Tissues, Pathology, and Diagnostic Microscopy

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Porcine adenovirus detected in urothelial cell culture isolated from porcine urinary bladder

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Cell cultures are an important research tool frequently used in life science today. Advances in cell cultivation and development of various *in vitro* models have found a number of applications in studying tissue development and function in health and disease. However, to provide reliable results, cell cultures must be free of pathogens. Contamination with bacteria and fungi usually causes visible effects on cell cultures, in contrast, mycoplasmas and viruses are difficult to detect visually and usually require special detection methods. Therefore, when establishing cell cultures from animal tissue, special caution should be taken as the animal tissue could be a potential source of contamination with pathogens.

For harvesting primary and subsequent urothelial cell (UC) cultures porcine urinary bladders were cut in large segments and UCs were gently scraped from urothelium, seeded onto plastic tissue flasks and grown in UroM medium [1]. After 3 weeks in culture, UC cultures were prepared for transmission electron microscopy [2]. Unexpectedly, in ultrathin sections of embedded cells, large paracrystalline structures in nuclei were found. Virus particles were also seen in the cytoplasm of infected cells ″Figure 1.".

For negative staining UC cultures were frozen, thawed three times and clarified by centrifugation at 1000 x *g*. Clarified supernatant was ultracentrifuged at 100 000 x *g* for 1h. Resulting pellet was resuspended in saline solution, placed onto formvar coated grids and negatively stained using phosphotungstic acid. The grids were examined with transmission electron microscope at 80 kV. From characteristic icosahedral shape morphology and size measurements between 70-90 nm, adenoviruses were identified ″Figure 2.".

Molecular diagnostic tests to detect human adenovirus as possible agent of UC contamination were performed and were negative. In addition, broader reaction primers were used to confirm adenovirus infection of UC cultures. Adenovirus was detected using PCR amplification of adenovirus hexon gene as previously described [3]. The presence of *Mastadenovirus* was confirmed by sequence analysis of hexon gene showing the highest similarity to recently described novel porcine adenovirus genotype (strain PAdV-W1) [3].

Since human tissue is usually difficult to obtain, majority of research is still done using cell cultures of animal origin. However, when establishing primary and subsequent UC cultures from animal tissue, the microbiological screening of urinary bladder tissues and UC cultures for potential pathogens is highly recommended.

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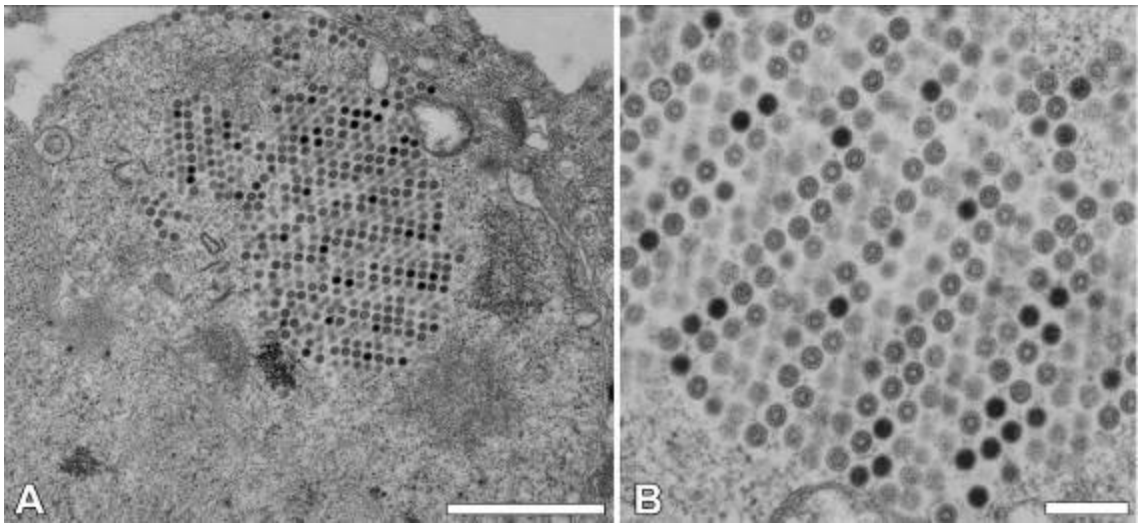

Figure 1. Transmission electron micrographs of porcine adenovirus in urothelial cell culture isolated from porcine urinary bladder. Epon resins. Scale bars, 1 µm (A) in 200 nm (B).

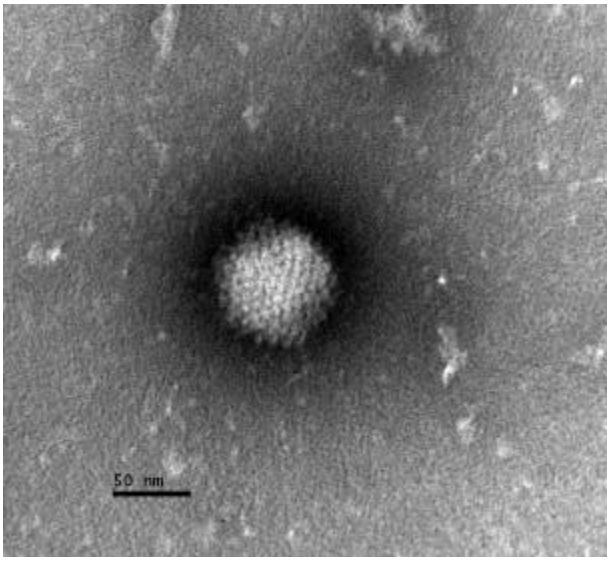

Figure 2. Transmission electron micrograph of negative stained adenovirus from porcine urothelial cell culture suspension. Scale bar 50 nm.