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FLI
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Federal Research Institute for Animal Health

Gene signatures associated with foot-and-mouth disease virus infection and persistence

Part I: Persistent FMDV infection in a three-dimensional model of the bovine soft palate

Freiburg, August 24th 2018

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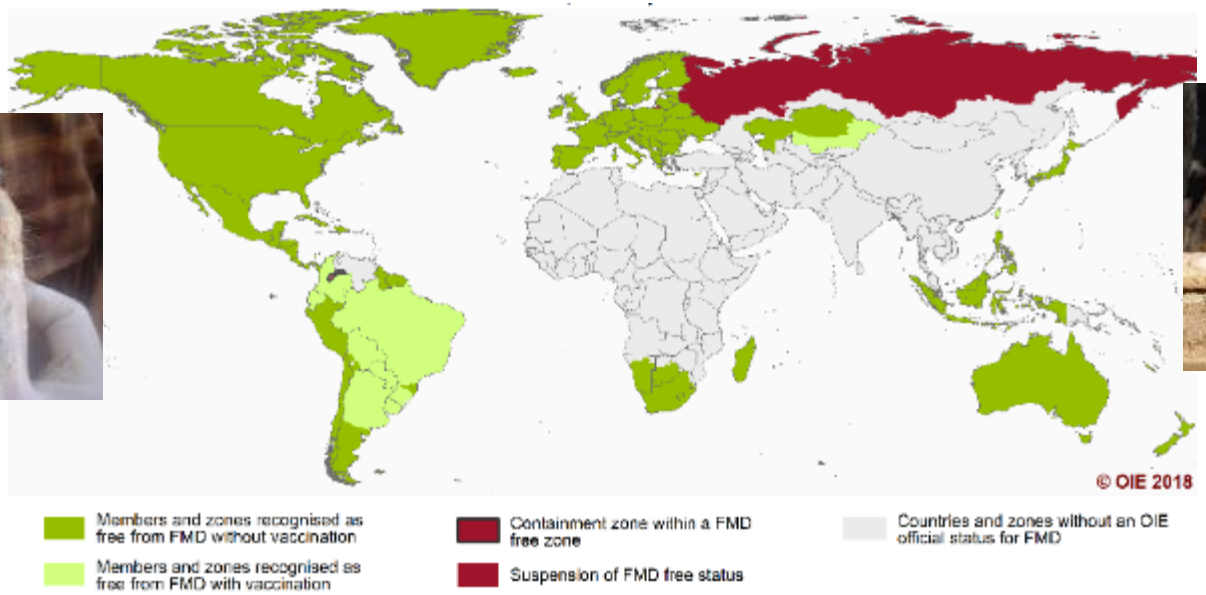
[*] Contributed equally

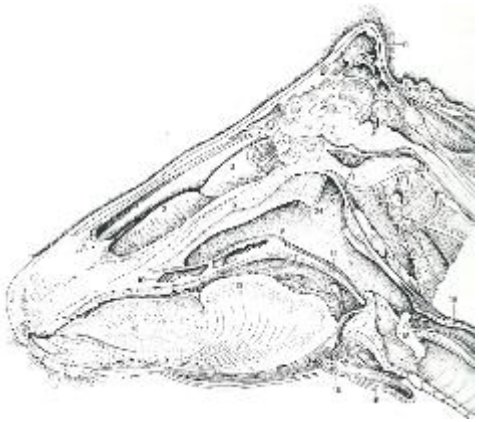
Foot-and-mouth disease

- Countries classified with and without official status:
  - free of FMD with and without vaccination
- Persistence in bovine oropharynx slows down recovery to FMDV-free status
  - prevent access to the most profitable trade

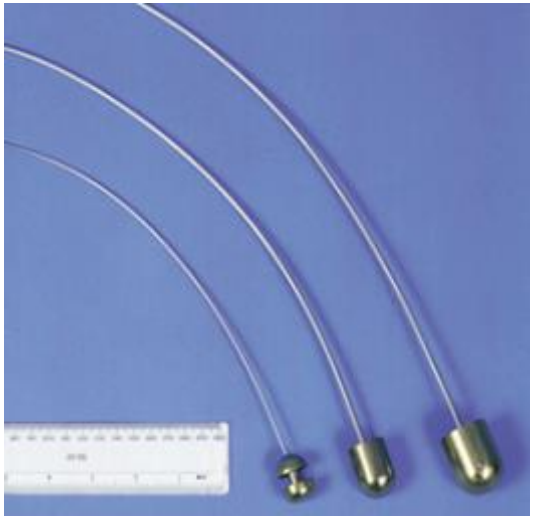


Foot-and-mouth disease virus

- Positive-sensed, single stranded RNA virus
- Genus *Aphtovirus*, family *Picornaviridae*
- 30 nm sized naked capsids with high antigenic variation
  - seven serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia 1)
    - multiple subtypes and lineages
    - animals that harbor FMDV after day 28 are defined as carriers

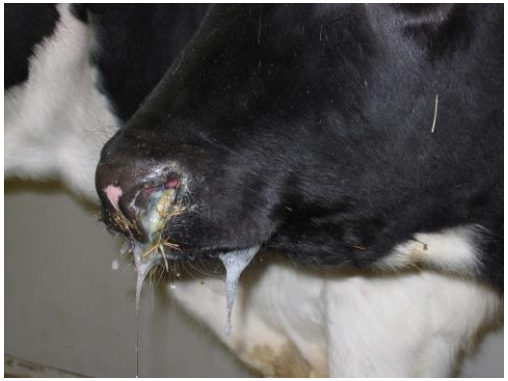


Dyce 1993



Foot-and-mouth disease virus

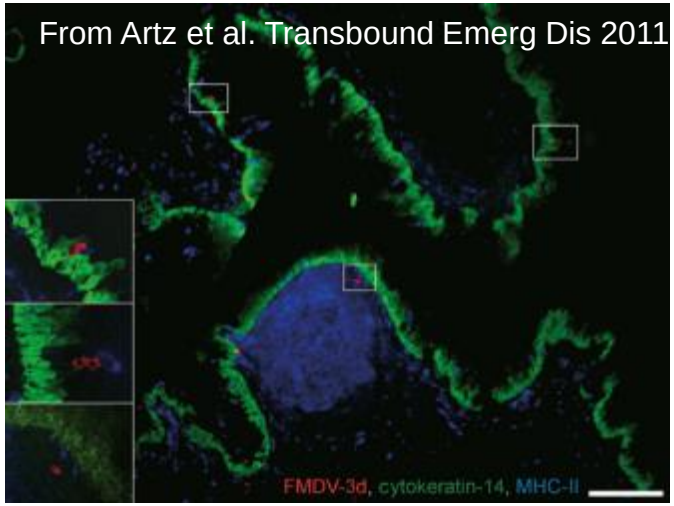
- Persistent virus in up to 50% of infected cattle
  - replicates in follicle-associated epithelium in the nasopharynx and soft palate
    (Alexandersen et al., 2002; Arzt et al., 2011; Stenfeldt et al., 2016)
    - cytokeratin-negative/ weakly positive cells in basal cell layers
      (Arzt et al., 2011; Pacheco et al., 2015)
    - highly cytokeratin-positive cells in the upper layers
      (Stenfeldt et al., 2016)
  - inactive form stored in the germinal centers of nasopharyngeal lymph nodes
    (Juleff et al., 2008; Maree et al., 2016)



Foot-and-mouth disease virus

- Previous studies:
  - animals
  - monolayers of pharyngeal cells
  - cell lines (BHK-1, IBRS-2 and MDBK)

    - viral suppression of host immune responses
      (Pacheco et al., 2015; Stenfeldt et al., 2016; Stenfeldt et al., 2017)
    - adaptation of host cells to the virus
      (de la Torre et al., 1988; Martin Hernandez et al., 1994)
    - mutations in the viral genome leading to functional changes
      - immune escape
        (Gebauer et al., 1988)
      - change in the use of receptors
        (O'Donnell et al., 2014)

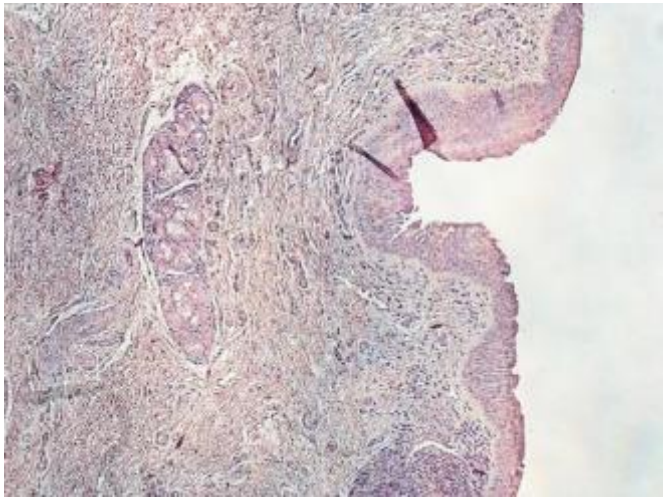


Aim

- Establish model of persistent infection in bovine soft palate multilayer cells at the air interface

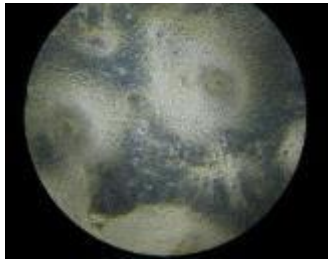
  - future studies of virus-host interactions:
    - viral changes/clearance in absence of selective pressure from the immune system
    - information on the pathways modulated by the virus in soft palate cells
    - screening of molecules to detect or interfere in persistence

- spare experimental animals

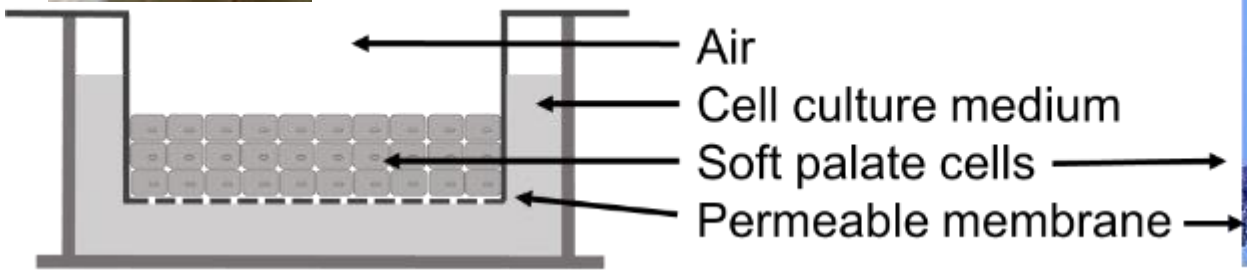


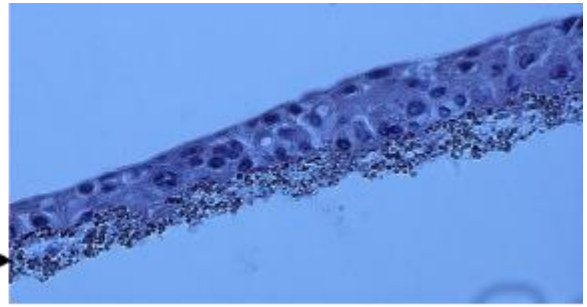
Establishement of the model

- Epithelial tissue from dorsal soft plate collected after commercial slaughter:
  - protease digestion and filtration
  - removal of unwanted cells by adherence
  - seeding in collagen-coated membrane inserts with 3.0 µm pores
  - growth with hepatocyte-growth factor (mitogenic to keratinocytes/ inhibits fibrosis)
  - upper compartment dried when cells were confluent
  - washed upper cell layer/ changed culture medium every 2-3 days



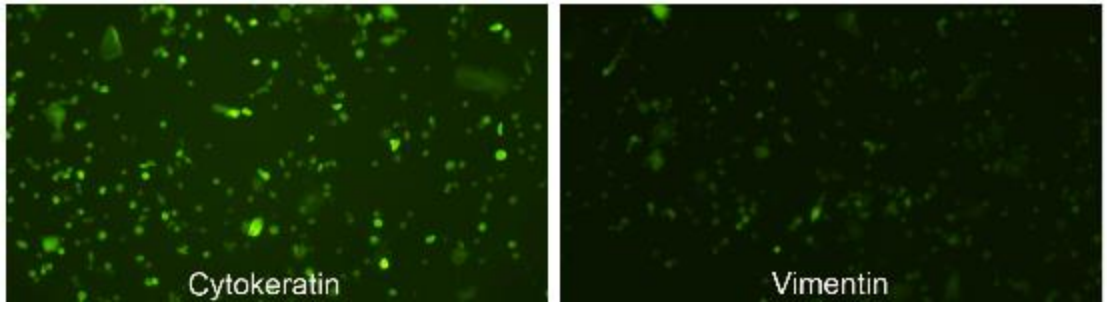
- Possibility to keep at least 3 months without passage



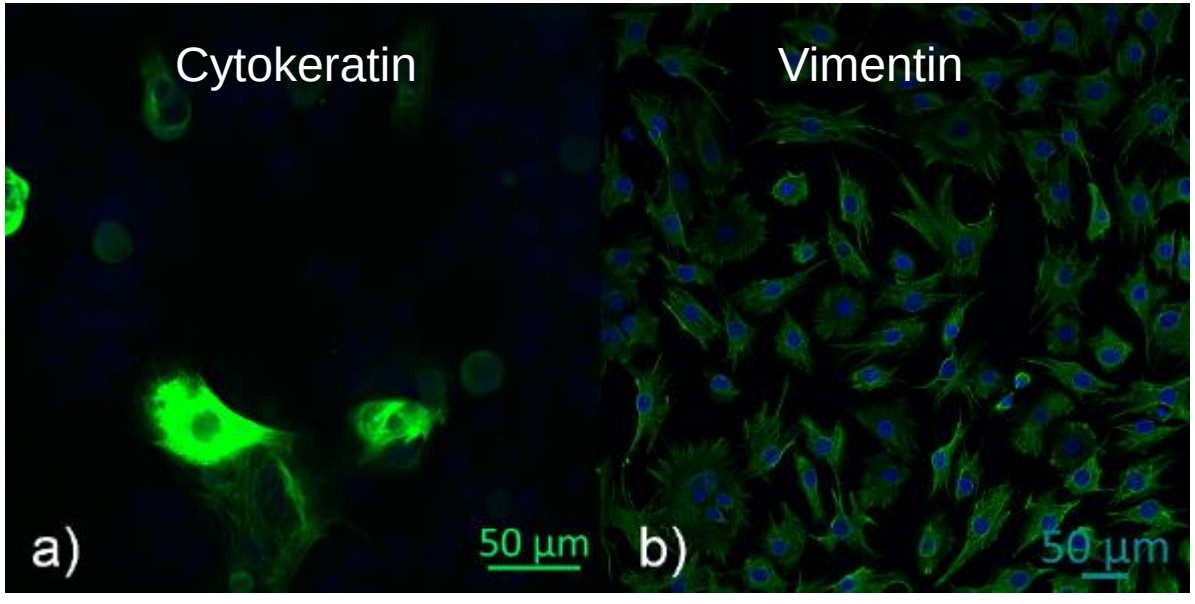


Cell characterisation

- Before passage: expression of vimentin intermediate filaments less extensive than cytokeratin



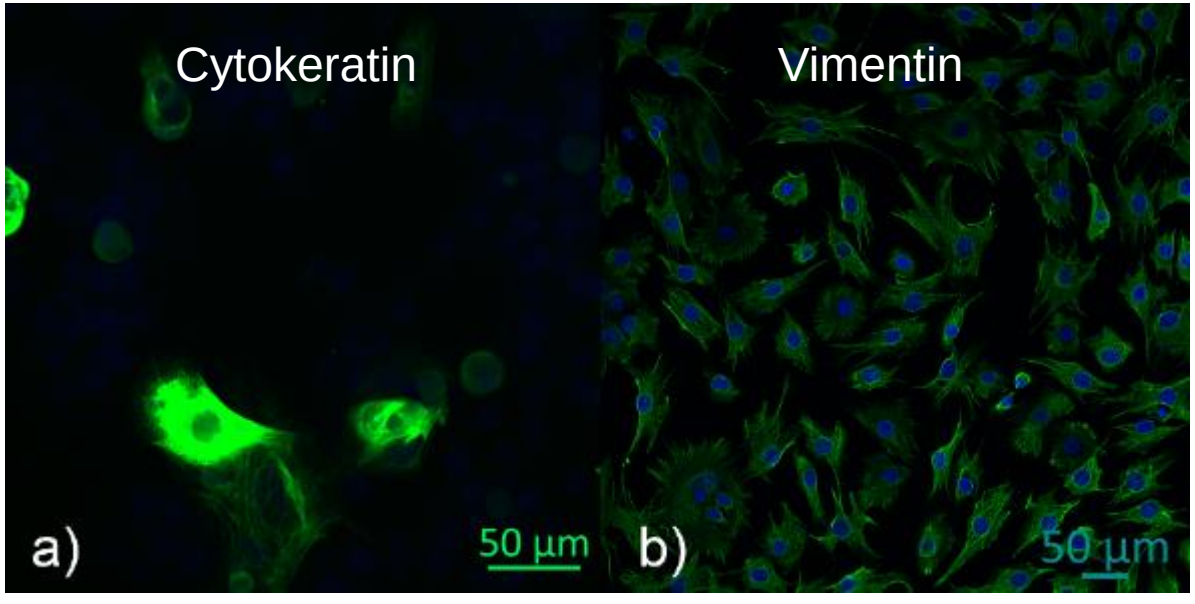
- After five passages: larger number of cells expressed vimentin. Cells were mostly polygonal, round or flat



Cell characterisation

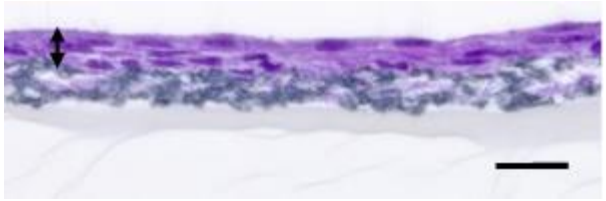
- Vimentin: fibroblast marker induced in cultured epithelial cells
(Pieper et al., 1992).
- Vimentin/cytokeratin: differential/simultaneous expression in epithelial cells
(Rogel et al., 2011; Kasper and Stosiek, 1990; Mendez et al., 2010; Eriksson et al., 2009).

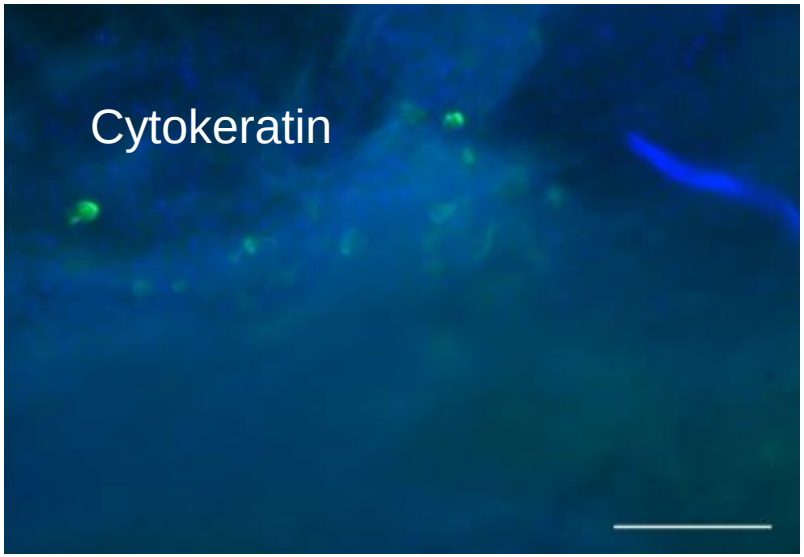
  - ➢ epithelial to mesenchymal transition (reversible process)
  (Rogel et al., 2011; Mendez et al., 2010)

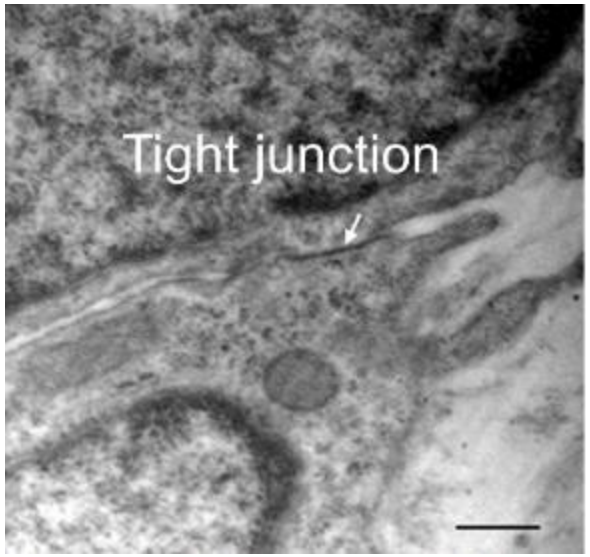


Cell characterisation

- Multilayers after five weeks, before infection
  - Staining from top of membrane:
    - subsets of cells expressed cytokeratin
    - most or all cells were vimentin positive
  - EM of cross-section:
    - ~20 % of cells: polygonal morphology and tight junctions
  - Impermeable to cell culture medium

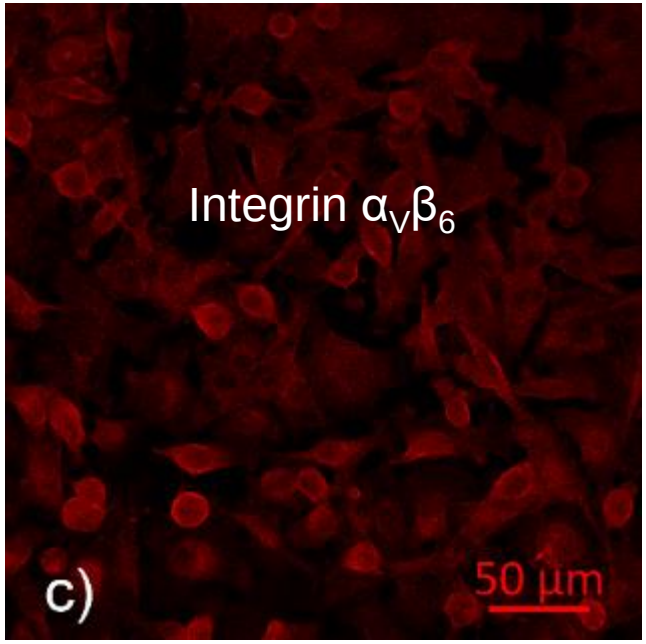






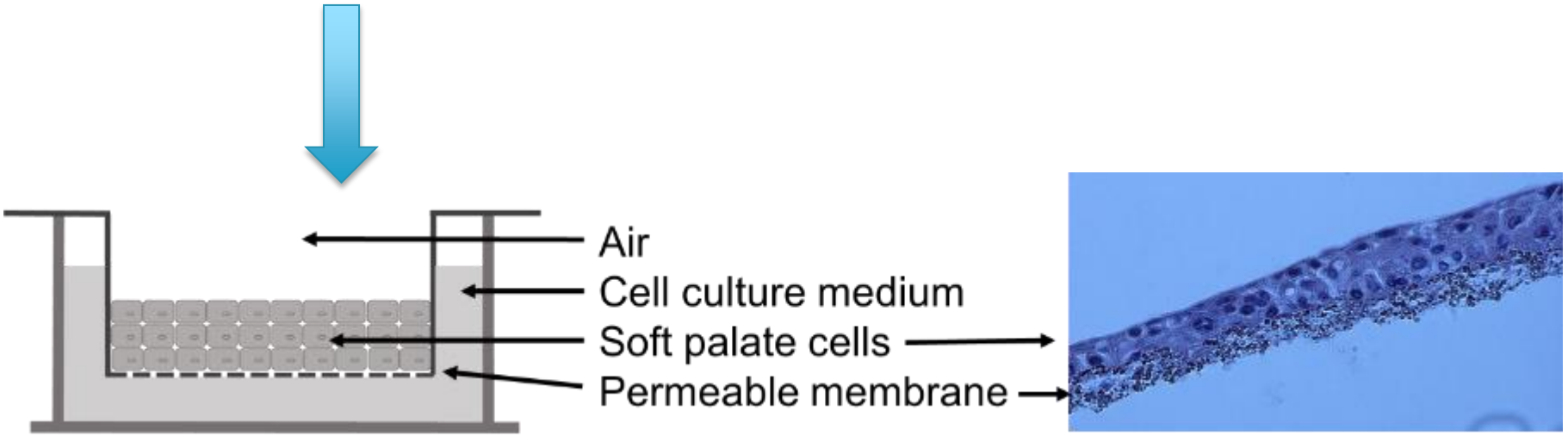
Cell characterisation

- Expressed integrin $\alpha_V\beta_6$ to varying extent (a FMDV receptor) in monolayers but not in multilayers

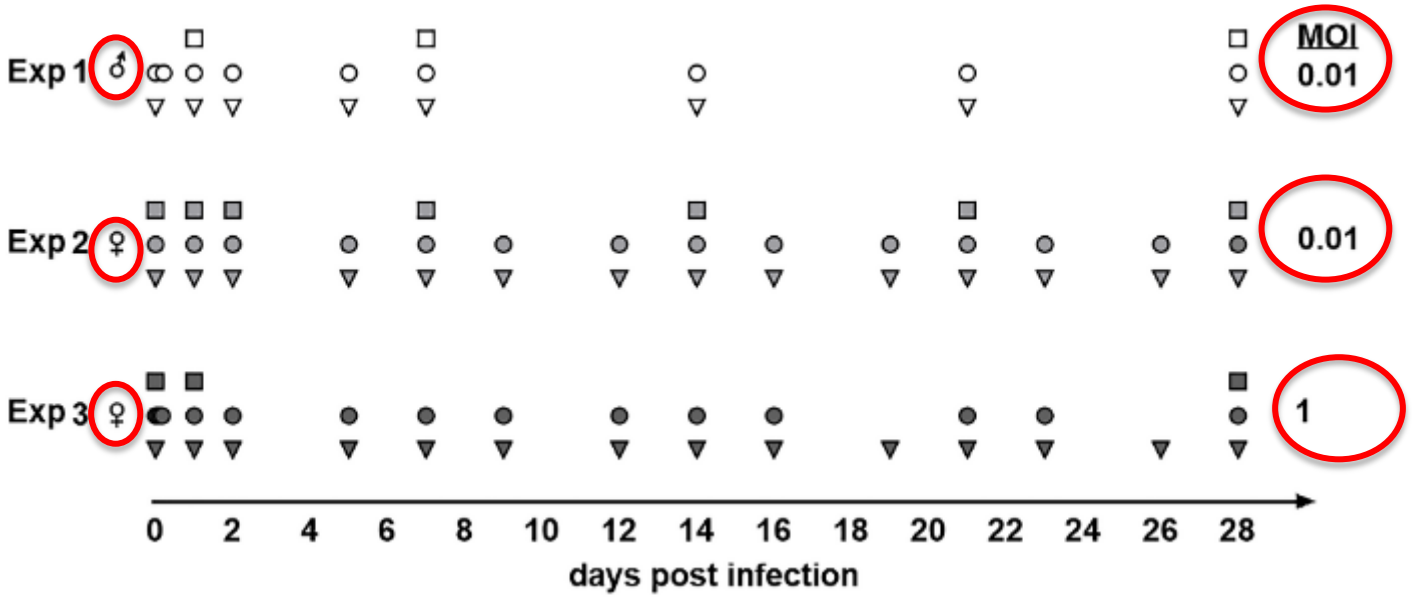


Persistent FMDV in SP cells

- Infection after 5 weeks of culture in multilayers, without further passage
  - viral clone derived from the FMDV O/FRA/1/2001 strain
  - wash of upper cell layer and change of culture medium every 2-3 days



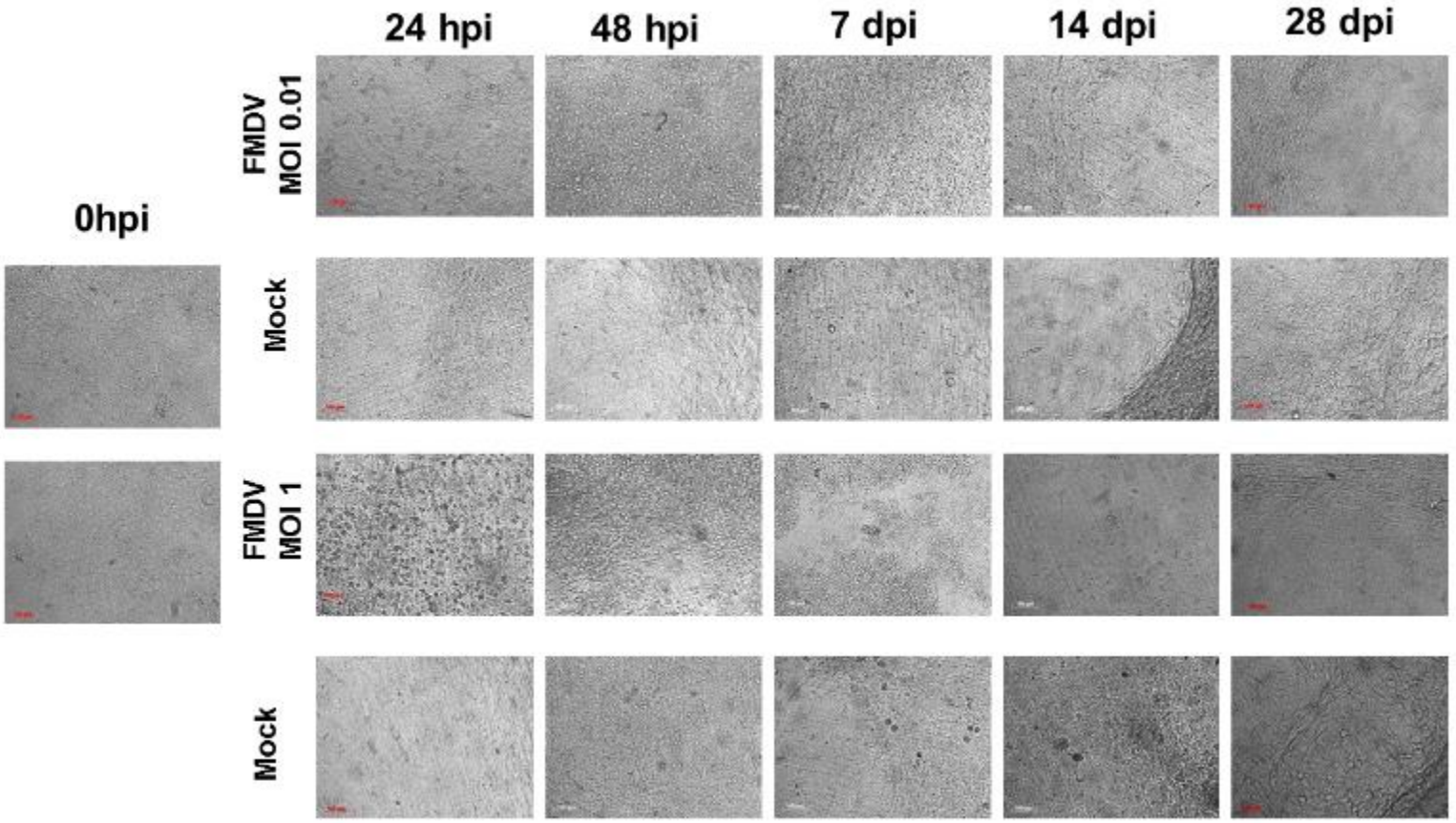
Persistent FMDV in SP cells



- Immunohistochemistry: made on cross sections of membranes
- RT-qPCR: } made on washes of cell surfaces
- virus isolation: } with cell culture medium

Persistent FMDV in SP cells

- Limited CPE in upper cell layers (peak at 24-48 hours)
- Recovery



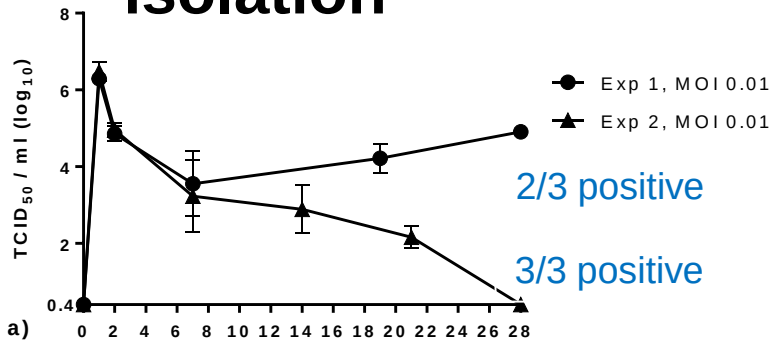
Persistent FMDV in SP cells

- 24-48 hours; peak of FMDV RNA and live virus
- day 28: FMDV isolated from 6 out of 8 cultures (undiluted)

isolation

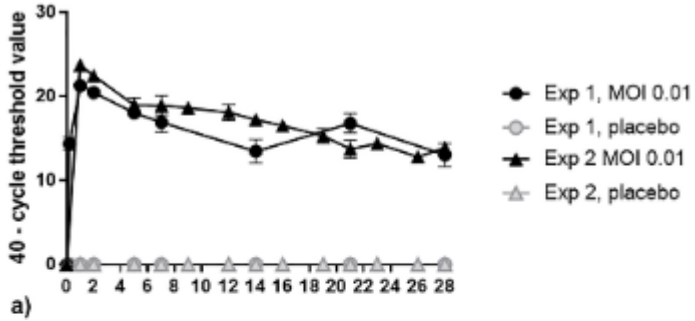
RT-qPCR

MOI 0.01

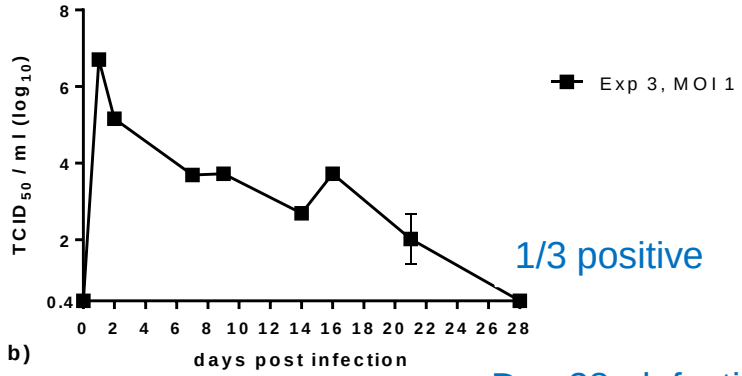


2/3 positive

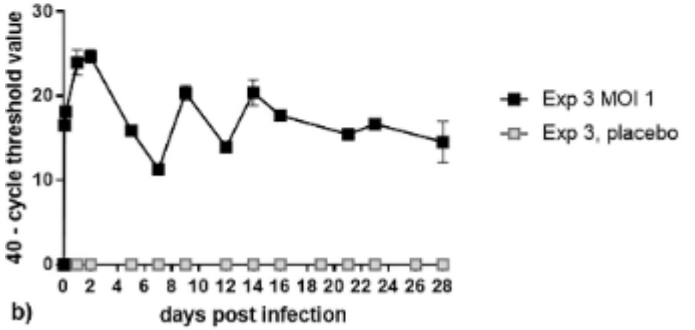
3/3 positive



MOI 1



1/3 positive



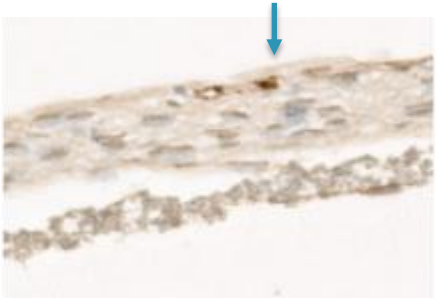
Day 28: defective virus particles?
inhibition of live virus, by innate immune molecules?

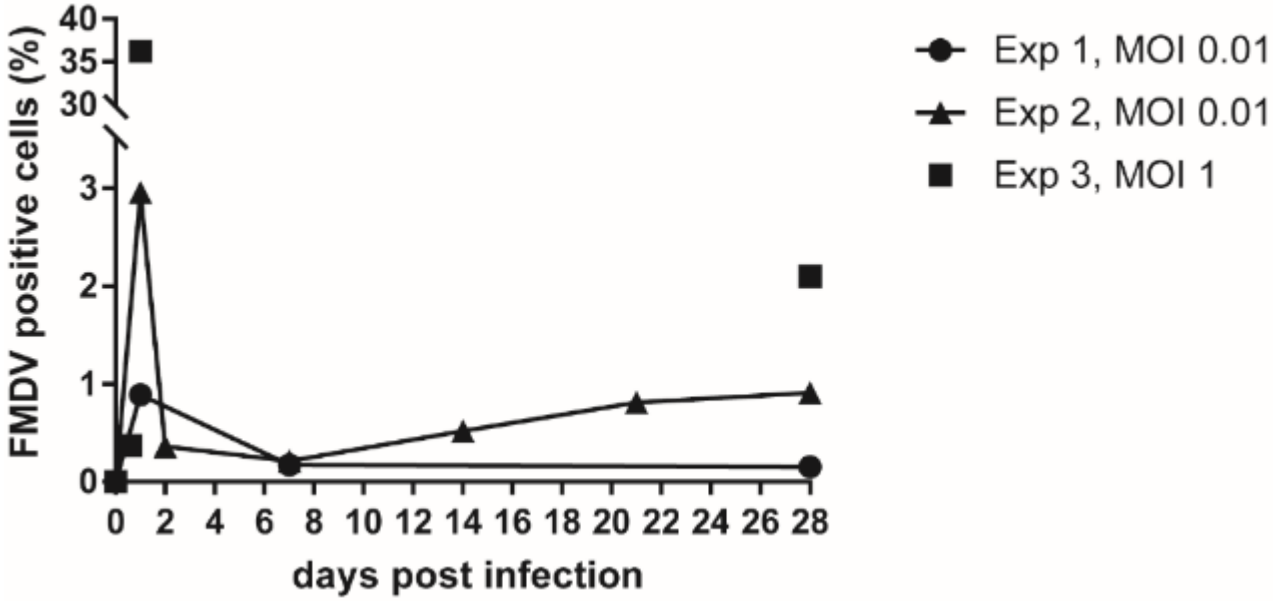
Persistent FMDV in SP cells

- The proportion of infected cells was highest at 24 hours
  - 3 % of cells at an MOI of 0.01
  - 36 % of cells at an MOI of 1

- At day 28, FMDV antigen was detected in 0.2% - 2.1% of cells, in all layers

Persistent FMDV





Conclusion

- Development of a model of FMDV persistence in multilayer of bovine soft palate cells grown at the air interface
    - mimicking what is observed *in vivo*
- Virus was recovered without visible CPE, 28 days post infection in 0.2-2% of cells. More work is required to characterise these cells.
- Possibility to study mechanisms underlying persistence
    - develop ways to control and diagnose persistent FMDV

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Animal Health and Welfare ERA-Net