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Alternative cells for isolation, detection and propagation of capripox viruses

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Introduction: Capri pox viruses are the causative agents of serious animal diseases in cattle, sheep and goats. They are categorizes as notifiable due to their potential for rapid spread and substantial socio-economic impact. Vaccination is the most effective way to control the spread of CaPVs. Only live attenuated vaccines are currently used. Currently, primary lamb kidney or primary lamb testis cells are the most commonly used for CaPVs proliferation and vaccine production. However, primary cells preparation is a heavy and complicated process. Several authors tried to adapt CaPVs on continuous cell lines such as Vero, MDBK, OA3. Ts cells. Nevertheless, they have low viral titer. The validation of suitable continuous cell line for Capri poxvirus is based on their use for virus isolation, propagation and replication and their application for immunostaining methods for infection confirmation.

Objective: Our work focuses on the comparison of the sensitivity of 4 primary cells of different tissues (heart, skin, kidney and testis) with Vero, OA3.Ts and ESH-L cell lines to three capripoxviruses LSDV, SPPV and GTPV.

Cells and viruses: Primary lamb testis cells LT were obtained by castration of a healthy three-month-old male. Heart, skin and kidney primary cells were prepared from three-month-old fetus lamb tissues. Vero cells and OA3. Ts cells were provided by ATCC. ESH-L cells were obtained from FLI. Fifteen PCR positive field samples representing inflammatory tissues, nodules and crusts taken in the acute phase of CaPVs diseases were used for viral detection. Three Capripox viruses, attenuated strains, were used for growth kinetic in this study: LSDV, SPPV, and GTPV.

Virus isolation and cell sensitivity evaluation: Specimen were diluted in PBS, centrifuged for 20 min and the supernatant was retained for inoculation on confluent cells. After 45 min of adsorption, inoculated cells were incubated and observed daily for CPE presence. The Evaluation of cell sensitivity was based on virus titration and PCR. The infectivity titer of the Capripox viruses' suspension was determined using three methods: the TCID50 titration method based on direct CPE observation the immunoperoxidase monolayer assay (IPMA) and immunofluorescence assay (IFA)

Cell growth: Primary embryonic and testis lamb cells were seeded with 40 000 cell/cm2 , which allow to obtain confluent cells after 3–4 days with a cell density of 180 000 cell/cm2 for embryonic heart, 160 000 cell/ cm2 for embryonic skin, 120 000 cell/cm2 for testis, and 100 000 cell/ cm2 .

Virus isolation: Samples were tested using LT cells and ESH-L cells, CPE was observed on 15 samples passed on LT and 13 samples passed on ESH-L cells.

Virus propagation and kinetic: All CaPVs developed a CPE starting the second day of incubation and picking between the 3 and 5 days. For the three viruses, the highest titer is obtained on Heart primary cells, followed by ESH-L cells with no significant difference between LSDV and SPPV. Virus titration in ESH-L cells and in the supernatant showed high amount of intracellular virus comparatively to extracellular virus.

Discussion: ESH-L cells were more specific than primary testis cells in virus isolation. In addition, the use of ESH-L cells may increase virus isolation efficacy when dealing with field samples. Among primary cells, heart origin cells were the most sensitive to the virus replication, followed by LT cells. Among the three cell lines tested, ESH-L were able to generate the highest level of virus.

The anti-serum immunostaining methods allowed easier and earlier visualization of the viral effect. The immunostaining method is specific and highly sensitive when used on ESH-L cells. Titration on primary cells is not convenient by this technic because of low cellular yield at advanced level of passages. During replication cycle in cells, the virus growth exclusively intracellular, very few particles are excreted in the media. Finally on ESH-L cells, the virus can be harvested after 3–5 days of incubation rather than 4–6 days on heart cells, which is a benefit.

Conclusion: ESH-L cells are an effective alternative to primary cells for growing Capripoxviruses and their diagnosis. Until now, no work was carried out to evaluate the efficacy of this cell line for isolation, growth and titration of CaPVs comparatively to primary cells.

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