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Enteropathogenic *Escherichia coli* Prevalence in Laboratory Rabbits

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Abstract

Rabbit-origin enteropathogenic *E. coli* (EPEC) causes substantial diarrhea-associated morbidity and has zoonotic potential. A culture-based survey was undertaken to ascertain its prevalence. EPEC was isolated from 6/141 (4.3%) commercially-acquired laboratory rabbits. Three of these did not have diarrhea or EPEC-typical intestinal lesions; they instead had background plasmacytic intestinal inflammation. Asymptomatically infected rabbits may function as EPEC reservoirs.

Keywords

Escherichia coli infections; laboratory animals; rabbits; diarrhea; zoonoses; inflammation

1. Introduction

Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhea in both animals and humans (Abba *et al.*, 2009; Garcia *et al.*, 2010; Hill *et al.*, 1991; Nguyen *et al.*, 2006). Rabbits infected with rabbit- and human-origin strains have also been used as experimental models of human *E. coli* infection (Cantey & Blake, 1977; Garcia *et al.*, 2002, 2006; Moon *et al.*, 1983; Shringi *et al.*, 2012). In a recent report, we described 10.5% EPEC-associated morbidity and 1.44% mortality in a large laboratory rabbit cohort (Swennes *et al.*, 2012). Disease presentation coincided with recent shipment, and the O145:H2 strain responsible was sensitive to the second-generation fluoroquinolone enrofloxacin. That strain's high associated morbidity, high subclinical infection prevalence (20%), and zoonotic potential prompted us to investigate laboratory rabbit EPEC prevalence.

2. Materials and Methods

2.1 Bacterial culture

Fecal samples were obtained from adult 141 New Zealand white rabbits acquired from 5 commercial vendors and housed at 5 research institutions. All rabbits were clinically healthy except for 3 that had diarrhea. Samples were collected in *Brucella* broth containing 10%

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glycerol, homogenized, streaked on blood and MacConkey agar plates, and grown at 37 °C under aerobic conditions. Lactose-positive colonies were sub-cultured based on morphology and speciated using API 20E identification test strips (bioMérieux, Marcy l'Etoile, France). All isolates were tested for antibiotic sensitivity using the Kirby-Bauer method as previously described (Swennes *et al.*, 2012).

2.2 Molecular characterization

DNA was extracted from pure *E. coli* cultures using the High Pure PCR Template Preparation Kit (Roche Applied Science, Indianapolis, IN). Isolates were differentiated using repetitive sequence-based PCR (REP-PCR) and PCR-tested for *eae*, *stx1*, and *stx2* as previously described (Swennes *et al.*, 2012). Representative isolates were serotyped by the Pennsylvania State University *E. coli* Reference Center (University Park, PA).

2.3 Histopathology

Pieces of duodenum, jejunum, ileum, cecum, ampulla, transverse colon, and descending colon were fixed in 10% neutral buffered formalin, paraffin embedded, cut into 5 μm sections, mounted on glass slides, and stained with hematoxylin and eosin. Slides were examined by a board-certified veterinary pathologist.

3. Results

E. coli isolates (n = 163) were obtained from 86 (61%) of the rabbits sampled. Of the 163 isolates, 13% were sensitive to ampicillin, 56% to amoxicillin-clavulanic acid, 4% to ephalothin, and 41% to gentamicin. All isolates were sensitive to trimethoprim-sulfamethoxazole and enrofloxacin. Isolates were differentiated by repetitive sequence-based PCR (REP-PCR), revealing 13 distinct banding patterns each corresponding to a single serotype (Table 1). Virulence factor-based PCR testing was performed for *eae*, the gene encoding the adhesin intimin that is involved in host epithelial association, as well as *stx1* and *stx2*, which encode the shiga-like toxins which have been previously identified in rabbit-origin *E. coli* strains (Garcia & Fox, 2003; Garcia *et al.*, 2002). Isolates from 6 rabbits (4.3%) tested *eae*-positive and *stx*-negative, the EPEC-characteristic genetic profile (Table 1). Two *eae*-positive strains were isolated from these rabbits, including an O145:H2 strain previously associated with rabbit diarrhea and a novel OM:H1 (multiple O-reactive) strain (Swennes *et al.*, 2012). EPEC-positive rabbits were obtained from 2 vendors, each harboring either EPEC strain. Of rabbits presenting with diarrhea, 3 of 3 (100%) harbored the O145:H2 strain, while 3 other EPEC-positive rabbits harbored either the O145:H2 or OM:H1 strain and had no associated medical history. The diarrheic potential of the OM:H1 strain merits further investigation.

Four of 6 EPEC-positive rabbits, 2 diarrheic and 2 asymptomatic, were available for necropsy following the initial survey. In all cases, the infecting EPEC strain was re-isolated from the cecal contents postmortem. Grossly, the 2 diarrheic rabbits had perineal, tail, and hindlimb accumulation of foul-smelling fecal material. Cecal and colonic serosal reddening suggestive of hemorrhage was evident, and the large intestine was distended with gas and watery feces. Histologically, EPEC-typical intestinal epithelial ulceration with mixed heterophilic/eosinophilic and plasma cell infiltrates was present (Heczko *et al.*, 2000; Peeters *et al.*, 1984; Swennes *et al.*, 2012). The 2 non-diarrheic EPEC-infected rabbits, which harbored either the O145:H2 or OM:H1 strain, displayed no gross lesions or EPEC-typical histologic changes. Instead, an increase in mucosal lamina propria cellularity was noted between intestinal crypts and consisted primarily of plasma cells and few heterophils (Figure 1). Relatively few lymphocytes were found primarily in aggregates. Apoptotic cells were also noted within the mucosal epithelium.

4. Conclusions

The survey performed here indicates that diverse *E. coli* strains are frequently found in the clinically healthy rabbits' normal intestinal flora. Of the rabbits surveyed, 4.3% harbored EPEC, indicating that these bacteria persist at low levels in commercially-acquired rabbits. Of the infected rabbits, half did not have diarrhea or intestinal histologic changes consistent with EPEC-associated disease. Instead, they had intestinal plasmacytic infiltration similar to lymphocytic-plasmacytic inflammatory bowel disease of dogs and cats (Day *et al.*, 2008). A prior study identified comparable intestinal plasmacytic infiltration in 53 of 102 (52%) rabbits used for various experimental purposes (Li *et al.*, 1996). It is likely that these rabbits had underlying immune-mediated intestinal disease with secondary EPEC infection. This data infers that laboratory rabbits may frequently have idiopathic chronic intestinal inflammation and display no overt clinical signs.

Pathogenic *E. coli* have been associated with chronic intestinal inflammatory disease in a variety of species. In one report, cotton-top tamarins with EPEC-positive fecal cultures were 2.7 times more likely to have active colitis (Mansfield *et al.*, 2001). Additionally, adherent/invasive *E. coli*, a heterogeneous pathotype possessing few conventional virulence genes, have been isolated from the ileal mucosa of Crohn's disease patients (Darfeuille-Michaud *et al.*, 2004) and from the colonic mucosa of Boxer dogs with granulomatous colitis (Simpson *et al.*, 2006). Population-level analyses also indicate that Crohn's disease patients' ileal mucosa contains numerous *E. coli* expressing various virulence genes, including *eae* (Baumgart *et al.*, 2007).

These reports suggest that the inflamed intestine presents a favorable niche for pathogenic *E. coli* such as EPEC. In this context, laboratory rabbits with intestinal inflammatory conditions may become infected and remain undetected within rabbit populations until other host or environmental factors, such as *Lawsonia intracellularis* (Schauer *et al.*, 1998) or rotavirus (Thouless *et al.*, 1996) infection, low dietary fiber intake (Gidenne & Licois, 2005), shipment (Swennes *et al.*, 2012), or increased stress level (Everest, 2007) potentiate fulminant disease. High subclinical EPEC prevalence (20%) was noted in our recent investigation (Swennes *et al.*, 2012). However, this report suggests that without an inciting cause to promote fecal shedding, baseline laboratory rabbit infection prevalence is likely much lower, i.e. 4.3%. Subclinical EPEC infection is at least 5.9% prevalent in children (Afset *et al.*, 2004; Knutton *et al.*, 2001) and may be 2.5% prevalent in the general human population (Robins-Browne *et al.*, 2004).

Laboratory rabbit enzootic EPEC infection is also significant because these strains are potentially zoonotic. Animal- and human-origin EPECs possess similar virulence factors and share similar clonal origins (Moura *et al.*, 2009). In addition to rabbits, EPEC O145:H2 strains have been identified in non-diarrheic sheep (Vettorato *et al.*, 2009), diarrheic human infants (Gonzalez *et al.*, 1997), and wastewater samples (Doughari *et al.*, 2011). Also, piglets and rabbits are routinely experimentally infected with EPEC and enterohemorrhagic *E. coli* (EHEC) strains from humans and other animals, indicating that infection is not host species-dependent (Garcia *et al.*, 2006; Moon *et al.*, 1983). EHEC transmission from cattle to wild rabbits to humans has also been associated with human hemorrhagic diarrhea and hemolytic uremic syndrome cases (Bailey *et al.*, 2002; Pritchard *et al.*, 2001). Our findings indicate that non-diarrheic rabbits function as reservoirs that potentiate EPEC-associated diarrheal disease and zoonotic transmission. Laboratory rabbit users and suppliers should monitor for and exclude potentially pathogenic *E. coli* strains.

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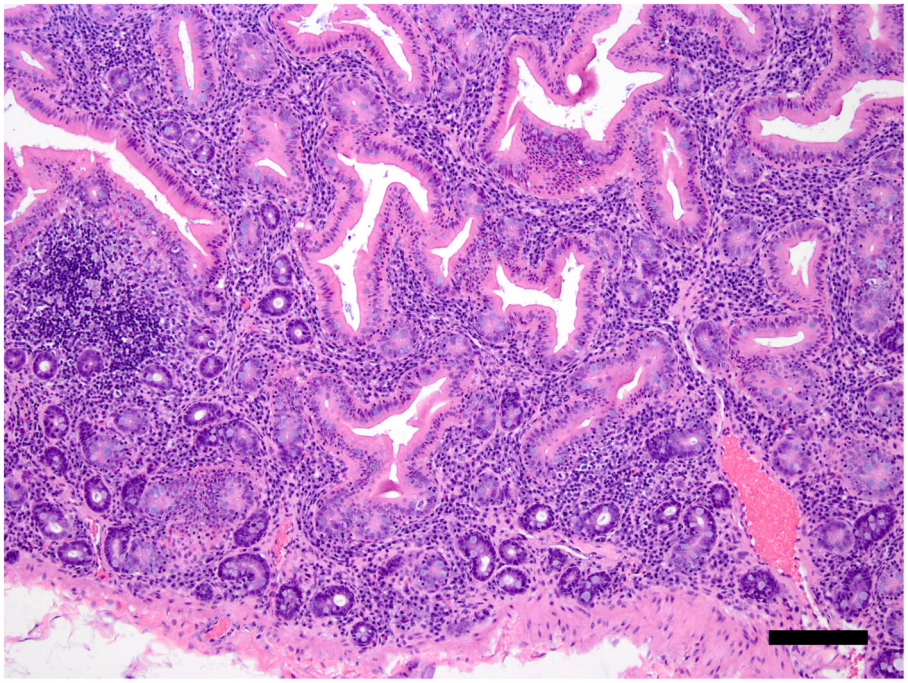

Figure 1.
Ileum from an EPEC-positive rabbit with no clinical signs. A diffuse increase in cellularity in the mucosal lamina propria, comprised primarily of plasma cells, was noted. Occasional lymphoid aggregates are also seen. Hematoxylin and eosin stain. Bar = 100 μm.

Table 1

E. coli isolates of each REP-PCR genotype and corresponding serotype obtained.

REP-PCR genotype	Serotype	*eae* PCR	*n*[1]
1	O2:H1	−	3
2	OM:H1	+	1
3	O-:H7	−	1
4	O7:H7	−	14
5	O8:H10	−	3
6	O8:H49	−	1
7	O103:H7	−	8
8	O145:H2	+	5
9	O170:H5	−	14
10	O174:H28	−	41
11	O13:H4	−	1
12	O103:H16	−	1
13	O18:H7	−	1

[1]Number of rabbits harboring the indicated REP-PCR genotype/serotype.