

## EPIDEMIOLOGY

### FIRST CONFIRMED ZONOTIC TRANSMISSION OF *ENTEROCYTOZOON BIENEUSI*

(V. Cama, J. Pearson, L. Cabrera, R. Gilman, S. Meyer, Y.R. Ortega, and L. Xiao)

Initially described in 1985 as a significant pathogen in AIDS patients, *Enterocytozoon bieneusi* also affects other immunosuppressed populations, as well as travelers, children, and the elderly, throughout the world. While immunocompetent individuals often have asymptomatic infection or self-limiting diarrhea, AIDS patients experience chronic diarrhea with accompanying weight loss and increased mortality rates. The transmission routes for human microsporidiosis are still poorly understood, and the role of animals as sources of human infections has been speculated. Recent reports seem to support an association between microsporidia positive animals and human infections. *E. bieneusi* has been reported in several species of domestic and wildlife animals including dogs, cats, pigs and cattle. The zoonotic potential of microsporidia was also suggested in a report from Uganda where humans and gorillas sharing the same natural habitats had the same genotype of *E. intestinalis*. In this study, additional support for the zoonotic potential of *E. bieneusi* was found.

A household was identified in which the stools of a 25-month old male cohort participant and a guinea pig tested positive for microsporidia. Follow-up stool specimens were collected from the child and other animals in the household to confirm infection. The child was microsporidia-positive over a ten-day period, had diarrhea on the first day of the infection-episode, and remained microsporidia-negative throughout the next 32 weeks in the study. This is the first case of zoonotic transmission of *E. bieneusi* from animals to human demonstrating that this parasite can indeed cross the host barrier.

### ENUMERATION OF *E. COLI* O157:H7 IN SPINACH OUTBREAK SAMPLES

(L. Ma, G. Zhang, P. Garner-Schmidt, and M. P. Doyle)

During August and September of 2006, more than 200 persons from 26 states were infected by a single strain (isolates indistinguishable by pulsed-field gel electrophoresis [PFGE]) of *E. coli* O157:H7. Fresh spinach was identified as the vehicle of the outbreak and *E. coli* O157:H7 with a PFGE pattern matching the outbreak strain was isolated from many open packages of fresh spinach consumed by patients. The objective of this study was to enumerate *E. coli* O157:H7 in outbreak-associated spinach samples obtained from patient refrigerators. A 3-tube Most Probable Number (MPN) method in combination with selective plating, multiplex PCR (targeting genes: *gad*, *eae*, *bfp*, *stx1*, and *stx2*) screening, confirmation by *E. coli* O157 latex agglutination assay, and PFGE pattern matching was used for the isolation and enumeration of the outbreak strain in the spinach samples. The estimated cell numbers in positive samples ranged from 0.61 MPN/g to 21 MPN/g. Multiplex PCR analysis revealed absence of the *stx 1* amplicon in the outbreak isolate.

### ISOLATION AND ENUMERATION OF *E. COLI* O157:H7 FROM POSSIBLE CONTAMINATED GROUND BEEF SAMPLES

(L. Ma, G. Zhang, P. Gerner-Smidt, and M. P. Doyle)

Direct plating on selective media (TC-CHROMagar O157) and a 3-tube Most Probable Number (MPN) method were used for the isolation and enumeration of *E. coli* O157:H7 from ground beef associated with an outbreak. Presumptive-positive isolates of *E. coli* O157:H7 were confirmed molecularly (multiplex PCR targeting five genes: *gad*, *eae*, *bfp*, *stx1*, and *stx2*) and immunologically (*E. coli* O157 latex agglutination assay). Subtyping by MLVA and PFGE confirmed the isolates were indistinguishable to the outbreak strain. Three out of eight of the original samples were positive for *E. coli* O157:H7, with cell numbers of the pathogen in the positive samples being generally low, ranging from <0.3 MPN/g to 24 MPN/g by the MPN method, or from <50 CFU/g to ca. 50 CFU/g by direct enumeration. Also, multiplex PCR revealed that all of the *E. coli* O157:H7 isolates contained *eae*, *stx1* and *stx2* genes. One sample contained a large number of colonies that had the same morphology (mauve) as *E. coli* O157 on TC-CHROMager O157 and reacted positively by the *E. coli* O157 latex agglutination assay; however, these isolates were later identified as *Serratia liquefaciens* by the API 20E test.

## ISOLATION AND ENUMERATION OF *SALMONELLA* FROM POT PIES FROM OREGON

(G. Zhang, L. Ma, V. Phelan, P. Gerner-Smidt, and M.P. Doyle)

During 2007, at least 272 isolates of *Salmonella* I 4,[5],12:i:- with an indistinguishable pulsed field gel electrophoresis (PFGE) subtype were obtained from ill persons in 35 states. At least 65 people were hospitalized and pot pies were determined to be the vehicle of the outbreak.

Three pot pies (2 chicken pies, 1 turkey pie) were received from the Oregon Department of Public Health and assayed for *Salmonella*. Universal preenrichment broth for preenrichment and Rappaport-Vassiliadis broth (RV) and Tetrathionate broth (Hajna) (TT) for enrichment was employed for detection and enumeration of *Salmonella*. All samples were also analyzed with the BAX<sup>®</sup> Detection System (DuPont Qualicon).

The two chicken pot pies were negative for *Salmonella* whereas the turkey pie was contaminated with *Salmonella* at 11 MPN/g. Three isolates from the pie were serotyped and PFGE subtyped at CDC and were identified as *Salmonella* 4,5,12:i:- that was indistinguishable from the pot pie-associated outbreak isolate.

## THE WIDE OCCURRENCE OF *CRYPTOSPORIDIUM BOVIS* AND THE DEER-LIKE GENOTYPE IN BOVINES

(Y. Feng, Y. Ortega, G. He, P. Das, X. Zhang, R. Fayer, W. Gatei, V. Cama, and L. Xiao)

Recent studies in the United States reported that of animals positive for *Cryptosporidium*, approximately 85% of preweaned dairy calves were infected with zoonotic *C. parvum* whereas only 1% of postweaned calves and 1-2 year-old heifers were infected. *C. bovis* and the deer-like genotype were much more prevalent in the postweaned animals. It is not clear whether the disproportionately high prevalence of *C. parvum* in preweaned calves is influenced by intensive animal production methods in the United States or is primarily a parasite-host age-related phenomenon. To determine whether the same *Cryptosporidium* infection pattern was present in other geographic areas, the genotypes of *Cryptosporidium* specimens collected from two farms in China and India were compared to specimens collected from farms in Georgia, USA. *C. bovis* was the most common species found in pre- and post-weaned calves in all three areas. In Georgia, the deer-like genotype was found frequently in pre- and post-weaned calves, and *C. andersoni* was found in one weaned calf. Both *C. bovis* and the deer-like genotype was found in the a few milking cows examined in Georgia. There were no differences in the small subunit rRNA gene sequences obtained from *C. bovis* or deer-like genotype among the three areas. One adult yak in China, however, was infected with a species similar to *C. bovis*, with only three nucleotide mutations in the target gene. All four common bovine *Cryptosporidium* spp. could be differentiated from each other by restriction fragment length polymorphism analysis with enzymes *SspI* and *MboII*. Thus, both *C. bovis* and the deer-like genotype are found in all age groups of cattle in diverse geographic areas and host adaptation of *C. bovis* might have occurred in yaks.

## GENOTYPIC AND INTRAGENOTYPIC ANALYSES OF *GIARDIA DUODENALIS* IN DAIRY CATTLE

(Y. Feng, Y. Ortega, V. Cama, and L. Xiao)

To characterize the transmission of bovine giardiasis, 58 *Giardia duodenalis*-positive fecal specimens were genotyped and subtyped by sequence analysis of the triosephosphate isomerase (TPI) gene. Both the livestock-specific assemblage E and the potentially zoonotic assemblage A were found, with the former detected in 86% of the specimens. A high degree of genetic polymorphism was evident within assemblage E, with 11 distinct subtypes identified, eight of which represented new subtypes. Three subtypes were identified in assemblage A, with the subtype A2 transiently found in calves and cows on one farm. All farms had multiple assemblage E subtypes circulating in cattle at each sampling, and concurrent infection with mixed subtypes or genotypes occurred in 24% of animals. Thus, the high intensity of *G. duodenalis* transmission is not only reflected by the high prevalence of the infection but also exemplified by the high intragenotypic diversity and concurrent occurrence of mixed infections. The zoonotic potential of bovine *G. duodenalis* needs to be further studied by extensive characterization of assemblage A specimens at the subtype level.