

Characterization of an O26:H11 *Escherichia coli* Strain that Makes Shiga toxin (Stx) type 1a and Stx2a and is Highly Virulent in a Mouse Model of Infection

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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 late 2015, an STEC fast-food outbreak was traced to an *stx*_{1a}+ *stx*_{2a}+ O26:H11 STEC strain. In this study, we evaluated the virulence of a clinical isolate from that outbreak *in vitro* and *in vivo*.

Methods: Two clinical isolates of O26:H11 (*stx*_{1a}+, *stx*_{2a}+) were used for these studies. One isolate, 2015C-4944 (4944), came from the fast-food outbreak, while the other (strain TW08571) was from a sporadic case of illness unrelated to the outbreak. The level of cytotoxicity and degree of inducibility of toxin expression from the strains was measured on Vero cells after growth in Luria broth with or without 15 ng/mL ciprofloxacin (Cip). *In vivo* studies were done in streptomycin (Str, 5g/L in drinking water)- or Str- and Cip- (1.5 ug/mouse administered by intraperitoneal injection) treated male BALB/c mice. Moribund (loss of $\geq 15\%$ of initial body weight) 4944-infected mice were euthanized, and kidneys were collected, fixed in formalin, embedded in paraffin, sectioned, and affixed to slides. Hematoxylin and eosin-stained sections were examined to determine kidney morphology. Passive immunization of mice with antibodies against Stx1 or Stx2 was done by tail-vein injection. Stx levels were measured by immuno-dot blot.

Results and Conclusions: *In vitro* toxin production was induced 10-fold from strain 4944 after growth with Cip. In the Str-treated mouse model, we unexpectedly observed morbidity and mortality in the absence of Cip treatment in animals infected with 4944. Strain 4944 is the first clinical *stx*_{1a}+, *stx*_{2a}+ STEC we have found to be pathogenic in the Str-treated mouse model. We also observed kidney lesions in 4944-infected mice that were similar to lesions seen in mice injected with Stx2. Administration of neutralizing antibody against Stx2 to the Str-treated and 4944-infected mice was protective. Protection was not seen in 4944-infected mice given a Stx1-neutralizing antibody. Furthermore, we found that strain 4944 produced more Stx2 *in vitro* than did TW08571. The latter strain was avirulent in Str-treated mice unless the animals were injected with Cip. We further found that Str-treated, 4944-infected mice had higher toxin levels as measured on Vero cells in feces collected after infection than did animals infected with strain TW08571. Together, these data suggest that the higher the initial uninduced level of Stx2a made by an O26:H11 STEC strain, the more likely that death will occur in a Str-treated mice fed the organism.