Isolation of *Salmonella enterica* and Shiga-Toxigenic *Escherichia coli* O157 from Feces of Animals in Public Contact Areas of United States Zoological Parks

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The fecal prevalence of subclinical *Salmonella enterica* and Shiga-toxigenic *Escherichia coli* O157 among animals in human-animal contact exhibits at institutions in the United States accredited by the Association of Zoos and Aquariums was estimated to assess public health risk. The prevalence was less than 0.6% for both zoonotic pathogens among 997 animals sampled at 36 exhibits.

Animal exhibits are popular sources of entertainment and educational enrichment that provide opportunities for direct and sometimes close human-animal contact. Zoonotic enteric human disease outbreaks associated with animal exhibits have increased in the past decade in North America and Europe. These outbreaks are usually attributable to the protozoan *Cryptosporidium parvum* and to nontyphoid *Salmonella enterica* and especially to Shiga-toxigenic *Escherichia coli* (STEC) O157 bacterial infections (5, 18). At least 17 animal exhibit-associated (agricultural fair, petting zoo, or open farm) STEC O157 outbreaks have occurred in the United States since 1999, and these outbreaks have affected 1,317 people, caused 69 hemolytic-uremic syndrome cases, and killed two persons (5, 6, 8, 9, 11, 12, 13, 18, 21). Since 1990, there have been at least four animal exhibit *Salmonella enterica* outbreaks in the United States attributable to *Salmonella enterica* serovars Typhimurium and Enteritidis (5). The *Salmonella* serovar Enteritidis outbreak, which was associated with visiting a temporary exhibit of a Komodo dragon at a metropolitan zoo, affected 65 persons, mostly children (15). Exhibit-associated outbreaks, real or alleged, are costly to affected individuals and their families, affected venues and their insurance underwriters, and health service providers. They also represent a source of legal vulnerability to exhibitors.

The Association of Zoos and Aquariums (AZA) is a nonprofit organization of 211 (in 2005) zoos, aquariums, and wildlife centers in North America. The AZA inspects and accredits member institutions every 5 years for adequacy of facilities, veterinary care, safety, security, collection management, finances, research, and other factors (1, 2). AZA member zoos and aquariums attract ~142 million visitors annually, employ ~46,000 people, and maintain collections of ~800,000 animals (3). About half of AZA-accredited institutions have human-animal contact areas (e.g., children’s zoos or similar types of interaction settings).

Human-animal contact exhibits are heterogeneous. They vary in hygiene and sanitation practices, degree of supervision, extent of animal contact permitted, numbers and types of animals displayed, nature of exhibits (temporary, recurring, or permanent), facility design, and visitor management (10, 18). Human-animal contact areas at AZA-accredited institutions are probably more similar to each other than they are to non-AZA exhibits due to the standardization inherent in accreditation. The motivation for this study was to estimate the unknown fecal shedding prevalence of zoonotic *Salmonella enterica* and STEC O157 in animal populations in the relatively homogeneous AZA human-animal contact settings. We hypothesized that the fecal shedding prevalence of both bacteria would be lower in animals in AZA human-animal contact areas than that in animals in production or agricultural fair environments. Commercial beef and dairy cattle and livestock displayed at agricultural fairs frequently have high (10% or greater) summer prevalence of both STEC O157 and *Salmonella* (4, 7, 14, 17; T. E. Wittum, J. E. Keen, G. Hansen, D. Mollenkopf, J. A. Funk, J. R. Dunn, J. L. Bono, and M. E. Fontenot, 84th Conf. Res. Workers Anim. Dis., abstr. 61, 2003).

AZA-accredited institutions in the United States with human-animal contact exhibits (typically children’s zoos) were recruited to participate voluntarily and confidentially. Freshly (i.e., observed) voided or rectal feces acquired digitally (~50 g, if available) were collected from a census of all animals in contact exhibits by institution staff. Fecal culture for both *Salmonella* and STEC O157 was initiated within 24 to 36 h of collection. Samples were collected in the summers of 2003 and 2004, the peak visitor season at most participating zoos and the peak period of *Salmonella* and STEC O157 shedding in livestock in general (4).

For *Salmonella* isolation, feces samples (up to 10 g, as available) were preenriched in tetrazionate broth (TTB) containing 0.1% brilliant green solution for 24 h at 37°C, followed by selective enrichment of 100 μl of TTB in 10 ml Rappaport-Vassiliadis R10 broth (Difco Laboratories, Detroit, MI) for 24 h at 37°C. R10 broth was then dual streak plated (10 μl) onto EF-18 agar (Neogen Corp., Lansing, MI) and Rambach agar (CHROMagar, Paris, France) (20, 22). Plates were incubated for 24 h at 37°C. Up to five colonies per plate exhibiting

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The low prevalence of *Salmonella* and STEC O157 at AZA-accredited institutions could result from the standardized management and facility conditions, routine isolation and quarantine procedures, generally high hygiene levels, low animal stress due to exhibit permanency (e.g., lack of transport stress), and low rate of new animal introductions and animal mixing compared to temporary or reoccurring types of animal exhibits or production livestock settings. Most human-animal contact exhibits at AZA-accredited institutions are permanent venues, and AZA zoological parks frequently possess more human and agricultural fair livestock.

The prevalence typical for either pathogen in production or agricultural fair livestock.

The prevalence at AZA-accredited institutions was much lower than the prevalence typical for either pathogen in production or agricultural fair livestock.

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TABLE 2. Antibiotic resistance of *Salmonella enterica* serovars and STEC O157 isolated from animals in human-animal contact areas at AZA-accredited zoological parks against 11 antimicrobials* as determined by disk diffusion

<table>
<thead>
<tr>
<th>Zoo</th>
<th>Animal</th>
<th><em>Salmonella enterica</em> serotype (serogroup) or E. coli</th>
<th>Antibiotic resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Goat</td>
<td>Serotype Infantis (C1)</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>8</td>
<td>Yak</td>
<td>STEC O157:H7</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>18</td>
<td>Horse</td>
<td>Serotype Javiana (D1)</td>
<td>Ampicillin, azithromycin</td>
</tr>
<tr>
<td>29</td>
<td>Goat</td>
<td>Serotype Newport (C2)</td>
<td>Ampicillin, tetracycline, azithromycin</td>
</tr>
<tr>
<td>29</td>
<td>Giraffe</td>
<td>Serotype Rubislaw*</td>
<td>Ampicillin, tetracycline</td>
</tr>
<tr>
<td>36</td>
<td>Goat</td>
<td>Serotype Rubislaw*</td>
<td>Ampicillin, tetracycline</td>
</tr>
<tr>
<td>36</td>
<td>Zebu calf</td>
<td>Serotype Javiana (D1)</td>
<td>Ampicillin, tetracycline</td>
</tr>
<tr>
<td></td>
<td>Serotype Muenchen (C2)*</td>
<td></td>
<td>Ampicillin, tetracycline</td>
</tr>
</tbody>
</table>

* Antimicrobials and their levels in disks (in micrograms) were as follows: ampicillin, 10; chloramphenicol, 30; streptomycin, 10; sulfisoxazole, 300; tetracycline, 30; trimethoprim, 5; cefotiofur, 30; ciprofloxacin, 5; gentamicin, 10; neomycin, 30; and azithromycin, 15.

Differences in the compositions of animals examined in the present zoo study versus production or agricultural fair livestock surveys may also have impacted the findings. Adult cattle and swine are frequent targets of zoonotic enteric pathogens at AZA-accredited zoological parks and aquariums (and accreditation standards), 2006 ed. Association of Zoos and Aquariums, Silver Spring, MD. http://www.aza.org/Accreditation/Documents/AccredGuide.pdf. Accessed 19 June 2006.


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