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Oral and Fecal Shedding of Epizootic Hemorrhagic Disease Virus, Serotype 1 from Experimentally Infected White-tailed Deer

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ABSTRACT: Epizootic hemorrhagic disease (EHD), one of the most important infectious diseases of white-tailed deer (*Odocoileus virginianus*), is vectored by species of midges in the genus *Culicoides*. Although vector borne, fecal shedding of EHD virus, serotype 2 has been reported from infected deer in a previous study. To evaluate the potential for fecal and oral shedding, oral and rectal swabs were obtained on day 8 post-inoculation from white-tailed deer fawns experimentally infected with EHD virus, serotype 1 (EHDV-1). Eight deer were viremic for EHDV-1; virus was detected in oral swabs from three (38%) and in rectal swabs from four (50%). The ability to isolate EHDV-1 in oral secretions or feces was not dependent on being able to detect clinical disease. These results indicate that in a relatively large proportion of EHDV-1 infected deer, virus can be detected in feces and oral secretions. Although more work is necessary, such shedding may be important in experimental studies or pen situations where deer-to-deer contact is prevalent and intense.

Key words: Epizootic hemorrhagic disease virus, EHD, hemorrhagic disease, *Odocoileus virginianus*, viral shedding, white-tailed deer.

Hemorrhagic disease (HD) caused by orbiviruses (Reoviridae) in the epizootic hemorrhagic disease (EHD) virus or bluetongue (BLU) virus serogroups, is one of the most important infectious diseases of white-tailed deer (*Odocoileus virginianus*) (Nettles and Stallknecht, 1992). Although it is known that these viruses are vectored by species of midges of the genus *Culicoides* (Gibbs and Greiner, 1983), previous research has suggested that alternate routes of transmission might exist. Ditchfield et al. (1964) reported isolation of EHD virus, serotype 2 (EHDV-2) from feces from deer orally inoculated with EHDV-2. Orally infected deer were febrile on day 5 to 7 post-inoculation, but other signs of clinical disease were not apparent. Contact transmission of an unknown serotype of BLU virus between white-tailed deer also has been reported (Thomas and Trainer, 1970). In the apparent absence of arthropods, we recently observed contact transmission with deer experimentally infected with EHDV-2. In this case, seroconversion to EHDV-2 was detected in a negative control deer that was in direct contact with three EHDV-2 infected deer for 31 days. Active surveillance for biting arthropods was not undertaken, but the experimental infection occurred indoors and in October, after the first frost. All four deer were negative for EHD and BLU viruses as well as for antibodies to EHD and BLU viruses at the onset of the experiment. Despite monitoring for viremia and clinical disease every other day, neither was detected in the negative control deer. The objective of this research was to determine if deer experimentally infected with EHD virus, serotype 1 (EHDV-1) shed virus in feces and oral secretions.

Nine, 4- to 6-mo-old white-tailed deer fawns were used in this study. All fawns were negative for antibodies to EHD virus and BLU virus by agar gel immunodiffusion (Veterinary Diagnostic Technology, Inc., Wheatridge, Colorado, USA) and by serum neutralization (Stallknecht et al., 1995). Eight fawns were experimentally infected with $10^{7.1}$ tissue culture infective doses ($TCID_{50}$) of EHDV-1. Half of the 1 ml inoculum was administered subcutaneously and half intradermally at multiple sites on the neck. The virus used in this study was originally isolated from a blood sample obtained from a clinically ill white-tailed deer from Walton County, Georgia

(USA) in 1999 and was propagated on baby hamster kidney cells (BHK_{21} cells) (American Type Culture Collection, Rockville, Maryland, USA). The remaining fawn served as an uninfected control, was housed separately, and was inoculated with 1 ml of minimum essential media (MEM). For inoculation, deer were sedated with approximately 0.5 mg/kg xylazine (Xylazine-100®, Butler Company, Columbus, Ohio, USA) intramuscularly (IM). After inoculation sedation was reversed with 2–5 mg yohimbine (Yobine®, Lloyd Laboratories, Shenandoah, Iowa, USA) administered IM.

On post-inoculation day 8 (PID 8), deer were manually restrained and visually examined for signs of clinical disease. Blood was collected in acid citrate by jugular venipuncture. Oral and rectal swabs (Pur-Wraps®, Hardwood Products Company, Guilford, Maine, USA) were collected and placed into individual vials containing 1 ml of viral transport media consisting of MEM with antibiotics (100,000 units penicillin G, 100 mg streptomycin, 25 mg gentamycin, 50 mg kanamycin, 2.5 mg amphotericin B / ml [SIGMA Chemical Company, St. Louis, Missouri, USA]). Virus isolation from blood was performed as previously described (Quist et al., 1997) using cattle pulmonary artery endothelial (CPAE) cells (American Type Culture Collection). Swab samples were vortexed, centrifuged at 2,880 x G for 5 min, and 100 µl of supernatant was inoculated onto BHK_{21} cells for virus isolation. Viral titers of all blood samples were determined by endpoint titration. All viruses isolated from blood, oral swab, and fecal swab were identified by virus neutralization against all North American EHD virus and BLU virus serotypes as previously described (Quist et al., 1997). All fawns were humanely euthanatized and necropsies were performed on PID 15.

Virus was isolated from the blood of all eight experimentally infected fawns on PID 8 with blood viral titers ranging from $10^{4.6}$ to $10^{5.26}$ $TCID_{50}$. Virus was isolated from oral swabs from three (38%) of the fawns and from rectal swabs from four (50%). In total, virus was detected in oral secretions and/or feces in six of eight fawns (75%). The control fawn remained negative on serology and virus isolation.

On PID 8, five of the eight experimentally infected fawns (63%) had clinical signs of hemorrhagic disease ranging from mild depression and erythema of ear pinna and nares to severe depression with congestion of mucous membranes, oral ulceration, and submandibular edema. Virus was detected in fecal or oral swabs from four of five (80%) clinically ill fawns and two of three (67%) fawns with undetectable clinical signs.

These data demonstrate that EHDV-1 infected white-tailed deer may shed virus in feces or oral secretions, or both. The pathogenesis of fecal and oral shedding is unknown, but it may occur via hemorrhage of viremic blood into the gastrointestinal lumen or oral cavity. Although clinical signs were not detected on PID 8 in two fawns in which virus was detected in feces or oral secretions, both of these fawns did exhibit postmortem lesions on necropsy performed on PID 15, suggesting gastrointestinal or oral hemorrhage may have been present on PID 8 and not detected. The fact that virus was detected from feces, oral secretions, or both, in six of eight (75%) infected fawns demonstrates that this type of shedding is not uncommon. The epidemiologic significance of these findings is unclear. However, when viewed in the context of prior reports of oral infection of domestic sheep (Jochim et al., 1965) and white-tailed deer (Ditchfield et al., 1964) with BLU-10 and EHDV-2, respectively, these findings suggest that contact transmission of EHDV-1 could occur via orally ingested virus shed in feces and oral secretions. If contact transmission of EHD and BLU viruses actually occurs it could have considerable implications regarding viral transmission in experimental settings or between penned deer stocked at high densities. To fully evaluate the po-

tential for contact transmission, the duration and titers of fecal and oral viral shedding and the minimum oral inoculation dose needed to infect white-tailed deer need to be determined.

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