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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 Foot-and-mouth disease (FMD) is a contagious and devastating animal disease affecting artiodactyls. The persistence of Foot-and-mouth disease virus (FMDV) in ruminants after clinical recovery constitutes an important issue. It is defined as the presence of infectious virus in the host beyond 28 days post-infection (dpi). This persistence has been evidenced in cattle and small ruminants but not in swine, which are described as unlikely to be competent long-term carriers of infectious FMDV. Persistent FMDV has been found in dorsal soft palate (DSP) epithelial cells in ruminants. While the DSP is one of the primary sites of FMDV replication in both ruminants and swine, no persistent infectious virus was found in swine DSP. Only viral RNA could be detected in vivo over 60 dpi.
The lack of suitable in vitro models is an obstacle to knowledge progress regarding FMDV and especially FMDV persistence. Although we have developed a bovine DSP cells model adapted to FMDV study, no model derived from porcine tissues of interest are currently available. Indeed, the porcine cells used for FMDV research and diagnosis are essentially kidney cells such as IBRS-2, PK15 and SK-6.
Methods Primary cells were isolated from DSP samples collected from 4-6 month old swine. These cells were then processed to maintain and isolate the epithelial cells. Both the presence of epithelial markers in these cells, such as occludin, and the presence of FMDV-specific integrin receptors were verified by immunofluorescence. Molecular analyses were performed to check that the cells were free of three viruses commonly found in swine. Once characterised, the cells were infected in monolayers and in multilayers at the air-liquid interface (ALI) with a type-O FMDV. Samples were regularly collected up to 115 dpi and were tested for the presence of infectious virus, viral RNA and viral antigen.
Results We confirmed that these cells were epithelial, possessed FMDV-specific receptors and were free of some common porcine viruses, namely Porcine Reproductive and Respiratory Syndrome Virus and Porcine Circovirus 2 and 3. Experimental infection revealed that they were less sensitive to FMDV infection than the bovine DSP model we previously established. Analysis of collected supernatants identified infectious virus up to 14 dpi, as well as viral RNA up to 60 dpi, consistent with in vivo observations. Multilayer (ALI) cell culture mimicked a multi-layered epithelium more similar to the biological system. Although viral RNA were detected up to 35 dpi, no infectious virus were detected in the supernatants after infection of the multilayers.
Conclusion & Perspectives Consistent with what was observed in vivo, no evidence of FMDV persistence was observed in this cell model as no infectious viruses could be detected after 28 dpi. The relevance of this model having been confirmed by the results obtained, it would be considered to immortalise these cells in view of developing a line of FMDV-sensitive porcine epithelial cells that could be used for research. Moreover, this model might be considered as a potential tool for FMDV-differential diagnosis, including Seneca Valley virus and Swine Vesicular Disease virus.