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Source: Journal of Wildlife Diseases, 16(1) : 117-123

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-16.1.117

EXPERIMENTAL INFECTION OF NEONATAL STRIPED SKUNKS *(Mephitis mephitis)* WITH INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

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Abstract: Experimental infection of neonatal skunks *(Mephitis mephitis)* with infectious bovine rhinotracheitis virus (IBRV) caused fatal systemic infection. Virus isolation and immunofluorescence tests were used to demonstrate a direct association between IBRV and the lesions. Histopathologic studies revealed multiple focal necrosis in the liver and the adrenal glands.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is a cause of respiratory, genital and nervous system infections of cattle.[12] As is characteristic of other herpesviruses, IBR virus (IBRV) has a broad tissue tropism and a broad host range. Natural infections with IBRV have been described in goats,[18] swine,[8,24,32] water buffalo,[36] wildebeest,[14] mink and ferrets.[27] Infections have been experimentally induced in mule deer,[7] goats,[21] swine,[23,38] ferrets,[33] laboratory rabbits [2,5,6,15,26,34] and eastern cottontail rabbits.[20] Serologic studies indicate that IBRV infections may exist in additional species of animals.[1,4,9,11,13,19,28,30,31,35,37]

The objective in the present research was to determine the pathogenicity of IBRV in the neonatal skunk *(Mephitis mephitis).*

MATERIALS AND METHODS

Virus

The Cooper strain of IBRV used[2] had been passaged eight times before receipt and then was passaged two times in bovine lung (BLU) cells. The stock preparation used for inoculation contained 2.0×10^7 plaque forming units (PFU)/ml.

Animals

A pregnant, adult skunk was live-trapped, its scent glands were surgically removed and it was housed in an individual cage until 10 days post partum. The neonatal skunks (n = 6) were transported in the litter box and maintained with their mother in an isolation pen throughout the experiment.

Animal Inoculation

Neonatal skunks (n = 5) were inoculated by intraperitoneal administration of 0.5 ml IBRV stock preparation. A control neonatal skunk (n = 1) was inoculated in the same manner with Eagle's minimum essential medium (MEM). The control skunk was maintained in contact with inoculated skunks.

Virus Isolation Procedures

Monolayers of BLU cells were established in 24-well plastic tissue culture plates for virus isolation. Samples of lung, liver, spleen, brain, kidney and adrenal glands were collected from skunks at necropsy. Separate pools were made of each organ-type and tissue

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suspensions (10%) in MEM were prepared by homogenization with Ten-Broeck tissue grinders. Serial 10-fold dilutions of tissue suspensions were titrated on monolayers of BLU cells. After a 90-min adsorption period, the monolayers were washed with MEM and an overlay containing 1% agarose, [a] MEM, 5% bovine fetal serum, 0.5% lactalbumin hydrolysate and antibiotics (100 IU penicillin and 100 μg streptomycin sulfate per ml) was added. Cultures were incubated at 37 C in a 5% CO_2 atmosphere for 72 h. The cultures were then fixed with 10% buffered formalin, the agarose overlay was removed and the cell monolayers were stained with crystal violet. Plaques were enumerated and the virus titer was determined.

Fluorescent Antibody and Histopathologic Examination

Frozen sections of lung, liver, spleen, kidney, brain and adrenal glands were cut, air-dried, and fixed in acetone. The sections were flooded with fluorescein-conjugated bovine anti-IBRV serum incubated for 30 min at 37 C in a moist chamber. After the sections were washed with 0.01 M phosphate-buffered saline solution (pH 7.4) and distilled water, they were mounted with buffered glycerol (pH 8.6) and examined by fluorescent microscopy. Various tissue specimens collected at necropsy were fixed in 10% buffered neutral formalin for microscopic examination.

RESULTS

Skunks developed a systemic infection which resulted in death on post inoculation day 5. Prior to death, the growth rate of infected skunks was noticeably less rapid than that observed in the control skunk. No other clinical signs of disease were observed.

Gross lesions at necropsy included enlarged adrenal glands, hyperemia of the intestine and pale discoloration of large discrete areas of the liver. Virus was isolated from pools of adrenal glands (8.0 $\log_{10}$ PFU/gm), liver (4.9 $\log_{10}$ PFU/gm), lung (4.0 $\log_{10}$ PFU/gm), brain (5.0 $\log_{10}$ PFU/gm) and kidney (4.3 $\log_{10}$ PFU/gm). Diffuse IBRV-specific fluorescence (Fig. 1) involved essentially all adrenal cortex tissue except cells in the zona glomerulosa. The liver contained multiple focal areas of IBRV-specific fluorescence which corresponded with microscopic lesions (Fig. 2). Nuclei of hepatocytes contained IBRV-specific fluorescence. A large band of IBRV-specific fluorescence was observed in the renal pelvis. Specific fluorescence was not observed in the lungs, brain, spleen or tissues from the control skunk.

Microscopic examination revealed a generalized peritonitis characterized by necrosis and infiltration with leukocytes. The inflamed serosal surfaces were adhered to visceral organs and the diaphragm. Lesions in the adrenal glands consisted of multiple focal areas of coagulative necrosis in the zona reticularis and zona fasciculata (Fig. 3). The lesions were remarkable in that virtually every cell within the focus contained an intranuclear inclusion (Fig. 4). These foci also contained cellular debris and were infiltrated by leukocytes. Hepatic lesions consisted of multiple focal areas of coagulative necrosis (Fig. 5). Foci contained cellular debris, leukocytes and occasional hepatocytes with intranuclear inclusions.

DISCUSSION

Results of this experiment indicate that IBRV infection can be established in neonatal skunks. This finding confirms preliminary evidence (Lupton, unpubl.)

[a] Seakem ME, Marine Colloids Division, FMC Corporation, Rockland, Maine 04841, USA.

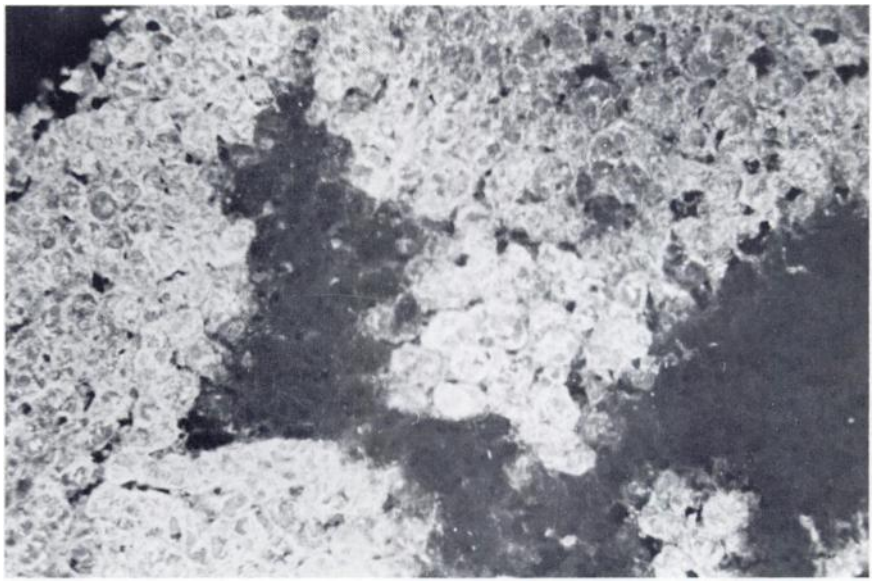

FIGURE 1. IBR virus specific immunofluroescence of adrenal gland 5 days after virus inoculation (× 150).

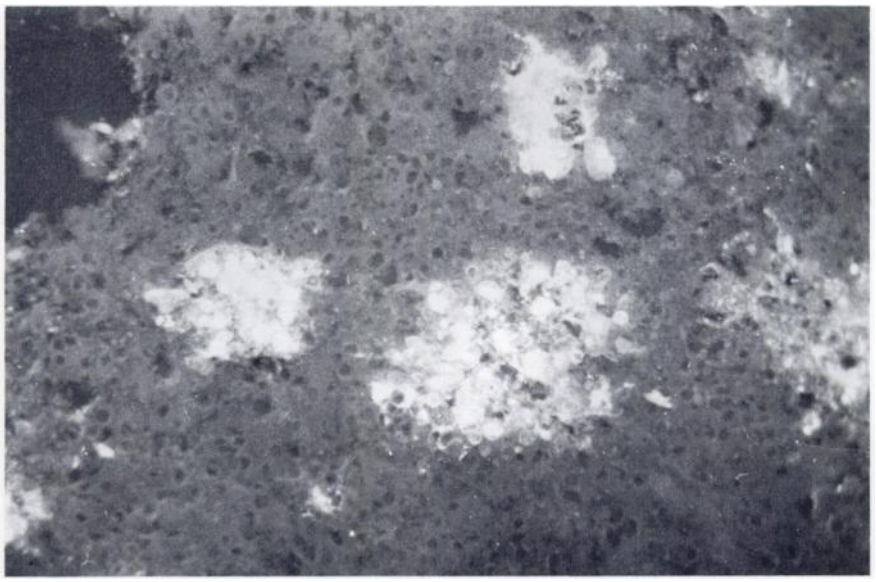

FIGURE 2. Multiple focal areas of IBRV-specific fluorescence in the liver of neonatal skunks 5 days after inoculation of IBR virus. Note IBRV-specific fluorescence of hepatocyte nuclei. (× 150).

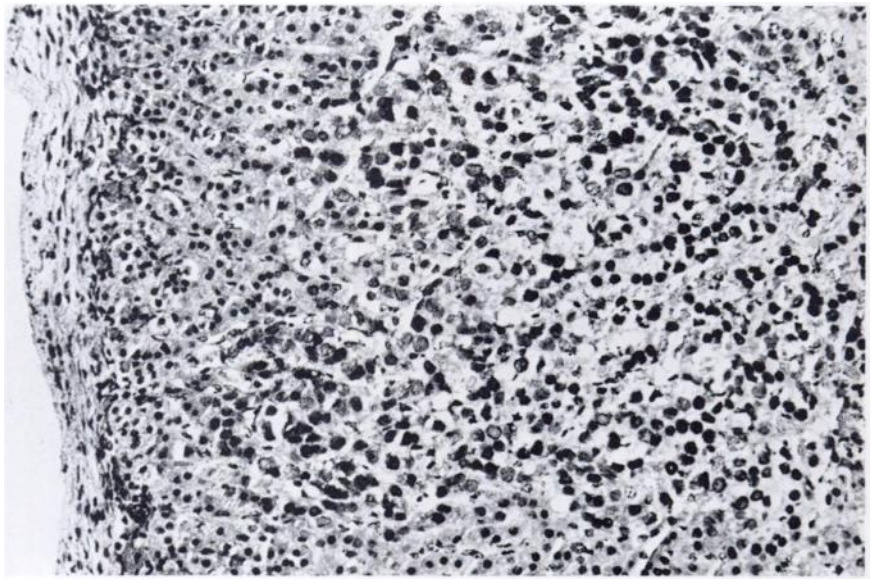

FIGURE 3. Focus of coagulative necrosis in zona reticularis and zona fasciculata of IBR virus infected neonatal skunk. Note large number of nuclear inclusion bodies. (H&E stain; × 150).

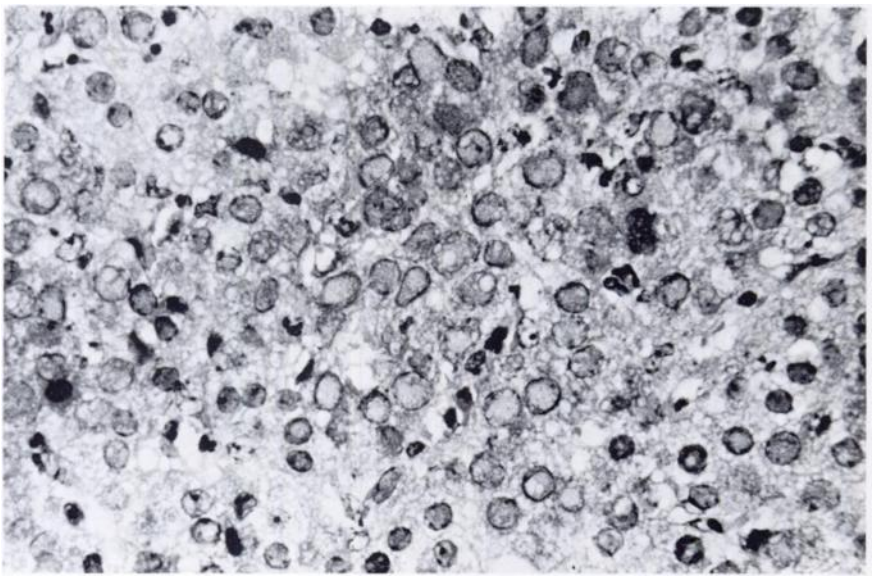

FIGURE 4. Intranuclear inclusion bodies in adrenal gland cells of IBR virus infected neonatal skunk. (H&E stain; × 380).

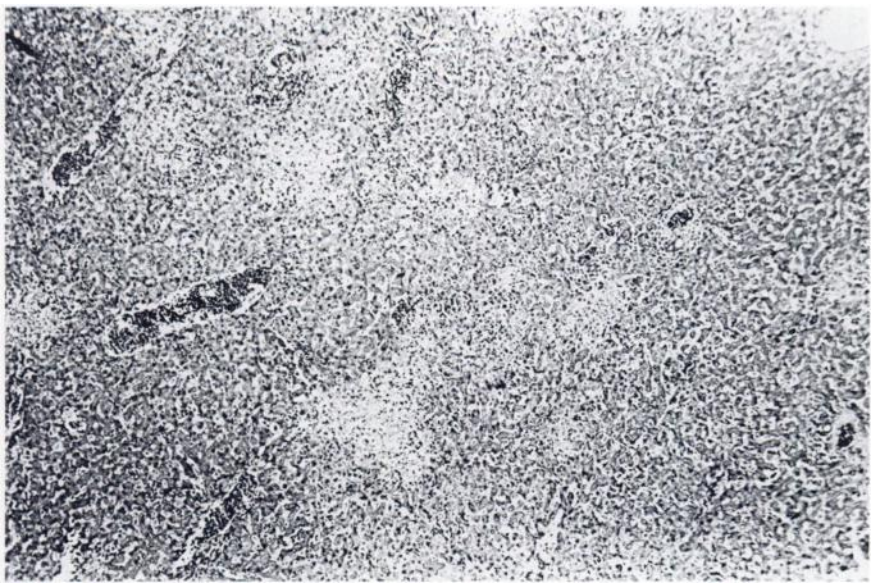

FIGURE 5. Multiple foci of coagulative necrosis of liver of IBR virus infected neonatal skunk. (H&E stain; × 60).

that experimental IBRV infection of an adult skunk resulted in shedding of IBRV for 10 days in ocular secretions and seroconversion. These findings extend the range of known susceptible hosts for IBRV.

System IBRV infection characterized by focal adrenal and hepatic necrosis has been described previously in laboratory rabbits,[15] eastern cottontail rabbits[20] and calves.[3,10,16,17,22,25,29] In the present study, systemic infection of neonatal skunks resulted in necrotic foci of the adrenal glands and liver. A significant characteristic of the adrenal gland lesions was that cells with IBRV inclusion bodies were the dominant feature. In comparison, inclusion bodies are readily found in IBRV infected rabbits but are difficult to find in infected calves.

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Received for publication 20 July 1979