Short communication: Detection of Shiga toxin-producing *Escherichia coli* (STEC) in healthy cattle and pigs in Lima, Peru¹

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ABSTRACT

The aim of this study was to determine the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in cattle and pigs as a possible STEC reservoir in Lima, Peru. One hundred and fourteen cattle and 112 pigs from 10 and 4 farms, respectively, were studied. Five *E. coli* colonies per culture were studied by a multiplex real-time PCR to identify Shiga toxin-producing (*stx1*, *stx2*, *eaeA*), enterotoxigenic (*lt*, *st*), enteropathogenic (*eaeA*), enteroinvasive (*ipaH*), enteroaggregative (*aggR*), and diffusely adherent *E. coli* (*daaD*). Shiga toxin-producing *E. coli* were isolated from 16 cattle (14%) but none from pigs. *stx1* was found in all bovine isolates, 11 of which also carried *eaeA* genes (69%); only 1 sample had both *stx1* and *stx2*. Thirteen *stx*-positive strains were classified as Shiga-toxigenic (81%) using an enzymatic immunoassay, 2 STEC strains were from serogroup O157 (13%), and 7 were sorbitol negative (44%). Enteropathogenic *E. coli* were detected more frequently in cattle (18%, 20/114) than in pigs (5%, 6/112). To our knowledge, this is the first study on the prevalence of STEC in farm animals in Peru using molecular methods. Further studies are needed in a large number of farms to determine the relevance of these findings and its consequences for public health.

Key words: Shiga toxin-producing *Escherichia coli*, cattle, pig, Peru

Short Communication

Shiga toxin-producing *Escherichia coli* (STEC), also called verotoxin-producing *E. coli* (VTEC), is a group of foodborne pathogens that can cause severe human disease, such as hemolytic uremic syndrome (HUS),

particularly when the serotype is O157:H7. The most commonly assayed virulence factors of STEC are the 2 phage-encoded cytotoxins, called Shiga toxin 1 and 2 (encoded by the *stx1* and *stx2* genes, respectively), and the protein intimin (encoded by the chromosomal gene *eae*), which is responsible for the intimate attachment of the bacteria to intestinal epithelial cells.

Shiga toxin-producing *E. coli* have been found in the fecal flora of a wide variety of animals, including cattle, sheep, goats, pigs, cats, dogs, chickens, and gulls (Caprioli et al., 2005). The most important animal species in terms of human infection are cattle and pigs (Caprioli et al., 2005). High rates of colonization of STEC isolates have been found in bovine herds in many countries (Karmali et al., 2010). These rates are typically in the range of 10 to 25%. Shiga toxin-producing *E. coli* strains are usually isolated from healthy animals but may be associated with an initial episode of diarrhea in young animals, followed by asymptomatic colonization. The isolation rates of O157 are much lower than those of non-O157 serotypes (Karmali et al., 2010).

Although STEC strains that cause human infections belong to a large number of O:H serotypes, most outbreaks and sporadic cases of hemorrhagic colitis and HUS have been attributed to strains belonging to the enterohemorrhagic serotype O157:H7. As non-O157 STEC are more prevalent in animals and as contaminants in foods, humans are probably exposed more often to these strains. However, environmental samples including stool samples from farm animals are not routinely evaluated for these pathogens in veterinary laboratories outside the research setting.

In Peru, STEC is responsible for 0.4% of watery diarrhea cases in children in the community setting (Contreras et al., 2011), and 9% of bloody diarrhea cases in children at the emergency room in Lima (A. Llanos, Universidad Peruana Cayetano Heredia, Lima, Peru, personal communication). The most common STEC serotype in Peru is O26:H11; and the most common seropathotype is B (45%; Contreras et al., 2011). Ad-

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ditionally, in our experience, HUS is common in Peru; we have identified 188 HUS cases in a retrospective case series during the past 10 yr at one pediatric hospital in Lima (T. Ochoa, unpublished data). However, no information exists on the prevalence, virulence factors, or serogroup of STEC strains isolated from farm animals. Therefore, the aims of this study were (1) to determine the prevalence of STEC in healthy cattle and pigs from Lima, Peru, (2) to determine the critical virulence factors (*stx1*, *stx2*, and *eae*) of the isolated STEC strains, and (3) to determine the serogroup O157 distribution of the STEC isolated strains.

This was a prospective, cross-sectional, descriptive study. Between November and December 2010, samples were collected from 114 cattle (2 mo to 8 yr old) and 112 pigs (2 to 24 mo old). Cattle were screened from 10 dairy farms in the Quilmaná and San Fernando Districts, both in Cañete Province (average of 11 cattle per farm). Pigs were screened from 4 farms in Puente Piedra District in Lima Province (average of 28 pigs per farm). These cattle and pig farms were geographically separate. All animals in this study were healthy and without treatment with antibiotics or other drugs. Both the dairies and pig farms were exposed to the same weather and were fed similar diets. Rectal swabs of rectal feces were collected following previously described procedures (Rice et al., 2003) and placed in a Cary-Blair transport medium.

Samples were cultured on MacConkey agar plates for isolation of *E. coli* colonies. Detection of STEC and other diarrheagenic *E. coli* was done at the Tropical Medicine Institute Alexander von Humboldt in Lima. Five lactose-positive *E. coli* colonies per animal were isolated from MacConkey agar plates and analyzed by a multiplex real-time PCR method using previously validated specific primers for each pathotype: enteroaggregative (*aggR*), enterotoxigenic (*lt*, *st*), enteropathogenic (*eaeA* alone), enteroinvasive (*ipaH*), diffusely adherent *E. coli* (*daaD*), and STEC (*stx1*, *stx2*, *eaeA*; Guion et al., 2008). The STEC or Enteropathogenic *E. coli* (EPEC) isolates were biochemically confirmed to be *E. coli* by conventional biochemical tests. The somatic (O) antigen 157 of STEC strains was determined by conventional serological methods using an *E.*

coli antisera kit (Difco Laboratories Inc., Detroit, MI) following the manufacturer's instructions. Additionally, all STEC-positive colonies per animal were cultured on MacConkey sorbitol agar plates and all of 5 colonies, as pool, were tested for Shiga toxin expression with the Premier enterohemorrhagic *E. coli* (EHEC) enzymatic immunoassay Shiga-toxin test kit (Meridian Diagnostics Inc., Cincinnati, OH) following the manufacturer's instructions. The study was approved by the Animal Ethics Research Institutional Committee at Universidad Peruana Cayetano Heredia, Lima, Peru. We found STEC in 16 out of 114 (16%) cattle stool samples and none from pig stool samples. Shiga toxin-producing *E. coli* isolation varied from 0 to 33% in each farm (Table 1). The STEC strains tended to be isolated more frequently in cattle younger than 24 mo of age than in older cows (20 vs. 6%); however, this difference was not significant. The *stx1* gene was found in all STEC isolates, 11 of which also carried *eaeA* genes (69%). Only 1 sample was found to have both Shiga-toxin genes (*stx1* and *stx2*; Table 2). Among PCR-positive STEC strains, 13 *stx*-positive strains were classified as Shiga-toxigenic (81%) using an enzymatic immunoassay. Only 2 STEC strains were positive for the O157 serogroup (13%); 1 strain was *stx1*+/*eae*− and the other *stx1*+/*eae*+. Both *E. coli* O157 isolates were Shiga-toxigenic. Seven STEC-positive samples were sorbitol negative (44%).

Enteropathogenic *E. coli* were detected more frequently in cattle (18%, 20/114) than pigs (5%, 6/112; $P < 0.01$; Table 1). Enteropathogenic *E. coli* tended to be detected more frequently in cattle younger than 24 mo of age than in older cows (21% vs. 13%). None of the other categories of diarrheagenic *E. coli* were detected.

The prevalence of STEC in dairy cattle from other countries of the world is highly variable. Caution is necessary when comparing prevalences because variations may be due to multiple factors, including number of animals screened, type and age of animals, sampling method, methodology used for processing of samples, and season in which the study is performed (Faith et al., 1996). In addition, fecal shedding by dairy cattle may be related to management strategies, including hygiene, diet, husbandry, and changes in housing or structure of the herd (Menrath et al., 2010).

Table 1. Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolation in cattle and pigs in Lima, Peru

Farm animal	Location	Number of farms	Number of animals	STEC [n (%)]	EPEC [n (%)]
Cattle	San Fernando District, Cañete Province	7	61	13 (21)	14 (23)
	Quilmaná District, Cañete Province	3	53	3 (6)	6 (11)
	Total	10	114	16 (14)	20 (18)
Pig	Puente Piedra District, Lima Province	4	112	0	6 (5)

Table 2. Genotypic and phenotypic characteristics of Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from cattle in Lima, Peru

Strain	Virulence gene ¹			Shiga-toxin production ²	O157 serogroup ³	MacConkey sorbitol negative
	<i>stx1</i>	<i>stx2</i>	<i>eae</i>			
CE-004	+	–	+	+	–	+
CE-005	+	–	+	+	–	–
CE-006	+	–	+	+	–	–
CE-009	+	–	+	+	–	–
CE-012	+	–	+	+	–	–
CE-016	+	–	+	–	–	–
CE-022	+	+	–	+	–	–
CE-025	+	–	–	+	+	+
CE-041	+	–	–	–	–	–
CE-044	+	–	+	+	–	+
CE-048	+	–	+	+	+	+
CE-053	+	–	+	+	–	+
CE-054	+	–	+	+	–	+
CE-085	+	–	+	–	–	+
CE-106	+	–	–	+	–	+
CE-115	+	–	–	+	–	+
Total (n = 16)	16 (100%)	1 (6%)	11 (69%)	13 (81%)	2 (13%)	9 (56%)

¹*stx1* = Shiga-toxin 1 gene; *stx2* = Shiga-toxin 2 gene; *eae* = intimin gene; + indicates that the gene was detected by PCR; – indicates that the gene was not detected by PCR.

²Shiga-toxin production tested by Premier enterohemorrhagic *E. coli* (EHEC) enzymatic immunoassay (Meridian Diagnostics Inc., Cincinnati, OH).

³Determination of the somatic (O) antigen type using an *E. coli* antisera kit (Difco Laboratories Inc., Detroit, MI).

In this study, STEC prevalence (14%) was lower than found in some Latin American countries such as Argentina (43%; Borie et al., 1997) and Chile (34.5%; Fernández et al., 2009). However, there are countries with lower prevalence, including Brazil (10%; Aidar-Ugrinovich et al., 2007) and Venezuela (1.9%; Narváez-Bravo et al., 2007). In the current study, STEC strains tended to be isolated more frequently from cattle younger than 24 mo of age (20%). Calves have been considered the bovine category with the highest prevalence of STEC (Nielsen et al., 2002), although some have found the highest prevalence in heifers (Cobbold and Desmarchelier, 2000). The reasons for this difference between age groups are unknown but might reflect differences in ruminant development, immune response, diet, aspects of cattle management, and other factors (Heuvelink et al. 1998).

Although some Shiga toxin 1-positive STEC isolates have previously been associated with severe disease (Brooks et al., 2005), epidemiological studies have demonstrated that Shiga toxin 2 is the most important virulence factor associated with severe human disease and is significantly associated with an increased risk of HUS in persons infected with O157 or non-O157 STEC (Brooks et al., 2005). In the present study, only 2 STEC strains harbored the *stx2* gene. This finding is consistent with our previous studies in Peruvian children in which STEC strains expressing only Shiga toxin 1 are highly prevalent in children with diarrhea (Contreras et al., 2011).

We detected EPEC in 18% of cattle and 5% of pigs. It is known that *stx* genes are carried by lysogenic bacteriophages and can be acquired by horizontal gene transfer (Muniesa and Jofre, 2000). Therefore, it has been suggested that the emergence of enterohemorrhagic *E. coli* strains has resulted from successive horizontal transfers of virulence factors between STEC and EPEC (Caprioli et al., 2005). It is known that fecal pollution of water is one of the most important routes of STEC transmission. Thus, improved wastewater treatment could be appropriate for reducing the survival of STEC and other *eae*+ *E. coli*. Contamination of the environment, followed by uptake of STEC by farm animals on pasture, maintains the epidemiological cycle of STEC and is a public health concern.

Our study has some methodological limitations. We did not use a method to improve the detection of STEC from stool samples, such as subculture on cefixime tellurite sorbitol MacConkey (CT-SMAC) agar or immunomagnetic separation, so it is possible that our findings underestimate the number of O157 STEC strains. Because this was a cross-sectional study focused on a short time interval, we cannot address STEC prevalence during other months or seasonality. In summary, STEC were isolated more frequently from younger cattle; Shiga toxin 1 was the most common toxin type, and only 13% were O157 positive. Shiga toxin-producing *E. coli* were not isolated from pigs. These data are relevant in estimating the local STEC prevalence in farm animals. Further studies are needed

to determine the prevalence in a larger number of samples and to implement strategies to minimize the risk of infection spreading to humans.

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