Novel Prion Protein in BSE-affected Cattle, Switzerland

To the Editor: Bovine spongiform encephalopathy (BSE) is a feed-borne prion disease that affects mainly cattle but also other ruminants, felids, and humans (1). Currently, 3 types of BSE have been distinguished by Western immunoblot on the basis of the signature of the proteinase K–resistant fragment of the pathologic prion protein (PrPres): the classic type of BSE (C-BSE) and 2 so-called atypical types of BSE with higher or lower molecular masses of PrPres (H-BSE and L-BSE, respectively) (2). C-BSE is transmitted to cattle by ingestion of contaminated meat-and-bone meal, a feed supplement produced from animal carcasses and by-products. H-BSE and L-BSE have been identified by active disease surveillance, and incidence in aged cattle is low; but little is known about their epidemiology, pathobiology, and zoonotic potential (3). We describe 2 recent cases of BSE in aged cattle in Switzerland in which a PrPres phenotype distinct from those of C-, L- and H-BSE was unexpectedly displayed.

In April 2011, an 8-year-old cow (cow 1) died of accidental injury, with no apparent precedent clinical signs, on a farm in the canton of St. Gallen, Switzerland. In the context of active surveillance for BSE, the medulla oblongata was tested and found to be BSE positive by using the PrioStrip test (Prionics AG, Schlieren, Switzerland), a lateral-flow immunochromatographic assay for detection of PrPres. One month later, another cow (cow 2), 15 years of age, in the canton of Berne, Switzerland, was slaughtered because of a hind limb fracture. Information on this animal’s health status before death was unavailable. Statutory testing of the medulla oblongata gave a BSE-positive result by using the Prionics Check Western, a rapid Western blot technique (4). Medulla oblongata samples from the 2 animals were forwarded to the National Reference Laboratory for confirmatory testing.

In accordance with the guidelines of the World Organisation for Animal Health (5), BSE was confirmed for each animal by positive test results in independent, approved screening tests, of which 1 must be a Western blot (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/18/1/11-1225-Techapp.pdf). Because the tissues were severely autolysed, target structures for the diagnosis of BSE could not be identified, and histopathologic and immunohistochemical results were inconclusive.

The Prionics Western blot detected a similar 3-band PrPres glycoprofile with molecular masses of roughly 16, 20, and 25 kDa for each animal, lower than equivalent PrP protein bands detected in animals with C-BSE (Figure). Sequencing of the open reading frame of the PRNP gene of cow 2 (which was unsuccessful for cow 1) indicated that the encoded protein was identical to the common bovine PrP amino acid sequence (as translated from GenBank accession no. AJ298878) and therefore was not likely to account for the differences observed by Western blot testing.

We next investigated which region of the prion protein was present in these abberant PrPres fragments by probing with a panel of antibodies in the Western blot that bind to different regions of the prion protein (online Technical Appendix). PrPres in cows 1 and 2 was readily detected by antibodies Sha31, 94B4, and JB10. By contrast, antibody 9A2, which maps to the PrPres N terminus, bound only to PrPres in samples from animals with C-, L- and H-BSE but not in samples from cows 1 and 2.

Figure. Molecular typing of the pathologic prion protein from 2 cows with bovine spongiform encephalopathy (BSE), Switzerland. A) Epitope mapping using antibodies 9A2, Sha31, 94B4, and JB10. B) Confirmatory Western blotting using antibody 6H4. C) Comparison PrPres in cow 2 and H-BSE with (+) and without (–) deglycosylation with PNGaseF (antibody 94B4, sodium sulfate–polyacrylamide gel electrophoresis) with NuPAGE MES instead of NuPAGE MOPS running buffer [Invitrogen, Carlsbad, CA, USA]). PrPres 1 and 2 in H-BSE samples are shown at positions 192 and 208 (-GlcNAc). C-BSE, classic BSE; cow 1, an 8-year-old BSE-positive cow; cow 2, a 15-year-old BSE-positive cow; PrPres, proteinase K–resistant fragment of the prion protein; H-BSE, atypical BSE with higher molecular mass of PrPres; L-BSE, atypical BSE with lower molecular mass of PrPres; Lane 1, C-BSE; Lane 2, cow 1; Lane 3, cow 2; Lane 4, H-BSE; Lane 5, L-BSE; lane 6, negative. A description of the methods is provided online (www.cdc.gov/EID/article/18/1/11-1225-F1.htm).
The molecular masses of the PrPres moieties from the 2 cows were also clearly distinct from those from controls with L- and H-BSE (Figure). For samples from animals with H-BSE, enzymatic deglycosylation demonstrated PrPres subtypes, 1 and 2, the latter being a C-terminal PrPres fragment of ~12–14 kDa (6). To investigate whether the novel PrPres type corresponds to PrPres subtype 2, we compared samples from cow 2 with those from the H-BSE control by Western blot. The PrPres type from the 2 cows reported here and PrPres subtype 2 from the H-BSE control were indeed distinct (Figure).

We report a novel PrPres signature in 2 cows with BSE diagnoses determined according to established criteria. Combining Western blot analysis with an epitope mapping strategy, we ascertained that these animals displayed an N terminally truncated PrPres different from currently classified BSE prions (Figure). The interpretation of these findings remains difficult because neuropathologic and systematic clinical data for the 2 cases are not available. Moreover, the tissue samples were autolyzed, and the question of whether this affected the PrPres molecular signature is of concern. Nonetheless, our findings raise the possibility that this cattle were affected by a prion disease not previously encountered and distinct from the known types of BSE. To confirm this possibility and to assess a potential effect on disease control and public health, in vivo transmission studies using transgenic mouse models and cattle are ongoing. Until results of these studies are available, molecular diagnostic techniques should be used so that such cases are not missed.

Acknowledgments

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References


Hantavirus in Bat, Sierra Leone

To the Editor: Hantaviruses (family Bunyaviridae) are transmitted from rodent reservoirs to humans. These viruses cause life-threatening human diseases: hantavirus cardiopulmonary syndrome in the Americas and hemorrhagic fever with renal syndrome in Asia and Europe (1). Since 2006, indigenous hantaviruses were reported also from Africa. Sangassou virus was found in an African wood mouse (Hylomyscus simus) in Guinea (2). Discovery of newer African hantaviruses, Tanganya virus and recently Azagny virus, was even more surprising because they were found in shrews (3,4).

The detection of hantaviruses in small mammals other than rodents, such as shrews and also moles (4), increasingly raises questions regarding the real hantavirus host range. Bats (order Chiroptera) are already known to harbor a broad variety of emerging pathogens, including other bunyaviruses (5). Their ability to fly

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Technical Appendix

Table 1. Details of 2 cows with bovine spongiform encephalopathy, Switzerland, 2011

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Year of birth</th>
<th>Breed</th>
<th>Laboratory confirmation test results</th>
<th>PrioStrip [RDU]* 1st/2nd run</th>
<th>Check Western†</th>
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<tr>
<td>2011/1</td>
<td>2003</td>
<td>Brown Swiss</td>
<td></td>
<td>438/418</td>
<td>Positive</td>
</tr>
<tr>
<td>2011/2</td>
<td>1995</td>
<td>Swiss Fleckvieh</td>
<td></td>
<td>243/252</td>
<td>Positive</td>
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</tbody>
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*Prionics AG, Schlieren, Switzerland. Cutoff value 90 relative density units (RDU).
†Prionics AG. Antibody used was 6H4.

Table 2. Prion protein–specific antibodies used in bovine spongiform encephalopathy testing, Switzerland, 2011*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Epitope*</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
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<tr>
<td>9A2</td>
<td>WNK</td>
<td>1 µg/mL</td>
<td>(1)</td>
</tr>
<tr>
<td>6H4</td>
<td>YEDRYYREN</td>
<td>0.2 µg/mL</td>
<td>(2)</td>
</tr>
<tr>
<td>Sha31</td>
<td>YEDRYYRE</td>
<td>1:10†</td>
<td>(3)</td>
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<tr>
<td>94B4</td>
<td>HTVTTTK</td>
<td>1 µg/mL</td>
<td>(4)</td>
</tr>
<tr>
<td>JB10</td>
<td>QRESQAYY</td>
<td>0.6 µg/mL</td>
<td>S. McCutcheon, unpub. data</td>
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</tbody>
</table>

*Amino acids and positions according to the bovine prion protein sequence (www.uniprot.org, accession no. P10279).
†Bio-Rad TeSeE Western kit (Hercules, CA, USA), concentration unknown.

References


