Enteropathogenic Escherichia coli O80:H2 in Young Calves with Diarrhea, Belgium

Damien Thiry, Marc Saulmont, Shino Takaki, Klara De Rauw, Jean-Noël Duprez, Atsushi Iguchi, Denis Piérard, Jacques G. Mainil

Author affiliations: University of Liège, Liège, Belgium (D. Thiry, S. Takaki, J.-N. Duprez, J.G. Mainil); Association Régionale de Santé et d’Identification Animales, Ciney, Belgium (M. Saulmont); Université Libre de Bruxelles, Brussels, Belgium (K. De Rauw, D. Piérard); University of Miyazaki, Miyazaki, Japan (S. Takaki, A. Iguchi).

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Serogroup O80 was detected in 40% of 104 enteropathogenic Escherichia coli isolates from calves with diarrhea from 42 farms in Belgium during 2008–2015. These isolates harbored the eae-ξ and fliCξ genes, similar to the O80 attaching-effacing Shigatoxinogenic E. coli isolates found in humans in France. This strain might be emerging.

We further tested the calf EPEC isolates and 3 human Shiga toxin 2–encoding gene (stx2)–positive AE-STE C O80 isolates from the STEC National Reference Center (Brussels, Belgium) by PCR for fliCξ and eae-ξ genes found in human AE-STE C O80 strains. For amplifying eae-ξ, we used previously published PCR conditions (6), and for amplifying fliCξ, we used primers H2_F (5’TGATCCGACATCTCCTGATG-3’) and H2_R (5’TGGTCATCAACAATGACGC-3’) and the following thermocycler conditions: initial denaturation at 94°C for 1 min; 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 58°C, and elongation for 1 min at 72°C; and final elongation at 72°C for 2 min. All 42 calf EPEC and 3 human AE-STE C isolates tested positive by both PCRs.

Among the 104 calf EPEC isolates, O80:H2 was frequently found (40% were PCR positive) and, thus, could be considered emerging. Indeed, the EPEC O80 isolates were isolated from calves from 42 farms. The yearly EPEC O80:H2 isolation rate varied from 12% in 2009 to 40%–50% during 2010–2013 to as high as 73% for the
first 6 months of 2015 (Table). In comparison, the rate for EPEC O26 serotype was 5%–25%. Although prevalence data before 2009 are lacking, EPEC O80 isolates have been found infrequently in animals: 8 in dead poultry (7,8), 1 in a piglet with diarrhea (9), 1 in a healthy cow (9), and 5 in lambs with diarrhea (10). However, even fewer AE-STE C O80 isolates have been found: 2 in healthy cattle (6); 1 in a calf with diarrhea in January 1987 (J.G. Mainil, unpub. data); and 1 in raw cow’s milk cheese (9). According to the literature, the O80:H2 serotype might be emerging in France, where human cases of AE-STE C O80:H2 have been reported (9).

Molecular virulotyping results indicate that our calf EPEC O80 isolates appeared to be more closely related to human AE-STE C (because they all harbored eae- and fliC (let) than to ovine and poultry EPEC O80 (which usually harbor eae-β and fliC (lcp)) (J.G. Mainil, unpub. data) (8,10). Further studies are needed to characterize these calf EPEC O80:H2 isolates, and the isolation rate of EPEC O80:H2 in calves with diarrhea must be tracked. Additional PCR virulotyping should be performed with our isolates to identify, if present, other EPEC-related virulence genes and extraintestinal E. coli–related virulence genes. Some genes could be located on plasmids, like those that were found in AE-STE C O80:H2 patients with bacteremia and internal organ infections (9), although the infected calves from which our isolates were taken did not show evidence of septice mia before or after necropsy. The relationship among calf EPEC O80:H2 isolates (which are all independent isolates, not constituting a single strain until proven otherwise) and between calf and human isolates needs to be further characterized with pulsed-field gel electrophoresis and whole-genome sequencing. The prevalence of O80:H2 EPEC and AE-STE C in healthy cattle at slaughterhouses and farms in Belgium should be examined. Finally, we need to determine whether these calf EPEC O80:H2 isolates are true EPEC, AE-STE C derivatives that have lost str genes, or AE-STE C precursors that could acquire str genes in the future. This work will aid in the detection, prevention, and control of this potentially emerging pathogen.

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Dr. Thiry is an assistant professor at the Bacteriology Laboratory at the Faculty of Veterinary Medicine of the University of Liège. His areas of research are the identification of enteropathogenic and Shigatoxigenic Escherichia coli isolated from young diarrheic calves and bacteriophages as an alternative treatment for bacterial infections (Escherichia coli and Staphylococcus aureus) in cattle.

References


**Table.** Comparison of yearly isolation rates of EPEC O80:H2 and O26 from calves with diarrhea, Belgium, January 2009–June 2015

<table>
<thead>
<tr>
<th>Year</th>
<th>Total no.</th>
<th>O80:H2, %</th>
<th>O26, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>17</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>2010</td>
<td>19</td>
<td>47</td>
<td>5</td>
</tr>
<tr>
<td>2011</td>
<td>12</td>
<td>50</td>
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<td>2012</td>
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<td>33</td>
<td>22</td>
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<td>2013</td>
<td>15</td>
<td>47</td>
<td>20</td>
</tr>
<tr>
<td>2014</td>
<td>20</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2015</td>
<td>11</td>
<td>73</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>41</td>
<td>16</td>
</tr>
</tbody>
</table>

*In 2008, only isolates collected in November and December were studied, and 1 EPEC was identified. EPEC, enteropathogenic Escherichia coli.
Incentives for Bushmeat Consumption and Importation among West African Immigrants, Minnesota, USA

Emily Walz, David Wilson, Jacob C. Stauffer, Danushka Wanduragala, William M. Stauffer, Dominic A. Travis, Jonathan D. Alpern

Author affiliations: University of Minnesota, Minneapolis, Minnesota, USA (E. Walz, W.M. Stauffer, D.A. Travis, J.D. Alpern); African Career, Education, and Resources, Inc., Brooklyn Park, Minnesota, USA (D. Wilson); Stillwater High School, Stillwater, Minnesota, USA (J.C. Stauffer); Minnesota Department of Health, St. Paul, Minnesota, USA (D. Wanduragala)

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The knowledge, attitudes, and practices surrounding bushmeat consumption and importation in the United States are not well described. Focus groups of West African persons living in Minnesota, USA, found that perceived risks are low and unlikely to deter consumers. Incentives for importation and consumption were multifactorial in this community.

Bushmeat hunting and butchery are risk factors for zoonotic disease transmission (1–3). However, less is known about health risks to those who consume products that are already butchered when purchased. Bushmeat in this report refers to meat from wild African animals such as rodents, hoofed animals, carnivores, primates, and bats (3). Thousands of pounds of bushmeat are illegally imported into the United States annually (4), mostly from West Africa (5). A previous study of bushmeat consumption by African immigrants in the United States described mixed perceptions regarding the risks and benefits of consuming bushmeat (5). Improved understanding of the complex social drivers of these practices is needed to better characterize risk and formulate communication strategies.

To identify the cultural perspectives and knowledge, attitudes, and practices surrounding bushmeat importation and consumption, we held focus groups with members of the Liberian community living in the Minneapolis–St. Paul area of Minnesota, USA. Minneapolis–St. Paul has the largest Liberia-born population in the United States, and ranks fifth in overall African populations in US metropolitan areas (6). Recognizing the history of stigmatization associated with increased risk for Ebola virus among persons from West Africa, we engaged a community-based organization to partner in the planning and execution of this study (7,8). Creating a comfortable environment where participants share personal experiences and insights freely is a key tenet of focus group methodology (9); this partnership was essential in gaining trust and maintaining cultural sensitivity.

Inclusion criteria for participant selection included: 1) minimum age 18 years, 2) self-identification as West African, and 3) willingness to discuss bushmeat in a group setting. The partner organization recruited community members by using a combination of purposeful sampling and social media advertisement and facilitated 3 focus groups (10–12 participants, each for 90 min) in January and February 2016; a designated research team member attended each session. A standard guide for questions was used for each session (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/12/17-0563-Techapp1.pdf). The University of Minnesota Institutional Review Board approved this study.

Sessions were audio recorded and transcribed; participants were not identified. Nonverbal cues (i.e., gestures, emotions, points of hesitation, nods of agreement) and other participant interactions were added to the transcript by a notetaker. We analyzed the collected data by using a modified grounded theory method with inductive analysis as previously described (10). Two authors (E.W., J.D.A.) analyzed each transcript by using an open and selective coding approach. Subsequently, all transcripts were analyzed together by using axial coding further describing relationships among themes (Table); representative quotes from participants were selected to exemplify a relationship or common theme (9) (Table). We supported validity of findings by using member-checking, triangulation of findings with multiple sources, and peer debriefing (9). Many themes were repeated in all groups; however, this study was limited by inability to confirm that we had reached saturation of perspectives. According to Creswell, it is ideal...