

## Title

### **Enterotoxins of F4+ *Escherichia coli* induce IL17 in the pig small intestine and enhance colonization with F18ac+verotoxigenic *E. coli***

**Authors** E. Cox<sup>1</sup>, Y. Luo<sup>2</sup>, M. Atef Yekta<sup>3</sup>, M. Loos<sup>4</sup>, A. Coddens<sup>5</sup>, S. Arnouts<sup>6</sup>, W. Van den Broeck<sup>7</sup>, U. Lundberg<sup>8</sup>, B. Devriendt<sup>1</sup>.

<sup>1</sup> Ghent University, Lab of Immunology- Fac Vet Med, Merelbeke, Belgium.

<sup>2</sup> Jinhua Polytechnic, Jinhua, China.

<sup>3</sup> Sanofi Pasteur, Toronto, Canada.

<sup>4</sup> Delaval NV, Drongen, Belgium.

<sup>5</sup> Ablynx NV, Zwijnaarde, Belgium.

<sup>6</sup> Provaxs, Ghent University, Merelbeke, Belgium.

<sup>7</sup> Ghent University, Morphology, Merelbeke, Belgium.

<sup>8</sup> Valneva Austria GmbH, Vienna, Austria.

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## **Abstract**

Enterotoxigenic *E. coli* (ETEC) play a major role in post-weaning diarrhoea in pigs. Upon weaning, F4+ETEC rapidly colonize the small intestine and produce LT, STa and/or STb enterotoxins which induce diarrhoea. Colonisation with F18+verotoxigenic *E. coli* (VTEC) is more variable. We examined if the LT enterotoxin might change the small intestinal barrier in such a way that it might enhance colonization with F18+VTEC. Furthermore, we studied the effect of enterotoxins on IL-17A, a cytokine that has been implicated in protection of the host against bacteria.

In first experiments we analysed the effect of different concentrations of LT on fluid secretion and mucus coverage in 20 cm long small intestinal segments (3 pigs/group). A dose of 10 µg LT induced fluid secretion and decreased the mucus coverage, comparable to this of an infection with 10<sup>8</sup> CFU F4+ETEC. Since the length of the small intestine of newly weaned pigs is approximately 8 meter, 40 times higher LT doses were administered in vivo (n=11), whereafter pigs were inoculated with F18ac+VTEC. LT administration significantly increased colonization with F18+VTEC in comparison with pigs receiving PBS (n=7) indicating that infection with an F4+ETEC strain can increase susceptibility to infection with F18+VTEC.

In a second series of experiments, the effect of different enterotoxins was analysed on IL-17A using among others mutant strains expressing one or more enterotoxins. A strong IL-17A expression was induced by STb. Surprisingly IL-17A was first seen in enterocytes, followed by goblet cells. Blocking experiments showed that this STb-mediated epithelial IL-17A controls the expression of genes involved in maintaining the epithelial barrier, such as β-defensin-2, mucins and pIgR. On top of this epithelial response, ETEC infection induced an influx of CD3 IL17A cells in the intestinal lamina propria. Our findings unravel a role of IL-17A in innate immunity against ETEC infection and show this might be a therapeutic target.