

PRESENCE OF *ESCHERICHIA COLI* O157:H7 IN FECES OF GRAZING LAMBS READY FOR SLAUGHTER IN URUGUAY

P. Rovira^{1*}, G. Brito¹ and F. Montossi¹

¹Programa Nacional de Carne y Lana, Instituto Nacional de Investigación Agropecuaria (INIA), Uruguay
*provira@tyt.inia.org.uy

Abstract – *Escherichia coli* O157:H7 is an important human foodborne pathogen being cattle the main reservoir. Recent studies have indicated that sheep are also potential carriers. The aim of this study was to detect the presence of *E. coli* O157:H7 and associated virulence factors in feces of grazing lambs intended for slaughter in Uruguay. Individual fecal samples were collected from grazing lambs (n=220) in four research units of the National Institute of Agricultural Research (INIA) and sent refrigerated to the microbiology unit of the Technological Laboratory of Uruguay (LATU). Samples were analyzed for the presence of *E. coli* O157:H7 and genes encoding virulence factors (*eae*, *stx*₁, *stx*₂) following standard procedures. Samples positives for *E. coli* O157:H7 were identified in two of the four research units participating in the study. Overall, 9 of 220 (4.1%) fecal samples were *E. coli* O157:H7-positive. All isolates carried genes encoding intimin and Shiga toxins 1 and 2. This study revealed the presence of pathogenic *E. coli* O157:H7 in the feces of grazing lambs ready for slaughter in Uruguay. It suggests that feces can be a source of contamination of lamb carcasses with *E. coli* O157:H7.

I. INTRODUCTION

Escherichia coli O157:H7 is an important human foodborne pathogen that can cause syndromes such as bloody diarrhea, vomiting, hemolytic uremic syndrome, and in some cases, death [1]. *E. coli* O157:H7 strains commonly carry virulence factors such as Shiga toxins (encoded by *stx*₁ and *stx*₂ genes) and factors for attachment to the host mucosa, including intimin (encoded by the *eae* gene) [2]. Early studies have indicated that cattle represent the main reservoir of *E. coli* O157:H7 [3, 4]; however, recently sheep have been proposed as carriers and sources for human infection on a number of occasions [5, 6].

Due to the lack of competitiveness of extensive sheep production systems, traditionally oriented to wool production, high

quality meat derived from lambs has gained in importance among sheep producers in Uruguay. This product is characterized by a young animal at slaughter (milk teeth female or male lamb), with a minimum of 34 kg of live weight and 3.5 value in the corporal condition scale from 1 to 5 [7]. In the last agricultural year (July 2012-June 2013) Uruguay exported 25.000 tons of mutton and lamb meat, which ranks it only behind Australia and New Zealand globally [8]. The aim of this study was to detect the presence of *E. coli* O157:H7 and associated virulence factors in feces of grazing lambs intended for slaughter in Uruguay.

II. MATERIALS AND METHODS

Four Research Units (RU) of the National Institute of Agricultural Research (INIA) finishing lambs on pastures were visited once between July 2012 and June 2013. In three RU sixty lambs (30-40 kg live weight) were chosen at random for sampling while in the remaining RU forty lambs were sampled (n=220). Fecal samples were collected rectally from each animal, placed in sterile bags, and transported refrigerated to the microbiology unit of the Technological Laboratory of Uruguay (LATU).

Fecal samples pools (25 g) were created by combining 5 g of individual samples from 5 lambs within each RU (n=44). Pooled samples were enriched, incubated and processed through the polymerase chain reaction (PCR) BAX System assay (Q7 Qualicon Dupont). Pools with positive results were open and individual samples integrating the pool were subjected to immunomagnetic separation (Dynal) and plated onto CHROMagar 157. Plates were incubated overnight (37°C) and typical mauve colored *E. coli* O157 colonies were picked up and tested for agglutination with *E. coli* O157:H7 latex agglutination test reagents (Oxoid) followed by biochemical confirmatory tests. Finally, confirmed *E. coli*

O157:H7 isolates were tested by PCR using specific primers for the presence of genes encoding toxins (*stx1*, *stx2*) and adherence factors (*intimin*).

III. RESULTS AND DISCUSSION

Lamb fecal samples from a total of four research units of INIA located in the East (n=2), North and South-West of Uruguay were analyzed for the presence of *E. coli* O157:H7. Samples positives for *E. coli* O157:H7 were identified in two of the four research units participating in the study. Overall, 9 of 220 (4.1%) fecal samples were *E. coli* O157:H7-positive. Previous studies in different countries have reported prevalence estimates in sheep between 0.2% and 8.7% [9, 10]. The sample size in the present study was inadequate to definitively estimate the prevalence of the pathogen at the national level in the lamb population. While cattle are generally regarded as the main reservoir of *E. coli* O157:H7 infection, the results of this study indicate that lambs may also be a significant contributing source. Most farmers that keep lambs also have cattle which may contribute to the survival and recycling of *E. coli* O157:H7 within grazing herds [5].

Different virulence genes, such as *stx₁* and *stx₂* and their variants which encode Shiga toxins, and *eae* which encodes the bacterial outer-membrane protein intimin, have been targeted to assess the presence of pathogenic *E. coli* O157:H7. In the present study, all confirmed isolates (n=9) carried genes encoding intimin and both Shiga toxins. Particularly important was the presence of *stx₂* which appears to be more cytopathic than *stx₁* in animal and in vitro models and is more frequently associated with severe forms of human morbidity [11].

IV. CONCLUSION

This study revealed the presence of pathogenic *E. coli* O157:H7 in the feces of grazing lambs ready for slaughter in Uruguay. It suggests that feces can be a source of contamination of lamb carcasses with *E. coli* O157:H7. By improving the understanding of the on-farm epidemiology of *E. coli* O157:H7, methods of prevention and/or control at the pre-slaughter/harvest level can be identified in order to increase the safety and

competitiveness of the Uruguayan red meat chain.

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