Adherence of Non-O157 Shiga Toxin–Producing *Escherichia coli* to Bovine Recto-anal Junction Squamous Epithelial Cells Appears to Be Mediated by Mechanisms Distinct from Those Used by O157

Indira T. Kudva,¹ Carolyn J. Hovde,² and Manohar John³

Abstract

This study presents evidence that the pattern (diffuse or aggregative) of adherence of clinically relevant non-O157 Shiga toxin–producing *Escherichia coli* (STEC) to bovine recto-anal junction squamous epithelial cells is similar to that of *E. coli* O157, although the mechanisms of adherence appear to be distinct. Our results further suggest that novel adhesins, and not Intimin, are likely involved in non-O157 STEC adherence to bovine recto-anal junction squamous epithelial cells. These findings have important implications for the development of efficacious modalities for blocking adherence of non-O157 STEC to bovine gastrointestinal epithelial cells.

Introduction

SHIGA TOXIN–PRODUCING *Escherichia coli* (STEC) comprise a diverse collection of serotypes, capable of causing hemorrhagic colitis and the often fatal sequelae, hemolytic uremic syndrome, in humans (Griffin, 1995; Griffin *et al.*, 1998; Kaper *et al.*, 1998; Griffin *et al.*, 2000; Gyles, 2006; Paton *et al.*, 1998). Of these foodborne pathogens, *E. coli* O157 (O157) is the most researched, considering that it causes an estimated 63,153 illnesses, 2138 hospitalizations, and 20 deaths annually in the United States alone (MMWR Report 2011; Scallan *et al.*, 2011). Although non-O157 STEC frequently cause human disease, few have been implicated in major outbreaks with the exception of serotypes O145, O111, O104, and O26 (<http://www.cdc.gov/ecoli/outbreaks.html>; Bradley *et al.*, 2012; Muniesa *et al.*, 2012). In fact, the Centers for Disease Control and Prevention report that 64% of all STEC infections in the United States are caused by non-O157 STEC, accounting for about 113,000 illnesses and 300 hospitalizations (Scallan *et al.*, 2011; Batz *et al.*, 2012; Hoffman *et al.*, 2012). The recent proactive directive from the U.S. Department of Agriculture–Food Safety and Inspection Service declaring six non-O157 serotypes as food adulterants is now encouraging the much-needed close scrutiny of these potent human pathogens, which include the serotype O26, O103, O111, O121, O145, and O45 (FSIS Notice 40-12, 2012).

Ruminants (cattle and sheep) are primary reservoirs for STEC and hence, developing optimal pre-harvest strategies in these animals to control entry of non-O157 STEC into the food chain is as critical as it is for O157 (Griffin, 1995; Kaper *et al.*, 1998; Griffin *et al.*, 2000; Gyles 2006). In this context, understanding host–pathogen interactions is essential to the development of such strategies. We recently demonstrated that O157 colonization of the recto-anal junction (RAJ) of cattle not only involves bacterial microcolony formation on the lymphoid follicle-associated columnar epithelial cells but also involves diffuse adherence to the adjacent RAJ squamous epithelial (RSE) cells (Kudva *et al.*, 2011). We subsequently developed an adherence assay using RSE cells that was able to successfully differentiate between O157 and other non-pathogenic *E. coli* strains as well as between strongly adherent and moderately adherent O157 strains from various sources (Kudva *et al.*, 2011; Kudva *et al.*, 2012). The RSE adherence assay was also extrapolated to differentiate between *Shigella* serotypes (Kudva, 2012).

Since the non-O157 STEC serotypes are closely related to O157 (Kaper *et al.*, 1998; Gyles, 2006), we hypothesized that these human pathogens would also interact with bovine gastrointestinal cells in a similar manner, and that studies ascertaining such interactions would likely provide insights into the colonization dynamics of these STEC in their

¹Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa.

²School of Food Science, University of Idaho, Moscow, Idaho.

³Pathovacs, Inc., Ames, Iowa.

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ruminant reservoirs. Hence, we (1) evaluated interactions of the non-O157 STEC serotypes with the RSE cells using the RSE cell adherence assay; (2) determined the role of Intimin, a well-characterized, primary STEC adhesin, and other proteins encoded along with Intimin on the locus of enterocyte effacement (LEE) in adherence of non-O157 STEC to RSE cells via adherence-inhibition assays; and (3) evaluated the ability of hyperimmune sera generated in cattle against O157 to inhibit adherence of non-O157 STEC to RSE cells to determine cross-protection across the STEC serotypes. Our results demonstrate that the pattern of adherence of non-O157 to bovine RSE cells is similar to that of O157; however, non-O157 STEC appear to utilize RSE cell adherence mechanisms that are distinct from those employed by O157 to bind the same cells at the primary site of colonization in the bovine gastrointestinal tract.

Materials and Methods

Bacterial strains and sera

Bacteria. STEC serotypes used in this study are listed in Table 1. Both clinical and environmental strains were included for each serotype except O121 due to lack of availability. For each non-O157 STEC serotype, strains A and B were used as test strains and strains C were used as the "verification strain," to verify the adherence patterns observed (Table 1). O157 strain EDL 933 (Perna *et al.*, 2001) was included for comparative purposes.

Pooled antisera. Rabbit antisera targeting the recombinant LEE-encoded proteins, Tir, EspA, and EspB, and Intimin (National Animal Disease Center Stocks, NADC, Ames, IA) were combined together to prepare the pooled antisera sus-

pension (Kudva *et al.*, 2012) for testing at 1:5 to 1:100 dilutions. The specificity of each fraction comprising the pooled antisera was confirmed by reacting against both O157 cell lysates and the cognate protein in Western blotting experiments (data not shown). Rabbit sera (Sigma-Aldrich, St. Louis, MO) from healthy animals (normal rabbit sera), at a 1:5 dilution, were used as a control in each experiment.

Pooled hyperimmune bovine sera. Hyperimmune bovine sera was generated by challenging cattle with oral doses containing a mixture of O157 strains of bovine and human origin, including strain EDL 933 (Rice *et al.*, 2003). For use in this study, sera collected from nine cattle that had shed O157 in feces for up to 2 months in that study were pooled together to constitute the pooled hyperimmune bovine sera (Rice *et al.*, 2003). Studies of the quality of the pooled hyperimmune bovine sera using dot immunoblotting and Western blotting revealed robust immunoreactivity against a variety of O157 components, including O157 lipopolysaccharide, previously identified LEE-encoded proteins, and several novel proteins that have been identified by us in our ongoing studies (Kudva *et al.*, 2006; Kudva *et al.*, 2010; Kudva *et al.*, unpublished data). Sera from healthy cattle not challenged with O157 and being used as "blood donors" at the NADC, Ames, IA (normal bovine sera) were used as control at a 1:5 dilution, in each experiment.

RSE cell assays

RSE cells were obtained from the recto-anal junctions of the gastrointestinal tracts of healthy cattle as described previously (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012) and used in the following assays.

Adherence assays and patterns. Adherence of O157 to the RSE cells was previously demonstrated and developed

TABLE 1. SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) STRAINS USED IN THIS STUDY

Serotype	Strain: number	Characteristics			Source
		Shiga toxin 1	Shiga toxin 2	Intimin	
O26:U	A: NADC 3108	+	–	+	Calf isolate; NADC strain collection (NADC)
O26:U	B: NADC 3145	–	–	+	Calf isolate; NADC
O26:H11	C: DEC 10B	+	–	+	Patient isolate; HC associated; STEC Center
O113:U	A: NADC 3128	+	+	–	Calf isolate; NADC
O113:U	B: NADC 547	–	–	–	Patient isolate; diarrhea associated; NADC
O113:H21	C: CL-3	–	+	–	Patient isolate; HUS associated; STEC Center
O103:U	A: NADC 3358	+	–	+	Calf isolate; NADC
O103:U	B: NADC 1526	–	–	–	Patient isolate; diarrhea associated; NADC
O103:H6	C: TB154A	+	–	+	Patient isolate: diarrhea associated; STEC Center
O121:H19	A: NADC 5705	+	+	–	Patient isolate; HUS associated; NADC
O45:U	A: NADC 3802	+	–	+	Calf isolate; NADC
O45:H16	B: NADC 1057	–	–	–	Patient isolate; diarrhea associated; NADC
O45:H2	C: DEC 11C	+	–	+	Patient isolate; HC associated; STEC Center
O145:U	A: NADC 3196	–	–	+	Calf isolate; NADC
O145:U	B: NADC 3419	+	–	+	Calf isolate; NADC
O145:NM	C: NADC 768	–	–	–	Patient isolate; diarrhea associated; NADC
O111:U	A: NADC 3309	+	–	+	Calf isolate; NADC
O111:H8	B: CL-37	+	–	+	Patient isolate; HUS associated; STEC Center
O111:H8	C: DEC 8B	+	+	+	Patient isolate; HC associated; STEC Center
O157:H7	EDL 933: 43895	+	+	+	Meat isolate; HC-outbreak associated; ATCC

Number, numbers assigned to the non-O157 STEC strains by the source institutions; U, unknown/untypeable; NADC, National Animal Disease Center/United States Department of Agriculture, Ames, IA; HUS, hemolytic uremic syndrome; HC, hemorrhagic colitis; STEC Center, The Thomas S. Whittam Shiga-toxin producing *Escherichia coli* Center, East Lansing, MI; ATCC, American Type Culture Collection, Manassas, VA.

into an adherence assay in our laboratory (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012). In this study, the assay was used to study the interactions of non-O157 STEC with RSE cells. Each of the RSE adherence assays was conducted in eight technical and two biological replicates as described previously (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012) for the test strains, and as eight technical replicates for the verification strain. Briefly, RSE cells were washed and resuspended in DMEM-No Glucose (Invitrogen, Carlsbad, CA) with 2.5% D+Mannose, to a final concentration of 10^5 cells/mL. Bacterial pellets from overnight cultures in DMEM-Low Glucose, incubated at 37°C without aeration, were washed and mixed with RSE cell suspensions to a final bacteria:cell ratio of 10:1 and incubated with aeration (37°C, 110 rpm, for 4 h). At the end of 4 h, the mixture was pelleted and washed thoroughly, once with 14 mL DMEM-NG and twice with 14 mL of sterile, distilled water (dH₂O) before reconstituting in 100 μ L dH₂O. Eight 2- μ L drops of this suspension were placed on Polysine (Thermo Scientific Pierce, Logan, UT) slides and dried overnight under direct light to quench non-specific fluorescence, before fixing in cold 95% ethanol for 10 min. The slides were then stained with 1% toluidine blue, or with fluorescence-tagged antibodies that specifically target O157 or *E. coli* (Kierkegaard Perry Laboratories, Gaithersburg, MD) and the RSE cell cytokeratins as described previously (Kudva *et al.*, 2011). Each experiment was then done in duplicate.

O157 adherence patterns on RSE cells were recorded as either diffuse or aggregative (clumps) for all positive interactions that involved direct association with the cells, as described previously (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012). Bacteria not adhering to cell membranes were considered to be negative for adherence (nonadherent) to the epithelial cells (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012). For a total of 100–160 well-dispersed RSE cells (10–20 cells per drop or chamber) analyzed per slide, the percent RSE cells with and without bacteria adhering to them were determined, and the average percents with standard error of means between trials was calculated using the GraphPad Prism5 software (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012). If more than 50% of RSE cells had >10 bacteria attached, the adherence was recorded as strongly positive. For >50% RSE cells with 1–10 adherent bacteria, the adherence was recorded as moderately positive. For less than 50% RSE cells with 1–5 adherent bacteria, the result was recorded as nonadherent.

Adherence-inhibition assay. The ability of pooled rabbit polyclonal antisera and bovine hyperimmune sera to interfere with and inhibit STEC adherence to RSE cells was evaluated. The assay was performed as before except that the bacterial cells were resuspended in sterile saline with or without antisera (“no sera” control), and incubated at 37°C for 30 min before mixing with RSE cells (Kudva *et al.*, 2012). Both sera were tested at dilutions ranging from 1:5 to 1:100. Adherence patterns were recorded as described above.

HEp-2 cell adherence and adherence-inhibition assay

The HEp-2 cell adherence assays were performed with non-O157 STEC as described previously with O157 (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012) and under conditions

used for RSE cell assays above. Each assay was conducted in duplicate, and in six chambers (technical replicates) of the chamber slides per run. Slides were stained with fluorescence-tagged antibodies that specifically target O157 or *E. coli* (KPL, Gaithersburg, MD), and the HEp-2 cell actin filaments as described previously (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012) and adherence patterns recorded as for RSE cells (see above).

Results and Discussion

Non-O157 STEC that were not exposed to any sera, irrespective of their serotypes or clinical/environmental derivation, adhered to RSE cells as well as the HEp-2 cells (Figs. 1A and 2A). Interestingly, the non-O157 STEC adherence patterns were similar to that of O157 strains as seen in this (O157 strain EDL 933) and previous studies (O157 strain 86-24) (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012). Of the seven non-O157 STEC serotypes tested, O145 and O111 showed a distinct aggregative binding pattern, while the other serotypes demonstrated diffuse adherence to RSE cells, similar to O157 strains EDL 933 and 86-24, respectively. However, all STEC adhered to HEp-2 cells in a diffuse manner. The differential adherence patterns are shown in Figs. 1A and 2A.

As seen with O157 (Kudva *et al.*, 2012), Intimin and other LEE-encoded proteins did not play a role in non-O157 STEC adherence to the RSE cells (Table 2 and Fig. 1B). The LEE-encoded proteins, which include EspA, EspB, Tir, and Intimin, have been shown to be required for efficient O157 adherence to the follicle-associated epithelium at the RAJ (Naylor *et al.*, 2003; Naylor *et al.*, 2005). However, in our recent studies, we demonstrated that these proteins were not involved in O157 adherence to the RSE cells also found at the RAJ (Kudva *et al.*, 2012). Likewise, in this study, we found the non-O157 STEC adhered to RSE cells in the presence of pooled antisera containing antibodies blocking the Intimin, EspA, EspB, and Tir proteins (Table 2 and Fig. 1B). This was observed at all antisera dilutions tested. In fact, O113, O145, and O111 serotypes adhered strongly and in aggregative patterns in the presence of this pooled antisera, which may be indicative of other adhesins being better exposed by the masking of Intimin and the LEE proteins by this antisera (Kudva *et al.*, 2012). Adherence to the RSE cells was also observed irrespective of the type of Intimin expressed (β -Intimin in serotype O45) (Oswald *et al.*, 2000), or the lack of Intimin expression in some of the O113, O103, O121, O45, and O145 strains tested (Table 1), supporting a role of additional adhesins (Kudva *et al.*, 2012).

Surprisingly, despite similarities in RSE adherence, the pooled hyperimmune bovine sera had opposite effects on the various STEC serotypes. A cross-inhibitory effect was anticipated with the pooled hyperimmune bovine sera that were generated in cattle experimentally inoculated with multiple strains of O157, considering the existing similarities between the STEC (Kaper *et al.*, 1998; Gyles, 2006). However, these sera demonstrated serotype specific inhibition by blocking only the adherence of O157 to RSE cells and none of the non-O157 STEC (Table 2 and Fig. 1C), at all dilutions tested.

The HEp-2 adherence assays are routinely used to evaluate adherence potential of O157 and non-O157 STEC (Kaper

FIG. 1. The different Shiga toxin-producing *Escherichia coli* adherence patterns, as represented by serotypes O157, O26, and O145, on recto-anal junction squamous epithelial cells in the presence of D+Mannose, and in the presence or absence of sera are shown. **A:** In the absence of sera (No sera). **B:** In the presence of "pooled antisera" against locus of enterocyte effacement, Intimin and flagellar H7 proteins, at a 1:100 dilution. **C:** In the presence of hyperimmune bovine sera from cattle challenged with O157, at a 1:100 dilution. The immunofluorescence-stained slides are shown at 40 $\times$ magnification. Shiga toxin-producing *Escherichia coli* have green fluorescence, cytokeratins of recto-anal junction squamous epithelial cells have orange-red fluorescence, and their nuclei have blue fluorescence. White arrows in each panel indicate bacteria adherent to RSE cells.

FIG. 2. The different Shiga toxin-producing *Escherichia coli* adherence patterns, as represented by serotypes O157, O26, and O145, on HEp-2 cells in the presence of D+Mannose, and in the absence or presence of sera are shown. **A:** In the absence of sera (No sera). **B:** In the presence of "pooled antisera" against locus of enterocyte effacement, Intimin and flagellar H7 proteins, at a 1:100 dilution. **C:** In the presence of hyperimmune bovine sera from cattle challenged with O157, at a 1:100 dilution. The immunofluorescence-stained slides are shown at 40 $\times$ magnification. Shiga toxin-producing *Escherichia coli* have green fluorescence, actin filaments of HEp-2 cells have orange-red fluorescence, and their nuclei have blue fluorescence. White arrows in each panel indicate bacteria adherent to HEp-2 cells.

TABLE 2. QUANTITATION OF RECTO-ANAL JUNCTION SQUAMOUS EPITHELIAL (RSE) CELLS WITH ADHERENT BACTERIA IN THE ABSENCE AND PRESENCE OF ANTISERA

STEC serogroup tested	Percent mean $\pm$ standard error of mean, of RSE cells with adherent bacteria in the ranges shown ^a (MOI = 10^6 bacteria: 10^5 RSE cells)					
	No antisera		Pooled antisera		Pooled hyperimmune bovine sera	
	Bacterial adherence pattern	1–10 ^b	Bacterial adherence pattern	1–10	Bacterial adherence pattern	1–10
O26	Diffuse, moderate	0.7 $\pm$ 0.7	Diffuse, moderate	32.9 $\pm$ 12.9	Diffuse, moderate	2 $\pm$ 0.6
O103	Diffuse, moderate	2.0 $\pm$ 0.5	Aggregative, strong	83 $\pm$ 3	Aggregative, strong	81.5 $\pm$ 3.5
O103	Diffuse, moderate	0	Diffuse, moderate	0	Diffuse, moderate	0
O121	Diffuse, moderate	0	Diffuse, moderate	10.3 $\pm$ 2.3	Diffuse, moderate	0
O45	Diffuse, moderate	0	Diffuse, moderate	0	Diffuse, moderate	0
O145	Aggregative, moderate	14.4 $\pm$ 13.1	Aggregative, strong	68 $\pm$ 4	Aggregative, strong	82 $\pm$ 4
O111	Aggregative, strong	72.1 $\pm$ 7.1	Aggregative, strong	66 $\pm$ 4	Aggregative, strong	82.4 $\pm$ 2.4
O157	Aggregative, moderate	0	Aggregative, moderate	33 $\pm$ 2	Non-adherent	0
						42.6 $\pm$ 4.6

^aAverage of two trials conducted for 2 strains, A and B, of each serotype, except for serotype O121:H19 for which only one strain was available, is shown. Each trial had one slide per bacterial group. Each slide in turn had eight technical replicates spotted on it; 10–20 well-dispersed RSE cells were evaluated per spot. Percent means for ranges used to determine “moderate or strong” adherence, in the absence of any antisera, are shown in bold.

^bNumber of bacteria adhering to each RSE cell is shown as a range of >10, and 1–10. Number of RSE cells without bacteria is not shown. STEC, Shiga toxin-producing *Escherichia coli*; MOI, multiplicity of infection.

et al., 1998; Vidal *et al.*, 2008), and have provided useful information in the context of the human host. Although the inability of these assays to reflect O157 interactions with the bovine RSE cells was already established (Kudva *et al.*, 2011; Kudva *et al.*, 2012), we included the HEP-2 cell adherence assay to compare results obtained with non-O157 STEC and the RSE cells. No differences were observed in the inhibitory effects of the pooled antisera or hyperimmune bovine sera on the STEC adherence to HEP-2 cells, irrespective of the serotype and sera dilutions used. Sera generated against O157 blocked adherence of various STEC serotypes to HEP-2 cells, which was in accordance with results reported by other researchers as well (Asper *et al.*, 2007; Moradi-Bihendi *et al.*, 2010). In fact, all isolates were effectively inhibited from adhering to HEP2 cells by both the pooled antisera (Table 3 and Fig. 2B), and the hyperimmune bovine sera (Table 3 and Fig. 2C), which was in contrast to the observations made with the RSE cells (Table 2).

Non-O157 STEC adhered to RSE cells as well as or better than O157, which may reflect upon the close relatedness of these STEC serotypes with O157 (Gyles, 2006). In this context, three significant observations were made in this study, including the following: (1) Intimin and other LEE-encoded proteins are not involved in the adherence of non-O157 STEC to RSE cells as reported for O157 (Kudva *et al.*, 2012), (2) hyperimmune cattle sera prevents adherence of homologous but not heterologous STEC serotypes to RSE cells, and (3) STEC interactions with cells derived from natural reservoir/animal hosts are not accurately reflected by those seen with human-derived HEP-2 cells as previously reported (Kudva *et al.*, 2011; Kudva, 2012; Kudva *et al.*, 2012), which dictates exercising caution in extrapolating inferences from results obtained using human cell-based assays when it comes to developing host-specific management modalities.

The central of the three observations made in this study (namely, that a natural O157 infection [or suboptimal experimental inoculation] does not elicit immune responses capable of abrogating adherence of heterologous non-O157 STEC to bovine RSE cells) has important implications for development of efficacious modalities, including broadly protective STEC vaccines. It has been well documented that constituents of such vaccines, with highly conserved proteins and domains that include conserved epitopes, are subject to pathogen decoy mechanisms because they serve important functions to the pathogen (Harty *et al.*, 1988; Embers *et al.*, 2002; Crane *et al.*, 2006; Bianchi *et al.*, 2005; Gambhira *et al.*, 2007; Sollner *et al.*, 2008; Alphs *et al.*, 2008; Chootong *et al.*, 2010). The end result of exertion of such decoy mechanisms is that highly conserved proteins/epitopes are rendered sub-immunodominant during natural infection or suboptimal vaccination (Crane *et al.*, 2006). Hence, such highly conserved proteins/epitopes targeted by host immune responses (Corti *et al.*, 2011) engender only subthreshold antibody responses that are ineffective in mediating heterologous protection. Interestingly, however, when such conserved antigens are identified and delivered optimally to the immune system (as in an efficacious vaccine), they reportedly confer excellent cross-protection (Crane *et al.*, 2006). Therefore, the obvious challenge is to identify such protein components of broadly protective STEC vaccines. We are currently addressing this challenge by adopting innovative strategies, including systems-based approaches to dissect alternate mechanisms of

TABLE 3. QUANTITATION OF HEP-2 CELLS WITH ADHERENT BACTERIA IN THE ABSENCE AND PRESENCE OF ANTISERA

STEC serogroup tested	No antisera			Pooled antisera			Pooled hyperimmune bovine sera		
	Bacterial adherence pattern			Bacterial adherence pattern			Bacterial adherence pattern		
	>10	1–10 ^b		>10	1–10		>10	1–10	
O26	1.5 ± 1.5	86.2 ± 13.9		0.8 ± 0.8	38.2 ± 10.2		3.57 ± 2.1	27.4 ± 0.4	
O113	0.7 ± 0.7	79.35 ± 4.4		0	23.7 ± 5.7		0	17.3 ± 5.6	
O103	0	59 ± 4		2.1 ± 2.1	42.5 ± 5.9		0	34.65 ± 5.4	
O121	0	81.2 ± 15.2		1.4 ± 1.4	40.8 ± 0.8		0	37.5 ± 7.5	
O45	3.2 ± 3.2	54.3 ± 0.7		4.2 ± 4.2	18.44 ± 4.9		5.4 ± 5.4	17.5 ± 7.5	
O145	0	75.5 ± 7.5		1.5 ± 1.5	43.15 ± 5.2		3.6 ± 3.6	26.4 ± 11.4	
O111	0	58.5 ± 6.5		1.3 ± 1.3	17.5 ± 2.5		1.4 ± 1.4	17.1 ± 2.1	
O157	0	78.3 ± 9.3		0	26 ± 4		0	37.6 ± 6.2	

^aAverage of two trials conducted for two strains, A and B, of each serotype, except for serotype O121:H19 for which only one strain was available, is shown. Each trial had one slide per bacterial group. Each slide in turn had six technical replicates in separate chambers on it; 10–20 well-dispersed HEp-2 cells were evaluated per chamber. Percent means for ranges used to determine “moderate or strong” adherence, in the absence of any antisera, are shown in bold.

^bNumber of bacteria adhering to each HEp-2 cell is shown as a range of > 10, and 1–10. Number of HEp-2 cells without bacteria is not shown. MOI, multiplicity of infection; STEC, Shiga toxin-producing *Escherichia coli*.

adherence used by these STEC to bind RSE cells with the objective of identifying novel shared adhesins.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Indira T. Kudva, PhD

Food Safety and Enteric Pathogens Research Unit

National Animal Disease Center/ARS/USDA

1 North, Bldg. 20, 1121

Ames, IA 50010

E-mail: Indira.Kudva@ars.usda.gov