Outbreak investigation: STEC O157

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Shiga-toxigenic Escherichia coli 0157:H7 (STEC 0157) is a zoonotic pathogen. Although it is most frequently thought of as a foodborne pathogen, it can also be transmitted via environmental reservoirs1 or animal contact, especially contact with cattle or goats.2,3 In recent years, a number of STEC 0157 outbreaks have been associated with petting zoos and state and county fairs.

Primary isolation of STEC 0 1 57 is an important part of any epidemiological investigation. Standard methods for the isolation of STEC 0157 from humans in clinical settings typically involve spreading fecal swabs on Sorbital MacConkey (SMAC) media.4 These methods, however, are frequently inadequate in outbreak investigations for isolation of STEC 0157 from environmental sources, such as dirt or animal feces.2,5 Sick humans commonly shed more than IfJ5 cfu/g.6 In contrast, STEC 0157 is not a pathogen for ruminants and, when present, is only a minority component of ruminant feces or of the soil microbio ta. So, methods that are sufficient for culture from clinically ill humans result in overgrowth of the competing microflora from animal feces or environmental samples.

Selective enrichment and IMS

The use of a selective enrichment, combined with immunomagnetic separation and plating on CHROMagar with antibiotics works well to isolate STEC 0 157 from a variety of animal and environmental matrices.2'5 The first step in the procedure is the selective enrichment for STEC 0157. The goal is to enrich for the E coli, while inhibiting the growth of the background organisms that may also be present in the sample. One broth that works well is 1 .5X (60 g/L) Brilliant Green Bile Broth (BGB). For each gram (g) of sample, 9 milliliters (mL) of 1.5X BGB is added, up to a maximum of 10 g of sample with 90 mL of 1 .5X BGB. After manual homogenization of the sample with the 1 .5X BGB, enrichments should be incubated at 37°C for six hours.

The second step is the use of immunomagnetic separation (IMS) using E coli 0 157 serotype-specific beads. This step concentrates the small number of STEC 0 157 cells that are present in the enrichment broth. For each enrichment, 20 pL of Dynal anti-0157 paramagnetic beads should be combined with 1

mLof enrichment culture washed twice with PBS (pH 7.4) containing 0.05% Tween 20 detergent and re-suspended in a final volume of 50 µL of wash buffer.

The third step is plating on CHROMagar 0157 (CHROMagar, Paris, France) containing 18 microliter (pL) per liter of 3.5% potassium tellurite solution (TCA). After IMS, 60 µ?, of beads should be spread on TCA, and incubated at 37°C for 24 hours. Suspect STEC Ol 57 colonies appear mauve-pink on TCA, while most competing background flora have blue or white colonies. TCA is preferred over SMAC for this application because the clearly contrasting colony morphology of STEC 0 1 57 compared to the background flora on TCA allows for the quick identification of even single suspect colonies in a near-lawn of non-target colonies, and because the colony morphology remains stable over time (unlike SMAC, on which the STEC 0157 colony morphology may change over time). The tellurite concentration suggested here was specifically formulated and evaluated for maximum recovery of STEC 0 157 from animal feces and environmental samples, and differs from the standard concentrations recommended for use by the manufacturer.

Stock show outbreak

These techniques were used to isolate STEC 0157 from four samples collected as part of an outbreak investigation at a livestock show. Samples were collected into individual Whirl-Pak bags and shipped at room temperature overnight to the laboratory for culture. Samples were logged and weighed upon arrival, and were composed of dirt and manure weighing between 0.73 g and 10 g each.

Ten colonies were picked from each TCA plate, for a total of 40 suspect isolates. All suspects were serotyped for the 0157 and H7 antigens with an enzyme immunoassay using anti-E coli 0157 and anti-£" coli H7 monoclonal antibodies and characterized by PCR for Shiga-toxin and virulence genes.7 Representative isolates from each of the four samples were characterized by DNA fingerprinting, and all were exact matches to the DNA fingerprint patterns of the human cases.

Using these methods can isolate STEC 0 157 from all four environmental samples, including samples that weigh less than 1 g. These methods are robust and can be an effective addition to the STEC 0 157 culture techniques currently employed for epidemiological investigations.

References

1 . Bopp DJ, Sauders BD, Waring AL, Ackelsberg J, et al. Detection, isolation, and molecular subtyping of Escherichia co//D157:H7 and Campylobacter jejuni associated with a large waterborne outbreak. J Clin Microbiol. 2003;41:174-180.

2. Durso LM, Reynolds K, Bauer N Jr, Keen JE. Shiga-toxigenic Escherichia coli 0157:H7 infections among livestock exhibitors and visitors at a Texas county fair. Vector Borne Zoonotic Dis. 2005;5:193-201.

3. Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM. Shiga-toxigenic Escherichia coli 01 57 in agricultural fair livestock, United States. Emerg Infect Dis. 2006;1 2:780-786.

4. Slutsker L, Ries AA, Greene KD, Wells JG, et al. Escherichia coli 0157:H7 diarrhea in the United

States: Clinical and epidemiologic features. Ann intern Med. 1997;126:505-513.

5. Goods B, O'Reilly C, Dunn J, Fullerton K1 et al. Outbreak of Escherichia coliQ] 57:H7 infections after petting zoo visits. North Carolina State Fair, October-November 2004. Arch Pediatr Adolesc Med. 2009;163:42-48.

6. Holland JL, Louie L, Simor AE, Louie M. PCR detection of Escherichia coli 0157-.H7 directly from stools: Evaluation of commercial extraction methods forpurifytng fecal DNA. J Clin Microbiol. 2000;38:41 08-41 13.

7. PatonAW, Paton JC. Detection and characterization of shiga toxigenic Escherichia coli by using muyltiplex PCR assays for stri stx2, eaeA, enterohemorhaglc E coli WyA, rfb^sub 0111^ and rfb^sub 0l57^. J Clin Microbiol . 1998;36:598-602.

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