TITLE:PHYLOGENETIC AND VIRULENCE FEATURES SHARED BY CANINE AND HUMAN ATYPICAL EPEC ISOLATES OF THE SAME SEROTYPE: A ZOONOTIC RISK?

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ABSTRACT:

Atypical EPEC (aEPEC) isolatesare emerging childhood diarrhea agents in many developing countries. This group is characterized by the presence of the locus LEE and absence of pEAF, a plasmid encoding BFP in the typical EPEC. Many aEPEC strainsare more related to STEC and have a wide host diversity, serotype and virulence genes. The occurrence of the same serotypes in humans and animals is a common finding, but the true zoonotic potential is unknown. We compared the phylogenetic and virulence features of aEPEC strains of the four serotypes(O51:H40, O4:H16, O49:H10 and O11:H16) isolated from dogs and children withdiarrhea. A total of 10 strains, 5 of dogs and 5 of children of corresponding serotypes, were studied for virulence markers by PCR, MLST and PFGE profiles, and adhesion/invasion assays in HEp-2, Caco-2 and T84 cells. The occurrence of virulence genes was similar among canine and human isolates of the same serotype. All strains presented the ecpA gene, while nleB and nleE were detected only in strains of O51:H40 and O4:H16 serotypes. Other genes, such as hcpA, efa-1, tccP, lpfa2, astA, fimH, toxB and several nlegenes were also detected in some canine and human strains. All isolates of O51:H40, O4:H16 and O11:H16 serotypes were identified as ST10; and O49:H10 strains were classified as ST206. The phylogenetic tree based on the concatenated sequences of the seven housekeeper genes showed a closer relationship with STEC strains instead of the EPEC prototype strain (O126:H7). The analysis of PFGE profiles allowed the grouping of human and canine strains of the same serotype, although the occurrence of common clones to both hostshas not been observed. "LAL" was the predominant adherence phenotype in HEp-2 cells after six hours of interaction. Furthermore, the strains of serotype O51:H40, isolated from both hosts, showed an invasive behavior in assays with Caco-2 and T84 cells compared with the virulent Salmonella Typhimurium strain. Our results showed that aEPEC strains of the same serotype isolated from dogs and children with diarrhea are phylogenetically similar, share several virulence genes in their accessory genome and exhibit a common pathogenic behavior when interacting with epithelial/ intestinal cells, including cell invasion. These findings point to the role of dogs as a source of aEPEC potentially pathogenic to humans.

Keywords: aEPEC, dogs, childhood diarrhea, zoonosis, virulence

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