Development of a primary cell model derived from porcine dorsal soft palate for foot-and-mouth disease virus research and diagnosis

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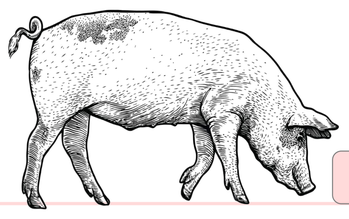
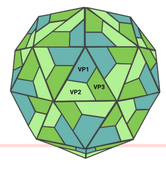
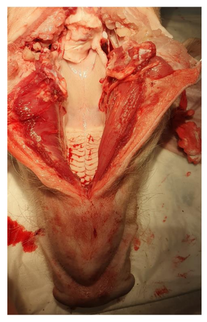


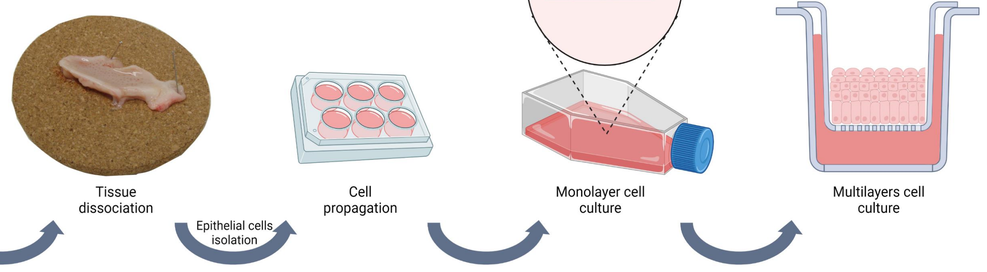


INTRODUCTION & OBJECTIVES

- **Foot-and-mouth disease** is a highly contagious disease affecting wild and domestic artiodactyls, caused by a Picornavirus named Foot-and-mouth disease virus (FMDV).
- **FMDV** is able to **persist (i.e. infectious virus detected after 28 days post-infection (dpi))** for several years in the pharyngeal epithelium of ruminants after resolution of clinical signs[1]. However, this is not the case in **pigs** where only **viral RNA** could be detected in vivo for **more than 60 dpi** in the **oropharyngeal tonsils** and **dorsal soft palate**[2,3]**.**
- **No porcine cell model from a tissue of interest** is currently available.

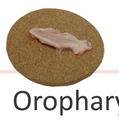
➢ **Aim of the study: To develop a specific cellular model to study FMDV infection in porcine cells.**





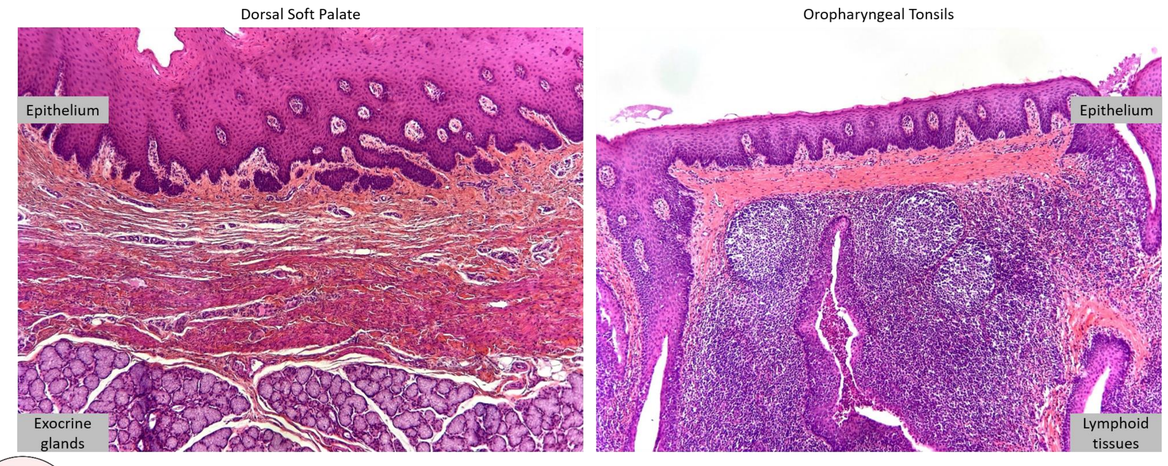
Workflow

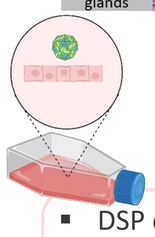
- Swine oropharyngeal tonsils and dorsal soft palate (DSP) were sampled.
- Epithelial structures were identified and isolated ①.
- Epithelial cells derived from DSP were propagated in monolayers, caracterized ②, then infected with FMDV ③.
- Epithelial cells were then grown in multilayers at the air-liquid interphase to mimic a pluristratified epithelium ④.



① Epithelial structures caracterization

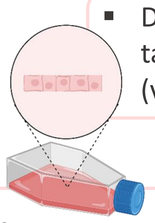
- Oropharyngeal tonsils and DSP samples were stained using HES (hematoxylin-eosin-saffron) in order to localize epithelial structures.
- Epithelium were identified in both oropharyngeal tonsils and DSP samples



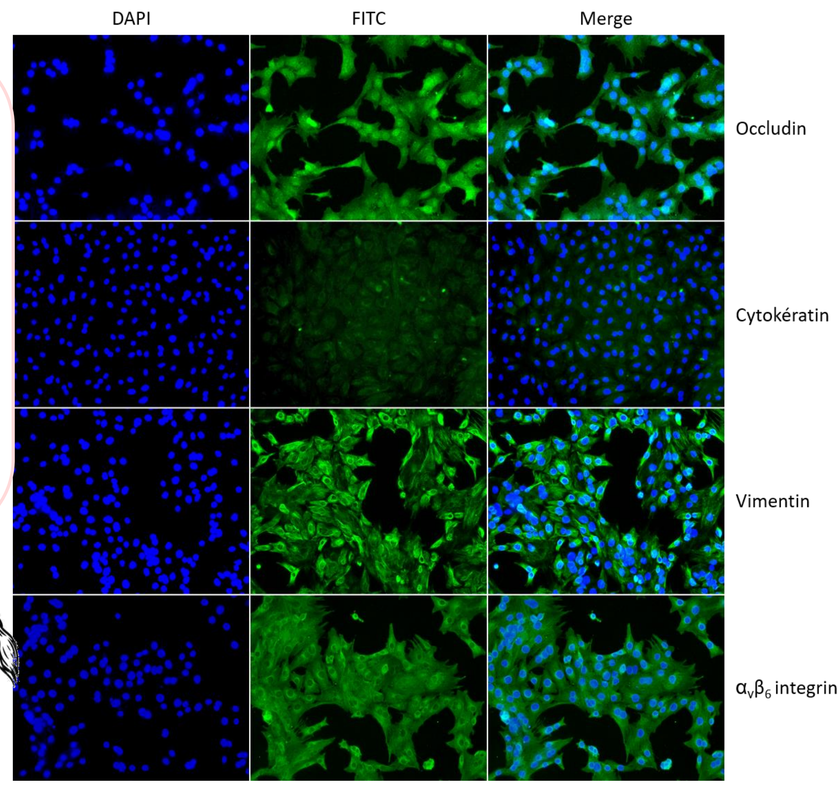


② DSP cells caracterization

- DSP cells were cultured in monolayers, fixed, then labelled with antibodies targeting epithelial markers (occludin, cytokeratin), fibroblastic markers (vimentin) and FMDV receptor ($\alpha_v\beta_6$ integrin).

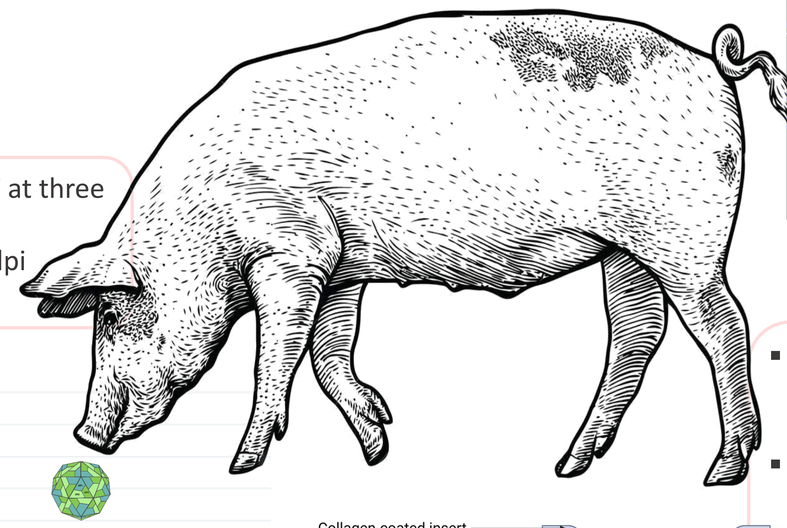


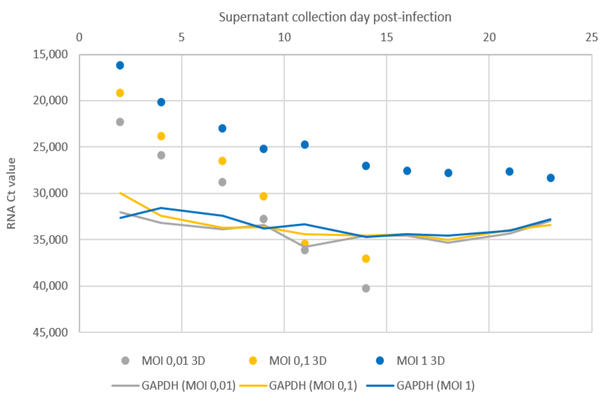
- Significant expression of occludin was detected, indicating an epithelial nature.
- Greater expression of vimentin than cytokeratin was detected, indicating an epithelial-mesenchymal transition.
- High expression of the major receptor for FMDV was detected.

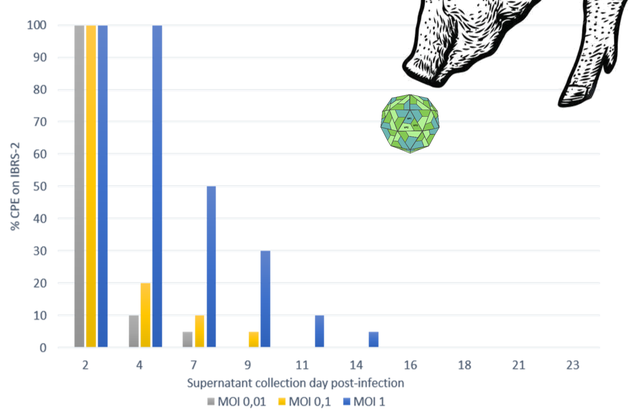


③ Infection of DSP monolayers

- DSP cells were grown in monolayers and infected with a type-O FMDV at three multiplicity of infection (MOI 0.01; 0.1; 1).
- Culture supernatants were collected every two or three days for 112 dpi and analyzed for infectious virus, antigens and viral RNA.



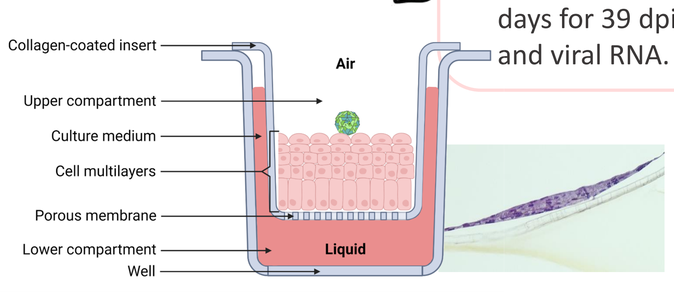


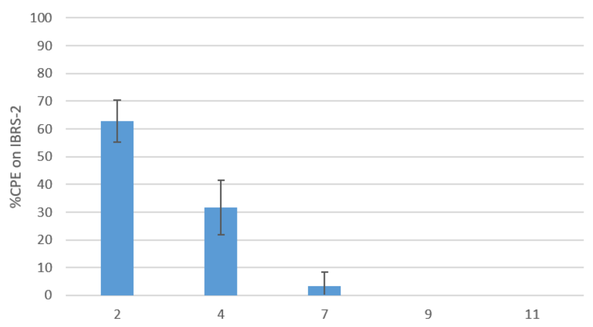


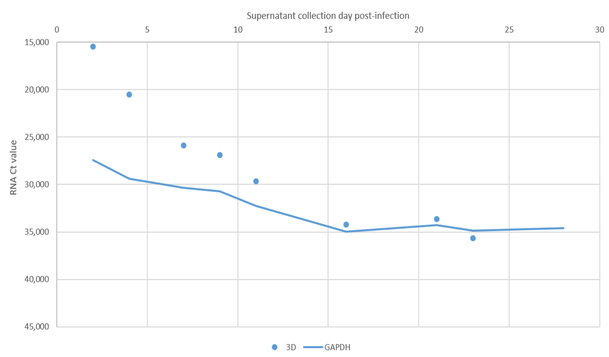
- MOI 1 was considered as the optimal infection condition for porcine DSP cells.
- Infectious virus were detected by viral isolation up to 14 dpi in culture supernatants.
- Viral antigen were detected up to 16 dpi by immunofluorescence.
- Viral RNA were detected up to 60 dpi by RTqPCR..

④ Infection of DSP multilayers

- DSP cells were grown in multilayers, on collagen membrane, at the air-liquid interface and then infected with a type-O FMDV at MOI 1.
- Culture supernatants were collected every two or three days for 39 dpi and analyzed for infectious virus, antigen and viral RNA.







- Multilayer depth was determined using HES staining.
- Infectious virus were detected by virus isolation up to 7 dpi in culture supernatants.
- Viral antigen were detected up to 9 dpi by immunofluorescence.
- Viral RNA were detected up to 23 dpi by RTqPCR.

Conclusion & perspectives

- We have succeeded in developing a primary porcine dorsal soft palate cell model susceptible to FMDV infection.
- A similar model based on oropharyngeal tonsil cells, could not be developed since these cells did not maintain in culture.
- Consistent with what has been observed in vivo in pigs, no evidence of FMDV persistence could be detected in our cell model (no infectious virus detected after 28 dpi).

➢ Porcine DSP cells could be immortalized to obtain a cell line suitable for FMDV research and diagnosis.
➢ These cells could constitute a privileged tool in the differential diagnosis of FMDV, if susceptible to the other viruses.

References

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- 3. Stenfeldt C, Pacheco JM, Smoliga GR, Bishop E, Pauszek SJ, Hartwig EJ, et al. Detection of Foot-and-mouth Disease Virus RNA and Capsid Protein in Lymphoid Tissues of Convalescent Pigs Does Not Indicate Existence of a Carrier State. Transboundary and Emerging Diseases. 2016;63(2):152-64.

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