

PREVALENCE OF SHIGATOXIGENIC *Escherichia coli* IN BOVINE CATTLE: A SURVEY IN THE NORTH REGION OF PORTUGAL

Ballem, A.^{1,2,4,5,6}; Gonçalves, S.¹; Ferreira, A. I.¹; Fernandes, C.⁴; Saavedra, M. J.^{2,4}; Pinto, C.¹; Oliveira, H.³; Almeida, G.¹; Almeida, C.¹

¹INIAV, IP- National Institute for Agrarian and Veterinary Research, Vairão, Vila do Conde, Portugal.

²Veterinary Science Department, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.

³CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal

⁴CITAB- Centre for the Research and Technology of Agro-Environmental and Biological Science, UTAD, Vila Real, Portugal.

⁵ CIMO- Mountain Research Centre, ESA-Polytechnic Institute of Bragança, Bragança, Portugal.

⁶IFFARR-Farroupilha Federal Institute, São Vicente do Sul, Brazil.

Shiga toxin-producing *Escherichia coli* (STEC) strains are food-borne pathogens that can be transmitted to humans through many different routes, but mainly through consumption of contaminated foods. STEC strains are characterized by the production of Shiga toxins (Stx) that are encoded by two genes *stx1* and/or *stx2*. Each toxin can be subdivided into subtypes and, currently, there are three known subtypes for *stx1* (*stx1a*, *stx1c*, and *stx1d*) and seven for *stx2* (*stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f*, and *stx2g*). Other virulence factors, such the production of intimin protein, encoded by *eae* gene, are associated with severe outcomes of STEC-associated diseases. The most common STEC serotype implicated worldwide is *E. coli* O157:H7, but many other STEC strains are associated with severe human diseases. Ruminants, especially cattle, are a major reservoir for O157 and non-O157 STEC. Information on STEC prevalence in Portuguese dairy cattle is limited, so we analysed for the presence of STEC 329 faecal specimens collected from the rectum of healthy dairy cattle. The samples were collected from adult lactating cows (n=194) and from heifers (n=135) with ages among 6 to 18 months, between March and June 2019, at milk farms (n=17) in the North region of Portugal. After enrichment, in modified TSB with novobiocin, samples were analysed by real time PCR to detect the presence of *stx1*, *stx2* and *eae* in accordance to ISO/TS 13136:2012(E). In addition, conventional PCR for the detection of *sxt1* and *stx2* gene subtypes was performed according to the guidelines of the VTEC European Union Reference

Laboratory. A total of 139 isolates were recovered from 108 positive animals (dairy cows and heifers). The STEC prevalence was significantly higher in heifers (68/135; 50.4%) than in adult cows (40/194; 20.6%) ($p < 0.05$, Fisher exact test statistic value is <0.00001). STEC harbouring only *stx2* (67/139; 48.2%) were the most common strains, followed by both genes *stx1* and *stx2* (40/139, 28.8%) and *stx1* (32/139, 23%). Of the 139 STEC isolates, 35.3% have also the *eae* gene. Subtyping of *stx1* (72) showed that *stx1a* was the most prevalent (100%), followed by *stx1c* (88.9%) and *stx1d* (6.9%). *Stx1a* and *stx1c* subtypes occurred alone or combination with other subtypes. However, subtype *stx1d* was only found in combination with subtype *stx1a* or *stx1c*. Concerning strains with *stx2* (107), the *stx2a* subtype was the most common and was found in 84.1% of the isolates, followed by *stx2d* (76.6%), *stx2c* (74.8%), *stx2g* (23.4%), *stx2b* (4.7%), *stx2e* (3.7%) and *stx2f* (0.9%). No strains carried *stx2b* or *stx2f* alone. For isolates carrying *stx1* and *stx2* simultaneously, combinations of subtypes *stx1a*, *stx1c*, *stx2a*, *stx2c* and *stx2d* were the most common. Furthermore, in 42 isolates (30.2%) 4 or more *stx* subtypes were detected simultaneously. These results show that STEC prevalence in dairy cattle is high, and that most isolates present a diverse combination of Shiga-toxin genes. Future strategies are needed to mitigate the presence of STEC in cattle and then reduce the possible contamination of food and, thus, humans.

Keywords: Food safety, Shiga toxin, Pathogens, *E. coli*, Cattle.

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