Replication of bluetongue virus and epizootic hemorrhagic disease virus in pulmonary artery endothelial cells obtained from cattle, sheep, and deer

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Objective—To compare replication of bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) in pulmonary artery endothelial cells (ECs) obtained from juvenile cattle, sheep, white-tailed deer (WTD; *Odocoileus virginianus*), and black-tailed deer (BTD; *O hemionus columbianus*).

Sample Population—Cultures of pulmonary artery ECs obtained from 3 cattle, 3 sheep, 3 WTD, and 1 BTD.

Procedure—Purified cultures of pulmonary artery ECs were established. Replication, incidence of infection, and cytopathic effects of prototype strains of BTV serotype 17 (BTV-17) and 2 serotypes of EHDV (EHDV-1), and (EHDV-2) were compared in replicate cultures of ECs from each of the 4 ruminant species by use of virus titration and flow cytometric analysis.

Results—All 3 viruses replicated in ECs from the 4 ruminant species; however, BTV-17 replicated more rapidly than did either serotype of EHDV. Each virus replicated to a high titer in all ECs, although titers of EHDV-1 were significantly lower in sheep ECs than in ECs of other species. Furthermore, all viruses caused extensive cytopathic effects and a high incidence of cellular infection; however, incidence of cellular infection and cytopathic effects were significantly lower in EHDV-1-infected sheep ECs and EHDV-2-infected BTD ECs.

Conclusions and Clinical Relevance—There were only minor differences in replication, incidence of infection, and cytopathic effects for BTV-17, EHDV-1, or EHDV-2 in ECs of cattle, sheep, BTD, and WTD. It is not likely that differences in expression of disease in BTV- and EHDV-infected ruminants are attributable only to species-specific differences in the susceptibility of ECs to infection with the 2 orbiviruses. (*Am J Vet Res* 2003;64:860–865)

Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are closely related orbiviruses that are transmitted to domestic and wild ruminants by biting midges of the genus *Culicoides*.[1,2] The genome of each virus consists of 10 double-stranded RNA segments that encode 7 structural and 4 nonstructural proteins.[3] Bluetongue virus and EHDV are distinguished on the basis of serogroup-specific serologic tests.[3,4] Four serotypes of BTV (BTV-10, -11, -13, and -17) and 2 serotypes of EHDV (EHDV-1 and -2) are currently endemic in the United States and cause periodic outbreaks of a similar hemorrhagic disease syndrome in susceptible ruminants.[5]

The pathogenesis of BTV and EHDV infection is similar in cattle, sheep, and deer.[6-14] Following initial replication in lymph nodes draining the sites of inoculation, the viruses disseminate to other tissues, including the lungs and spleen, where replication occurs principally in **endothelial cells (ECs)** and mononuclear phagocytes.[11,12,15-17] Although the pathogenesis of BTV and EHDV infection is similar among ruminants, there are distinct differences in the severity of disease in BTV- and EHDV-infected cattle, sheep, and deer. Infection with BTV may cause severe disease in sheep and **white-tailed deer (WTD**; *Odocoileus virginianus*), whereas BTV infection of cattle and **black-tailed deer (BTD**; *O hemionus columbianus*) does not usually cause clinical signs of disease.[7,12,18-24] Disease attributable to EHDV infection is principally evident in WTD in the United States but is rare or undocumented in cattle, domestic breeds of sheep, and BTD.[8,24-30] Ibaraki virus, which is a genetically distinct strain of EHDV, is responsible for outbreaks of severe disease in cattle in Asia, whereas EHDV infection that does not cause clinical signs of disease is common in US cattle.[26,27,31-34]

Disease induced in ruminants by infection with BTV and EHDV is characterized by extensive vascular injury leading to hemorrhage, edema, disseminated intravascular coagulation, and tissue necrosis.[2,8-10,12,20,35,36] Pulmonary vascular injury with subsequent pulmonary edema is characteristic of many fulminant cases. Virus particles are found in the cytoplasm of ECs in the lungs of BTV- and EHDV-infected cattle, sheep, and deer, as

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determined by use of electron microscopy.[12,14,16] Thus, vascular endothelium, particularly that of the lungs, is a critical replication site for these viruses in susceptible ruminants, although the precise mechanism of injury to ECs is uncertain.[37-41]

Endothelial cells cultured from various species are heterogeneous, retain their phenotype in culture, and may react differently to the same stimuli.[37,38,41,42] Differences in the response of BTV-infected bovine and ovine ECs, including pulmonary artery ECs, have been described.[37,38-41] Thus, primary cultures of pulmonary artery ECs from cattle, sheep, BTD, and WTD were used in the study reported here to determine whether species-specific differences in the susceptibility of ECs to BTV and EHDV infection are responsible for the differing clinical manifestations of BTV and EHDV infection among ruminants.

Materials and Methods

Isolation, purification, and characterization of ECs—Segments of the pulmonary artery were obtained from juvenile (3 to 12 weeks old) animals (3 cattle, 3 sheep, 3 WTD, and 1 BTD). Cultures of ECs were established in accordance with methods described elsewhere for the isolation, propagation, and characterization of pulmonary artery ECs.[38,42]

Briefly, each animal was euthanatized, and the heart and lungs were obtained immediately thereafter. A segment of the pulmonary artery was aseptically removed, washed, and transported on ice in **Hanks' balanced salt solution (HBSS)** that contained penicillin-streptomycin, gentamicin, and amphotericin B. Each segment of pulmonary artery was filled with a solution of 2 mg of collagenase 1A-dispase[a]/mL of HBSS. After digestion for 5 minutes, ECs were removed by gently rinsing each segment with **minimal essential medium (MEM)**.[b] The isolated cells were then washed and stimulated with EC mitogen,[c] vascular endothelial growth factor,[d] β-endothelial growth factor,[e] or a combination of these products. The ECs were then grown in EC isolation medium (consisting of MEM, 10% irradiated fetal bovine serum, penicillin-streptomycin, MEM vitamins,[f] nonessential amino acids, heparin,[g] and hydrocortisone[h]) supplemented with a 5% filtered solution (0.2µM) of deer serum obtained from each donor WTD and BTD. The enzyme digestion procedure was repeated twice for each segment of pulmonary artery, and ECs from each digest were grown on tissue culture dishes coated with 1 µg of natural mouse laminin[i]/cm^2.

Cultures were incubated until colonies that had a cobblestone appearance characteristic of ECs were identified. Spindle-shaped contaminating cells were removed by aspiration. Individual EC colonies were then selected by use of trypsin-soaked cloning discs,[j] pooled, expanded, and labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindo-carbocyanine perchlorate conjugated to acetylated low-density lipoprotein.[k] Then, pooled ECs were further purified via **fluorescence-activated cell sorting (FACS)** by use of a FACS instrument,[l] as described elsewhere.[38]

After 3 successive cycles of FACS enrichment, the phenotype and purity of each EC culture were determined by use of **monoclonal antibodies (MAbs)** specific for epithelial and mesothelial cells (cytokeratin-specific MAb AE1/AE3),[m] pericytes (desmin-specific MAb D33),[n] and smooth muscle cells (actin-specific MAb 1A4),[o] as well as rabbit antiserum to **von Willebrand factor (vWF)**,[p] as described elsewhere.[38] Antibody binding was detected by use of **fluorescein isothiocyanate (FITC)**-conjugated secondary antibodies[q] and fluorescence microscopy. Only vWF-positive cultures devoid of contaminating cells were expanded in laminin-coated flasks containing EC maintenance medium (consisting of Dulbecco's MEM,[r] 10% irradiated fetal bovine serum, penicillin-streptomycin, nonessential amino acids, L-glutamine, and heparin). Cultures of ECs were screened for bovine viral diarrhea virus by use of immunofluorescence staining[43] and cryopreserved at low passage. All experiments were performed on EC cultures between passages 7 and 15.

Viruses—Cell-culture-adapted prototype strains of BTV serotype 17[s] and EHDV serotypes 1[t] and 2[u] were obtained. Each virus was reconstituted in MEM, passaged once in **baby-hamster kidney cells (BHK**; ie, BHK-21)[v] plaque-cloned twice from agar-overlaid Vero cells,[w] amplified once by passage in BHK-21 cells, and then further purified by ultracentrifugation through a 20% sucrose cushion to remove cellular debris from the inoculum. Virus pellets were resuspended in PBS-buffered lactose peptone solution[x] (consisting of 10% D-lactose and 0.2% peptone in PBS solution); resuspended cells were divided into aliquots and stored at –70°C. Lysed uninfected BHK-21 cells treated in the same manner were used as a control inoculum. Virus titers were determined by microtitration in BHK-21 cells. Results were expressed as $TCID_{50}$/mL, as described elsewhere.[44,45]

Virus replication in ECs—Replication of the various viruses (BTV-17, EHDV-1, and EHDV-2) was compared by 1-step growth analyses after infection of cattle, sheep, BTD, and WTD ECs. Confluent cultures of cattle, sheep, BTD, and WTD ECs of similar passage were inoculated simultaneously at a high **multiplicity of infection (MOI**; value of 3). Following incubation for 1 hour at 37°C, the inoculum was removed, and ECs were washed 3 times with Dulbecco's MEM and then supplemented with 1 mL of EC maintenance medium. Cells were harvested 1, 4, 12, 16, 24, 48, 72, and 96 hours after inoculation and examined microscopically for evidence of cytopathic effects. Virus titers ($TCID_{50}$/mL) were determined by microtitration in BHK-21 cells, as described elsewhere.[44,45]

Flow cytometric analysis—Incidence of cellular infection was compared in BTV- and EHDV-infected cattle, sheep, BTD, and WTD ECs by use of FACS analysis after immunofluorescence labeling of BTV proteins. Specifically, EC cultures of similar passage from each species were inoculated simultaneously with BTV-17, EHDV-1, or EHDV-2 at a high MOI (value of 3.0), and cells were harvested 1, 4, 12, 16, 24, 48, 72, and 96 hours after inoculation. Cells remaining in each flask were digested by use of trypsin, collected, added to the media, and then pelleted by centrifugation (400 × g for 5 minutes at 4°C). Cells were rinsed once in cold PBS solution, fixed in 90% methanol, incubated with 0.5% Triton-X 100, blocked with 10% normal goat serum, and then labeled with MAbs specific for virus protein 7 of BTV (ie, MAb 290),[46] virus protein 7 of EHDV-2 (ie, MAb 4F4.H1)[47,y] or protein A/G purified[z] polyclonal mouse anti-EHDV-1 ascitic fluid.[aa] Virus-infected cells were detected by the addition of FITC-conjugated goat anti-mouse[bb] or anti-rabbit[cc] immunoglobulin and subsequent FACS analysis with a FACS instrument,[dd] as described elsewhere.[44]

Percentage of necrotic ECs in cultures infected with BTV-17, EHDV-1, and EHDV-2 was quantitated in samples obtained 1, 12, 24, 48, 72, and 96 hours after inoculation. Determinations were based on exclusion of propidium iodide and FACS analysis, as described elsewhere.[38]

Data analysis—Data for the FACS analysis were analyzed by use of a software program,[ee] and statistical analysis was conduced by use of another software program.[ff] Each experiment was repeated at least 3 times, and data from replicate experiments were compared at each time point. Mean ± SD values were calculated for each variable,

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and differences among species were determined at each time point by use of a 1-way ANOVA and the Tukey multiple-comparison post-hoc tests. Values of $P \leq 0.05$ were considered significant.

Results

Characteristics of cultured BTD and WTD pulmonary artery ECs—Pulmonary artery ECs obtained from BTD and WTD were isolated and cultured. Immunofluorescent staining for vWF in perinuclear granules (Weibel-Palade bodies) confirmed that the cultured cells were ECs. Lack of immunofluorescent staining with MAbs directed against cytokeratin, smooth muscle actin, and desmin further confirmed purity of the cultures. All ECs had strong contact-inhibited characteristics and formed cobblestone monolayers that were maintained for up to 20 passages without contamination by other cell types.

Virus replication and kinetics in ECs—All viruses (BTV-17, EHDV-1, and EHDV-2) replicated in cultures of cattle, sheep, BTD, and WTD ECs that were inoculated at high MOI; however, BTV-17 replicated more rapidly in ECs of all 4 species, compared with results for either serotype of EHDV (**Fig 1**). Pulmonary artery ECs of cattle, BTD, and WTD were equally susceptible to infection by BTV-17, EHDV-1, and EHDV-2, and we did not detect significant differences in maximum virus titers in these cultures. The BTV-17 and EHDV-2 replicated to similar titers in sheep ECs, whereas titers of EHDV-1 were significantly lower in sheep ECs at 24 ($P = 0.021$), 48 ($P = 0.046$), and 72 ($P = 0.045$) hours

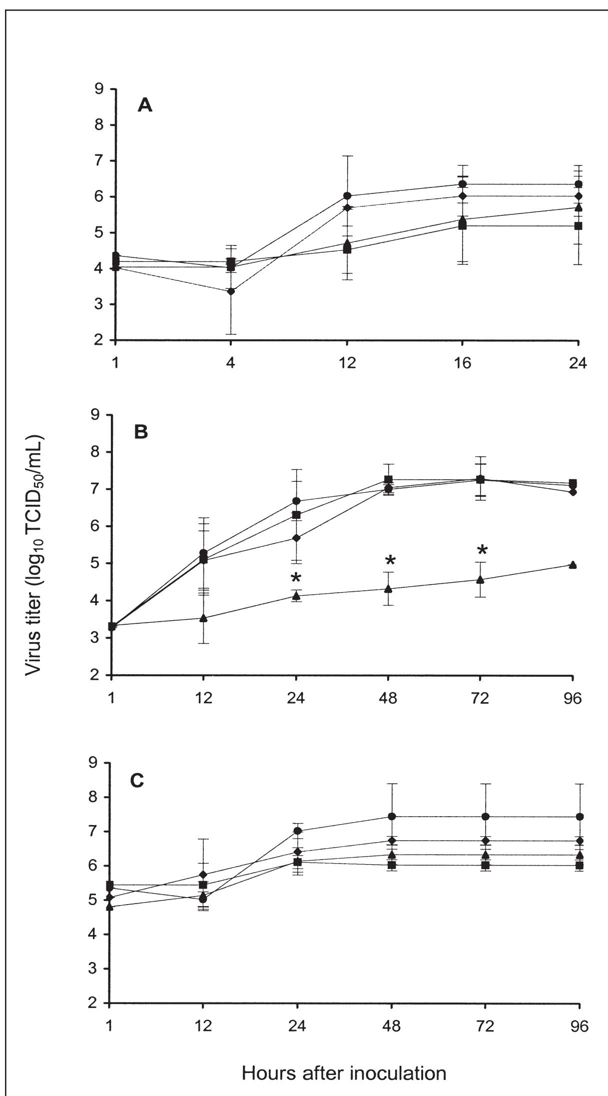

Figure 1—Mean ± SD viral titers in cultures of pulmonary artery endothelial cells (ECs) obtained from cattle (diamond), sheep (triangle), black-tailed deer (BTD; square), and white-tailed deer (WTD; circle) that were inoculated with bluetongue virus (BTV) or 2 serotypes of epizootic hemorrhagic disease virus (EHDV). The ECs were inoculated (time 0) with BTV-17 (A), EHDV-1 (B), or EHDV-2 (C). *Viral growth is significantly ($P < 0.05$) lower in EHDV-1-infected ECs of sheep, compared with viral growth in EHDV-1-infected ECs of cattle, BTD, and WTD at the same time point.

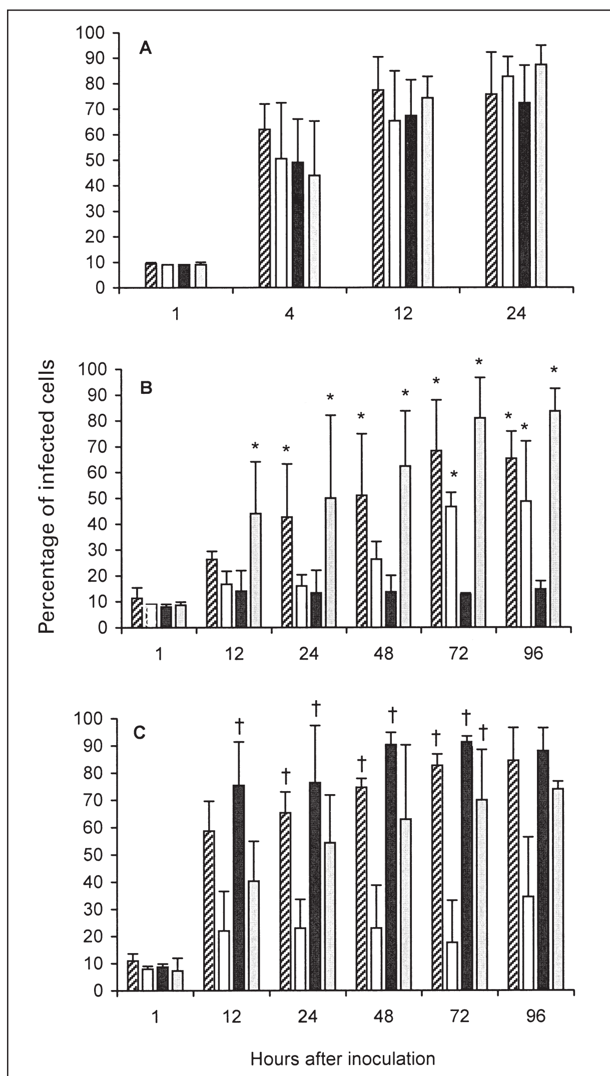

Figure 2—Mean ± SD values for flow cytometric analysis of the incidence of BTV-17 (A), EHDV-1 (B), or EHDV-2 (C) infection in cultures of pulmonary artery ECs obtained from cattle (diagonal-striped bar), sheep (black bar), BTD (white bar), and WTD (gray bar). The incidence of infection is similar in all BTV-17-infected ECs of the 4 species, but the incidence of EHDV-1 and -2 infection of ECs differed significantly among ECs of the 4 species. *Within a time point, value differs significantly ($P < 0.05$) from value for ECs obtained from sheep. †Within a time point, value differs significantly ($P < 0.05$) from value for ECs obtained from BTD.

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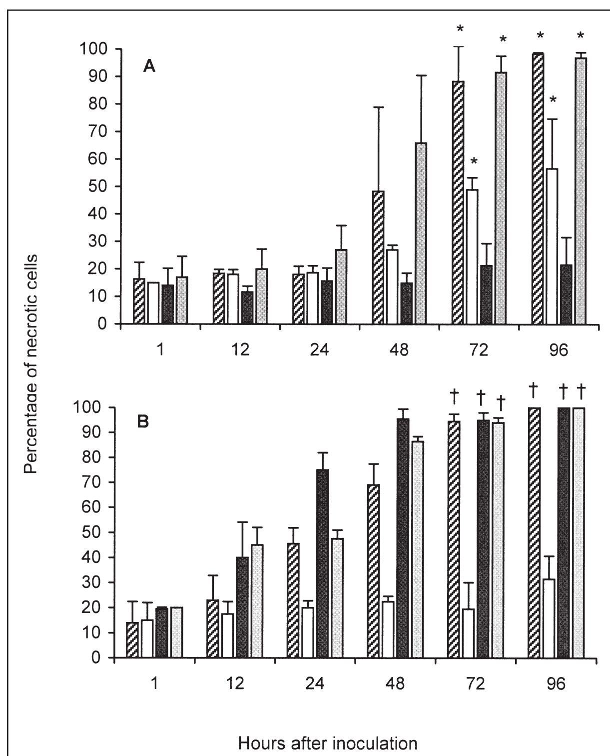

Figure 3— Mean ± SD percentage of necrotic cells attributable to infection with EHDV-1 (A) or EHDV-2 (B) in cultures of pulmonary artery ECs obtained from cattle (diagonal-striped bar), sheep (black bar), BTD (white bar), and WTD (gray bar). *See* Figure 2 for key.

after inoculation, compared with titers of EHDV-1 in pulmonary artery ECs of cattle, BTD, or WTD.

Incidence of infection and necrosis in BTV- and EHDV-infected ECs—The FACS analysis was used to compare the incidence of infection by BTV-17, EHDV-1, and EHDV-2 in pulmonary artery ECs of cattle, sheep, BTD, and WTD. Whereas there was not a significant difference in the incidence of BTV-17 infection in any of the EC cultures for the 4 species, the incidence of infection by EHDV-1 and -2 differed depending on the species of origin for the EC cultures **(Fig 2)**. A significantly ($P = 0.001$ to 0.038) lower percentage of virus-positive cells were detected in EHDV-1-infected sheep EC cultures 12, 24, 48, 72, and 96 hours after inoculation, compared with the percentages in EHDV-1-infected cultures of cattle, BTD, and WTD ECs at those same time periods. Furthermore, significantly ($P = 0.045$) fewer EHDV-1-infected ECs were detected 72 hours after inoculation for BTD ECs than for WTD ECs. Incidence of infection by EHDV-2 was similar in EC cultures of cattle, sheep, and WTD, but it was significantly ($P = 0.001$ to 0.037) lower 12, 24, 48, and 72 hours after inoculation in EC cultures of BTD.

Cytopathic effects caused by BTV-17, EHDV-1, or EHDV-2 were compared by microscopic evaluation (data not shown) and FACS quantitation of damage to EC membranes based on the uptake or exclusion of propidium iodide (c 3). All 3 viruses caused extensive cytopathic effects in EC cultures of each of the 4 species; however, cytopathic effects did not involve the entire monolayer in EHDV-2-infected ECs of BTD or EHDV-1-infected ECs of sheep, whereas complete necrosis (100%) was detected in EHDV-1- and EHDV-2-infected ECs of cattle and WTD.

Discussion

Although the pathogenesis of BTV and EHDV infection is similar in ruminants, there is marked variation in expression of disease in cattle, sheep, BTD, and WTD infected with these viruses. Virus-mediated EC injury and disseminated intravascular coagulation are central to expression of disease in BTV- and EHDV-infected ruminants.[9,16,37,38] Specifically, EC injury and death occur prior to the development of disseminated intravascular coagulation in susceptible ruminants, but the precise roles of direct virus-mediated EC injury, EC activation, and inflammatory and vasoactive mediators in expression of disease attributable to BTV or EHDV have not been adequately defined.[9,12,14,16,38-40] Thus, pure cultures of pulmonary artery ECs were used to determine whether inherent species-specific differences in the susceptibility of ECs are responsible for differences in the clinical manifestations of BTV and EHDV infections in cattle, sheep, BTD, and WTD.

Prototype strains of BTV-17, EHDV-1, and EHDV-2 used in the study reported here all replicated to high titers in ECs regardless of the species of origin, and there were only minor differences in the titers of each of these viruses in infected EC cultures of the various species. Specifically, EHDV-1 but not EHDV-2 replicated to lower titers in infected sheep ECs, and the incidence of infection was lower in EHDV-1-infected ECs of sheep and EHDV-2-infected ECs of BTD. Similarly, cytopathic effects involved the entire monolayer of EHDV-1- and -2-infected ECs of cattle and WTD, whereas complete cytopathic effects never developed in EHDV-1-infected ECs of sheep and EHDV-2-infected ECs of BTD. Together, these results indicate that although ECs of sheep and BTD are somewhat less susceptible to infection by EHDV than are ECs of cattle or WTD, differences in direct virus-mediated EC damage are unlikely to be solely responsible for the differences in disease expression in BTV- and EHDV-infected ruminants.

Infections attributable to hemorrhagic viruses may alter hemostasis through direct damage to ECs or activation of various inflammatory pathways.[48-50] These inflammatory pathways involve EC- and leukocyte-derived cytokines, such as interferon, **interleukin (IL)**-1, IL-8, and IL-6 that regulate the hemostatic activities of ECs, as well as EC-derived procoagulant factors, such as vWF, tissue factor, and thromboplastin and anticoagulant factors such as prostacyclin.[51,52] Similarly, BTV-induced EC-derived mediators clearly modulate the mechanism of cell death (necrosis or apoptosis) in cattle and sheep pulmonary ECs,[38] and the transcription of genes encoding the proinflammatory cytokines IL-1, IL-6, IL-8, and cyclooxygenase-2 was significantly increased in BTV-infected ECs of cattle, compared with transcription in ECs of sheep.[39,40] Furthermore, BTV-infected pulmonary ECs of cattle produced significantly higher amounts of prostacyclin than did similarly infected pulmonary ECs of sheep[38];

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these findings were subsequently confirmed by an in vivo study.[39] Thus, inflammatory and vasoactive mediators released by ECs and other cells likely influence disease expression in BTV- and EHDV-infected cattle, sheep, BTD, and WTD.

Results obtained in the study reported here contrast slightly with those reported in other studies.[38-41] In those studies, investigators reported that the consequences differed for BTV infection of ECs of sheep and ECs of cattle. However, investigators in 1 of those studies[41] used ECs cultured from the umbilical veins of fetal sheep and cattle, whereas we used pulmonary artery ECs derived from juvenile sheep, cattle, BTD, and WTD. It is clear that ECs derived from various tissues are phenotypically distinct and retain their phenotype in culture.[38,42] Similarly, differences in consequences of BTV infection of ECs of sheep and cattle described in other studies[38-40] were most evident in ECs derived from the pulmonary microvasculature and in cultures infected at low MOI in which virus-induced inflammatory and vasoactive mediators likely exerted a substantial effect. Russell et al[41] also used a low MOI for infection of ECs in their experiments. In contrast, we used a high MOI for infection of ECs to minimize the impact of these mediators. Lastly, it must be emphasized that primary EC cultures reflect the genetic constitution of the animal or animals from which they are derived. It is logical that future in vitro studies of the pathogenesis of infections attributable to BTV and EHDV in ruminants will use ECs derived from the pulmonary microvasculature of cattle, sheep, BTD, and WTD, because these are the most relevant target cells.

In the study reported here, pulmonary artery ECs obtained from cattle, sheep, BTD, and WTD provided a useful in vitro model for use in investigating the pathogenesis of BTV and EHDV infections in ruminants. However, analysis of data from this study suggests that direct virus-induced EC injury is only 1 aspect of the vascular damage caused by these viruses.

[a]Collagenase 1A-dispase, Sigma Chemical Co, St Louis, Mo.
[b]Minimum essential medium, Invitrogen, Carlsbad, Calif.
[c]Endothelial mitogen, Biomedical Technologies, Stoughton, Mass.
[d]Vascular endothelial growth factor, Sigma Chemical Co, St Louis, Mo.
[e]β-endothelial growth factor, Sigma Chemical Co, St Louis, Mo.
[f]MEM vitamin solution (100X), Invitrogen, Carlsbad, Calif.
[g]Heparin sodium salt from porcine intestinal mucosa, Sigma Chemical Co, St Louis, Mo.
[h]Hydrocortisone 98%, (cell culture tested), Sigma Chemical Co, St Louis, Mo.
[i]Natural mouse laminin, Invitrogen, Carlsbad, Calif.
[j]Cell cloning discs, Scienceware, Pequannock, NJ.
[k]DiI-Ac-LDL, Biomedical Technologies, Stoughton, Mass.
[l]MoFlo, Cytomation, Fort Collins, Colo.
[m]MAb Ae1/AE3, cytokeratin, Dako Corp, Carpinteria, Calif.
[n]MAb D33, desmin, Dako Corp, Carpinteria, Calif.
[o]MAb 1A4, actin alpha smooth muscle, Dako Corp, Carpinteria, Calif.
[p]von Willebrand Factor (factor VIII related antigen), Dako Corp, Carpinteria, Calif.
[q]Anti-mouse IgG (Fab specific)–FITC antibody produced in goat and anti-rabbit IgG (Fab specific)-FITC antibody produced in goat, Sigma Chemical Co, St Louis, Mo.
[r]Dulbecco's modified eagle medium, MediaTech, Herndon, Va.
[s]VR-875, American Type Culture Collection, Manassas, Va.
[t]VR-419, American Type Culture Collection, Manassas, Va.
[u]VR-982, American Type Culture Collection, Manassas, Va.
[u]CCL-10, American Type Culture Collection, Manassas, Va.
[w]CCL-81, American Type Culture Collection, Manassas, Va.
[x]D-lactose and peptone, Sigma Chemical Co, St Louis, Mo.
[y]Monoclonal antibody 4F4.H1 was provided by Dr. J. O. Mecham, USDA, Agricultural Research Service, Arthropod-Borne Animal Diseases Research Laboratory, Laramie, Wyo.
[z]ImmunoPure (A/G) IgG purification kit, Pierce Biotechnology, Rockford, Ill.
[aa]VR1234AF, American Type Culture Collection, Manassas, Va.
[bb]Anti-mouse IgG (Fab specific)–FITC antibody produced in goat, Sigma Chemical Co, St Louis, Mo.
[cc]Anti-rabbit IgG (Fab specific)-FITC antibody produced in goat, Sigma Chemical Co, St Louis, Mo.
[dd]FACscan, Becton Dickinson, San Jose, Calif.
[ee]CELLQuest, version 3.4.1, Becton Dickinson, San Jose, Calif.
[ff]SPSS 11.0 for Windows, SPSS Inc, Chicago, Ill.

References

1. Erasmus BJ. Bluetongue in sheep and goats. *Aust Vet J* 1975;51:165–170.
2. Hoff GL, Trainer DO. Bluetongue and epizootic hemorrhagic disease viruses: their relationship to wildlife species. *Adv Vet Sci Comp Med* 1978;22:111–132.
3. Gould AR, Hyatt AD. The orbivirus genus. Diversity, structure, replication and phylogenetic relationships. *Comp Immunol Microbiol Infect Dis* 1994;17:163–188.
4. Gorman BM. An overview of the orbiviruses. In: Walton TE, Osburn BI, eds. *Bluetongue, African horse sickness and related orbiviruses: proceedings of the second international symposium*. Boca Raton, Fla: CRC Press Inc, 1992;335–347.
5. Gibbs EP, Greiner EC. The epidemiology of bluetongue. *Comp Immunol Microbiol Infect Dis* 1994;17:207–220.
6. Barratt-Boyes SM, MacLachlan NJ. Dynamics of viral spread in bluetongue virus infected calves. *Vet Microbiol* 1994;40:361–371.
7. Barratt-Boyes SM, MacLachlan NJ. Pathogenesis of bluetongue virus infection of cattle. *J Am Vet Med Assoc* 1995;206:1322–1329.
8. Fletch AL, Karstad LH. Studies on the pathogenesis of experimental epizootic hemorrhagic disease of white-tailed deer. *Can J Comp Med* 1971;35:224–229.
9. Howerth EW, Greene CE, Prestwood AK. Experimentally induced bluetongue virus infection in white-tailed deer: coagulation, clinical pathologic, and gross pathologic changes. *Am J Vet Res* 1988;49:1906–1913.
10. MacLachlan NJ. The pathogenesis and immunology of bluetongue virus infection of ruminants. *Comp Immunol Microbiol Infect Dis* 1994;17:197–206.
11. MacLachlan NJ, Rossitto PV, Jagels G, et al. The pathogenesis of experimental bluetongue virus infection of calves. *Vet Pathol* 1990;27:223–229.
12. Mahrt CR, Osburn BI. Experimental bluetongue virus infection of sheep; effect of vaccination: pathologic, immunofluorescent, and ultrastructural studies. *Am J Vet Res* 1986;47:1198–1203.
13. Stallknecht DE, Howerth EW, Kellogg ML, et al. In vitro replication of epizootic hemorrhagic disease and bluetongue viruses in white-tailed deer peripheral blood mononuclear cells and virus-cell association during in vivo infections. *J Wildl Dis* 1997;33:574–583.
14. Tsai K, Karstad L. The pathogenesis of epizootic hemorrhagic disease of deer: an electron microscopic study. *Am J Pathol* 1973;70:379–400.
15. Barratt-Boyes SM, Rossitto PV, Taylor BC, et al. Response of the regional lymph node to bluetongue virus infection in calves. *Vet Immunol Immunopathol* 1995;45:73–84.
16. Howerth EW, Tyler DE. Experimentally induced bluetongue virus infection in white-tailed deer: ultrastructural findings. *Am J Vet Res* 1988;49:1914–1922.
17. Pini A. Study on the pathogenesis of bluetongue: replication of the virus in the organs of infected sheep. *Onderstepoort J Vet Res* 1976;43:159–164.
18. Bowne JG. Bluetongue disease. *Adv Vet Sci Comp Med* 1971;15:1–46.
19. Hourrigan JL, Klingsporn AL. Bluetongue: the disease in cattle. *Aust Vet J* 1975;51:170–174.

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20. Moulton JE. Pathology of bluetongue of sheep. *J Am Vet Med Assoc* 1961;138:493–498.

21. Shope RE, Lester MD, MacNamara G, et al. A virus-induced epizootic hemorrhagic disease of the Virginia white-tailed deer (*Odocoileus virginianus*). *J Exp Med* 1959;111:155–170.

22. Stauber EH, Farrell RK, Spencer GR. Nonlethal experimental inoculation of Columbia black-tailed deer (*Odocoileus hemionus columbianus*) with virus of epizootic hemorrhagic deer disease. *Am J Vet Res* 1977;38:411–412.

23. Vosdingh RA, Trainer DO, Easterday BC. Experimental bluetongue disease in white-tailed deer. *Can J Comp Med Vet Sci* 1968;32:382–387.

24. Work TM, Jessup DA, Sawyer MM. Experimental bluetongue and epizootic hemorrhagic disease virus infection in California black-tailed deer. *J Wildl Dis* 1992;28:623–628.

25. Gibbs EP, Lawman MJ. Infection of British deer and farm animals with epizootic haemorrhagic disease of deer virus. *J Comp Pathol* 1977;87:335–342.

26. House C, Shipman LD, Weybright G. Serological detection of epizootic hemorrhagic disease in cattle in the USA with lesions suggestive of vesicular disease. *Ann N Y Acad Sci* 1998;849:497–500.

27. Omori T, Inaba Y, Morimoto T, et al. Ibaraki virus, an agent of epizootic disease of cattle resembling bluetongue. I. Epidemiologic, clinical and pathologic observations and experimental transmission to calves. *Jpn J Microbiol* 1969;13:139–157.

28. Pirtle EC, Layton JM. Epizootic hemorrhagic disease in white-tailed deer—characteristics of the South Dakota strain of virus. *Am J Vet Res* 1961;22:104–108.

29. Thompson LH, Mecham JO, Holbrook FR. Isolation and characterization of epizootic hemorrhagic disease virus from sheep and cattle in Colorado. *Am J Vet Res* 1988;49:1050–1052.

30. Uren MF. Clinical and pathological responses of sheep and cattle to experimental infection with five different viruses of the epizootic hemorrhagic disease of deer serogroup. *Aust Vet J* 1986;63:199–201.

31. Abdy MJ, Howerth EE, Stallknecht DE. Experimental infection of calves with epizootic hemorrhagic disease virus. *Am J Vet Res* 1999;60:621–626.

32. Aradaib IE, Sawyer MM, Osburn BI. Experimental epizootic hemorrhagic disease virus infection in calves: virologic and serologic studies. *J Vet Diagn Invest* 1994;6:489–492.

33. Gould AR, Pritchard LI. Phylogenetic analyses of the complete nucleotide sequence of the capsid protein (VP3) of Australian epizootic haemorrhagic disease of deer virus (serotype 2) and cognate genes from other orbiviruses. *Virus Res* 1991;21:1–18.

34. Ohashi S, Yoshida K, Watanabe Y, et al. Identification and PCR-restriction fragment length polymorphism analysis of a variant of the Ibaraki virus from naturally infected cattle and aborted fetuses in Japan. *J Clin Microbiol* 1999;37:3800–3803.

35. Karstad L, Winter A, Trainer DO. Pathology of epizootic hemorrhagic disease of deer. *Am J Vet Res* 1961;22:227–234.

36. Thomas AD, Neitz WO. Further observations on the pathology of bluetongue in sheep. *Onderstepoort J Vet Sci Anim Ind* 1947;22:27–40.

37. Coen ML, Ellis JA, O'Toole DT, et al. Cytokine modulation of the interaction between bluetongue virus and endothelial cells in vitro. *Vet Pathol* 1991;28:524–532.

38. DeMaula CD, Jutila MA, Wilson DW, et al. Infection kinetics, prostacyclin release and cytokine-mediated modulation of the mechanism of cell death during bluetongue virus infection of cultured ovine and bovine pulmonary artery and lung microvascular endothelial cells. *J Gen Virol* 2001;82:787–794.

39. DeMaula CD, Leutenegger CM, Bonneau KR, et al. The role of endothelial cell-derived inflammatory and vasoactive mediators in the pathogenesis of bluetongue. *Virology* 2002;296:330–337.

40. DeMaula CD, Leutenegger CM, Jutila MA, et al. Bluetongue virus-induced activation of primary bovine lung microvascular endothelial cells. *Vet Immunol Immunopathol* 2002;86:147–157.

41. Russell H, O'Toole DT, Bardsley K, et al. Comparative effects of bluetongue virus infection of ovine and bovine endothelial cells. *Vet Pathol* 1996;33:319–331.

42. Meyrick B, Hoover R, Jones MR, et al. In vitro effects of endotoxin on bovine and sheep lung microvascular and pulmonary artery endothelial cells. *J Cell Physiol* 1989;138:165–174.

43. Corapi WV, Donis RO, Dubovi EJ. Monoclonal antibody analyses of cytopathic and noncytopathic viruses from fatal bovine viral diarrhea virus infections. *J Virol* 1988;62:2823–2827.

44. Barratt-Boyes SM, Rossitto PV, Stott JL, et al. Flow cytometric analysis of in vitro bluetongue virus infection of bovine blood mononuclear cells. *J Gen Virol* 1992;73:1953–1960.

45. MacLachlan NJ, Schore CE, Osburn BI. Antiviral responses of bluetongue virus-inoculated bovine fetuses and their dams. *Am J Vet Res* 1984;45:1469–1473.

46. Whetter LE, MacLachlan NJ, Gebhard DH, et al. Bluetongue virus infection of bovine monocytes. *J Gen Virol* 1989;70:1663–1676.

47. Mecham JO, Jochim MM. Development of an enzyme-linked immunosorbent assay for the detection of antibody to epizootic hemorrhagic disease of deer virus. *J Vet Diagn Invest* 2000;12:142–145.

48. Arnold R, Konig B, Galatti H, et al. Cytokine (IL-8, IL-6, TNF-alpha) and soluble TNF receptor-I release from human peripheral blood mononuclear cells after respiratory syncytial virus infection. *Immunology* 1995;85:364–372.

49. Chen JP, Cosgriff TM. Hemorrhagic fever virus-induced changes in hemostasis and vascular biology. *Blood Coagul Fibrinolysis* 2000;11:461–483.

50. Vercellotti G. Effects of viral activation of the vessel wall on inflammation and thrombosis. *Blood Coagul Fibrinolysis* 1998;9:S3–S6.

51. Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. *FASEB J* 1992;6:2591–2599.

52. Wu K, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. *Annu Rev Med* 1996;47:315–331.

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