolated rabbit anti-dog IgG (Sigma, No. D-8650; 1:500) was added and incubated for 2 hr. A strong cross-reactivity of about 80% between fox and dog IgG was shown by immunoelectrophoresis. The plates were washed again and the bound antibodies were incubated for 2 hr with 50 µl of an affinity isolated goat anti-rabbit globulin peroxidase conjugate (Sigma, No. A-6154; 1:2,000). After washing, the peroxidase activity was detected using OPD and hydrogen peroxide as substrate. The absorbance was determined at 492 nm using a MTF 10 spectrophotometer. Optical densities of at least twofold of the negative control (test without antigen) were

TABLE 1. Reciprocal antibody titers of 18 positive red fox sera tested against rabbit hemorrhagic disease virus and European brown hare syndrome virus by blocking ELISA, hemagglutination inhibition test, and sandwich ELISA.

Animal number	Blocking ELISA[a]		HIT[b]	Sandwich ELISA	
	RHDV	EBHSV		RHDV	EBHSV
1	10	—	—	—	—
2	10	—	160	—	—
3	10	—	40	—	—
4	10	—	160–320	—	—
5	10	—	160	—	—
6	10	—	—	—	—
7	10	—	—	—	—
8	100	—	320	1,600	800
9	10	—	—	—	—
10	10	—	—	—	—
11	10	—	—	—	—
12	10	—	—	—	—
13	10	—	—	800	400
14	100	—	—	6,400	6,400
15	10	—	—	—	—
16	10	—	80	3,200	400
17	10	—	80	200	100
18	1,000	—	>640	6,400	3,200

[a] ELISA = enzyme-linked immunosorbent assay.
[b] HIT = hemagglutination inhibition test.

classified as positive reactions. We compared the OD obtained for EBHSV and RHDV with sandwich ELISA for each fox by a Wilcoxon test (Sachs, 1992). The significance level was set at $\alpha = 0.05$.

Sera which were positive (>1:4) or suspected (1:4) in the blocking ELISA also were analyzed by the hemagglutination inhibition test (HIT), according to the methods of Mayr et al. (1977) and adapted to conditions specifically for RHDV. The incubation of serum and antigen was performed for 1 hr at room temperature and we used human (0)-blood cells, phosphate buffer (pH 6.5), inactivated RHDV antigen and for a positive control, a hyperimmune serum against RHDV (Federal Research Institute for Virus Diseases of Animals, Riems, Germany).

In addition, two positive samples (No. 14, No. 18) were investigated by Western blot analysis (Towbin et al., 1979). Firstly, EBHSV and RHDV were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the methods of Laemmli (1970). The separating gel and stacking gel were 10% and 5% acrylamide, respectively (Rotiphorese Gel 30, ROTH GmbH, Karlsruhe, Germany). For controls, lanes of EBHSV, RHDV, and marker proteins were stained with Coomassie Brillant Blue R-250 (Roti-Blue, ROTH GmbH, Karlsruhe, Germany). Molecular weight analysis and densitometry were performed by WINCAM 2.2. software (CYBERTECH, Berlin, Germany). Gels were blotted to nitrocellulose membranes as described by Towbin et al. (1979), with the following modifications. After blocking with 1% gelatin in Tris buffered saline/tween for 1 hr, the membrane was air-dried. The nitrocellulose strips were incubated simultaneously with positive fox sera (No. 14 and No. 18, dilution of 1:500) as well as with negative control serum (No. 1) for 2 hr at room temperature (RT), followed by a washing procedure. The strips were then incubated with rabbit anti-dog IgG (Sigma, No. D-8650; 1:1,000, 2 hr at RT) and after another washing procedure, they were incubated with 50 µl goat anti-rabbit globulin peroxidase conjugate (Sigma, No. A-6154; 1:2,000; 2 hr at RT). Staining was performed with diamino benzidine tetrachloride.

RESULTS

Antibodies against RHDV were detected by blocking ELISA in 18 of 352 (5%) foxes. Titers ranged from 1:10 to 1:1,000. (Table 1). Eight of the 18 positive samples, but none of the suspected samples in the ELISA, were positive in the HIT. Titers in the HIT ranged between 1:40 and 1:640. The positive serum which showed a titer

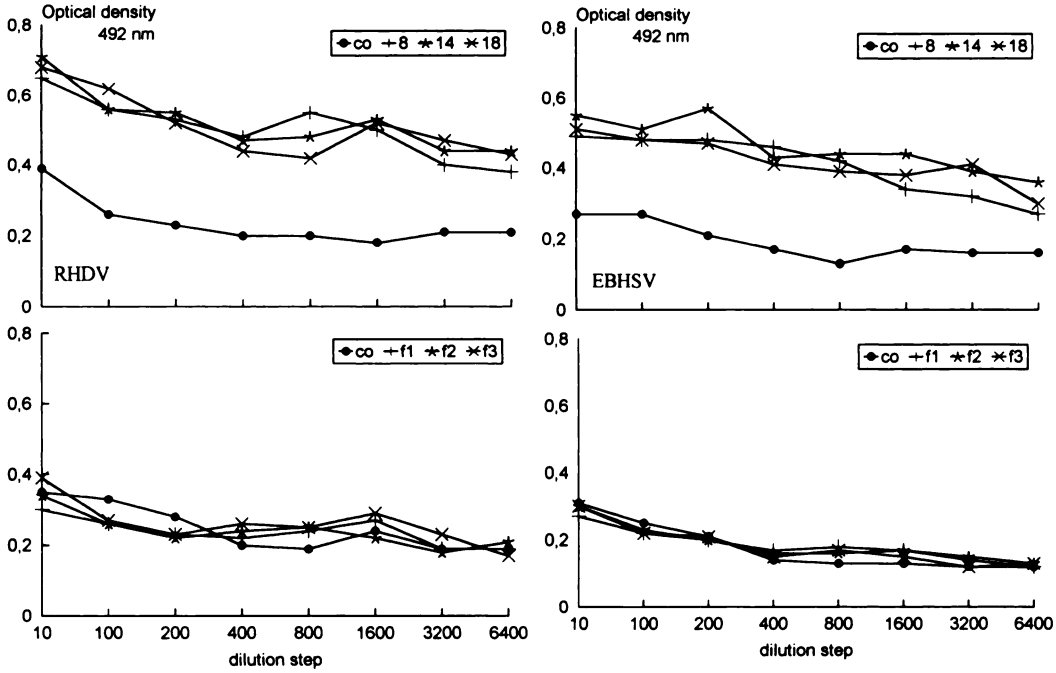

FIGURE 1. Sandwich enzyme immunoassay (sandwich ELISA) of red fox serum samples against rabbit hemorrhagic disease virus (RHDV—left) and European brown hare syndrome virus (EBHS—right). The upper diagrams represent the samples (8, 14, 18) which were positive in the blocking ELISA. Below are diagrams of optical densities of three negative external control samples (f1, f2, f3) from farm foxes. Internal controls (co) are without fox serum.

of 1:1,000 in the blocking ELISA had a titer >1:640 in the HIT. Antibodies against EBHSV were not detected by blocking ELISA (Table 1).

Antibodies against RHDV were found by sandwich ELISA in six samples. Titers varied between 1:200 and 1:6,400 (Table 1). Titers varied between the blocking ELISA and sandwich ELISA. Most of the foxes with titers of 1:10 in the blocking ELISA showed no positive reaction in the sandwich ELISA. In contrast to the blocking ELISA, all RHDV positive samples in the sandwich ELISA also reacted with EBHSV. In five of six foxes, titers against RHDV were higher than titers against EBHSV and in one animal (No. 14), the titers were identical. However, the extinctions in different dilutions against RHDV were significantly higher in only three of the six positive foxes and in one case, almost significantly higher than against EBHSV ($P = 0.0178$, $P = 0.0274$, $P = 0.0176$, $P = 0.0620$, $P = 0.1493$, $P = 0.2359$). Optical densities of negative control animals were similar to negative test controls (without antigen) for each dilution in the RHDV as well as in the EBHS assay (Fig. 1).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of virus antigens showed different polypeptide bands between 84 and 20 kDa (Fig. 2) for both viruses. The main proteins had molecular weights of 65 and 52 kDa (RHDV) and 67 and 57 kDa (EBHSV), respectively. In Western blot immunoassay samples, No. 14 and 18 showed clear positive reactions with these two main polypeptide bands of EBHSV and RHDV (Fig. 2). The areas below the peaks of the bands after immunoblotting indicated a stronger reaction with the two main polypeptides of RHDV than with the two main polypeptides of EBHSV with identical amounts of antigens

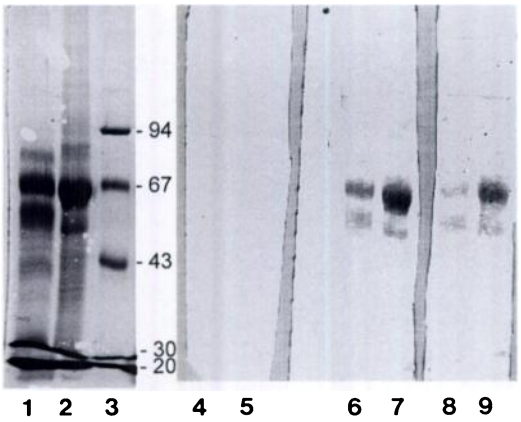

FIGURE 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (lanes 1–3) and Western blot immunoassay (lanes 4–9) of rabbit hemorrhagic disease virus (RHDV) and European brown hare syndrome virus (EBHSV) antigens. Lanes consist of the following: 1—EBHSV antigens, 2—RHDV antigens, 3—marker proteins which are, from top to bottom, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor, 4—EBHSV and 5—RHDV incubated in negative control serum, 6—EBHSV and 7—RHDV incubated in fox serum No. 14, 8—EBHSV and 9—RHDV incubated in fox serum No. 18.

(3.3 times more for No. 18 and 2.0 times more for No. 14).

DISCUSSION

Using four different methods, we detected antibodies against RHDV in free-ranging red foxes. We detected 18 positive sera in the ELISA in comparison to only eight positive samples in the HIT. Hemagglutination inhibition test seems to have failed to detect some sera with low concentrations of antibodies against RHDV compared to blocking ELISA, as demonstrated in other studies (Danner, 1995).

To verify the results of the blocking ELISA in which the detecting antibodies react with the bound antigen, a second indirect sandwich ELISA was developed. In contrast to the blocking ELISA, the detecting antibodies reacted directly with the fox antibodies bound to the antigen in the sandwich ELISA. Samples with titers ≥1:100 in the blocking ELISA were positive in the sandwich ELISA (Fig. 1). Differences between blocking ELISA and sandwich ELISA were possibly due to the incomplete antigenic identity of fox and dog immunoglobulins. By sandwich ELISA, all sera which had antibodies against RHDV also reacted with EBHSV antigen, but titers were lower in five of six animals. In three cases, the differences were significant and no samples negative for RHDV showed a positive reaction against EBHSV. Therefore, we have strong evidence that the immune response against RHDV was higher than against EBHSV. However, the results also suggest the existence of cross-reactivity between RHDV and EBHSV antigens. This confirms the findings of Capucci et al. (1995) and Marcato et al. (1989). Cross-reactivity between RHDV and EBHSV is shown also by the results of Western blot analysis.

Blocking ELISA and sandwich ELISA positive fox samples showed clear positive reactions with the two main peptide bands of EBHSV and RHDV (67–52 kDa), but a weaker reaction against EBHSV. The 67 kDa polypeptide was established as a major structural polypeptide of RHDV by Jin et al. (1989) and other authors reported main peptides between 60 kDa and 50 kDa (Du, 1991; Ohlinger et al., 1990; Wu et al., 1990) corresponding with our findings. The lower molecular weight proteins (20–30 kDa) showed no reactivity with RHDV or with EBHSV.

Only a few studies of caliciviruses in canines have been conducted. Leighton et al. (1995) gave red foxes an oral dose of homogenized liver from rabbits that died from RHD and these foxes developed a pronounced antibody response. In France, Simon et al. (1994) gave domestic dogs RHDV by parenteral infection. The dogs did not develop clinical signs, but they shed virus in the feces. Rabbits infected by virus isolated from the feces of these dogs developed RHD. However, no antibodies against RHDV were found in 75 free-ranging foxes by using HIT (Simon et al., 1994). Our results are the first indica-

tion of calicivirus exposure in free-ranging red foxes in Europe.

Antibodies against EBHSV were not detected by blocking ELISA. Therefore, the antibodies detected against caliciviruses appear to be induced by RHDV or other closely related viruses. It may be that foxes from this area mainly fed on rabbits rather than on hares. However, serologic data are difficult to interpret because of the possibility that closely related caliciviruses may be present in rabbit populations in Europe and may crossreact with RHDV. In principle, this is supported by investigations of Prato et al. (1977) performed in gray foxes (*Urocyon cinereoargenteus*) in California. They found antibodies against a calicivirus isolated from California sea lions (*Zalophus californianus*) in 7% of 85 gray foxes and suggested that caliciviruses of marine origin have a potentially broad range in both marine and terrestrial mammalian species. Their results indicate that gray foxes may be naturally infected with caliciviruses, but their role in transmission is unknown.

Approximately 5% of foxes tested had been in contact with RHDV presumably via infected rabbits. Contact probably was mainly 1 to 2 wk before sampling, because experimental studies showed that antibody titers diminished on average the second week after exposure (Leighton et al., 1995). Probably, the mode of exposure is mainly by feeding on rabbits which had died of RHD or by catching live viremic rabbits. However, the potential period for catching infected rabbits is rather short, because they survive for only 3 days on average.

Monitoring red foxes for antibodies against RHDV could be used to provide information on the presence of this disease in sympatric rabbit populations. Analogous investigations have been conducted by testing carnivores for antibodies against *Yersinia pestis* for monitoring the occurrence of plague in their prey (Clover et al., 1989). Carnivores are valuable sentinels in the detection of *Y. pestis* in wildlife populations (Willeberg et al., 1979). When coyotes, (*Canis latrans*) become infected with *Y. pestis*, they usually do not develop clinical signs, but develop antibody titers (Barnes, 1982). This makes coyotes an indicator species for plague. Thus, changes in prevalence of plague in the coyote population are likely related to changes in the prevalence of plague in the coyotes prey base, such as small mammals (Gese et al., 1997). Moreover, serologic surveys of carnivores for infectious diseases of their prey may lead to insights about their prey in discrete habitats.

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LITERATURE CITED

BARNES, A. M. 1982. Surveillance and control of bubonic plague in the United States. Symposia of the Zoological Society of London 50: 237–270.

CAPUCCI, L., G. FRIGOLI, L. RONSHOLT, A. LAVAZZA, E. BROCCHI, AND C. ROSSI. 1995. Antigenicity of the rabbit hemorrhagic disease virus studied by its reactivity with monoclonal antibodies. Virus Research 37: 221–238.

CLOVER, J. R., T. D. HOFSTRA, B. G. KULURIS, M. T. SCHROEDER, B. C. NELSON, A. M. BARNES, AND R. G. BOTZLER. 1989. Serologic evidence of *Yersina pestis* infection in small mammals and bears from a temperate rainforest of north coastal California. Journal of Wildlife Diseases 25: 52–60.

DANNER, K.-J. 1995. Untersuchungen zur Diagnostik und Epizootiologie der Heamorrhagischen Kaninchenseuche (RHD). Veterinär Medizinische Dissertation, Justus-Liebig-Universität, Gießen, Germany, 176 pp.

DEDEK, J. 1995. Mikrobiell bedingte Infektionen und Infektionskrankheiten des Rotfuchses (*Vulpes vulpes* Linnaeus 1758). Internationales Symposium über die Erkrankung der Zoo- und Wildtiere 37: 365–369.

DU, N. X. 1991. Molecular biology of the viral haemorrhagic disease virus of rabbits. Revue Scientifique et Technique Office International des Epizooties 10: 325–336.

FRÖLICH, K., H. H. D. MEYER, Z. PIELOWSKI, L. RONSHOLT, S. VON SECK-LANZENDORF, AND M. STOLTE. 1996. European brown hare syndrome in free-ranging hares in Poland. Journal of Wildlife Diseases 32: 280–285.

GAVIER-WIDEN, D., AND T. MÖRNER. 1993. Descriptive epizootiological study of European

brown hare syndrome in Sweden. Journal of Wildlife Diseases 29: 15–20.

GESE, E. M., R. D. SCHULTZ, M. R. JOHNSON, E. S. WILLIAMS, R. L. CRABTREE, AND R. L. RUFF. 1997. Serological survey for diseases in free-ranging coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming. Journal of Wildlife Diseases 33: 47–56.

JIN, L., W. Y., XU, AND N. X. DU. 1989. Identification of polypeptides of rabbit haemorrhagic disease virus. Bulletin of the Veterinary College of Peoples Liberation Army 9: 56–59.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

LEIGHTON, F. A., M. ARTOIS, L. CAPUCCI, D. GAVIER-WIDEN, AND J.-P. MORISSE. 1995. Antibody response to rabbit viral hemorrhagic disease virus in red foxes (*Vulpes vulpes*) consuming livers of infected rabbits (*Oryctolagus cuniculus*). Journal of Wildlife Diseases 31: 541–544.

LIU, S. J., H. P. XUE, B. Q. PU, AND N. H. QUIAN. 1984. A new viral disease in rabbits. Animal Husbandry and Veterinary Medicine 16: 253–255.

MARCATO, P. S., C. BENAZZI, M. GALEOTTI, AND L. DELLA SALDO. 1989. L'epatite necrotica infettiva dei leporidi. Rivista di Coniglicoltura 8: 41–50.

MAYR, A., P. A. BACHMANN, B. BIBRACK, AND G. WITTMANN. 1977. Virologische Arbeitsmethoden, Vol. 2. Gustav Fischer Verlag, Jena, Germany, 696 pp.

MORISSE, J. P., G. LE GALL, AND E. BOILLETOT. 1991. Hepatitis of viral origin in Leporidae: Introduction and aetiological hypotheses. Revue Scientifique et Technique Office International des Epizooties 10: 283–295.

OHLINGER, V. F., B. HAAS, G. MEYERS, F. WEILAND, AND H.-J. THIEL. 1990. Identification and characterization of the virus causing rabbit haemorrhagic disease. Journal of Virology 64: 3331–3336.

———, AND H.-J. THIEL. 1991. Identification of the viral hemorrhagic disease virus of rabbits as a calicivirus. Revue Scientifique et Technique Office International des Epizooties 10: 311–323.

PRATO, C. M., T. G. AKERS, AND A. W. SMITH. 1977. Calicivirus antibodies in wild fox populations. Journal of Wildlife Diseases 13: 448–450.

SACHS, L. 1992. Angewandte Statistik, 7th ed., Springer Verlag, Berlin, Germany, 846 pp.

SCHLÜTER, H. 1988. Hämorrhagische Septikämie der Kaninchen (HSK). Tiergesundheitsjahrbuch der DDR, 9: 330–331.

SIMON, M. C., R. MUGURUZA, J. L. ALONSO, J. L. MUZQUIZ, O. GIRONES, AND A. HAFFAR. 1994. Recherche du virus de la maladie hemorragique virale du lapin (RHD) chez le renard et role des canides domestiques dans la transmission de la maladie. Recueil de Medecine Veterinaire 170: 841–845.

SMID, B., L. VALICEK, L. RODAK, J. STEPANEK, AND E. JURAK. 1991. Rabbit hemorrhagic disease: An investigation of some properties of the virus and evaluation of an inactivated vaccine. Veterinary Microbiology 26: 77–85.

TOWBIN, H., T. STAEHELIN, AND J. GORDON. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceedings of the National Academy of Science, USA 76: 4350–4354.

WILLEBERG, P. W., R. RUPPANNER, D. E. BEHYMER, H. H. HIGA, C. E. FRANTI, R. A. THOMPSON, AND B. BOHANNAN. 1979. Epidemiologic survey of sylvatic plague by serotesting coyote sentinels with enzyme immunoassay. American Journal of Epidemiology 110: 328–334.

WU, A. Z., X. Z. XIA, Y. YANG, H. P. YANG, AND J. D. CHEN. 1990. Viral proteins of rabbit haemorrhagic disease virus and their immunogenicity. Chinese Journal of Virology 6: 332–335.

XU, W. Y. 1991. Viral hemorrhagic disease of rabbits in the People's Republic of China: Epidemiology and virus characterisation. Revue Scientifique et Technique Office International des Epizooties 10: 393–408.

XU, Z. J., AND W. X. CHEN. 1989. Viral hemorrhagic disease in rabbits: A review. Veterinary Research Communications 13: 205–212.

ZIMEN, E. 1981. The anthropogenic influence on near urban and country fox populations in southern Saarland. Verhandlungen der Gesellschaft für Ökologie. Göttingen 9: 311–319.

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