

Experimental infection of calves with epizootic hemorrhagic disease virus

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Objective—To determine whether experimental inoculation with a field strain of epizootic hemorrhagic disease virus serotype-2 (EHDV-2) suspected of causing clinical disease in naturally infected cattle would cause clinical disease in calves.

Animals—8 calves.

Procedure—A strain of EHDV-2 isolated from a white-tailed deer that died of hemorrhagic disease was passaged twice in deer and used to inoculate 6 calves SC and ID; the other 2 calves were used as controls. Physical examinations, CBC, lymphocyte blastogenesis assays, and coagulation assays were performed; rectal temperature, interferon production, and serum neutralizing antibody responses were measured; and virus isolation was attempted every other day for 21 days after inoculation and then every fourth day for another 30 days. Calves were euthanized on postinoculation day 51, and necropsy was performed.

Results—Calves inoculated with EHDV-2 became infected, as evidenced by development of viremia and seroconversion. However, the virus did not cause detectable clinical disease, clinicopathologic abnormalities, or gross lesions. Viremia was prolonged despite development of a serum neutralizing antibody response. A white-tailed deer inoculated with the same EHDV-2 strain developed clinical signs of epizootic hemorrhagic disease, demonstrating that the inoculum was virulent.

Conclusion—Calves experimentally infected with EHDV-2 developed viremia and seroconverted but did not develop detectable clinical disease. (*Am J Vet Res* 1999;60:621–626)

The epizootic hemorrhagic disease (EHD) and bluetongue (BT) viruses are closely related serogroups in the genus *Orbivirus*.¹ In North America, 2 EHD virus serotypes, EHD virus serotype-1 (EHDV-1) and EHD virus serotype-2 (EHDV-2) have been identified, whereas 5 BT virus serotypes, BT virus-2 (BTV-2), BT virus-10 (BTV-10), BT virus-11 (BTV-11), BT virus-13 (BTV-13), and BT virus-17 (BTV-17) have been recognized.² These viruses are transmitted by *Culicoides* midges and cause disease in wild and domestic ruminants. Bluetongue virus infection in cattle usually results in subclinical disease. Affected cattle may develop fever, lameness, stom-

atitis, or reproductive problems^{3,6}; however, severe clinical disease and death are rare. Reports of EHD viruses that cause clinical disease in cattle in the United States are rare, but there is evidence that EHD viruses were responsible for disease in Oregon in 1969, in Tennessee in 1972, and in Colorado in 1972.⁴ In addition, EHDV-2 was isolated from clinically normal cattle in Colorado during 1984.⁷ During the summer of 1993, a BT virus was suspected of causing laminitis and muzzle ulceration in cattle in West Virginia; however, viruses were not isolated from affected animals, and results of serologic tests suggested that the cattle were not infected with BT virus but with EHDV-2. At the same time, more than 200 white-tailed deer (WTD; *Odocoileus virginianus*) in the same locale as the affected cattle died of hemorrhagic disease (HD), and 1 of the authors of the present study (DES) isolated EHDV-2 from these deer. During the summer of 1996, cattle herds in southern Indiana were reported to be suffering from laminitis and stomatitis.⁸ Initially, BT, vesicular stomatitis, and bovine viral diarrhea were suspected, but diagnostic tests failed to confirm these suspicions. Epizootic hemorrhagic disease virus serotype-2 was isolated from 1 affected cow and from several WTD that died of HD in the same vicinity as the affected cows.⁸

Because of the uncertainty as to whether EHDV-2 can cause clinical disease in cattle, the purposes of the study reported here were to determine whether experimental inoculation with a field strain of EHDV-2 suspected of causing clinical disease in naturally infected cattle would cause clinical disease in calves and to evaluate cell-mediated, humoral, and innate (ie, production of interferon [IFN]) immune responses, alterations in blood coagulation and fibrinolytic systems, and duration of viremia in calves experimentally infected with EHDV-2.

Materials and Methods

Animals—Six crossbred beef calves and 2 Holstein steers (approx 136 to 227 kg) were used in the study. Prior to the study, all calves were seronegative for EHD and BT viruses, as determined by use of an EHD virus-BT virus agar gel immunodiffusion^b test.³ At the time calves were obtained, they were vaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, and bovine viral diarrhea virus^c and dewormed with 0.5% ivermectin^d applied topically. Throughout the study, calves were housed in individual, indoor, temperature-controlled stalls free of *Culicoides* spp.

Viral inoculum—A strain of EHDV-2 isolated from a WTD that died during the 1993 outbreak of HD in West Virginia was obtained and serially passaged through 2 juvenile WTD. These juvenile WTD had been raised in captivity and were seronegative for EHD and BT viruses, as determined by use of an EHD virus-BT virus agar gel immunodiffusion test. The first WTD was inoculated SC and ID with $1 \times 10^{5.3}$ medi-

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