Epizootic Hemorrhagic Disease in Cattle, Western Turkey

Ethem Mutlu Temizel, Kadir Yesilbag, Carrie Batten, Sezgin Senturk, Narender S. Maan, Peter Paul Clement Mertens, and Hasan Batmaz

In 2007, an outbreak of epizootic hemorrhagic disease (EHD) occurred in Turkey. On the basis of clinical investigation, 41 cattle were suspected to have EHD. Reverse transcription–PCR and sequence analyses indicated that the virus belonged to EHD virus serotype 6, thus confirming EHD virus infection of cattle in Turkey.

Epizootic hemorrhagic disease virus (EHDV) is a member of the genus Orbivirus, family Reoviridae, and is closely related to bluetongue virus (BTV). EHD often causes death in white-tailed deer and, less frequently, a bluetongue-like illness in cattle (1–3). Culicoides spp. act as vectors, transmitting EHDV between susceptible ruminant hosts (2). The clinical signs of EHD in cattle are fever, anorexia, dysphagia, ulcerative and necrotic lesions of the oral mucosa (Figure 1), hyperemia and edema of the conjunctival mucosae (Figure 2), sore muzzle, hyperemia of the teats and udder, hemorrhage, dehydration, and lameness (3). EHDV has been isolated from cattle throughout the world, including Africa, North America, Australia, Japan, and recently Israel (4–10). Recent outbreaks of EHDV in Israel during 2006 were attributed to EHDV-7 (6); outbreaks in Morocco and Algeria were similar to EHDV-6/EHDV-318. An initial suspicion of EHD, based on observation of clinical signs, can be confirmed by virus isolation and characterization, nucleic acid identification, or serologic testing. ELISA is a specific and sensitive method for detecting EHDV-specific antigens or antibodies and confirming the disease (2–5,8,11,12).

The Study

In July 2007, a 7-week outbreak of disease in cattle began in Mugla, Turkey. The disease was regarded as unusual or atypical for the region, and cases were reported to the Uludag University Faculty of Veterinary Medicine.

Similar reports were also received from Izmir, Canakkale, and Istanbul through the end of August 2007. The cattle had stomatitis, swelling of eyelids, respiratory distress, nasal and ocular discharge, redness and scaling of muzzle and lips, lameness, and udder erythema, and some were recumbent (Table 1). Body temperatures were elevated (39.7°C–41.1°C), except for 1 animal, whose temperature was 37.5°C, below the reference range for cattle (37.8°C–39.2°C). However, heart rates (mean 72 ± 3 beats/min) and respiratory rates (mean 24 ± 4 breaths/min) were within reference ranges of 60–80 beats/min and 10–30 breaths/min, respectively, for cattle with suspected disease. Cattle with EHD had tachycardia and tachypnea (Table 2). Causes of mucosal disease, stomatitis, and fever, including bovine viral diarrhea, foot and mouth disease, and infectious bovine rhinotracheitis, were considered, but the rate of spread and some of the clinical signs ruled out these diseases. However, the clinical signs of the disease were consistent with either EHD or BTV infection (6,8–10). These diseases were therefore considered as requiring further laboratory-based diagnostic assays.

A total of 41 blood samples were obtained from the affected cattle (35 Holsteins and 6 Brown Swiss, 2–5 years of age). Samples were obtained in tubes with and without EDTA. Complete blood analysis showed that 5 of the cattle with EHD had low leukocyte counts (Table, available from www.cdc.gov/EID/content/15/2/317-appT.htm). After use for hematologic analysis, samples were stored at –30°C until virologic and serologic tests could be performed. Samples from the 41 animals were tested by ELISA for bovine viral diarrhea virus antigens; results were negative. To isolate virus, we spread uncleotted blood samples onto baby hamster kidney–21 (BHK) cells.

Because EHDV had never been observed in Turkey, no diagnostic procedures were available. We therefore submitted selected samples (11 whole blood samples, 4 serum
samples, and 15 supernatant samples from the BHK cells) to
the World Organisation for Animal Health reference
laboratory for BTV (Institute for Animal Health, Pirbright,
UK) for virologic and serologic analysis. All samples were
tested for BTV by real-time RT-PCR and for EHDV by
conventional RT-PCR (13–15). All results were negative
for BTV. However, a conventional RT-PCR assay target-
ing genome segment 7 of EHDV (15) indicated that one
of the cell culture supernatants, from an early case from
Mugla, was positive for EHDV; this cow died 3 hours after
clinical examination and sample collection. The remaining
cell culture supernatants were negative for EHDV. It is
unusual to isolate EHDV by direct inoculation of BHK cells;
initial passage through eggs or the Culicoides variipennis
larvae cell line (KC cells) is usually required (15). The 4
serum samples were also tested for EHDV-specific anti-
bodies by ELISA (12); only 1 sample was found to contain
antibodies to EHDV.

Conventional RT-PCR of RNA extracted from the 11
original blood samples gave inconclusive results. Agarose
gel electrophoresis indicated no product of the expected
size. However, virus was isolated from 6 of the blood sam-
ple by using KC cells (dsRNA virus reference collection
at the Institute for Animal Health, reference collection nos.
TUR2007/01-06). These 6 samples and the 1 original posi-
tive cell culture were further tested by serotype-specific RT-
PCRs that targeted segment 2 for identification of EHDV
serotype. This analysis identified all viruses as EHDV-6,
sharing 95.7% nucleotide sequence identity (segment 2,
110–670 bp) with the EHDV reference strain 318.

Conclusions

Of the selected samples submitted for BTV and EHDV
testing, the positive identification of EHDV RNA supports
initial clinical identification of an EHD outbreak in Tur-
key. The negative results from the blood samples may have
resulted from degradation of viral RNA during transfer
to the laboratory or insufficient sensitivity in the conven-
tional RT-PCR. The propagation of another 6 virus isolates
(TUR2007/01–06) by passage through KC cells indicates
that virus was indeed present in the original blood samples,
although not detected by conventional RT-PCR.

That only 1 of the 4 original serum samples was posi-
tive for EHDV antibodies by ELISA can be explained by
time of sample collection. Antibodies to BTV can be de-
tected from 8 days after infection (11); these samples may
have been collected during the early stages of infection,
before development of the immune response.

This study confirms EHDV infection of cattle in Tur-
key. EHD needs to be considered in the differential diag-
nosis of cattle with clinical signs that include fever; stoma-
titis; lameness; salivation; redness and scaling of the nose

<table>
<thead>
<tr>
<th>Table 1. Clinical signs in cattle tested for EHD, Turkey, 2007*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHD status†</td>
</tr>
<tr>
<td>Suspected (n = 41)</td>
</tr>
<tr>
<td>PCR+ (n = 1)</td>
</tr>
<tr>
<td>Seropositive (n = 1)</td>
</tr>
<tr>
<td>Virus isolated (n = 6)</td>
</tr>
</tbody>
</table>

*EHD, epizootic hemorrhagic disease; CE, conjunctival edema; RM, reduced milk; UE, udder edema; RD, respiratory distress.
†PCR, ELISA, and virus isolation were performed on selected samples from the 41 samples (11 whole blood samples, 4 serum samples, and 15 supernatant samples from the baby hamster kidney cells). The virus-positive animals were PCR negative.
‡Nasal and ocular discharge
§Redness and scaling of nose and lips.
and lips; swelling of the tongue; and erosions of the pulvinus dentalis, palatinum, and nose. More detailed studies of EHDV infection in cattle are needed.

Dr Temizel is a veterinarian at the Uludag University Faculty of Veterinary Medicine, Department of Internal Medicine, Bursa, Turkey. His research interests are the infectious and metabolic diseases of cattle and small ruminants.

References


Address for correspondence: Ethem Mutlu Temizel, Uludag University Faculty of Veterinary Medicine, Internal Medicine, Gorukle, Bursa 16190, Turkey; email: ethem@uludag.edu.tr

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Table 2. Vital signs of cattle tested for EHD, Turkey, 2007*

<table>
<thead>
<tr>
<th>EHD status</th>
<th>Temperature, °C</th>
<th>Heart rate, beats/min</th>
<th>Respiratory rate, breaths/min</th>
<th>Mucous membranes</th>
<th>Enlarged lymph nodes</th>
<th>Rumen motility, contractions/5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected (n = 41)</td>
<td>37.5–39.2</td>
<td>72 ± 3‡</td>
<td>24 ± 4‡</td>
<td>Cyanotic (n = 2)</td>
<td>ND</td>
<td>0–12</td>
</tr>
<tr>
<td>PCR+ (n = 1)</td>
<td>40.5</td>
<td>110</td>
<td>52</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Seropositive (n = 1)</td>
<td>41.1</td>
<td>104</td>
<td>48</td>
<td>Hyperemic</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Virus isolated (n = 6)</td>
<td>39.7–40.6</td>
<td>68–86</td>
<td>32–56</td>
<td>Normal color</td>
<td>Submandibular</td>
<td>0–4</td>
</tr>
</tbody>
</table>

*EHD, epizootic hemorrhagic disease; ND, not detected.
†PCR, ELISA, and virus isolation were performed on selected samples from the 41 samples (11 whole blood samples, 4 serum samples, and 15 supernatant samples from the baby hamster kidney cells). The virus-positive animals were PCR negative.
‡Mean ± SEM.
### Appendix Table. Hematologic findings in cattle tested for epizootic hemorrhagic disease, Turkey, 2007*

<table>
<thead>
<tr>
<th>EPH status†</th>
<th>WBCs, ×10⁹/µL</th>
<th>Neutrophils, %</th>
<th>Lymphocytes, %</th>
<th>Monocytes, %</th>
<th>Eosinophils, %</th>
<th>Basophils, %</th>
<th>RBCs, ×10¹²/µL</th>
<th>HGB, g/dL</th>
<th>HCT, %</th>
<th>MCV, µm³</th>
<th>MCH, pg/cell</th>
<th>MCHC, g/dL</th>
<th>RDW, %</th>
<th>PLT, ×10⁹/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected (n = 41) ‡</td>
<td>4.3 ± 0.3</td>
<td>55.1 ± 2.7</td>
<td>33.2 ± 2.8</td>
<td>9.1 ± 0.8</td>
<td>0.3 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>6.4 ± 0.2</td>
<td>9.2 ± 0.2</td>
<td>25.7 ± 0.7</td>
<td>40.8 ± 0.9</td>
<td>15 ± 0.5</td>
<td>36.7 ± 0.8</td>
<td>22.9 ± 0.4</td>
<td>359.4 ± 32</td>
</tr>
<tr>
<td>PCR+ (n = 1)</td>
<td>1.99</td>
<td>66.5</td>
<td>22.8</td>
<td>8.59</td>
<td>0.224</td>
<td>1.82</td>
<td>5.42</td>
<td>9.27</td>
<td>24.8</td>
<td>45.8</td>
<td>17.1</td>
<td>37.3</td>
<td>20</td>
<td>168</td>
</tr>
<tr>
<td>Seropositive (n = 1)</td>
<td>5</td>
<td>30</td>
<td>56</td>
<td>6</td>
<td>8</td>
<td>0.8</td>
<td>6.43</td>
<td>8.5</td>
<td>24</td>
<td>41</td>
<td>13.4</td>
<td>35.2</td>
<td>19.8</td>
<td>150</td>
</tr>
<tr>
<td>Virus isolated (n = 6)</td>
<td>1.6–5.13</td>
<td>36.2–70.3</td>
<td>14.6–59.2</td>
<td>3.03–23.5</td>
<td>0–2.5</td>
<td>0.48–4.2</td>
<td>3.9–6.7</td>
<td>7.0–11.8</td>
<td>17.8–29.9</td>
<td>45.1–48.8</td>
<td>17.2–19.9</td>
<td>37.5–40.7</td>
<td>18.9–20.9</td>
<td>152–603</td>
</tr>
</tbody>
</table>

*EPH, epizootic hemorrhagic disease; WBCs, white blood cells; RBCs, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelets.
†PCR, ELISA, and virus isolation were performed on selected samples from the 41 samples (11 whole blood samples, 4 serum samples, and 15 supernatant samples from the baby hamster kidney cells). The virus-positive animals were PCR negative.
‡Mean ± standard error of the mean.