EXPERIMENTAL INFECTION OF WHITE-TAILED DEER WITH RINDERPEST VIRUS 1

Authors: HAMDY, F. M., DARDIRI, A. H., FERRIS, D. H., and BREESE, S. S.

Source: Journal of Wildlife Diseases, 11(4) : 508-515

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-11.4.508

EXPERIMENTAL INFECTION OF WHITE-TAILED DEER WITH RINDERPEST VIRUS[1]

F. M. HAMDY, A. H. DARDIRI, D. H. FERRIS and S. S. BREESE, Jr.,
U.S. Department of Agriculture, Agricultural Research Service,
Plum Island Animal Disease Center, Greenport, Long Island, New York 11944, USA

Abstract: White-tailed deer (*Odocoileus virginianus*) succumbed to experimental infection with virulent rinderpest (RP) virus that was also lethal to cattle and goats. The deer developed clinical signs typical of RP and died 5 and 6 days post-inoculation. Infection was confirmed by recovery of virus from blood before death, from lymph node tissue after necropsy, and demonstration of specific complement fixing antigen in those tissues. Electron micrographs of infected Vero cell cultures revealed extracellular virions and intracytoplasmic and intranuclear inclusions made of randomly distributed fibrillar strands.

INTRODUCTION

Rinderpest (RP) is an acute or subacute highly contagious disease of cattle and other ruminants characterized by a lymphoid and mucosal reaction syndrome and high mortality rate. RP is a classic example of a disease that kills both domesticated and wild animals. Because epizootics of RP in both types of animals occur in parts of Africa and Asia, the disease has worldwide significance and constitutes a threat to countries free of RP. Scott[15] presented lists of animals susceptible to RP, and the number of species involved was of a magnitude to suggest that most artiodactyls could be included. A number of species in the Cervidae have proven susceptible to natural or experimental RP.[2,4,5,14,15] Experimental infection of white-tailed deer (*Odocoileus virginianus*) with RP virus has not been reported. Because this indigenous species is numerous and widely dispersed in North America, its susceptibility to RP is a matter of obvious concern to wildlife and agricultural authorities.[6] Any information on this subject is of concern not only from the epizootiological point of view, but also from the practical standpoint of the possible role this species might play as a source of RP infection to other species.

This paper reports on the experimental infection of white-tailed deer with RP virus and the subsequent clinical picture, post-mortem findings, re-isolation of RP virus, and its identification.

MATERIALS AND METHODS

Virus:

The virulent Kabete "O" reference strain of RP virus was used. This strain was maintained for many years by passage in cattle in the Kabete Veterinary Laboratory, Kenya. It was received by the Plum Island Animal Disease Center in 1954 and maintained by passage in cattle and cell culture.[3] The virus was passaged once on Vero cell culture for these experiments.

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Vero Cell Culture:

The Vero cell line was obtained from the American Type Culture Collection. The medium used was Earle's balanced salt solution with 0.05% lactalbumin hydrolysate, 0.005% yeast extract, 0.0015% phenol red (LEY) supplemented with 5% fetal calf serum. One hundred units of penicillin and 100 μg of streptomycin per ml were incorporated at a final pH of 7.3.

Deer:

The deer were supplied by Dr. Frank A. Hayes, Director, Southeastern Cooperative Wildlife Disease Study, Athens, Georgia. Three white-tailed deer (*O. virginianus*) were infected with RP virus; two of these were used in a viral recovery experiment. Each of the three deer was inoculated intramuscularly with 1 ml of RP virus suspension containing 10^5 $TCID_{50}$. They were kept under observation in strict isolation during the course of the experiment. Several uninfected deer served as environmental controls.

Virus Isolation:

Defibrinated blood was collected from two deer 3 and 5 days post-inoculation (DPI) and centrifuged at 1,500 xg in an International Centrifuge for 15 min. The buffy coat cell (BCC)-rich fraction was separated, and 0.2 ml was inoculated on Vero cell cultures grown in 60 ml Falcon plastic flasks. Lymph nodes taken from the necropsied deer were tested for virus isolation. A 10% suspension in LEY medium was prepared and tested in the same way as with blood samples.

Cultures were incubated for 1 h at 37 C to allow seeding of leukocytes, after which the fluid maintenance medium was added. Medium was changed after 2 days and then at 3-day intervals until cytopathogenicity was observed.

Virus Neutralization:

Two viral isolates recovered from two deer 3 and 5 DPI were identified by virus neutralization. Virus suspensions were harvested at a stage of approximately 50% cell destruction by cytopathic effects (CPE). Cell-free virus preparations were tested for neutralization by goat RP immune serum. Normal control and RP-specific immune goat sera were diluted 1:5 in phosphate buffered saline and inactivated at 56 C for 30 min. They were then chilled, dispensed in test tubes, and mixed with equal volumes of a series of 10-fold virus dilutions. The virus-serum mixtures, as well as the virus and serum controls, were incubated at 37 C for 30 min., after which they were chilled and 0.2 ml of each mixture was inoculated in two Vero cell cultures grown in 60 ml Falcon plastic flasks. After 1 h adsorption at 37 C, the maintenance medium was added. A few Vero cell cultures were left uninoculated and served as controls. All cultures were incubated at 37 C and examined by light microscope for the development of CPE. Titers were recorded as neutralization indices representing the number of logs of virus neutralized by the serum.

Complement Fixation (CF) Test:

Antigens were prepared from lymph nodes (LN) collected from the dead deer. A 10% (wt/vol) suspension was made in veronal buffer diluent,[10] subjected to three cycles of freezing (—70 C), and thawing (37 C), and clarified by centrifugation at 2,000 xg for 1 h The CF method was essentially the same as that of the Laboratory Branch Task Force[10] except that C'titration was performed in the presence of the test antigen. Five C'H50 units were used.

Electron Microscopy (EM):

Vero cell cultures inoculated with deer BCC revealed CPE characteristic of RP, and were repassed in Vero cells and processed for EM. Normal control and infected Vero cell cultures were fixed *in situ* in 1% (wt/vol) glutaraldehyde in a 0.1 M sodium cacodylate buffer pH 7.2 for 15 min. at 4 C. After this initial fixation, cells were gently scraped from the plastic surface with a rubber policeman and centrifuged at 500 xg for 5

min. to form a soft pellet. This pellet was washed three times in cold 0.1 M cacodylate buffer for 1 h at 4 C. Cells were then post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, washed once in buffer, then dehydrated in a graded ethyl alcohol-propylene oxide series, embedded in Epon 812[11], and polymerized at 60 C for 24 h.

Thin sections were cut on a Porter Blum MT-2 ultramicrotome (Sorvall, Inc., Norwalk, Conn.) and stained with uranyl acetate and lead citrate.[18] Electron micrographs were taken with an RCA-EMU-3G electron microscope.

RESULTS

Deer inoculated with virulent Kabete "O" Reference strain of RP virus showed typical clinical signs of RP, similar to those in bovidae. Incubation period was as short as 2 days, after which a high body temperature (approximately 41 C) was reached. By the third day, the animals were obviously sick, less active, and depressed. Conjunctivitis with mucopurulent discharge was observed by the fourth day. Also, a nasal discharge and bloody diarrhea developed. The animals died 5-6 DPI after suffering severe prostration.

Necropsy revealed the following:

1. External Features—Eyes were dehydrated and sunken. A dried bloody discharge was around the anus.

2. Digestive System—Lesions were observed in the oral cavity as gingivitis, congested soft palate, very tiny punched-out erosions on the mucosa of the cheeks and base of the tongue, thick and greyish hemorrhagic discharge in the pharyngeal cavity, and diffuse hemorrhage in the esophagus and rumen. The rumenal mucosa under the papillae was congested. Catarrhal enteritis of the ileum was observed, with sloughing of the epithelial mucosa, originating at the duodenum and becoming more pronounced in the cecum and colon. Necrotic hemorrhagic enteritis was observed in the colon. Rectal mucosa had very pronounced zebra striping, accompanied by massive sloughing of the epithelium.

3. Respiratory System—Thick, greyish blood-tinged discharge was present in the pharyngeal cavity and extended to the congested larynx. The tracheal epithelium was congested, the lungs were edematous, and hepatized areas were observed in the apex and base of the diaphragmatic lobes.

4. Cardiovascular System—The heart contained extensive subendocardial petechial hemorrhages on the surface of the auricles and on top of the heart, and ecchymotic hemorrhages in the endocardium. The submaxillary LN was dark red, rigid, rubbery and edematous. The cut surface showed edema and petechiation. The prescapular LN was enlarged, edematous and very hemorrhagic. Mesenteric LNs were large and edematous; when cut, the surface exuded sanguinous fluid. Abdominal LNs were dark in color, resembling blood clots. The popliteal LN was enlarged. Mediastinal areas had scattered patches and areas of ecchymosis.

Virus Isolation:

Virus was recovered from blood samples taken 3 and 5 DPI and from LNs from two deer after necropsy; virus isolation from the third deer was not attempted. Vero cells inoculated with the BCC-rich fraction or LN suspension developed CPE approximately 8 DPI. The CPE consisted of cell rounding and polykaryocyte formation. It started as discrete foci and then spread outwards. Fig. 1 is a photomicrograph of Vero cells at 8 DPI. The inoculum was BCC preparation from deer blood at 3 DPI.

Virus Neutralization:

Two isolates from two deer recovered 3 and 5 DPI were identified by neutralization test in Vero cell cultures. Goat RP immune serum neutralized 5 and ≥ 4.3 $\log_{10}$ of the two virus isolates whereas normal goat serum did not have neutralizing activity.

Complement Fixation Test:

Antigens made from the deer LN samples after death were tested for CF activity against RP-specific goat antiserum. Results were positive with titers of 640 as calculated by net weight of original LN tissue. Control antigen from RP-negative baby deer LN tissue simultaneously tested was negative at its lowest dilution (10). No CF activity was detected when LN antigens from deer infected with RP virus were tested against normal control goat serum.

Ultrastructural Morphology:

Thin sections of infected Vero cells revealed viral forms at different developmental stages: fibrillar strands in the nucleus (Fig. 2); intracytoplasmic inclusions consisting of random arrays of fibrillar strands (Fig. 3); pleomorphic particles in the process of budding from the plasma membrane of infected cells; and mature virions extracellularly (Fig. 4). The shape and size of the virus particles varied widely. Some of the virions contained strands similar in morphology to those forming the cytoplasmic inclusions (Fig. 3). Others lacked these internal structures.

DISCUSSION

Susceptibility of white-tailed deer to experimental RP infection was demonstrated according to the clinical, pathological, immunologic, and morphologic data. The incubation period was short, as in *Cervus aristotelis* inoculated with virulent bovine RP virus.[8] Clinical signs and lesions in white-tailed deer were as severe as in the highly susceptible breeds of cattle. The infection expressed itself in

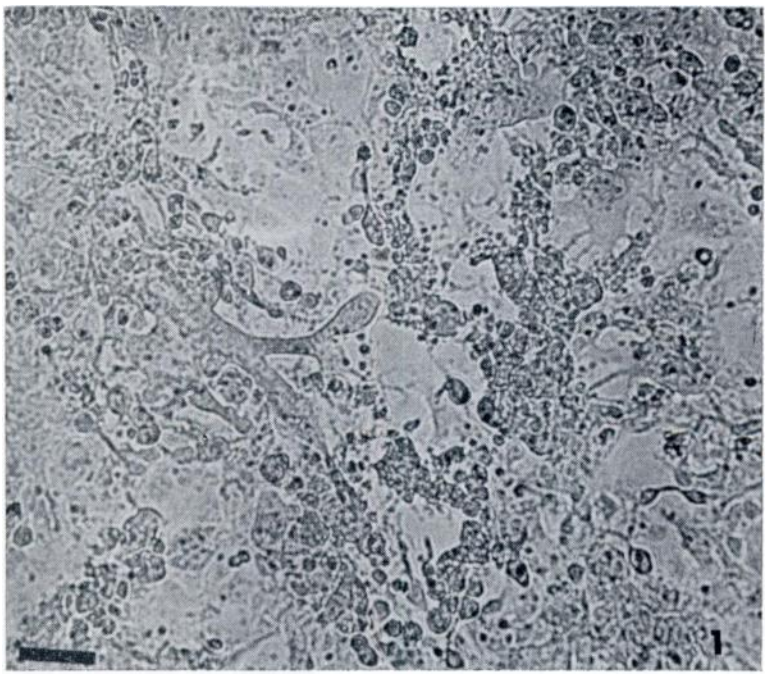
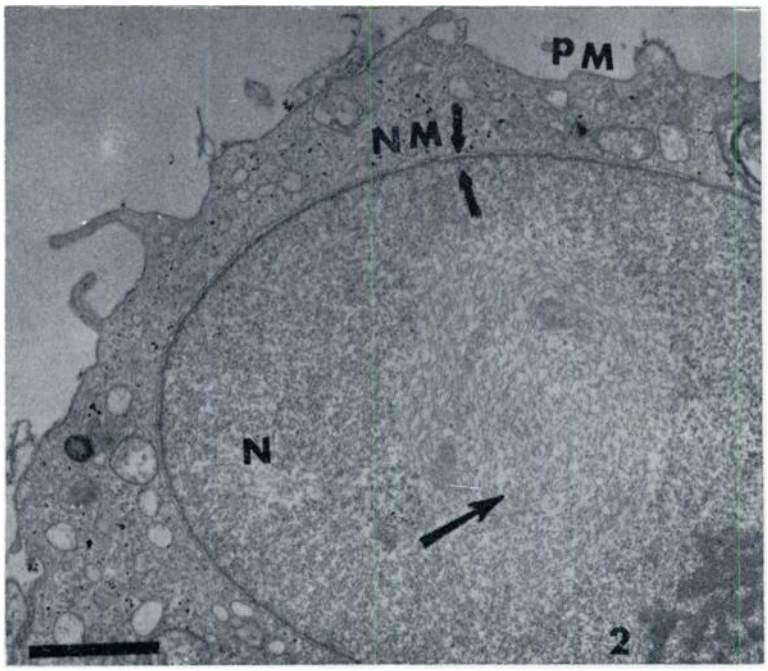


FIGURE 1. Photomicrograph of Vero cell culture 8 DPI with blood from deer inoculated with RP virus; cell culture shows CPE. Bar = 25 μm.

an acute form as the inoculated animals died after 5-6 days with lesions typical of RP. This fatal reaction was different from that in *C. aristotelis* in which only minor transient illness was recorded. The clinical picture and lesions were similar to those reported in natural RP infection in barking deer (*Cervulus muntjak*)[5], hog deer (*Cervus porcinus*),[5] and spotted deer (*Cervus axis*)[5] except that the course in white-tailed deer was shorter.

The brevity of the course of the disease and its fatal consequence indicates a higher relative susceptibility of white-tailed deer to RP virus, because the same amount of inoculated virus killed six steers (age 18 months) at 6 DPI and two goats (age approximately 10 months) at 10 DPI. CF titers of 640 were also detected in the six steers (F. M. Hamdy and A. H. Dardiri, 1974, unpublished data). This indicates that RP virus propagation in deer LN is comparable to cattle.

Virus isolation from deer blood, and its identification, leaves no doubt that infection was established. With the EM, additional evidence was obtained by demonstrating virus particles and virus related structures morphologically characteristic of RP virus.[1,16] The EM revealed a virus morphogenesis typical of myxoviruses and paramyxoviruses (cytoplasmic and intranuclear fibrillar strands,



FIGURES 2-4. Electron micrographs of thin section of Vero cell infected with RP virus, isolated from deer blood, fixed with glutaraldehyde-osmium and stained with uranyl acetate and lead citrate. N—nucleus; NM—nuclear membrane; PM—plasma membrane; and C—cytoplasm.

FIGURE 2. Intranuclear inclusions made up of random array of fine fibrillar strands (arrow) presumably viral RNA. Bar—1 μm.

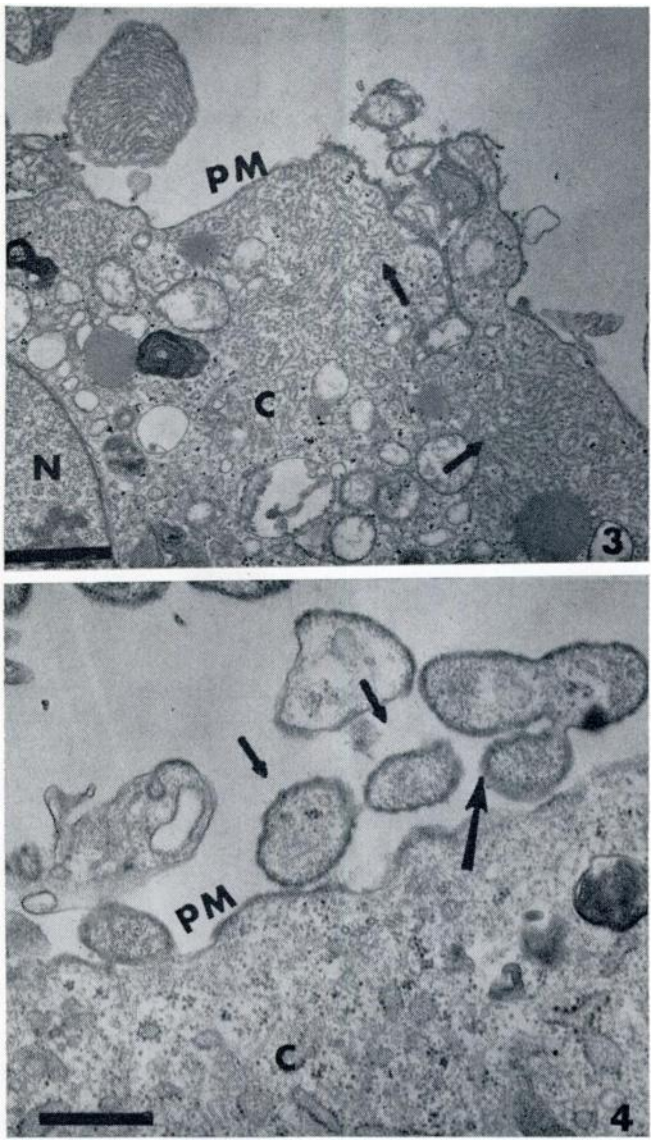


FIGURE 3. Intracytoplasmic inclusions made up of randomly distributed fibrillar strands (arrow) thicker than those in the nucleus (Fig. 2). These are presumably viral ribonucleoprotein. Bar—1 μm.

FIGURE 4. Extracellular pleomorphic virions (small arrows) assuming different size and shapes. Note the bizarre virion (large arrow). Bar = 0.5 μm.

virus budding from the plasma membrane of the infected cells and particle pleomorphism). Holland[7] and Kingsbury[9] proposed a scheme explaining the sequence of events for myxovirus and paramyxovirus replication. They concluded that replication occurred only in the cytoplasm and not in the nucleus or the nucleolus. We found that nuclei of some infected cells had arrays of fine strands. These were not observed in uninoculated control cells, indicating the nucleus may have some role in the synthesis of virus components. We propose that a differential course of infection involving nuclear functions may occur, besides the replicative cycle in the cytoplasm. The cytoplasmic inclusions are morphologically distinct from the nuclear inclusions in that the cytoplasmic inclusions are thicker and continuous. This was also observed in cells infected with "Peste des Petits Ruminants" (F. M. Hamdy, A. H. Dardiri and S. S. Breese, Jr., unpublished data). These nuclear structures were also demonstrated in lymphocytes from bovine LN infected with RP virus.[16]

The pleomorphism of RP virus particles, like some myxoviruses and paramyxoviruses prompts us to propose that virus assembly does not occur in highly ordered manner relative to the precise amount of RNP contained in the virions. Differences in the amount of RNP in the virions cause them to assume different sizes and configurations.

Acknowledgement

We would like to thank T. V. Franke, J. J. Giovanelli and S. J. Pyne for their able technical assistance.

LITERATURE CITED

1. BREESE, S. S., JR. and C. J. DeBOER. 1973. Ferritin-tagged antibody cross-reaction among rinderpest, canine distemper, and measles viruses. J. gen. Virol. 20: 121-125.
2. BRISTOWE, J. S. 1866. Supplementary report on the morbid anatomy of the cattle plague as it occurs in sheep, goats and deer. Cited by G. R. Scott (1970). In: *Infectious Diseases of Wild Mammals.* Iowa State Press, Ames, pp. 20-35.
3. DeBOER, C. J. 1961. Adaptation of two strains of rinderpest virus to tissue culture. Arch. ges. Virusforsch. 11: 65-67.
4. GRINER, L. 1974. Some diseases of zoo animals. Adv. vet. Sci. and comp. Med. 18: 251-271.
5. GUPTA, K. C. S. and N. S. VERMA. 1949. Rinderpest in wild ruminants. Indian J. vet. Sci. 19: 219-222.
6. HAYES, F. A. 1969. Foreign and emergency disease surveillance training in the southeastern United States. Trans., 59th Conv. int. Assoc. of Game, Fish and Conserv. Comm., New Orleans, Louisiana, pp. 65-67.
7. HOLLAND, J. J. 1974. Slow, inapparent and recurrent viruses. Sci. Amer. 230: 32-40.
8. JACOTOT, H. 1927. Transmission experimentale de la peste bovine a *Cervus aristotelis.* Compt. Rend. Seances Soc. Biol. 96: 1134. Cited by G. R. Scott (1970). In: *Infectious Diseases of Wild Mammals.* Iowa State Press, Ames, pp. 20-35.
9. KINGSBURY, D. W. 1972. Paramyxovirus replication. Curr. Top. Microbiol. Immunol. 59: 1-33.

10. LABORATORY BRANCH TASK FORCE (U.S. PUBLIC HEALTH SERVICE). 1965. Standardized diagnostic complement fixation method and adaptation to microtiter. Part 1. LBCF Method. Public Health Monograph No. 74. P.H.S. Publication Number 1228. U.S. Government Printing Office, Washington, D.C.

11. LUFT, J. 1961. Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. 9: 409-414.

12. ONO, S. and S. KONDO. 1923. Studies on rinderpest in deer (*Cervus sika*) and changes in the blood of infected animals. Author's English Extract. J. Jap. Soc. vet. Sci. 2: 158-161. Cited by W. Plowright (1968). In: Virol. Monogr. 3: 57.

13. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell. Biol. 17: 327-342.

14. SAINTE-HILLAIRE, M. A. G. 1865. Note sur le typhus contagieux au jardin d'acclimatation. Bull. Soc. Imp. Zool. Acclim., Paris 2: 685. Cited by G. R. Scott (1970). In: *Infectious Diseases of Wild Mammals.* Iowa State Press, Ames, pp. 20-35.

15. SCOTT, G. R. 1970. Rinderpest. In: *Infectious Diseases of Wild Mammals.* Ed. by John W. Davis, Lars H. Karstad and Daniel O. Trainer. Iowa State Press, Ames, pp. 20-35.

16. TAJIMA, M. and I. USHIJIMA. 1971. The pathogenesis of rinderpest in the lymph nodes of cattle. Amer. J. of Path. 62: 221-228.

Received for publication 17 March 1975