

In vivo study of O26:H11 Escherichia coli strains show differences in virulence and impact of neutralizing antibody

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Background: Stx-producing *E. coli* (STEC) cause food-borne outbreaks of diarrhea. The two major types of Stx produced by STEC are Stx1a and Stx2a. Although Stx1a is more toxic than Stx2a to Vero cells, Stx2a is more potent than Stx1a in mice. In addition, STEC strains that produce only Stx2a are more commonly linked to a serious sequela of disease, the hemolytic uremic syndrome (HUS), than isolates that make Stx1a or both Stx1a and Stx2a. Additionally other toxin variants are often associated with milder disease, with the exception of Stx2d. In this study, we evaluated the virulence of two O26:H11 strains. An isolate from the recent Chipotle outbreak and another clinical isolate were subtyped and used in a mouse model to study virulence and the effect of passive immunization of the mice with neutralizing antibodies to Stx1 and Stx2.

Methods: Two clinical isolates of O26:H11 were subtyped for presence of toxin variants. The isolate from the Chipotle outbreak (4994) was positive for stx1a, stx2a, and stx2b. Strain 1664 was positive for stx1a and stx2a. The level of cytotoxicity and degree of inducibility of toxin expression by the strains was measured on Vero cells without or with ciprofloxacin (Cip). *In vivo* studies were done in streptomycin (Str, 5g/L in drinking water)- or Str- and Cip- (5 or 15 ug/mouse administered by intraperitoneal injection) treated male BALB/c mice. Passive immunization of mice with antibodies against Stx1 or Stx2 was done by tail-vein or intraperitoneal injection.

Results: *In vitro* toxin production for both strains was induced 10-fold from an overnight culture of bacteria grown in Luria Bertani broth supplemented with Cip. In the mouse model, Cip-treatment was required for strain 1664 to cause weight loss and mortality. Mice infected with strain 4994 exhibited weight loss and death with or without Cip, indicating it is more virulent in mice. Administration of neutralizing antibody against Stx2 to the Str- and Cip-treated and infected mice was protective for both strains. Administration of antibody to Stx1 to mice infected with 4994 showed no effect. Mice displayed weight loss and death similar to negative controls. However, unexpectedly, when mice infected with 1664 treated only with Str were given antibody to Stx1, weight loss and mortality was observed (in the absence of Cip).

Conclusions: Our study shows that pathogenicity in a mouse model for two clinical O26:H11 isolates differ. Strain 1664 required Cip administration, most likely due to induction of toxin expression *in vivo*. However, when Stx1a was neutralized the strain became virulent in the absence of Cip, a finding that suggests Stx1a attenuates pathogenicity of this Stx1a+ Stx2a+ O26:H11 strain in mice. Conversely strain 4994 isolated from the Chipotle outbreak caused death and weight loss in the absence of Cip. This virulence was reduced when antibody against Stx2 were administered. We did not observe an effect of neutralizing Stx1 in this strain. We hypothesize that toxin levels *in vivo* may contribute to this difference.