

BEEF

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

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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.

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

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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

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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.