**TITLE:** NON-O157:H7 SHIGA TOXIN-PRODUCING *Escherichia coli* (STEC) ISOLATED FROM HEALTHY CALVES

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## ABSTRACT:

Shiga toxin-producing Escherichia coli (STEC) is a group responsible for great part of the gastrointestinal diseases in humans such as Hemolytic Uremic Syndrome (HUS) and Hemorrhagic Colitis (HC) and for the high calf mortality rate. Healthy animals, especially bovines, might have STEC strains and be reservoirs that can later affect humans. The infection can occur via ingestion of uncooked meat from infected animals or raw cow milk, or even by ingesting food and drinks contaminated with feces from cattle. In addition to the stx gene responsible for producing Shiga toxin, STEC strains have another virulence-associated factor responsible for the intimate adherence of enteropathogenic E. coli to epithelial cells causing attaching and effacing lesions in the intestinal mucosa, the eae gene. Seeing how colibacillosis can affect farms economics, the cattle is usually treated with antimicrobials both in prophylactic and therapeutic measures. This can cause STEC strains to develop resistance to the most common drugs. In this study, 284 E. coli strains were isolated from healthy calves' feces and tested for stx and eae genes using PCR. A total of 93 (32.7%) strains were positive for stx1 and/or stx2. With 55 (19.4%) being positive for eae+stx1, 27 (9.5%) for stx1, 9 (3.2%) for stx2, 2 (0.7%) for stx1+stx2 and none strain was positive for eae+stx2. Eight strains were positive eae. All strains positive either for stx1 or for stx2 were assayed on Vero cells for detection of Shigatoxin and 92/93 showed cytotoxic effect. Strains positive for stx1+eae were tested for O157 and H7 gene but none was positive. The 93 strains positive for stx were also tested for twelve bovine and human antimicrobials by disc diffusion test. It was found that 12 (13.3%) were resistant to amoxicillin, 11 (12.2%) to tetracycline and 7 (7.8%) to ampicillin. Also, five strains were classified as Multidrug Resistant (MDR, resistant to ≥ 3 antimicrobial classes), and one showed ESBL production. High frequency of STEC present in healthy calves' feces alerts for contamination risks in the milking environment, moreover when associated with multidrug resistant STEC strains. This raises concerns about workers involved with the animals and about milk's contamination.

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