Isolation of Shiga Toxin-Producing *Escherichia coli* from a South American Camelid (*Lama guanicoe*) with Diarrhea

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Shiga toxin-producing *Escherichia coli* belonging to serotype O26:H11 was isolated from a 2-month-old guanaco with severe watery diarrhea. *E. coli* colonies carried the stx_1 and *eae* genes, showed localized adherence to HEp-2 cells, and produced enterohemolysin. A serological response to lipopolysaccharide O26 was observed at the onset of diarrhea.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) is recognized as a cause of diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in human beings (17). Besides producing Stx, some STEC strains express intimin, a protein responsible for the strong attachment of bacteria to intestinal epithelial cells, causing a cytopathic attaching and effacing (A/E) lesion. Intimin is encoded by the *eae* gene, located in a chromosomal pathogenicity island referred to as the locus of enterocyte effacement (16). Localized adherence to epithelial cells, an adhesive property of enteropathogenic *E. coli* (EPEC) (8) and some STEC strains (6, 30), is closely related to the ability to produce intimin, though the mere presence of the *eae* gene does not confer the adherence phenotype upon any *E. coli* strain (16). A subset of STEC isolates with virulence-associated traits, such as a defined serotype, production of enterohemolysin (Ehly), and the presence of the *eae* gene, are recognized as enterohemorrhagic *E. coli* (EHEC) strains.

Ruminants, particularly cattle and sheep, are reservoirs of STEC. Although healthy animals normally carry and shed a variety of serotypes (3, 4, 5), natural outbreaks and experimental infections have demonstrated that STEC strains that induce the A/E lesion can also cause diarrhea and dysentery in young calves (13, 19, 23, 24, 29, 30).

Serotypes of frequently implicated STEC strains were O5: H−, O26:H11, O103:H2, O111:H−, and O145:H−. These and other virulence-associated traits, such as production of Ehly and localized and diffuse adherence patterns to epithelial cells, are shared with human STEC strains. The purpose of this study was to report the isolation and characterization of an STEC strain from a diarrheic guanaco (*Lama guanicoe*) cria and the concomitant increase of lipopolysaccharide (LPS) antibody levels.

A group of 18 1-month-old guanaco crias belonging to a breeding farm located in the Rio Negro Province (Patagonia region), Argentina, were transported to the Instituto Nacional de Tecnología Agropecuaria Castelar Experimental Station, Buenos Aires Province, Argentina, according to proper management practices for this species. The animals were dewormed at entry and placed in a corral with access to shelter. They were fed with bovine pasteurized milk three times a day and received potable water ad libitum. One month later, two crias were housed in an isolation facility for physiological studies. Eight days after confinement, one of the crias developed a severe case of green, watery diarrhea. The animal was orally rehydrated with an electrolyte solution. The diarrhea was autolimited 3 days after onset without antimicrobial therapy.

Fecal specimens of the confined crias were collected daily and screened for the presence of rotavirus (RV) antigen by enzyme-linked immunosorbent assay (ELISA) as described previously (7). In the case of the diarrheic animal, feces collected 1 day before diarrhea onset, during the peak of severe diarrhea, and 4 days after the onset of diarrhea were screened for *E. coli*, *Yersinia enterocolitica*, *Salmonella enterica*, and *Clostridium perfringens*. Isolations of *E. coli* and *Y. enterocolitica* were performed with MacConkey agar, whereas *Salmonella* organisms were isolated with salmonella-shigella and Wilson-Blair agars after an enrichment step in tetrathionate broth. *C. perfringens* was investigated by isolating it under an anaerobic atmosphere in Brewer and egg yolk agars. Standard biochemical tests were used for confirmation of suspected colonies (10). The stx_1, stx_2, and *eae* sequences were sought by PCR according to the protocol of Blanco et al. (3). Bacterial strains harboring *stx* sequences were tested on Vero cells to determine Stx production, as described previously (18). The HEp-2 cell adhesion assay and detection of the Ehly phenotype were previously described elsewhere (19). O and H antigens were examined according to standard procedures (20). Antisera for O26, O111, O157, H7, and H11 antigens were provided by the ANLIS Institute of Microbiology "Dr. C. G. Malbrán," Buenos Aires, Argentina.

Serum samples from all animals were collected upon arrival to the station and monthly up to 3 months postarrival. ELISA techniques were adapted for RV antibody detection in serum by previously described assays (21). Each sample was assayed in serial fourfold dilutions. Anti-LPS$_{O26}$ titers in serum were determined by a dot blot ELISA as described previously (9). We used purified LPS obtained from the isolated STEC O26 strain by the method of Westphal and Jann (28). Ten micrograms of LPS and serial twofold serum dilutions ranging from 1:15 to 1:480 were applied per well. Peroxidase-labeled goat

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FIG. 1. PCR amplification products corresponding to the *eae* gene from *E. coli* strains O26:H11 (lane 3), EDL933 (*eae* positive control) (31) (lane 2), and DH5α (*eae* negative control) (lane 1) and to the stx_1 gene from *E. coli* O26:H11 (lane 5), C600(933J) (stx_1 positive control) (31) (lane 6), and DH5α (stx_1 negative control) (lane 7). Sizes of *eae* and stx_1 amplicons are 815 and 894 bp, respectively. Lane 4 contains 100-bp molecular mass marker.

anti-llama immunoglobulin G (IgG) (H+L; Bethyl Laboratories, Inc.) was used as conjugate for both assays.

All animals were seropositive for RV at arrival, and two crias were shedding the virus asymptomatically, indicating its circulation in the herd. However, RV was not detected in the feces of the diarrheic animal, which also showed the lowest anti-RV titer of the group of camelids.

Among all pathogens investigated, the only one detected in the diarrheic animal was *E. coli*. Four out of six selected colonies were positive for the stx_1 and *eae* genes (Fig. 1), produced Stx according to the Vero cell culture assay, and showed the Ehly-positive phenotype. In agreement with the detection of the *eae* gene, the Stx-producing *E. coli* strains showed localized adherence (Fig. 2) in the HEp-2 cell adhesion assay. The isolates belonged to serotype O26:H11. Fecal samples collected 4 days after the onset of diarrhea were negative for STEC O26:H11, and three of six colonies corresponded to STEC O26:H11 on the sample collected 1 day before the animal became ill. Only the sample collected at the peak of diarrhea showed a watery aspect. An eightfold increase in the level (15 to 120 [the titer was expressed as the inverse of the maximal dilution with a positive signal]) of LPS_{O26} antibodies was detected in the serum sample collected during the acute period, and the level remained constant in the samples taken 1 and 2 months later. Further searching for LPS_{O26} antibodies in sera collected upon arrival at the Experimental Station from eight selected healthy animals showed titers equal to or higher than 80, which continued unaltered on the subsequent serum samples.

A limited number of reports concerning infectious diseases of South American camelids (SAC) has been published in recent years (1, 11, 14, 21, 22, 26, 27). Enteric pathogens, such as *C. perfringens*, *Salmonella* sp., RV, coronavirus, and certain parasites, such as *Cryptosporidium* sp., *Giardia duodenalis*, and coccidia, have been reported to be involved in outbreaks or sporadic cases of diarrhea in these animals (1, 21, 22). In particular, there is one report of a diarrheic outbreak where RV associated with *E. coli* or *Salmonella* sp. was detected in the

feces of newborn guanacos from the Argentinean Patagonia region (21).

In this study, an STEC strain was isolated from the feces of a 2-month-old SAC with diarrhea. Remarkably, we found STEC colonies in samples collected during the diarrheic episode and 1 day before the onset of diarrhea but not later. This isolate presented the typical characteristics of O26:H11 EHEC strains and seemed to lead to a seroconversion in the diseased guanaco. Surprisingly, a high titer of LPS_{O26} antibodies was observed in some of the young guanacos when they arrived at the research station. While these antibodies could be of colostral origin, an active immune response to previous infection in 1-month-old animals could not be discounted. It is well known that colostrum-deprived neonate animals, such as calves and pigs, are able to develop an active immune response against a natural or experimental infection early in life (2, 15). As the goat anti-llama IgG (H+L) used here was reported by the manufacturer to react also with light chains common to other llama immunoglobulins (IgM and IgA), the antibodies detected during the acute phase could be a primary IgM response or a secondary IgG response. However, because no reagent to detect camelid IgM and IgA antibodies was available, the isotype-related response to *E. coli* LPS could not be discriminated in the present study.

The source of contamination with STEC O26:H11 of the young camelid remains unclear. Previous contamination of the environment with bovine fecal bacteria could have served as a source of infection for the diarrheic animal. It is documented that some serotypes of STEC, such as O157:H7, O26:H11, and O111:H⁻, can survive in bovine feces for a long time, especially at 15°C (12). However, we cannot discount the existence of a resident population of serologically diverse STEC strains in guanacos, including serotypes such as O26:H11, which could be responsible for the LPS_{O26} antibodies detected in the animals when they arrived at the Experimental Station. Similarly, high levels of specific LPS_{O157} antibodies have been observed
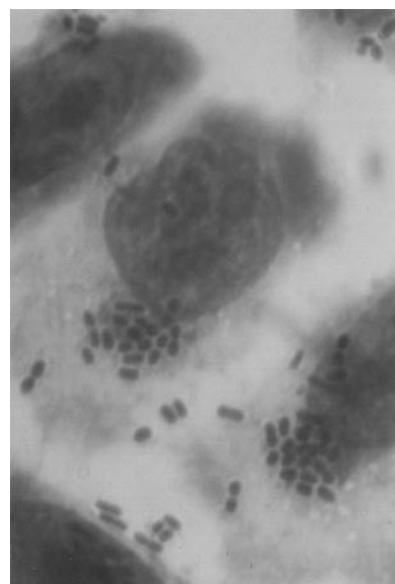

FIG. 2. Microphotography showing the localized adherence of *E. coli* O26:H11 to HEp-2 cells (magnification, ×1,250).

with human *E. coli* O157 disease, and a serological test was proposed to provide evidence of infection (25).

Taking into account that we were not able to demonstrate the presence of any other enteric pathogen in the stools of the diarrheic guanaco, our data suggest that STEC O26:H11 was responsible for the symptoms observed in the affected animal.

Although isolation of STEC strains from several animal species has been reported, most of these strains were not identified as typical EHEC strains. According to the virulence markers investigated, the present isolate was indistinguishable from EHEC associated with severe human and bovine disease. To our knowledge, this is the first documented isolation of *E. coli* O26:H11 with genetic determinants corresponding to Stx-producing strains with the ability to form A/E lesions in a SAC.

Since cattle and sheep, the principal reservoirs of STEC, are pregastric fermentors, there is a high risk that SAC, their close relatives, will become an important source of harmful human STEC, as well as EHEC, in the future. The housing of guanacos in the same environment as cattle will probably facilitate the transmission of pathogens from domestic species to SACs. Implementation of management practices will change living conditions for camelids. Indeed, factors such as diet, direct contact with soil and water contaminated with bovine feces, housing, and other activities or situations causing stress could give rise to carriage of STEC by these animals.

REFERENCES

1. **Adams, R., and F. B. Garry.** 1992. Gram-negative bacterial infection in neonatal New World camelids: six cases (1985–1991). J. Am. Vet. Med. Assoc. **201:**1419–1424.
2. **Barrington, G. M., and S. M. Parish.** 2001. Bovine neonatal immunology. Vet. Clin. North Am. Food Anim. Pract. **17:**463–476.
3. **Blanco, M., J. E. Blanco, J. Blanco, A. Mora, C. Prado, M. P. Alonso, M. Mouriño, C. Madrid, C. Balsalobre, and A. Juárez.** 1997. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Vet. Microbiol. **54:**309–319.
4. **Cerqueira, A. M. F., B. E. C. Guth, R. M. Joaquim, and J. R. C. Andrade.** 1999. High occurrence of Shiga toxin-producing *Escherichia coli* (STEC) in healthy cattle in Rio de Janeiro State, Brazil. Vet. Microbiol. **70:**111–121.
5. **Cobbold, R., and P. Desmarchelier.** 2000. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian dairy herds. Vet. Microbiol. **71:**125–137.
6. **Cookson, A. L., and M. J. Woodward.** 2003. The role of intimin in the adherence of enterohaemorrhagic *Escherichia coli* (EHEC) O157: H7 to HEp-2 tissue culture cells and to bovine gut explant tissues. Int. J. Med. Microbiol. **292:**547–553.
7. **Cornaglia, E. M., M. Barrandeguy, N. Fijtman, and A. Schudel.** 1989. Enzyme link immunosorbent assay, immunofluorescent test and electrophoretic analysis of rotaviral RNA in the diagnosis and characterization of the bovine rotavirus. Rev. Latinoam. Microbiol. **31:**59–62.
8. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. **3:**95–99.
9. **Dean-Nystrom, E. A., L. J. Gansheroff, M. Mills, H. W. Moon, and A. D. O'Brien.** 2002. Vaccination of pregnant dams with intimin$_{O157}$ protects suckling piglets from *Escherichia coli* O157:H7 infection. Infect. Immun. **70:**2414–2418.
10. **Edwing, W. H.** 1986. The genus *Escherichia*, p. 93–134. *In* W. H. Ewing (ed.), Identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
11. **Frank, N., L. L. Couetil, and K. A. Clarke.** 1998. *Listeria monocytogenes* and *Escherichia coli* septicemia in a 7-day-old llama. Can. Vet. J. **39:**100–102.
12. **Fukushima, H., K. Hoshina, and M. Gomyoda.** 1999. Long-term survival of Shiga toxin-producing *Escherichia coli* O26, O111, and O157 in bovine feces. Appl. Environ. Microbiol. **65:**5177–5181.
13. **Hall, G. A., D. J. Reinolds, N. Chanter, J. H. Morgan, K. R. Parsons, T. G. Debeney, A. P. Bland, and J. C. Bridger.** 1985. Dysentery caused by *Escherichia coli* (S102-9) in calves: natural and experimental disease. Vet. Pathol. **22:**156–163.
14. **Hewson, J., and C. K. Cebra.** 2001. Peritonitis in a llama caused by *Streptococcus equi* subsp. *zooepidemicus*. Can. Vet. J. **42:**465–467.
15. **Hodgins, D. C., S. Y. Kang, L. deArriba, V. Parreño, L. A. Ward, L. Yuan, T. To, and L. J. Saif.** 1999. Effects of maternal antibodies on protection and development of antibody responses to human rotavirus in gnotobiotic pigs. J. Virol. **73:**186–197.
16. **Jerse, A. E., J. Yu, B. D. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Natl. Acad. Sci. USA **87:**7839–7843.
17. **Karmali, M. A.** 1989. Infection by verocytotoxin-producing *Escherichia coli.* Clin. Microbiol. Rev. **2:**15–38.
18. **Konowalchuk, J., J. I. Speirs, and S. Stavric.** 1977. Vero response to a cytotoxin of *Escherichia coli.* Infect. Immun. **18:**775–779.
19. **Mercado, E. C., A. Gioffré, S. M. Rodríguez, A. Cataldi, K. Irino, A. M. Elizondo, A. L. Cipolla, M. I. Romano, R. Malena, and M. A. Méndez.** 2004. Non-O157 Shiga toxin-producing *Escherichia coli* isolated from diarrhoeic calves in Argentina. J. Vet. Med. B **51:**82–88.
20. **Ørskov, I., and F. Ørskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **14:**43–112.
21. **Parreño, V., V. Constantini, S. Cheetham, J. Blanco Viera, L. Saif, F. Fernández, L. Leoni, and A. Schudel.** 2001. First isolation of rotavirus associated with neonatal diarrhoea in guanacos (*Lama guanicoe*) in the Argentinean Patagonia region. J. Vet. Med. B **48:**713–720.
22. **Rulofson, F. C., E. R. Atwill, and C. A. Holmberg.** 2001. Fecal shedding of *Giardia duodenalis, Cryptosporidium parvum, Salmonella* organisms, and *Escherichia coli* O157: H7 from llamas in California. Am. J. Vet. Res. **62:**637–642.
23. **Sandhu, K. S., and C. L. Gyles.** 2002. Pathogenic Shiga toxin-producing *Escherichia coli* in the intestine of calves. Can. J. Vet. Res. **66:**65–72.
24. **Schoonderwoerd, M., R. C. Clarke, A. A. van Dreumel, and S. A. Rawluk.** 1988. Colitis in calves: natural and experimental infection with a verotoxin-producing strain of *Escherichia coli* O111: NM. Can. J. Vet. Res. **52:**484–487.
25. **Thomas, A., T. Cheasty, J. A. Frost, H. Chart, H. R. Smith, and B. Rowe.** 1996. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in England and Wales: 1992-4. Epidemiol. Infect. **117:**1–10.
26. **Tsur, I., A. Harmelin, I. Dvir, and J. Yanai.** 1996. Meningoencephalitis and brain abscessation due to *Escherichia coli* in a 2 week old alpaca cria. Aust. Vet. J. **74:**437–438.
27. **Uzal, F. A., R. A. Assis, and E. C. Reissig.** 2000. Malignant oedema in a guanaco (*Lama guanicoe*). Vet. Rec. **147:**336.
28. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides, p. 83-91. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry. Academic Press, New York, N.Y.
29. **Wieler, L. H., R. Bauerfeind, and G. Baljer.** 1992. Characterization of Shiga-like toxin producing *Escherichia coli* (SLTEC) isolated from calves with and without diarrhoea. Zentbl. Bakteriol. **276:**243–253.
30. **Wieler, L. H., E. Vieler, C. Erpenstein, T. Schlapp, H. Steinrück, R. Bauerfeind, A. Byomi, and G. Baijer.** 1996. Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of *eae* and other genes. J. Clin. Microbiol. **34:**2980–2984.
31. **Yu, J., and J. B. Kaper.** 1992. Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. **6:**411–417.