P4.82 - DETECTING ENTEROTOXIGENIC *ESCHERICHIA COLI* IN ANIMAL PRODUCTION: METHOD DEVELOPMENT AND VALIDATION

Maria Margarida Barros [1,2], Ricardo Oliveira [1,3,4(*)], Joana Castro [1,5], Ana Campos [1], Gonçalo Nieto Almeida [1], Daniela Araújo [1], Sónia Silva [1,5,6], Divanildo Outor-Monteiro [2], Carina Almeida [1,3,4]

[1] INIAV, I.P - National Institute for Agrarian and Veterinarian Research, Portugal

[2] Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

[3] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

[4] ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

[5] Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, Braga, Portugal

[6] LABBELS –Associate Laboratory, Braga/Guimarães

[(*)] e-mail: ricardo.oliveira@iniav.pt

Keywords: ETEC; colibacillosis; toxins; fimbriae; PCR.

ABSTRACT

Swine enteric colibacillosis is a disease characterized by an intestinal infection caused by enterotoxigenic *Escherichia coli* (ETEC). This infection mostly causes illness or death in neonatal and weaned pigs making it responsible for significant economic losses. Bacterial fimbriae (F4/F5/F6/F18/F41) are responsible for the adhesion to epithelial cells, and when both the immunological systems and the gut microbiota are poorly developed, ETEC colonizes and produces one or more enterotoxins (LT/STa/STb/Stx2e) that can have local and systemic effects. Therefore, it is of prime importance to monitor and characterize ETEC in the swine industry to develop mitigation strategies.

In this study, our aim was to develop a methodology to detect ETEC and its major virulence factors (*i.e.,* toxins/fimbriae) from swine. Firstly, we optimized a qPCR methodology using ETEC control strains for the screening of ST/LT/stx2 toxins. Thus, the efficiency of primers, sensitivity, and specificity were determined. Also, the limit of detection was performed in artificially contaminated samples. This methodology is intended to be applied to the initial screening of enriched liquid cultures. Secondly, to better characterize the virulence features of ETEC isolates, we developed a multiplex PCR to specifically determine both the fimbriae (F4/F5/F6/F18/F41), and toxins (LT/Sta/STb/Stx2e). To validate our method, we collected rectal swabs from pigs. Our results showed that the qPCR approach can detect between 20-400 DNA copies/µL depending on the toxin tested and is also highly specific and sensitive. Furthermore, we also optimized the multiplex PCR technique using the ETEC control strains. Regarding its validation, our preliminary data with porcine rectal samples showed a high prevalence of the above-mentioned toxins (namely ST) as well as the F4 and F18 fimbriae.

In sum, this methodology has the potential to be adopted as a routine technique for the rapid detection of ETEC strains in livestock, since the method exhibit a robust performance.

Acknowledgements: This work was financially supported by: Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and by FCT under the scope of the strategic funding of UIDB/04469/2020 unit (CEB). The authors are also grateful to FCT for the PhD Grant SFRH/BD/2023.04664.BDANA and for the CEEC Individual (2022.06886.CEECIND).