**In Vitro Effect on Piglet Gut Microbiota and In Vivo Assessment of Newly Isolated Bacteriophages against F18 Enterotoxigenic *Escherichia coli* (ETEC)**

Margaux Navez 1, Céline Antoine 1,2, Fanny Laforêt 1,2, Elizabeth Goya-Jorge 2, Caroline Douny 3, Marie-Louise Scippo 3, Marjorie Vermeersch 4, Jean-Noël Duprez 1, Georges Daube 5, Jacques Mainil1, Bernard Taminiau 5, Véronique Delcenserie 2 and Damien Thiry 1,\*

1Laboratory of Bacteriology, Department of Infectious and Parasitic Diseases, FARAH and Faculty of veterinary Medicine, University of Liege, 4000 Liege, Belgium; margaux.navez@uliege.be (M.N.); fanny.laforet@uliege.be (F.L.); celine.antoine@uliege.be (C.A.); jean-noel.duprez@uliege.be (J.-N.D.); jg.mainil@uliege.be (J.M.); damien.thiry@uliege.be (D.T.)

2Laboratory of Food Quality Management, Food Science Department, FARAH and Faculty of Veterinary Medicine, University of Liège, 4000 Liège, Belgium; egoya@uliege.be (E.G.-J.); veronique.delcenserie@ulg.ac.be (V.D.)

 3 Laboratory of Food Analysis, Department of Food Sciences, FARAH and Faculty of Veterinary Medicine, University of Liège, 4000 Liège, Belgium; cdouny@uliege.be (C.D.); mlscippo@uliege.be (M.L.S.)

4Center for Microscopy and Molecular Imaging, Electron microscopy laboratory, Gosselies, ULB, 6041, Belgium; Marjorie.vermeersch@ulb.be (M.V.)

5 Laboratory of Food Microbiology, Fundamental and Applied Research for Animals & Health (FARAH), Department of Food Sciences, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium; bernard.taminiau@uliege.be (B.T.)

Pig production is impacted by the negative effects of enterotoxigenic Escherichia coli (ETEC), which might result in post-weaning diarrhea (PWD) in piglets. F4 and F18 fimbriae are utilized by ETEC strains to adhere to the small intestinal epithelial cells of the host. If antibiotic resistance becomes a problem with ETEC infections, phage therapy would be an interesting alternative. Four bacteriophages, termed vB\_EcoS\_ULIM2, vB\_EcoM\_ULIM3, vB\_EcoM\_ULIM8, and vB\_EcoM\_ULIM9 in the present study, have been isolated against an O8:F18 E. coli strain (A-I-2110) and selected based on their host range. These phages have been investigated in-vitro and shown lytic activity throughout a pH (4-10) and temperature (25-45 °C) range. These bacteriophages have been categorized as Caudoviricetes according to genomic investigation. No lysogeny-related gene have been identified. Galleria mellonella larvae in vivo model revealed the therapeutic potential of one chosen phage, vB\_EcoS\_ULIM2, with a statistically significant increase in survival compared to untreated larvae. A static model of the piglet intestinal microbial environment was infected with vB\_EcoS\_ULIM2 for 72 hours in order to evaluate the impact of this phage on the piglet gut microbiota. In a Galleria mellonella model, this study confirms the effective replication of the phage and demonstrates the safety of the phage-based therapy for the piglet microbiota.