

TITLE: LEE-NEGATIVE SHIGA TOXIN-PRODUCING *Escherichia coli* ISOLATED FROM CALVES AND *Musca domestica* ON DAIRY FARM

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ABSTRACT:

In the typical enterohemorrhagic *Escherichia coli* (EHEC), lesions on intestinal epithelium are produced through the pathogenicity island Locus of Enterocyte Effacement (LEE) responsible for the attaching and effacing (AE) formation. However, in the last decade, LEE-negative Shiga toxin-producing *Escherichia coli* strains (LEE-negative STEC) have been attracting attention for association with outbreaks and severe illnesses. Mechanisms of infections of this STEC subgroup remain unknown. Here we report the detection of LEE-negative STEC isolated from healthy dairy calves' feces and the external surface of flies collected in milking environment. In total, 93 STEC were detected in 284 *E. coli* from 58 calves' feces by PCR (*stx1* and *stx2* genes) and *in vitro* cytotoxicity assay in Vero cells. Of 135 strains isolated from 57 *Musca domestica* flies, seven were STEC. Of 100 STEC, 45 were LEE-negative STEC (38 from calves and 7 from flies), only 8 harbored *saa* gene and 10 *hlyA*. We selected 22 LEE-negative STEC according to profiles of antimicrobial susceptibility by disk diffusion method and all seven strains from flies for serogroup typing by agglutination test, phylogroup by PCR, and biofilm production determination by tissue culture plate method. The strains from calves showed the serogroups O22 (18%), O7 (18%), O123 (9%), O126 (4.5%), O23 (4.5%), and O123 (4.5%), 82% were placed in phylogroup B1, 68% strains had weak biofilm and 32% non-biofilm formation. As for flies' strains, O-antigen was not typeable (ONT), 57% strains were placed in phylogroup B1, and 43% in E. Biofilm formation has not been determined yet. Two LEE-negative STEC strains from each source showed multidrug resistance profile (resistance \geq three antimicrobial classes). Considering the LEE-negative STEC association with gut infections and illness severe, our results provide data for monitoring of emergency of these strains in reservoir animals and warn for contamination concerns in the milking environment.

Keywords: dairy cattle, colibacillosis, STEC.

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