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Detection and Isolation of *Escherichia coli* O157:H7 in Beef from Food Markets and Fecal Samples of Dairy Calves in the Peruvian Central Highlands

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Abstract. Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a food and waterborne pathogen with severe public health implications. We report the first-time isolation of this pathogen in the Central Highlands of Peru through standardized culture procedures and polymerase chain reaction (PCR). *Escherichia coli* strains were cultured from rectal-anal swabs from dairy calves and beef from food markets. The latex agglutination test was used to detect O157 and H7 antigens, and multiplex real-time PCR was carried out to detect virulence-related genes. The STEC O157:H7 strains were isolated from 3.5% (1/28) of beef samples and from 6.0% (3/50) of dairy calves that also carried both *eaeA* and *stx1* genes. Therefore, this pathogen is a potential cause of food/waterborne disease in the region, and its surveillance in both livestock and their products should be improved to characterize the impact of its zoonotic transmission. From 2010 to 2020, *E. coli* was suspected in 10 outbreaks reported to the Peruvian Ministry of Health. Isolates from future outbreaks should be characterized to assess the burden posed by STEC O157:H7 in Peru.

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important food and waterborne pathogen that can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Among these diseases, HUS is a major cause of acute kidney injury, estimated to occur in 10% of infected children under the age of 5 years, and may lead to severe sequels such as kidney failure or neurological damage.[1–3] Cattle are considered the major reservoir of *E. coli* O157:H7; thus, the bacteria can live, grow, and multiply in the gastrointestinal tract without producing clinical signs or disease in the animal.[4] Therefore, beef carcasses can become contaminated during the removal of the hide and evisceration at the slaughterhouse.[5] Consumption of this undercooked contaminated meat and cross-contamination produced in the kitchen during its preparation are the main pathways of exposure, together with the consumption of contaminated unpasteurized milk or vegetables.[2,6] Outbreaks of STEC O157:H7 mainly occur sporadically when the population is exposed to contaminated food sources.[7] Despite it being recognized as one of the major foodborne pathogens worldwide, there is very limited information regarding STEC O157:H7 in the ruminant reservoir or as a cause of human infection in Peru. Therefore, the aim of this study was to elucidate the possible presence of STEC O157:H7 contamination in beef and dairy calves from the Central Highlands of Peru, where it had not been reported to date.

From November 2020 to May 2021, beef and fecal samples were collected from the two principal food markets and a local commercial dairy farm located in the Mantaro Valley in the region of Junin, respectively. The collected samples were processed following the Bacteriological Analytical Manual for diarrheagenic *E. coli* developed by the Food and Drug Administration.[8] Twenty-seven raw beef samples were collected randomly and were immediately transported to the laboratory in a cooler with gel packs to maintain a refrigeration temperature between 3 °C and 10 °C. For microbiological processing, 25 g of each meat sample was homogenized with 225 mL of R & F *E. coli* O157:H7 Enrichment Broth (R & F Products, Inc., Downers Grove, IL) for 2 minutes, and 10 mL of the homogenized broth was transferred to an assay tube for incubation at 37 °C for 18 hours. Subsequently, the enriched samples were submitted to immunomagnetic separation using DynaMag™-2 (Life Technologies AS, Oslo, Norway) with Dynabeads™ anti–*E. coli* O157 (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) and resuspended in 1 mL of phosphate-buffered saline solution with 0.1% Tween 20 Detergent.[9] On the other side, fecal samples from 53 dairy calves were collected through rectal-anal mucosal swab following the method described by Rice et al.[10] and processed at the laboratory immediately after sampling. The swab was inoculated into 10 mL Tryptone Soy Broth (Becton Dickinson GmbH, Heidelberg, Germany) and incubated at 37 °C for 24 hours. After pre-enrichment, an inoculum of the culture was streaked on MacConkey agar plates (Oxoid Limited, Thermo Fisher Scientific Bothell, WA, and Winsford, United Kingdom) to carry out the isolation of colonies and incubated at 37 °C for 18 hours. Subsequently, the suspect colonies were assessed by the following biochemical tests: indole, bromothymol blue indicator, citrate, and lactose fermentation tests.[11]

After the initial processing, all the resuspended meat samples and only the fecal samples identified as generic *E. coli* by the biochemical tests were streaked on sorbitol-MacConkey (Oxoid Limited, Thermo Fisher Scientific) and R & F *E. coli* O157:H7 chromogenic (R & F Products, Inc.) agar plates and incubated at 35 °C for 22 hours. Subsequently, the MUG and indole tests using Bactident *E. coli* (Merck KGaA, Darmstadt, Germany) were carried out on samples with the typical *E. coli* O157:H7–negative reactions for both sorbitol fermentation and the R & F chromogenic substrate.[12] Indole-positive colonies were evaluated using the latex agglutination test Remel RIM

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E. coli O157:H7 (Thermo Fisher Scientific) to detect both O157 and H7 antigens.[10] Afterward, the samples positive for *E. coli* O157:H7 by the conventional methods previously detailed were assessed by multiplex real-time polymerase chain reaction (qPCR) to evaluate the presence of virulence-related genes. The two regions amplified by multiplex qPCR were from the *stx1* and *eaeA* genes specific for STEC whose primers and probes were previously described by Sharma et al.[13] To carry out the amplification, a master mix was prepared in a final volume of 30 μL containing 10 μL DNA, 0.3 μM of each primer, 0.1 μM of each probe, and 1X TaqMan Environmental Master Mix 2.0 (Applied Biosystems, Thermo Fisher Scientific). The final reaction mixtures were transferred to a 96-well PCR plate, including synthetic DNA from *E. coli* O157:H7 strain 3081 (Integrated DNA Technologies, Inc., Coralville, IA) as a positive control in duplicate and two negative controls without template DNA. Afterward, the multiplex qPCR assay was performed in a QuantStudio 3 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) with the following temperature and time conditions: initial denaturation at 95 °C for 10 minutes and 50 cycles of denaturation at 94 °C for 20 seconds, annealing at 55 °C for 30 seconds, and polymerization at 72 °C for 40 seconds. This was followed by a final extension at 72 °C for 5 minutes and a hold at 4 °C.[14]

Among the samples processed by the conventional methods, all (28/28) the meat samples and 98% (49/50) of the fecal samples were positive for generic *E. coli*. Regarding the detection of *E. coli* O157:H7, 3.6% (1/28) of meat samples and 6% (3/50) of fecal samples were positive for the presence of both O157 and H7 antigens. The positive meat samples presented a typical negative reaction in the MUG test, whereas the positive fecal samples presented a positive reaction determined as *E. coli* O157:H7 atypical strains.[12] Furthermore, all the *E. coli* O157:H7–positive samples presented amplicons for both *stx1* and *eaeA* genes through multiplex qPCR. The presence of both genes indicates that the strains belong to the pathotype known as STEC.

In Peru, STEC O157:H7 was isolated for first time from a fecal sample of an infant in the southern coast of the country in 2001[15]; since then, STEC without subsequent serotyping has been reported as the etiological agent of 9.2% of bloody diarrhea produced in Peruvian children.[16] On the other hand, the presence of the serotype O157:H7 in meat samples has only been reported in the region of Lima to date.[1,9,17,18] The proportion of *E. coli* O157:H7–positive meat samples in this study is comparable to those observed in the region of Lima (1.5–4%). Furthermore, this study is the first to report *E. coli* with both O157 and H7 antigens in fecal samples from healthy cattle in Peru, where to date the presence of *E. coli* with only the O157 antigen had been reported in such cattle from Lima.[17,18] The presence of *E. coli* O157:H7 in fecal samples suggests a potential risk of microbial contamination of the meat mainly in slaughterhouses, especially in those with poor hygienic conditions, representing a risk to the public health.[11]

To more accurately describe the potential risk, the development of a quantitative microbial risk assessment study for *E. coli* O157:H7 in the Central Highlands is recommended. Improved surveillance in livestock is needed to characterize how much of this pathogen the population is being exposed to. This can be complemented with improved surveillance in children to further describe both the disease burden and the disability-adjusted life years produced by *E. coli* O157:H7 infections.[19] In this way, it may be possible to model the dynamic of transmission and estimate its impact on the Central Highlands. This will allow evaluation of the cost-effectiveness of various strategies to control this disease, such as the one health approach, which addresses the spread of zoonotic diseases by integrating the health of people, animals and the environment.[20] Finally, it is advised that future outbreaks attributed to *E. coli* include characterization of the isolates. In the 11-year 2010–2020 period, 10 such outbreaks were reported to the Peruvian Ministry of Health, but the suspected pathogen was further characterized in none of them.

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