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CYTOMORPHOLOGICAL TRANSFORMATIONS IN YADK-04 CELLS DURING INTERACTION WITH PESTE DE PETITS RUMINANTS VIRUS

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SUMMARY

The paper presents experimental study results of the cytopathic effect of peste de petits ruminants virus on a goat gonad continuous cell line (YaDK-04). The interaction of peste de petits ruminants virus with cells at different stages of its reproduction was shown using a combination of phase-contrast and luminescent microscopy. It was found that at the initial stage of interaction (20–24 hours) the cells became rounded and de-adhered, and the monolayer was partially loosened. On day 2 post reproduction the most part of the culture monolayer affected by the virus began to destruct, and the cell nuclei were displaced to periphery. At the terminal stage (72 hours) the destruction of monolayer cells and cytoplasmic matrix, deformation and partial lysis of the nuclei and cytoplasm, aggregation of detritus occurred. At the final stage of reproduction (96 hours) the peste de petits ruminants virus diffused into the culture medium, the fluorescence in the yellow spectrum decreased significantly, but the virus titer reached 6.89 lg TCD_{50}/cm^3.

Key words: YaDK-04 cell culture, peste de petits ruminants virus, cytopathic effect, luminescent and phase-contrast microscopy.

INTRODUCTION

The importance of studying cytomorphological transformations during interaction of viruses with cell lines *in vitro* has several aspects, the first of which are specific morphological transformations that could indicate the agent's species. Based on specific nature of lesions in cells we can collect information on possible lesions of certain tissues and organs of animals [1]. Using specific features of cytomorphological transformations is also helpful for refinement of conditions of virus cultivation and virological tests [5].

Peste des petits ruminants (PPR) virus belongs to the *Paramyxoviridae* family and is similar to sheep and goat pox virus in terms of its cultural properties (the virus reproduces in the same cultures and under the same conditions), but differs in its cytopathic effect on cells [3, 6].

The aim of the work was to study interaction of PPR virus with a goat gonad cell culture (YaDK-04) using luminescent and phase-contrast microscopy and determine the specificity of the effect of the given virus on YaDK-04 cell culture.

MATERIALS AND METHODS

Viral materials. "ARRIAH" production strain of PPR virus with infectivity of 5.89 ± 0.18 lg TCD_{50}/cm^3 was used along with "ARRIAH" production strain of sheep pox virus with infectivity of 6.25 lg TCD_{50}/cm^3.

Cell culture. The viruses used in the study were cultivated in YaDK-04 continuous cell culture. Incubation temperature of normal and infected cell cultures was 37.5–38.0 °C.

Nutrient media and solutions. The growth medium for YaDK-04 cell culture was a mixture of PSP nutrient media (parietal nutrient medium) and 199 at the ratio of 2:1 with 10% bovine serum treated with lanthanoids (pH of the medium – 7.0–7.2).

The maintenance medium for the virus was PSP nutrient medium with 2% bovine serum inactivated at 58 °C for 30 minutes (pH 7.3–7.5). Before inoculation of the virus, the monolayer of YaDK-04 cell culture was washed with Hank's salt solution with pH 7.1–7.2. Correction of pH of the medium was conducted with 7.5% solution of sodium bicarbonate.

Table 1
Dynamics of PPR virus accumulation in YaDK-04 continuous cell culture
(*n* = 3)

Cultivation time, h	Virus titre, lg TCD_{50}/cm^3
24	3.08 ± 0.14
48	4.25 ± 0.00
72	5.33 ± 0.18
96	5.89 ± 0.18
120	5.08 ± 0.18

Antibiotics were added in a common dosage (100 units/ml of kanamycin or 40 units/ml of gentamicin) to prevent bacterial growth in nutrient media and Hank's solution.

Glassware and apparatus. Laboratory cultivation of cells was conducted in Corning bottles (USA) with the growth surface of 25 cm^2.

Cytomorphological transformations in the culture during interaction with the virus were studied in a phase contrast with Olympus CKX41 microscope in 100x and 200x magnification and ML-2B luminescent microscope in 100× and 200× magnification. For luminescence microscopy native preparations grown on cover slips were stained with 0.01% solution of acridine orange [4].

The titre of the virus was calculated by Karber method in Ashmarin's modification and expressed in lg TCD_{50}/cm^3.

RESULTS AND DISCUSSION

References say that currently primary and continuous cell cultures are used to cultivate PPR virus [1, 3, 6]. One of the drawbacks of cultivating a virus in primary cell cultures is a quite demanding process of preparation of a cell monolayer and possible previous microbial contamination of the material used for trypsinization. For this reason, continuous cell lines constitute a promising and convenient way to prepare large volumes of viral raw materials with the advantage of enabling the creation of a cell bank with characteristics necessary for vaccine production.

The FGBI "ARRIAH" developed a technology of industrial cultivation of PPR virus on YaDK-04 continuous cell culture. The given cultivation system enables obtaining virus-containing raw materials with high infectivity.

In the present research the virus quantity was determined in dynamics at the interval of 24 h by titration in YaDK-04 cell culture. The results are shown in the table.

The table demonstrates that the level of PPR virus accumulation during the cultivation process increased reaching its maximum by 96 h of cultivation (5.89 ± 0.18 lg TCD_{50}/cm^3), with a subsequent drop of infectivity titre down to 5.08 ± 0.18 lg TCD_{50}/cm^3 after 120 h.

The sample of a viral material with optimum infectivity (96 h of cultivation) was taken for the upcoming work to fulfill the task of the research – to study cytomorphological transformations in YaDK-04 cells induced by PPR virus.

There is no single systematic approach to studying interaction of viruses with cell cultures. Researchers apply various methods to do that. The most well-known are methods involving radioactive labels, histochemical and serological methods, electronic microscopy, etc. [1, 2, 7]. The study concerned the effect of PPR virus on the morphology of a monolayer and individual cells of a constant YaDK-04 line in the course of their interaction using phase-contrast and luminescent microscopy.

All viruses are intracellular parasites. Uncoating, nucleic acid replication (DNA, RNA), synthesis of membranes and virion assembly take place once a virus penetrates a cell. Main replication processes occur in the cytoplasm of cells where viral particles conglomerate most often in a diffusion-like pattern. Since the virus uses the biomaterial of cells, it triggers conformational transformations of all organelles, cytoplasmic matrix and nucleus. These transformations within the cells caused by PPR virus are known as oxiophilic or eosinophilic inclusions, granules, etc. [2, 6]. Staining of native preparations and luminescence microscope used in the research helped to determine certain cytomorphological transformations during reproduction of PPR virus in YaDK-04 cell line.

A normal (intact) morphology of YaDK-04 cell culture is a large number of spindle-shaped cells and few cells of fibroblast type (Fig. 1, 5). Cells in the process of division have a spherical elongated shape and the nuclei are elliptic which conforms to the morphology of all cells. The cell culture in question had a minimum number of dividing cells and pH 6.8 at the end of the logarithmic growth phase.

On the first day of cultivation (20–24 h) the effect of PPR virus came with the appearance of rounded de-adhesed cells. Their maximum number was observed after 48 h (Fig. 2, 6). Certain cells aggregated in spherical symplasts. During that period affected cells and mini-symplasts had amorphous boundaries. Spiralization of chromatin of nuclei occurred inside the cells (karyopyknosis). Following the condensation of chromatin (intensified luminescence in green spectrum) the nuclei deformed to become sickle-shaped and moved to one of the cell edges.

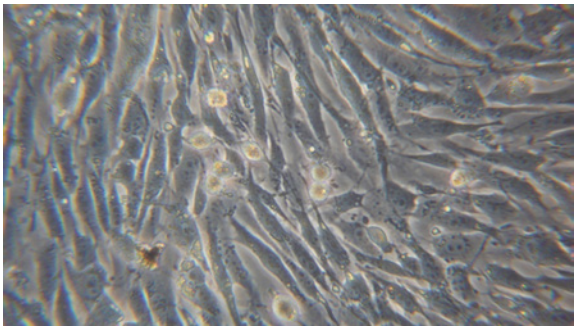

Fig. 1. Morphology of YaDK-04 cells before contamination with PPR virus (400× magnification)

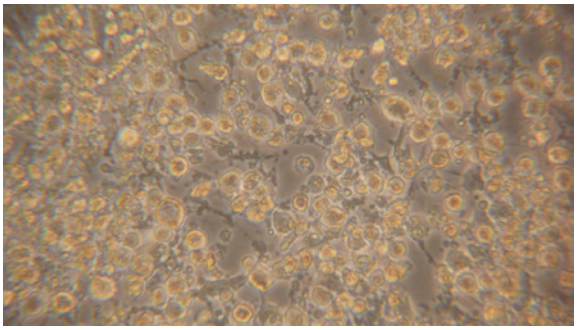

Fig. 2. Transformation of YaDK-04 cells 48 h after inoculation of PPR virus (400× magnification)

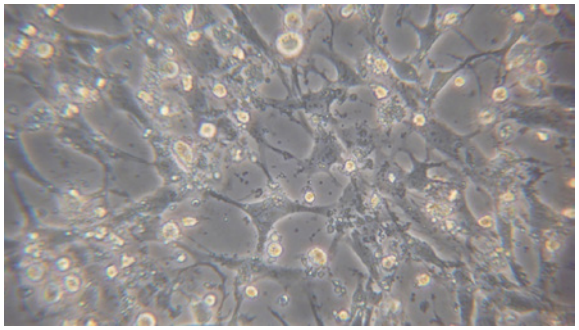

Fig. 3. Transformation of YaDK-04 cells 72 h after inoculation of PPR virus (400× magnification)

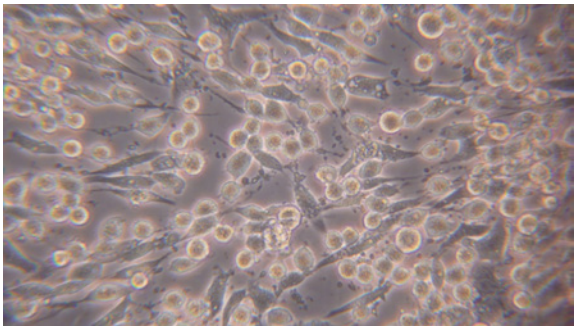

Fig. 4. Transformation of YaDK-04 cells 72 h after inoculation of sheep pox virus (400× magnification)

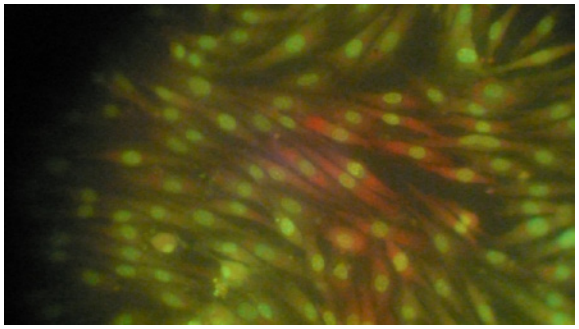

Fig. 5. Morphology of YaDK-04 cells before contamination with PPR virus (200× magnification), luminescence

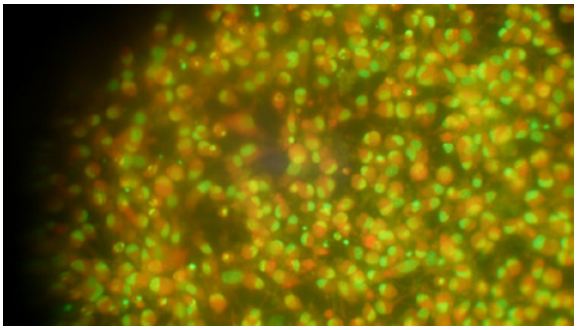

Fig. 6. Transformation of YaDK-04 cells 48 h after inoculation of PPR virus (200× magnification), luminescence

By this time, the cell cytoplasm remained intact and luminesced in orange colour which is common for RNA concentrates and corresponded to the localization of RNA-PPR virus (Fig. 6).

The next stage (after 72 h) was marked with observation of lysis of the major part of affected cells. The cell cytoplasm and the virus were diffused in a nutrient medium (Fig. 3, 7). The cell nuclei with supercoiled RNA fluoresced in green spectrum and were subjected to lysis last (Fig. 9). Up to the end of the cycle the substrate was observed to have 2–5% of not destroyed but affected cells (Fig. 8, 9).

In some exceptional cases, the interaction between PPR virus and YaDK-04 cell culture produced quite large symplasts comprising several dozens of cells, though they formed against the background of non-affected parts of the monolayer and did not cause a total cytopathic effect. Karyopyknosis and fragmentation of nuclei also occurred in large symplasts (Fig. 10). More than 90% lysis of cell membranes and diffusion of the cytoplasm contents into the nutrient medium were observed at the terminal stage of the cytopathic effect by PPR virus. With that, only pyknotic nuclei and insignificant number of non-destroyed cells contrasted in acridine orange (Fig. 8, 9). RNA cytoplasm component (probably, a viral one) was no longer stained.

All stages of PPR virus reproduction are based on transformations of a viral RNA which occur in a cell nucleus in the first place and then in the cytoplasm of infected cells [1]. The transformations observed in the cell culture were provoked by intensified synthesis of the viral RNA in the first 24–48 h. Figure 6 demonstrates that the cytoplasm of a small part of cells infected by the virus fluoresced in orange colour which attests to the increased number of viral RNA in the cytoplasm and to the start of the synthesis

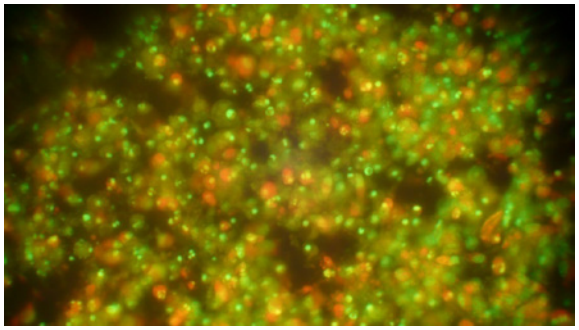

Fig. 7. Transformation of YaDK-04 cells 72 h after inoculation of PPR virus (200× magnification), luminescence

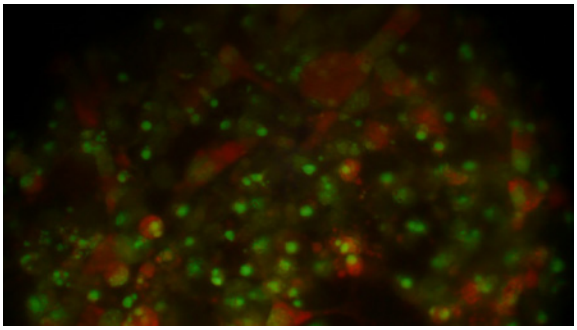

Fig. 8. Transformation of YaDK-04 cells 72 h after inoculation of PPR virus (400× magnification), luminescence

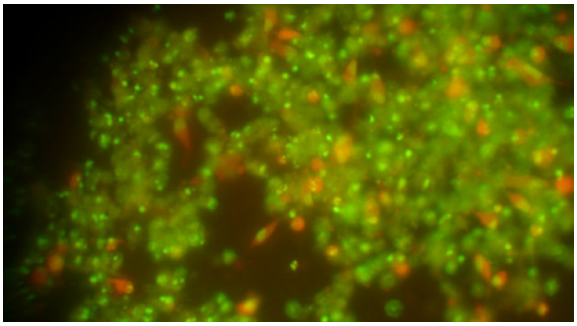

Fig. 9. Transformation of YaDK-04 cells 96 h after inoculation of PPR virus (200× magnification), luminescence

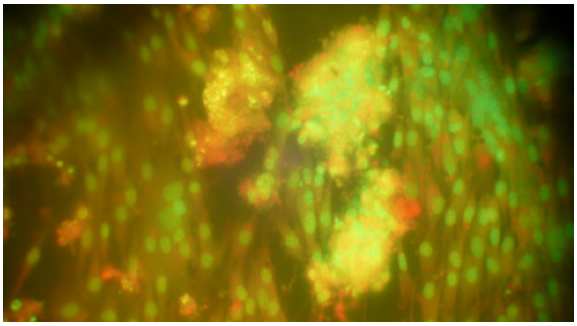

Fig. 10. Transformation of YaDK-04 cells 72 h after inoculation of PPR virus. Formation of symplasts (200× magnification), luminescence

of the viral protein. Reference materials indicate that in the next hours the viral RNA is transported to the cell cytoplasm where the synthesis of the protein and subsequent assembly of viral particles take place on that RNA. By the time of 72 h of cultivation orange fluorescence in the cytoplasm disappears as the viral RNA immediately transforms into a ribonucleoprotein and is then covered with a viral coating on the cell membranes which it simultaneously lyses [7]. This was clearly expressed by 72 h after infection. For that reason, this cultivation period was also marked with a cell lysis which corresponded to a pre-maximum accumulation of the virus in the culture, though a part of the virus was still within the cells. The formation of symplasts typical of PPR virus is rarely seen during infection of YaDK-04 cells with sheep pox virus [3]. Figure 4 presents a photograph of YaDK-04 cell culture infected with sheep pox virus by 72 h of incubation where neither formation of symplasts, nor cell lysis was observed. This is explained by the absence of the fusion protein in the structure of sheep pox virus which is responsible for the cell lysis and formation of aggregates and symplasts in case of PPR infection [1].

CONCLUSION

The study of the cytopathic effect of PPR virus in YaDK-04 cell culture was carried out in a phase contrast and using the method of luminescence microscopy and staining with 0.01% solution of acridine orange.

Initially, after analyzing the dynamics of the virus accumulation in YaDK-04 culture for 120 h of incubation, it was established that the maximum quantity of the virus appeared by 72–96 h of incubation and the infectivity titre decreased.

It was shown that the cytopathic effect of PPR virus at different stages of incubation had signs typical of the viruses of the group and differed from the similar effect of sheep pox virus.

Luminescence and phase-contrast microscopy contributed to a more detailed demonstration of the morphological transformation in the monolayer and in YaDK-04 cells at different stages of PPR virus incubation. These transformations unfolded consecutively. At the beginning the cells became rounded and aggregated, and then the nuclei were deformed inside the cytoplasm and moved to the edge of cells, and finally, a major part of the monolayer lysed and the contents of the cytoplasm burst into the medium. We identified the oxiophilic inclusions observed by some experts as a result of the interaction between PPR virus and constant cell lines as cell nuclei which were displaced to the periphery of the cells and much condensed which led to their destruction in the viral suspension.

Conflict of interest. The authors declare that there is no conflict of interest.

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