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Detection of Shiga Toxin Variants, Virulence Genes and the Relationship to Cytotoxicity of Shiga Toxin-Producing *Escherichia coli* (STEC) from Domestic Farm Animals

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Background: Shiga toxin-producing *Escherichia coli* (STEC) can cause foodborne illnesses ranging from diarrhea to life-threatening diseases such as hemorrhagic colitis, and hemolytic uremic syndrome in humans. In this study, we determined virulence genes, *stx*subtypes and we evaluated the cytotoxicity in STEC strains isolated from domestic farm animal feces. **Methods:** The STEC strains were analyzed by using PCR with sequence-specific primers to determine the presence of genes encoding intimin (*eae*), enterohemolysin (*ehxA*), serine protease (*espP*), catalase peroxidase (*katP*), the type II secretion system (*etpD*), subtilase cytotoxin (*subA*), autoagglutinating adhesion (*saa*), type III secreted effectors encoded in the genomic islands OI-122 (*ent/espL2*), OI-71 (*nleH1-2* and *nleA*) and the distinct variants of Shiga toxin (*stx_{1a}*, *stx_{1c}*, *stx_{1d}*, *stx_{2a}*, *stx_{2b}*, *stx_{2c}*, *stx_{2d}*, *stx_{2e}*, *stx_{2f}*, *stx_{2g}*). Also, we evaluated the cytotoxicity the STEC strains by use of a fluorescent Vero cell-based method. **Results:** Most (97%) of the 61 strains carried a *stx₂*gene. Moreover, the *stx_{2a}* subtype, which is related with severe illness, was present in 10 % in our STEC strains. The other *stx*subtypes identified were *stx_{1a}*, *stx_{1c}*, *stx_{2b}*, *stx_{2c}* and *stx_{2d}*. A total of 25 (46%) of the strains showed more than one *stx* subtype in its genome, and among them the most frequent combination was *stx_{1a}*, *stx_{1c}*, *stx_{2b}*. The virulence gene most frequently detected was *ehxA* with 93% (57/61). Other virulence genes such as *espP*, *katP* and *etpD* were more associated with the serotype O157:H7. Only 5 strains of the serotype O8:H19 were positive for the virulence genes *saa* and *subA*, which are located in the plasmid pO113. The cytotoxicity was higher in the non-O157 STEC strains with serotypes O8:H19, O73:NT, O111:H8, O146:H8, O146:H21 and ONT:NT. Moreover, the *stx* subtypes related to high cytotoxicity were *stx_{1a}/stx_{1c}/stx_{2b}* with a 69.6%, then *stx_{1a}/stx_{2a}/stx_{2c}* with the 21.8%, and 4.3% for *stx_{1a}/stx_{2b}* and *stx_{1a}* subtypes. **Conclusions:** The STEC strains analyzed from domestic farm animals were found to be diverse in their genetic composition and showed different virulence genes. Furthermore, the presence of more than one *stx*gene variant in the same isolate reflected a higher cytotoxicity in mammalian cells.

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