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Real-time replication of swine vesicular disease virus (SVDV) in cell culture systems *in vitro*

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Abstract

A swine vesicular disease virus (SVDV) replication assay in IB-RS-2, SK-6, and PK-15 cell cultures was performed using the xCELLigence system. The cell status was monitored by impedance measurement, expressed as cell index (CI). Proliferation of particular cells was examined at the beginning of the study. The cells exhibited the ability to form a monolayer, and the CI values increased with the cell culture growth. After about 23 h and while still in the growth phase, the cells were infected with decimal virus dilutions (10^{-1}–10^{-6}) containing from 100 000 to 1 median tissue culture infectious doses ($TCID_{50}$). SVDV replication in cell cultures induced a change in cell index; together with the occurrence of cytopathic effect (CPE), the CI values declined. A significant correlation between the concentration of the virus used and CPE occurrence was found. The results also enabled determination of cell sensitivity to SVDV infection. The highest sensitivity was exhibited by IB-RS-2, followed by SK-6. To conclude, the xCELLigence System was used effectively and evaluated as being an efficient tool for CPE detection and SVDV replication analysis in cell cultures. Compared to the standard method, it enabled a more precise assessment of viral replication based on the quantitative CI measurement, providing additional current information.

Keywords: swine vesicular disease, impedance, cell index, cytopathic effect.

Introduction

Swine vesicular disease (SVD) affects all breeds of domestic pigs and wild boars. It is listed by the World Organisation for Animal Health (OIE) as subject to notification and official control.

The aetiological agent of the disease is a virus (swine vesicular disease virus – SVDV) belonging to the family *Picornaviridae* in the *Enterovirus* species, and antigenically related to human pathogens Coxsackie B5 and A16. Only one serotype of the virus is known, but antigenically different isolates occur, varying in virulence and tropism (1, 6).

The disease was first recognised in Italy in 1966, where it is still detected, whereas in Poland the outbreaks were observed in the 1970s. The disease is characterised by vesicles developing on the limbs and, to a lesser extent, the oral mucous membranes; it is clinically indistinguishable from other diseases with vesicular lesions during their course, including the most serious and the most easily spread of them, foot-and-mouth disease. The presence of the virus or specific neutralising antibodies constitutes the basis for the detection of the disease. The methods recommended by the OIE are an isolation test and indirect sandwich ELISA for the detection and identification of the virus, PCR for the detection of its genetic material, and monoclonal antibody competitive ELISA (MAC-ELISA) and a standard neutralisation test for serology.

The methods involving the use of sensitive cells are among those with the highest sensitivity and specificity (7, 8). In the case of viral infections, studies involving cell cultures are considered the gold standard in diagnosis. SVDV proliferates in primary and secondary swine cell cultures and in the continuous cell lines IB-RS-2, SK-6, and PK-15. This is a cytopathogenic virus, whose replication in the above-mentioned cultures leads to morphological changes as a cytopathic effect (CPE) visible in

microscopy. CPE is a basis for studies using cell cultures. A novel method for the real-time assay of *in vitro* cells is real-time cell analysis (RTCA) (4, 5, 9, 10, 11, 14), also applied to diagnosis of viral diseases (2, 3, 12, 13), and it creates a possibility for improvement in that area. The aim of this experiment was to evaluate the applicability of RTCA in CPE detection in SVDV-infected cell cultures.

Material and Methods

Cell lines. The porcine kidney cell lines IB-RS-2, SK-6, and PK-15 were propagated in 75 cm^2 bottles in Eagle's minimal essential medium (MEM) (Sigma-Aldrich, USA), supplemented with 10% foetal bovine serum (FBS) (Gibco, part of Thermo Scientific, USA) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin, Sigma-Aldrich, USA). IB-RS-2 cells were obtained from the Pirbright Institute (UK), whereas SK-6 and PK-15 originated from the Department of Swine Diseases at the National Veterinary Research Institute in Pulawy, Poland.

Virus adaptation to cell cultures. The cultures were infected with a laboratory-developed strain of SVDV. They were passaged two or three times until they showed 90%–100% CPE.

Impedance and cell index measurement. The measurements were performed using the xCELLigence system (Roche Applied Science, ACEA Biosciences, USA) which consisted of the RTCA dual-plate (DP) station for three 16-well culture plates (E-plate 16 and E-plate View 16), combined with an analyser and a computer with integrated software. Specially designed plates with a glass bottom are coated with gold microelectrodes, which take up approximately 80% of the plate well surface. The system enables dynamic monitoring of adherent cells in real time through a quantitative impedance measurement in each plate well, expressed as a cell index (CI) value. CI is a non-dimensional parameter which provides information on the biological status of the cells, their changing numbers, morphology, and adhesion; it rises with an increasing number of cells or stronger adhesion, and declines with their decreasing number or weaker adhesion.

Prior to the assay, the RTCA DP station was placed in an incubator at 37ºC (±1ºC) with a controlled atmosphere of 5% CO_2 (±0.5%), and connected to a computer located outside the incubator. Next, 100 µL of growth medium was added to each plate well and the plates were placed inside the station in order to measure the background that is the cell-free signal. One plate was designated for each type of cells. After the measurements, 100 µL of a particular cell suspension was added, at about 50 000 cells per well. The plates were left at room temperature for 30 min and then placed inside the station, still remaining in the incubator. CI measurement was registered every 30 min. After about 23 h of cell growth, the cultures on plates were inoculated with a virus adapted to a particular cell line, in tenfold increasing dilutions of 10^{-1}–10^{-6} (100 000–1 median tissue culture infectious doses ($TCID_{50}$)), at 50 µL per well. Two wells were designated for each dilution, whereas the control consisted of uninfected cultures. After the plates were placed in the RTCA DP station, the CI measurements were continued.

Simultaneously, a standard assay was performed following the infection of cell cultures in Nunc 96-well flat bottom plates (Nunc, part of Thermo Scientific, USA). The cytopathic effect was microscopically inspected only during working hours.

Results

The obtained results are presented in Figs 1–3. Fig. 1 shows the dynamics of SVDV replication in the IB-RS-2 cell culture. The virus in doses of 100 000, 10 000, 1 000, 100, 10, and 1 $TCID_{50}$ induced a decline of the CI values after 4, 5, 8½, 11, 12, and 18 h respectively. In the SK-6 cell culture infected with the virus in doses of 100 000, 10 000, 1 000, 100, and 10 $TCID_{50}$, the CI values declined after 5, 6, 8, 12, and 19 h respectively (Fig. 2). Finally, in the PK-15 cell culture, the CI values declined only after inoculation with the virus in doses of 100 000, 10 000, and 1 000 $TCID_{50}$, after 6, 7, and 9½ h respectively (Fig. 3). In the SK-6 culture infected with a 1 $TCID_{50}$ dose of the virus and in the PK-15 culture inoculated with doses 100, 10, and 1 $TCID_{50}$, CPE was not detected. These cells continued to grow, and in the case of PK-15 the CI values were almost identical to the control. The cytopathic effect in IB-RS-2 cell culture on an E-plate View 16 and on a standard plate is shown in Figs 4, 5.

Discussion

The study demonstrated the efficacy of xCELLigence for CPE detection in IB-RS-2, SK-6, and PK-15 cell cultures infected with SVDV. First, cell proliferation was assayed. The cells showed the ability to form monolayers, and CI values increased due to culture growth. Eagle's MEM as used was appropriate for impedance measurement, from which data were collected in real time, in both numerical and graphic form. In microscopic examination, satisfactory culture growth in the plate wells (E-plate View 16) was observed, comparable with that obtained on the plate used in the standard method. The cells were infected with SVDV doses ranging from 100 000 to 1 $TCID_{50}$ while still in the growth phase. Monitoring of the infection kinetics followed, based on the changes of the cell index value. Viral replication induced the occurrence of CPE in the cell

culture. Each curve was characterised by a cell growth phase correlated with an increase in CI value, and a consecutive CI drop, reflecting cytopathic effect. These were dependent on the dose of the virus used, which indicates the possibility of SVDV titration using the xCELLigence system. A clear correlation between the $TICD_{50}$ doses of the virus used for infection and the occurrence of CPE was observed. In the cultures infected with higher SVDV concentrations, the drop in CI value due to viral replication occurred earlier and continued until reaching zero, which indicated the death of the infected cell culture. When infected with lower concentrations, CI values dropped later or the cells continued to grow, similarly to uninfected cells. The ultimate results of CPE detection in cell cultures using the xCELLigence system and by means of the standard method were comparable, which implies that this new technique is appropriate for measuring the changes in cellular parameters during infection. This study has also enabled researchers to assay sensitivity of the cells used to SVDV infection; the highest sensitivity was exhibited by IB-RS-2, followed by SK-6. Few authors have published the results of studies similar in scope. Interesting data emerged from CPE monitoring in Vero cell cultures infected with Saint Louis encephalitis virus and West Nile virus, both of the *Flaviviridae* family (2). Furthermore, the study conducted by Golke *et al.* (3) showed the efficacy of the new technique for monitoring a primary murine neuron culture and equine dermal (ED) cell lines infected with equine herpesvirus type 1 from the *Herpesviridae* family. The system of real-time cell analysis was also used for monitoring CPE in a Madin–Darby canine kidney (MDCK) cell culture infected with strains of A/H1N1 influenza virus (12), as well as for titration of the virus from the *Poxviridae* family in a human embryo kidney 293 cell culture (13).

Summing up, the xCELLigence system was used effectively and assessed as being an efficient tool for CPE detection and analysis of SVDV replication in IB-RS-2, SK-6, and PK-15 cell cultures. Compared to the standard method, it enabled a more precise assessment of viral replication in real time based on the CI measurement and provided additional current information on the predominance of either viral or cellular activity in cell–virus interaction. A further advantage was the possibility of constant control over the quality of cells during the whole course of the study. With a view to improving and expanding laboratory diagnostics of swine vesicular disease, further investigations using the xCELLigence system based on more comprehensive material are justified, with regard to detection of the virus through isolation and viral antibodies by seroneutralisation.

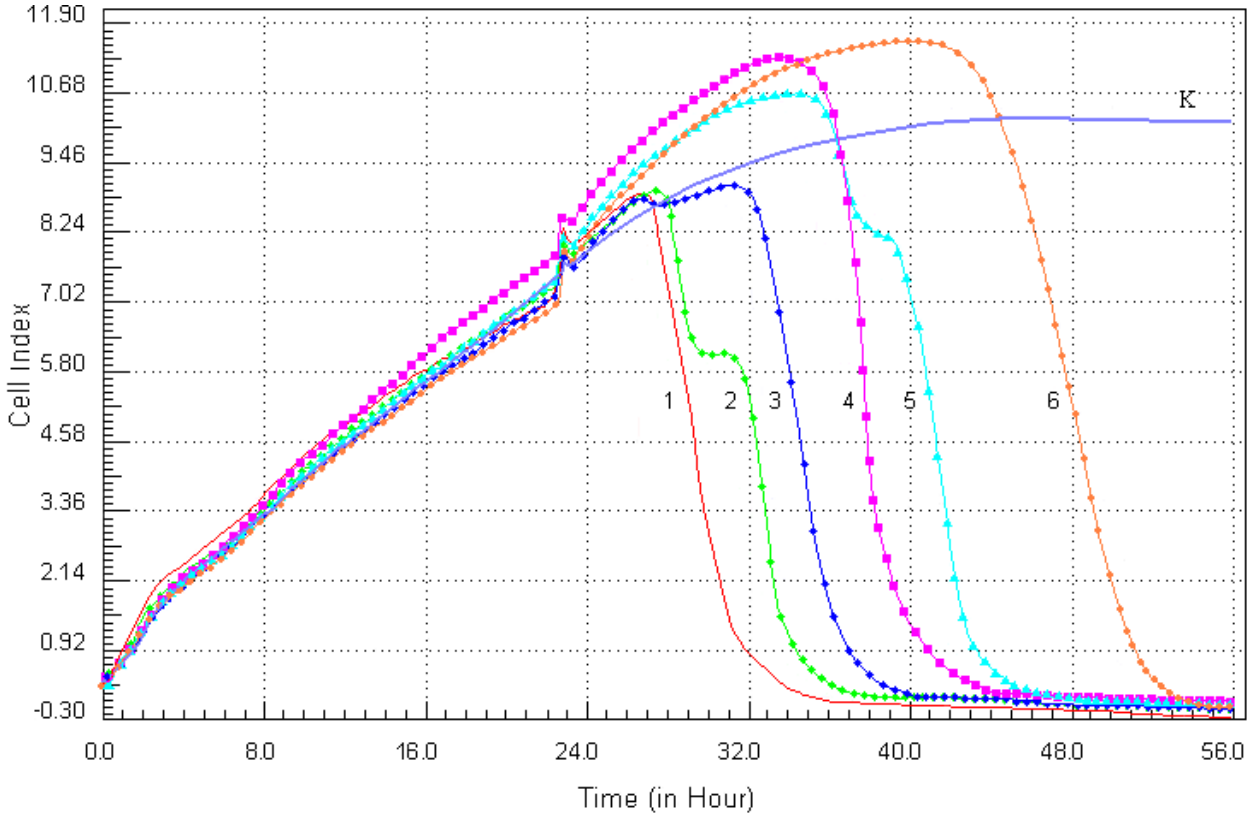

Fig. 1. SVDV replication in IB-RS-2 cell culture. Curves 1, 2, 3, 4, 5, and 6 match the virus doses of 100 000, 10 000, 1 000, 100, 10, and 1 $TCID_{50}$. K – control, uninfected cell culture

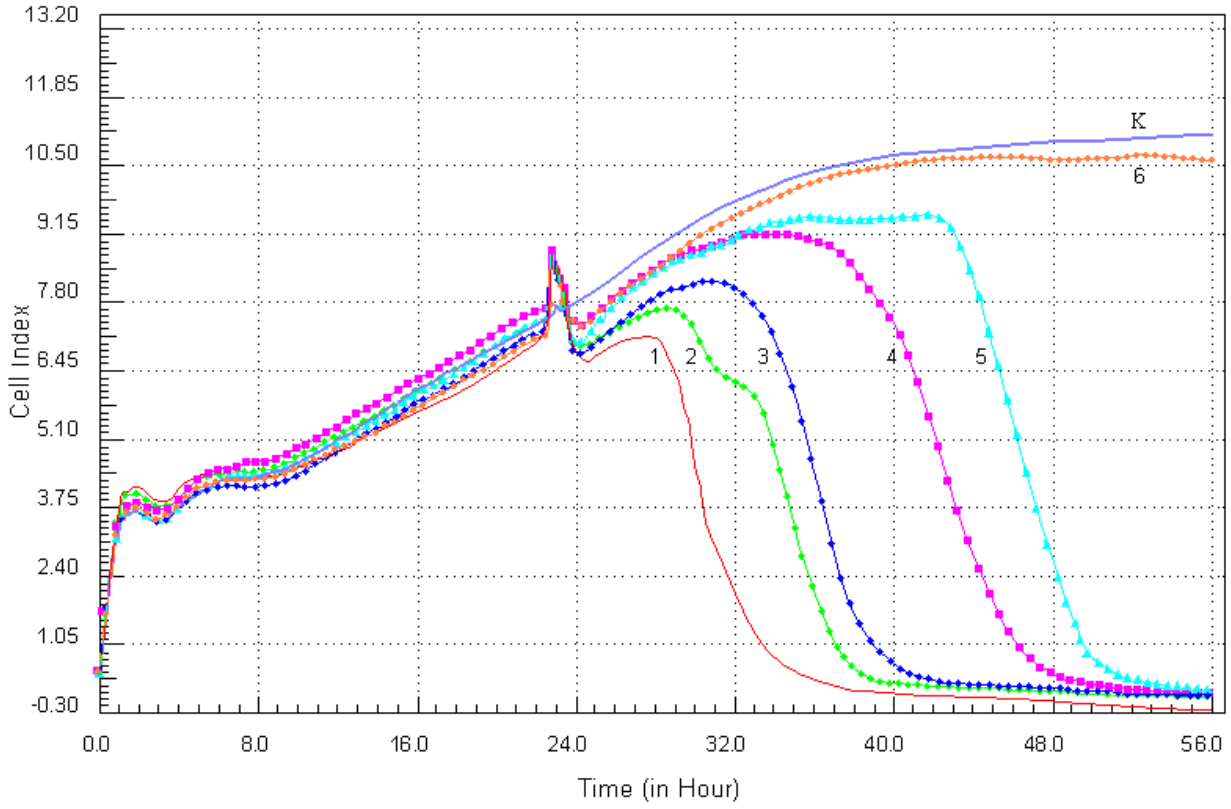

Fig. 2. SVDV replication in SK-6 cell culture. Curves 1, 2, 3, 4, 5, and 6 match the virus doses of 100 000, 10 000, 1 000, 100, 10, and 1 $TCID_{50}$. K – control, uninfected cell culture

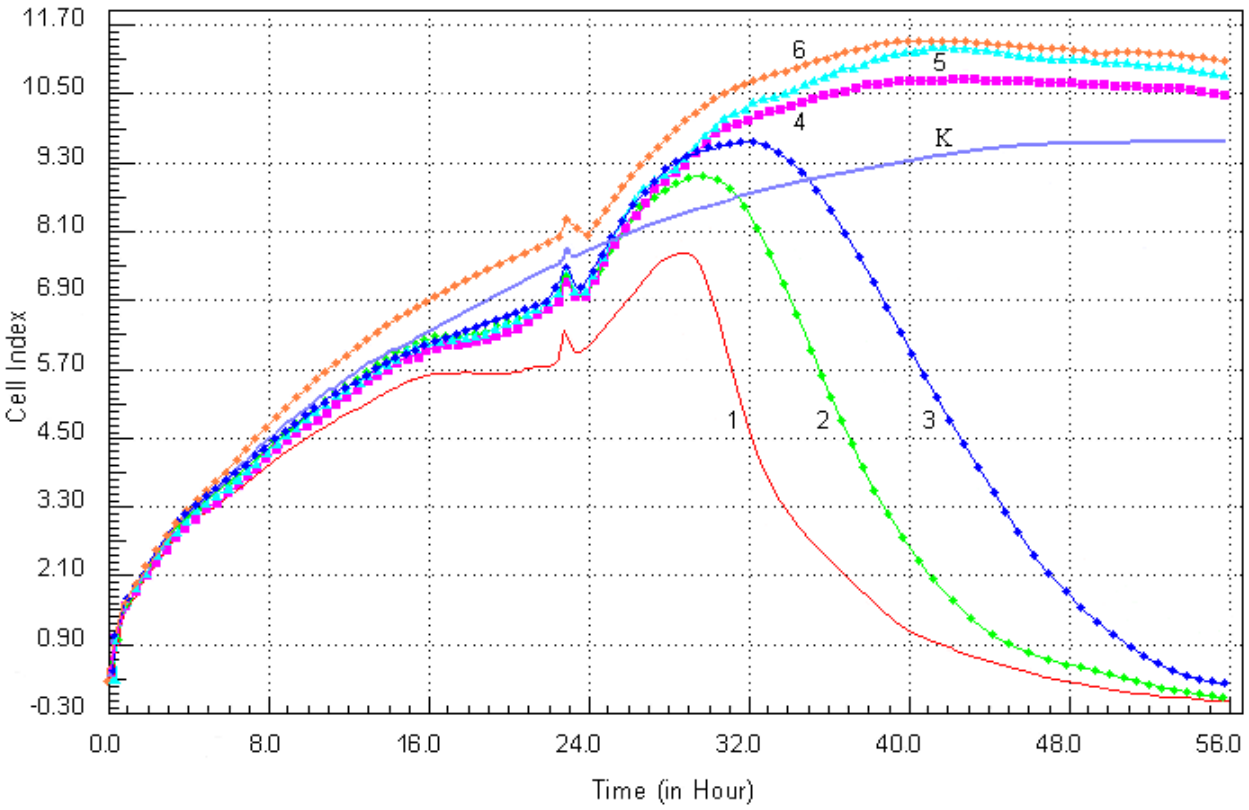

Fig. 3. SVDV replication in PK-15 cell culture. Curves 1, 2, 3, 4, 5, and 6 match the virus doses of 100 000, 10 000, 1 000, 100, 10, and 1 $TCID_{50}$. K – control, uninfected cell culture

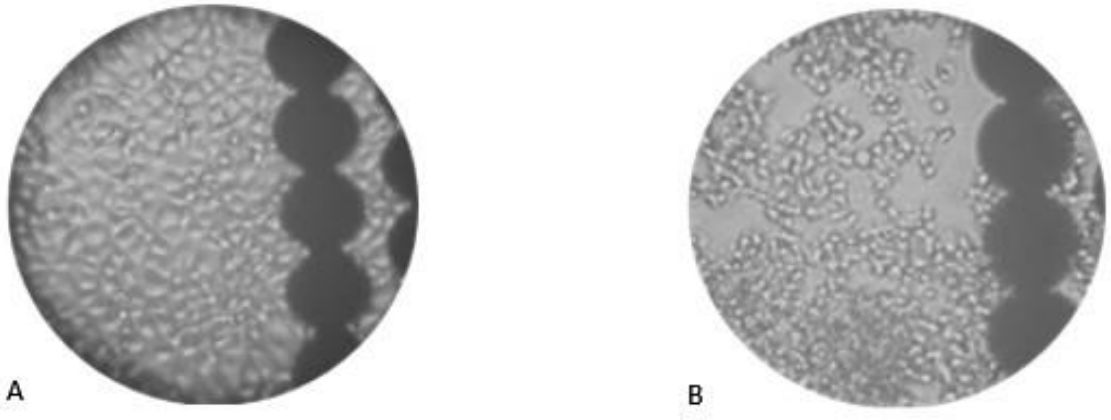

Fig. 4. Cytopathic effect in IB-RS-2 cell culture on an SVDV-infected E-plate View 16. A – control, uninfected cell culture; B – cell culture at 24 h after 1 $TCID_{50}$ infection

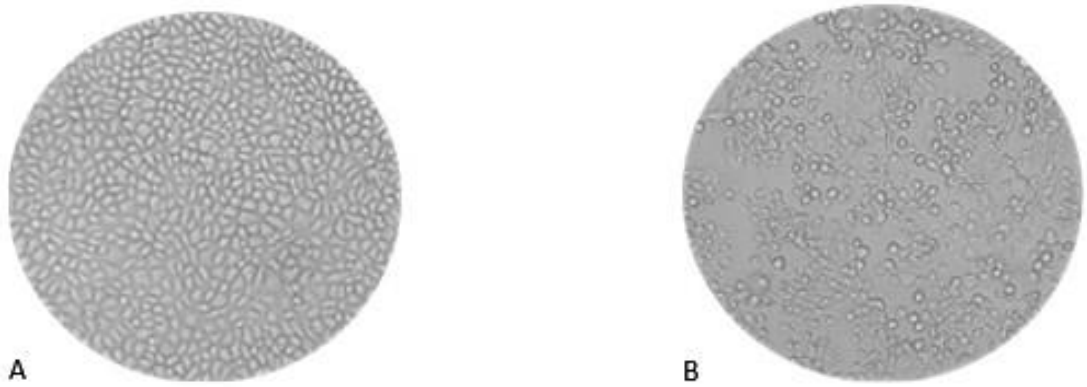

Fig. 5. Cytopathic effect in IB-RS-2 cell culture on an SVDV-infected 96-well flat bottom plate. A – control, uninfected cell culture; B – cell culture at 24 h after 1 $TCID_{50}$ infection

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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