Healthy carriage and fecal shedding by ruminants of enterohemorrhagic *Escherichia coli* strains causing food-borne infections

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Enterohemorrhagic *Escherichia coli* (EHEC), also known as Shiga toxin–producing *Escherichia coli* (STEC), cause diarrhea and hemorrhagic colitis, which can lead to severe complications such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (PROULX et al., 2001).

1 – EPIDEMIOLOGY

Since 1982, large-scale outbreaks (several hundreds or even thousands of cases) in the United States, Japan, and also in Europe, have alerted the authorities to the potential dangers of these bacteria. Between 1982 and 2000, 238 outbreaks involving the O157:H7 serotype were documented in the United States (RANGEL et al., 2005). The most striking occurred in 1993, and affected over 500 people, 45 of whom developed HUS (3 deaths). Other sizeable outbreaks were recorded in Japan in July 1996 (over 9000 cases, 12 deaths), Scotland in December 1996 (512 cases, 18 deaths), the United States in August 1999 (921 cases, 2 deaths), and Canada in May 2000 (over 2000 cases, 7 deaths). While large outbreaks are often well documented, it is much more difficult to obtain data on sporadic cases, many of which are due to non-O157 EHEC (BROOKS et al., 2005).

France has experienced only two real outbreaks, which occurred in October-December 2005. The first was due to *E. coli* O157:H7, and was linked to the eating of deep-frozen ground beef in South-West France. Sixty-nine people were affected, 57 of them children under 13 years of age. One adult and 16 children presented with HUS. The second outbreak, in Normandy, was caused by *E. coli* O26 in camembert cheese made from unpasteurized milk. Seventeen children aged under six presented with HUS (www.invs.sante.fr). In 1996, the National Institute for Public Health Surveillance, together with pediatric nephrologists, set up a network to monitor HUS in children under 15 years of age.
Since then, 62% of 859 recorded cases of HUS have been attributed unambiguously to EHEC strains. The annual incidence of HUS ranges from 0.59 to 1.01 per 100,000 children under 15. In 2005, 122 cases, 89 of which were sporadic, were recorded (table 1).

Table 1
French cases of HUS in children under fifteen

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cases</th>
<th>Incidence (for 10^5 children under 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>81</td>
<td>0.66</td>
</tr>
<tr>
<td>1997</td>
<td>94</td>
<td>0.77</td>
</tr>
<tr>
<td>1998</td>
<td>76</td>
<td>0.59</td>
</tr>
<tr>
<td>1999</td>
<td>93</td>
<td>0.76</td>
</tr>
<tr>
<td>2000</td>
<td>78</td>
<td>0.64</td>
</tr>
<tr>
<td>2001</td>
<td>74</td>
<td>0.61</td>
</tr>
<tr>
<td>2002</td>
<td>73</td>
<td>0.60</td>
</tr>
<tr>
<td>2003</td>
<td>80</td>
<td>0.66</td>
</tr>
<tr>
<td>2004</td>
<td>87</td>
<td>0.72</td>
</tr>
<tr>
<td>2005</td>
<td>122</td>
<td>1.01</td>
</tr>
<tr>
<td>Total</td>
<td>859</td>
<td>0.70</td>
</tr>
</tbody>
</table>

The principal reservoir of EHEC is the gastrointestinal (GI) tract of ruminants, and principally cattle. Bovine fecal matter is the source of most cases of human contamination. Several modes of transmission are possible: foodborne, mostly via ground beef, but also unpasteurized dairy products, or fruit and salad that have been in contact with manure. Reported cases of contamination by drinking or bathing water are increasingly frequent. Interhuman contamination and transmission through direct contact with animals are also possible. The mean infectious dose appears to be extremely low: a few dozen microorganisms (50 bacteria).

2 – VIRULENCE FACTORS

EHEC adheres to the colonic mucosa, where it causes highly characteristic attaching and effacing (AE) lesions, which occur through effacement of enterocyte microvilli and intimate attachment of the bacterium to a pedestal-like structure (figure 1). The genes responsible for this phenotype are carried by a pathogenicity island called the locus of enterocyte effacement (LEE). The LEE codes for a type III secretion system, which enables the bacterium to inject effector proteins directly into the host cell. These proteins, most of which are also encoded by the LEE, interfere with cell signaling, leading to the formation of the pedestal. The LEE also codes for an adhesin, called intimin, which enables intimate attachment of the bacterium to the enterocyte (GARMENDIA et al., 2005). The great majority of O157:H7 strains carry the LEE, but this is not so in
certain nonpathogenic non-O157:H7 strains. Other genes, not carried by the LEE, code for factors involved in the adhesion of bacteria to epithelial cell lines in vitro, or influence the colonization of the GI tract in animal models (lpf, iha, efa-1, saa). Some are present in O157:H7 and non-O157 strains, while others are solely in LEE-negative non-O157 strains. Their role in the adhesion to the colonic epithelium has not, however, been demonstrated.

Shiga toxins are responsible for the hemorrhagic symptoms of the infection, and for serious complications (HUS and TTP). STEC produces two types of toxins, Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). Their primary sequences present 60% identity, but they have different immunologic properties. Epidemiologic studies show that the strains producing only Stx2 are more frequently associated with serious diseases than the strains producing only Stx1, or both toxins simultaneously. These toxins comprise five B subunits, which allow binding to the cell receptor, globotriaosylceramide (Gb3), and one A subunit, which is catalytic. Shiga toxins are produced by the STEC in the intestinal lumen. Two routes have been proposed for their transport across the epithelial barrier: either intracellular, although enterocytes do not express Gb3, and the mechanism is incompletely elucidated; or paracellular, in which translocation of toxins is favored by increased cellular permeability due to destruction of the tight junctions, and the inflammatory response induced by the infection. Endothelial cells of the blood vessels are targeted by Shiga toxins, particularly those of the intestine, kidney, and brain, which are rich in Gb3. After binding to the receptor, the toxin is endocytosed and transported in a retrograde manner from the Golgi apparatus to the endoplasmic reticulum. The A subunit has N-glycosidase activity, which alters 28S ribosomal RNA, resulting in inhibition of protein synthesis and cell death. This stimulates adhesion of neutrophils to the endothelium, blood coagulation, platelet aggregation, and fibrin deposition. There follow thrombocytopenia and mechanical lysis of erythrocytes (O’LOUGHLIN and ROBINS-BROWNE, 2001).
3 – HEALTHY CARRIAGE OF EHEC STRAINS BY RUMINANTS

Ruminants transiently carry EHEC strains in their GI tract, without being affected by them, and excrete the bacteria in the feces. Animals vary considerably in how much EHEC is excreted in the feces, and for how long. After natural or experimental infection, some animals eliminate the EHEC in under one week, others in one month, and others in more than two months. The reasons for these individual differences are unclear, but may include the animal’s immunologic status, the physicochemical conditions of the GI contents, or the composition and activity of the indigenous microflora. The GI site where EHEC persist also seems to vary from one individual to another. Certain studies indicate that it is the rumen, whereas others refer to the cecum and colon, or the rectum. Although EHEC adhere in vitro to bovine colonic mucosa, forming AE lesions, they are very rarely associated with tissues in animal carriers, but are generally isolated from the gut contents (GRAUKE et al., 2002). EHEC do not, therefore, seem to colonize the gut mucosa, except for the anorectal mucosa, which has been described as the preferred colonization site, but for O157:H7 strains only (NAYLOR et al., 2003). Certain bacterial factors that favor the persistence of EHEC in the GI tract, or the colonization of the anorectal mucosa, have been identified, such as the adhesion factor Efa-1, type III secretion system, intimin and its receptor (SHENG et al., 2006; DZIVA et al., 2004; STEVENS et al., 2002).

4 – INFLUENCE OF RUMINANT DIET ON PERSISTENCE OF EHEC IN THE GASTROINTESTINAL TRACT

By influencing the physicochemical conditions of the ruminant’s gut contents, diet affects the duration and level of EHEC shedding. Although individual variability is high, a fiber-rich diet increases fecal shedding of *E. coli* O157:H7 both in duration and quantity, compared with a diet rich in cereals. Perturbations of indigenous microflora by a sudden dietary change, or fasting, also favor the growth and fecal shedding of EHEC (KUDVA et al., 1997; BROWN et al., 1997; HARMON et al., 1999).

To study the influence of biotic and abiotic factors on survival of EHEC in the gut contents of ruminants, we collected rumen fluid and prepared fecal samples from sheep fed different diets. These samples were inoculated with *E. coli* O157:H7, whose growth was determined after 18 hours of anaerobic culture. We found that the type of diet affects the growth of *E. coli* O157:H7 in the rumen by altering the physicochemical conditions: a slightly acid pH combined with a high concentration of volatile fatty acids, resulting from a diet rich in cereals, does not favor survival of this pathogenic strain (BOUKHORS et al., 2002). Furthermore, the ruminal microflora strongly inhibits its development. Conversely, the fecal medium is favorable to the growth of *E. coli* O157:H7, independently of the animal’s diet, and the fecal flora has no barrier effect (CHAUCHEYRAS-DURAND et al., 2006).
Epidemiologic studies show that the infectious dose of EHEC is very low, which indicates that these strains are highly acid-resistant, since they can survive the extremely acid conditions in the human stomach. Acid resistance mechanisms are often induced during exposure to a moderately acidic environment. The rumen contents of an animal fed a cereal-rich diet could be just such an environment favorable to the induction of acid resistance in EHEC. Few in vivo studies have been done, and their findings are contradictory (GRAUKE et al., 2003; HOVDE et al., 1999; RUSSELL et al., 2000). We evaluated the acid resistance of EHEC strains by measuring their survival in abomasums fluid (pH 2.5), after growth in the rumen contents of animals fed different diets. The results
indicate that resistance to the acidity of the abomasum is influenced by the physiologic state of the bacteria in the rumen, and by the physicochemical conditions that they encounter there. When STEC are exposed to rumen fluid from animals fed a maize-rich diet, they develop mechanisms of resistance to acid shock, which enable them to survive exposure to abomasum, whereas they do not develop these mechanisms when they are exposed to rumen fluid from animals fed hay (figure 3) (BOUKHORS et al., 2002).

6 – CONCLUSION

While abrupt dietary changes, or fasting, clearly favor the growth of EHEC in the GI tract, it is presently difficult to recommend a diet to limit the healthy carriage of EHEC by ruminants. Most observations indicate that a cereal-rich diet shortens the period during which EHEC are excreted in the feces. However, such a diet may favor the emergence of acid-resistant EHEC, which will survive better in acid conditions and in the human stomach. These pathogens will therefore pose a greater risk in terms of food contamination and human health. Research is now focusing on the utilization of probiotics to reduce both the carriage and the acid resistance of EHEC.

Figure 3

Resistance of STEC cells to the acidic conditions of the abomasum. Cells were incubated for 5 h in anaerobiosis in LB, rumen juice from hay-fed animal, or rumen fluid from hay + wheat-fed animals. The acid challenge was performed by incubating 1x10^5 to 5x10^5 cells for 2 h in abomasum fluid pH 2.5
REFERENCES


