PARASITES

FIRST CONFIRMED ZOONOTIC 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.

THE WIDE OCCURRENCE OF *CRYPTOSPORIDIUM BOVI*S 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 *Ssp*I and *Mbo*II. 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.

DETECTION OF *CRYPTOSPORIDIUM, GIARDIA*, AND *CYCLOSPORA* IN WATERS FROM GEORGIA

(Y.R. Ortega)

Protozoan parasites have been associated with gastrointestinal infections in animals and humans. In the U.S., food and waterborne infections of cryptosporidiosis, cyclosporiasis and giardiasis have been well recognized. *Cryptosporidium, Giardia* and *Toxoplasma* are zoonotic parasites that infect domestic and farm animals. Surface water may become contaminated via the entry of infectious oocysts by agricultural run-off from adjacent farm animals or by accidental contamination from human sewage. The ubiquitous nature of these parasites, resistance to environmental conditions, small size, and low sedimentation rate make water or moist environments an optimal matrix where the oocysts can remain viable and infectious for long periods of time. These parasites can cause zoonotic infections and have a low infectious dose. They are resistant to sanitizers and disinfectants commonly used in the water and produce. Because of these factors, parasites and water quality are a priority for food production and processing.

The role man and domestic/farm animals may play in contaminating recreational waters (lakes and rivers) and the subsequent contamination of our food supply in Georgia is unknown. In 2007, rivers and lakes of the central regions of Georgia were sampled and examined for the presence of parasites. Of those 18 environmental water samples, 4 had *Cryptosporidium*, 9 *Giardia* and 1 *Eimeria*. The locations with more parasites were the Flint and Appalachian Rivers.

Parasites identified in waters suggest that human and animal waste are present in these rivers and that they may play a role in contaminating animals and crops that use this water for irrigation. More studies are needed to evaluate the impact of these parasites in irrigation waters.

EFFICACY OF GASEOUS CHLORINE DIOXIDE AS A SANITIZER AGAINST *CRYPTOSPORIDIUM PARVUM, CYCLOSPORA CAYETANENSIS*, AND *ENCEPHALITOZOON INTESTINALIS* ON PRODUCE

(Y.R. Ortega, A. Mann, M.P. Torres, and V. Cama)

Parasites have frequently been identified in fresh produce or vegetables and have caused several foodborne outbreaks. *Cryptosporidium parvum*, a parasite often linked to waterborne transmission, has also been reported causing disease through the consumption of unpasteurized milk or apple cider and by eating unwashed contaminated vegetables and fruits. Most cases of cyclosporiasis have been almost exclusively associated with the consumption of contaminated fresh vegetables, such as raspberries, lettuce, basil, mixed greens, and snow peas. Recently, chlorine dioxide gas has been evaluated for the killing of *Salmonella*, yeast and molds on berries as an alternative to rinse sanitizers for fruits and vegetables eaten raw.

The efficacy of gaseous chlorine dioxide to reduce parasite and bacterial burden in produce was investigated in this study. Basil and lettuce leaves were inoculated with *C. parvum* and *Cyclospora cayetanensis* oocysts, *Encephalitozoon intestinalis* spores, and a cocktail of two isolates of nalidixic acid-resistant *Escherichia coli* O157:H7 and subsequently treated for 20 minutes with gaseous chlorine dioxide at 4.1 mg/l. *Cryptosporidium, Encephalitozoon*, and *E. coli* loads were significantly reduced (2-4 log), although *Cyclospora* was resistant to the treatment. Our findings demonstrate that *Cyclospora* oocysts are resistant to gaseous chlorine dioxide treatment but other pathogens such as *Cryptosporidium, Encephalitozoon* and *E. coli* can be inactivated using gaseous chlorine dioxide, therefore providing an alternative treatment for safer vegetables.