[D&PA 42]

Detecting Enterotoxigenic *Escherichia coli* in animal production:method development and validation

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

[1]National Institute for Agrarian and Veterinarian Research (INIAV), 4485-655 Vairão, Portugal
[2]Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, 5000-801 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]Center of Biological Engineering (CEB), Campus de Gualtar, University of Minho, 4710-057 Braga, Portugal

**e-mail margarida.barros@iniav.pt*

Swine enteric colibacillosis is a disease characterized by an intestinal infection caused by the colonization of enterotoxigenic Escherichia coli (ETEC). This infection mostly causes illness or death in neonatal and weaned pigs making it responsible for significant economic losses worldwide [1,2]. Bacterial fimbriae (F4/F5/F6/F18) allow the adhesion of the bacteria to epithelial cells, and when both the immunological systems and the gut microbiota are poorly developed, ETEC colonizes and producesone or more enterotoxins (LT/STa/STb) that can have local and systemic effects [3,4]. Therefore, it is ofprime importance to monitor and characterize ETEC in the swine industry to develop mitigation strategies.

In this study, we aimed to isolate and characterize ETEC strains from faecal porcine swabs. As such, itwas developed a methodology to detect the presence of ETEC and their major virulence factors (toxins/fimbriae). This procedure was divided into two phases, firstly the collected swabs were enriched and then then screened for the presence of genetic determinants of toxins (ST/LT/stx2) by real-time PCR (qPCR). Secondly, the positive enrichments were plated in Tryptone Bile X-glucuronide (TBX) agarand incubated at 37 ºC for 24 hours. Fifty characteristic *E. coli* colonies were then extensively screened for the presence of toxins (Sta/STb/LT/stx2e) and fimbriae (F4/F5/F6/F18/F41) by multiplex-PCR.

The development of both qPCR/multiplex-PCR methods, as well as the optimization of the enrichment step, was done using ETEC controls harbouring the above-mentioned toxins/fimbriae. Nonselective andselective (with novobiocin) enrichment in TSB was performed by using ETEC inoculated faeces samples; with the 24h-selective enrichment providing higher ETEC recovery rates. Optimized qPCR conditions for toxins detection were as follow: 95 ºC for polymerase activation/denaturation, 60 ºC for annealing/extension during 40 cycles, and an internal control (pUC19 DNA) was used in each reaction.Multiplex-PCR was optimized through the conditions, 95 ºC initial denaturation and 35 cycles of 94 ºC denaturation, 60 ºC for annealing and 72 ºC for extension/final extension.

In sum, this methodology has the potential to be adopted as a routine technique for the rapid detectionof ETEC strains in livestock.

Acknowledgments: This work was financially supported by: Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) andby 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).

References:

[1] M. Rhouma, J. M. Fairbrother, F. Beaudry, and A. Letellier, “Post weaning diarrhea in pigs: Risk factors and non- colistin-based control strategies,” *Acta Veterinaria Scandinavica*, vol. 59, no. 1. BioMed Central Ltd., May 19, 2017. doi: 10.1186/s13028-017-0299-7.
[2] A. Luppi, “Swine enteric colibacillosis: Diagnosis, therapy and antimicrobial resistance,” *Porcine Health Management*, vol. 3. BioMed Central Ltd., Aug. 08, 2017. doi: 10.1186/s40813-017-0063-4.
[3] J. M. Fairbrother and É. Nadeau, “Colibacillosis,” in *Diseases of Swine*, 11th ed., J. J. Zimmerman, L. A. Karriker,
[4] A. Ramirez, K. J. Schwartz, G. W. Stevenson, and J. Zhang, Eds. John Wiley & Son, 2019, pp. 807–834.
[5] C. L. Gyles and J. M. Fairbrother, “*Escherichia coli*,” in *Pathogenesis of bacterial infections in animals*, 4th ed., C.
[6] L. Gyles, J. F. Prescott, J. G. Songer, and C. O. Thoen, Eds. Wiley-Blackwell, 2010, pp. 267–279.