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COMPARATIVE ANALYSIS OF PROPAGATION PROPERTIES OF ASFV ODINTSVO 02/14 ISOLATE IN PRIMARY CELL CULTURES

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SUMMARY

Odintsovo 02/14 isolate of African swine fever virus was tested for its propagation properties in primary cell cultures with adding different sera to the nutrient medium. The test results showed that the virus propagation was the most successful in porcine bone marrow and porcine kidney cells when fetal bovine serum and FS FetalClone II, multipurpose serum substitute were added.

Key words: African swine fever, isolate, virus titre, porcine primary cell culture, serum, fetal serum.

INTRODUCTION

African swine fever (ASF) is a highly dangerous transboundary contagious viral disease of pigs of all species and ages characterized by fever, skin cyanosis and extensive hemorrhages in internal organs of infected animals.

So far, no highly effective means for specific ASF prevention have been developed. Therefore, early disease diagnosis and high biosecurity in pig industry are of importance for combating disease. Sensitivity of used laboratory diagnostic methods depends first of all on the virus concentration in tested samples of pathological materials.

In some cases it is necessary to passage the recovered ASFV isolate several times in different continuous and primary porcine cell cultures for ASF virus accumulation at diagnostic concentrations. The virus-containing material yielded from different cell cultures is used for diagnostic tests and for testing ASFV isolates for their biological, molecular-genetic properties [1, 3, 5, 8, 10, 11].

ASF virus *in vitro* propagates in hematopoietic cells, macrophages/monocytes of different tissue origins. They include porcine alveolar macrophages (AM), bone marrow cells (BMC) kidney cells (KC), splenocytes (SC) and testicle cells (TC). Many authors note that ASF agent actively propagates in highly adhesive cell fraction of porcine bone marrow macrophage pool and less actively propagates in lymphocytes, neutrophiles and endothelial cells [6, 10, 11].

Viable ASF virus is capable to induce specific modulation of infected cell membranes and erythrocyte adsorption onto surface of infected cells (hemadsorption).

Heterogeneous ASF virus population produces hemadsorption in primary cell cultures manifested simultaneously in two or three variants: fluffy – infected cells adsorb up to 20 erythrocytes; intermediate – 20-40 erythrocytes; dense – 40-80 erythrocytes onto their surface [5].

It was shown that FS FetalClon II, multipurpose serum substitute with additional components for cells, that contains highly purified heat treated bovine serum albumin, bovine trasferrin, bovine insulin and does not contain growth factors – steroid hormones, glucocorticoids and cell adhesion factors, can be used for *in vitro* cultivation of the virus instead of porcine and bovine sera [8].

There is no sufficient evidence on propagation of current ASFV isolates in porcine primary cell cultures (BMC, leukocytes, AMC, KC) supplemented with different propagation stimulants [1, 2, 5, 6, 8] in national and foreign literature Therewith, tests of ASF viruses recently isolated in the Russian Federation for the said properties are of current importance.

MATERIALS AND METHODS

Animals. Two-four month-old piglets (body weight- 20-30 kg) obtained from infectious disease-free farms located in the Vladimir Oblast were used.

Tubular bones as well as kidneys, spleen and testicles were collected from the piglets for tests after their complete bleeding [6, 9].

Table
Infectivity of ASF virus propagated in different primary cell cultures supplemented with various sera (n=5)

Cell culture	Hemadsorbing ASFV titres in primary cell cultures supplemented with different sera, lg $HAdU_{50}/cm^3$		
	FBS No.2	NPS	FS FetalClon II
BMC	6.25±0.43	6.00±0.46	7.25±0.56
SC	6.50±0.58	2.75±0.25	3.50±0.36
KC	6.25±0.25	5.75±0.25	6.00±0.25

Cell cultures. Cell suspension was added to wells of 96-well culture plate, plastic 25 cm^2 culture flasks. Thereat, the nutrient medium was supplemented with 20% of: 1) fetal bovine serum (FBS; No.1 – PAN BIOTECH, USA; No. 2 – FBS, PAA, Austria); 2) normal porcine serum (NPS); 3) FBS clone II (FS FetalClon II, HyClone, USA).

The cell cultures were infected with Odintsovo 02/14 isolate of ASF virus recovered from wild boar in the Moscow Oblast in 2014. Multiplicity of infection was 0.1–0.01 HAdU/cell.

Cell counting was performed with both standard methods (counting in Goryaev chamber) and with Countess™ automated cell counter (Invitrogen™, South Korea).

Infected cell cultures were cultivated in CO_2-incubator with 5% CO_2.

Haemadsorption test and direct immunofluorescence test were used for control of ASF virus propagation in primary cell cultures.

Infectivity titre of ASF virus was determined by its titration in different primary cell cultures with haemadsorption test (HAdT). A 2-day monolayer of cell cultures grown in wells of 96-well microplate was used for ASFV titration. Haemadsorption test was performed with a classical method using working porcine erythrocyte suspension [7]. The infectivity titre of the virus was calculated with Karber method (1931) modified by I.P. Ashmarin (1959, 1962) 5-7 days after infection of primary cell cultures and expressed in lg $HAdU_{50}/cm^3$.

Presence of ASFV in all primary cell cultures used for tests was confirmed by direct immunofluorescence test (DIF test). Specific FITC-immunoglobulins for immunofluorescence diagnosis of ASF (State Scientific Institution «National Research Institute of Veterinary Virology and Microbiology of the Russian Academy of Agricultural Sciences», Pokrov, RF) as well as 18 BG3-FITC, FITC-labeled monoclonal antibodies prepared against vp72 protein, (INGENASA, Spain) were used for direct immunofluorescence test. The cell cultures were observed for cytopathogenic effect (CPE) under luminescent inverted ULWCD 0.30 Olympus microscope, CKX41 Model.

RESULTS AND DISCUSSION

It was required to select optimal seeding density at one of the stages of preparation of comparable specimens of different primary cell cultures. During tests with similar cell seeding density the number of BMC attached to plastic flask bottom was higher as compared to SC. The seeding density of KC used for dense cell monolayer preparation varied from 500 ths/cm^3 to 2 mln/cm^3 that was four times higher than the required TC seeding density. TC monolayer formed 24-48 hours after seeding whereas KC monolayer formed 72-96 hours after seeding with the same seeding density.

BMC and SC cultures were seeded with density of 5-6 mln/cm^3 and their monolayers formed 48-72 hours after seeding.

Cell culture yield at working concentration derived from organs collected from one piglet was as follows: for BMC – 400-500 ml, for SC – 150-200 ml, for TC – 200-300 ml, maximum yield was obtained for KC and was 600-800 ml.

Thus, KC culture was the most technological since its yield was the highest, as BMC and TC yields were lower and SC yield was the lowest.

ASF virus propagation in primary BMC, SC, KC, TC cultures was accompanied with characteristic morphological changes in infected cells: disadhesion of some cells resulted in cell rounding and then cell pyknosis.

Characteristic inclusions specific for the virus that was confirmed by direct immunofluorescence test using FITS-conjugated monoclonal antibodies against ASFV vp72 were observed in cytoplasm of infected cells 18-36 hours after infection during examinations. Then, cells fused together and formed multinuclear giant cells (Fig. 1-2 , P. 6), and later cell destruction resulted from the virus-induced apoptosis was observed.

Fig. 3, 5, 7, 9 (P. 6-8) show images of intact cells (negative control), and Fig. 4, 6, 8, 10 (P. 6-8) – primary TC, KC, BMC and SC cultures infected with ASF virus, respectively.

Test results showed that hemadsorption of various types occurred in BMC, KC, SC, TC cultures infected with ASF virus (Odintsovo 02/14 isolate) (Fig. 11, P. 8): dense (in 65-80% of infected cells), intermediate (in 10-15% of infected cells) and fluffy (in 5-10% of infected cells).

Analysis of speed of ASF virus detection with direct immunofluorescence test showed that hemadsorption appeared in SC cultures 12-24 hours after infection (minimum period), in BMC and KC cultures 48 hours after infection and in TC cultures 48-72 hours after infection.

Therefore, it is reasonable to use SC culture to increase speed and effectiveness of diagnostic tests, whereas other cell cultures (BMC, KC and TC) can be used for yielding virus amounts required for diagnostic tests.

Tests of ASF virus for its propagation properties in primary cell cultures supplemented with different sera (FBS, NPS) and FS FetalClon II, multipurpose serum substituent, showed that the virus accumulated at the highest titres (7.25±0.56 lg $HAdU_{50}/cm^3$) in BMC culture supplemented with FS FetalClon II 120-168 hours after virus inoculation. Calculated infectivity titres of ASF virus propagated in different cell cultures supplemented with the above-mentioned sera are given in Table below.

It was shown that ASF virus titre was 7.25±0.56 lg $HAdU_{50}/cm^3$ in BMC culture supplemented with FS FetalClon II that was on average 1lg higher than that one in BMC supplemented with FBS No.2 (6.25±0.43 lg $HAdU_{50}/cm^3$) and NPS (6.00±0.46 lg $HAdU_{50}/cm^3$). In SC culture ASF virus titres were the highest when the SC culture was supplemented with FBS No. 2 (6.50±0.58 lg $HAdU_{50}/cm^3$), that was 3 lg higher than that one in SC culture supplemented with FS FetalClon II (3.50±0.36 lg $HAdU_{50}/cm^3$) and 4 lg higher than that one in SC culture supplemented with NPS (2.75±0.25 lg $HAdU_{50}/cm^3$).

FBS seems to be the most effective at maintaining splenocytes (SC) that is reflected in significant differences in titres. For KC culture, the best results (6.25±0.25 lg $HAdU_{50}/cm^3$) were observed when FBS No.2 was used. When KC culture was supplemented with FS FetalClon II and NPS the results were slightly worse, 6.00±0.25 lg $HAdU_{50}/cm^3$ and 5.75±0.25 lg $HAdU_{50}/cm^3$, respectively.

CONCLUSION

Based on the analysis of the test results it was suggested to use SC culture to improve diagnostic test speed and effectiveness. BMC, PK and TC cultures can be used to prepare virus amounts required for diagnostic tests.

When Odintsovo 02/14 isolate of ASF virus was tested for its propagation properties in BMC culture the highest titres were detected in BMC culture supplemented with FS FetalClone II (7.25±0.56 lg $HAdU_{50}/cm^3$) and FBS (6.25±0.43 $HAdU_{50}/cm^3$).

Thus, the test results confirm that FBS maintains SC, KC and BMC cultures the most sustainably and effectively whereas FS FetalClone II is the most effective at maintaining BMC culture only.

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