Mammal intestinal organoids for studying zoonotic pathogens.

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Intestinal organoids are self-organized three dimensional (3D) structures composed of a layer of polarized intestinal epithelial cells surrounding a hollow lumen. They recapitulate *in vitro* the intestinal multicelular composition, architecture and physiology.

The aim of this work was to set up organoid models for studying zoonotic pathogens such as *Salmonella* and *Toxoplasma gondii*.

T. gondii's sexual cycle is restricted to felid's intestines, which are characterized by an excess of linoleic acid given by the lack of delta-6-desaturase activity. "Felinized" murine intestinal organoids were generated for triggering *T. gondii*'s sexual differentiation *in vitro*. For this purpose, murine intestinal organoids from C57BL/6 mice were established from crypt isolated intestinal stem cells (2D or 3D) and incubated in the presence of 20 µM delta-6-desaturase inhibitor and 200 µM linoleic acid. Under these conditions no cytotoxicity of felinizing compounds was observed until 5 days of incubation. To optimize *T. gondii's* infection, intestinal organoids were incubated with tachyzoites (at three distinct multiplicities of infection, MOIs) and evaluated by immunofluorescence assays (IFAs) at three time points post-infection.

In order to set up a *Salmonella* infection model, intestinal organoids from farm animals (cow and sheep) were established and characterized by light microscopy and RT-PCR of specific markers. Forward steps will involve bovine intestinal organoids exposure to *Salmonella enterica* reporter strains at different MOIs, and bacteria invasion/proliferation evaluation at two time points after infection by extra and intracellular bacteria quantification and IFAs.

Our results highlight the versatile uses of intestinal organoids as a powerful in *vitro* tool for modeling zoonotic diseases, contributing to the principle of reducing the use of experimental animal models.