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# The Results of Kazakh of Cultivation of Strain Chlamydia Psittaci in Cell Culture


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**ABSTRACT**

The paper presents the data of electron microscopic studies of Kazakh strain of Chlamydia psittaci, been grown cell culture

**KEYWORDS** Chlamydia, strain, cell culture, electron microscopic examination.

**Background**

Chlamydia infection is a classic anthropozoonosis widespread all over the world and representing a constant threat to humans and animals due to polyphagia and expressed plasticity of the disease agent [1, 2, 3].

According to some researchers infection among agricultural animals in the farms where the disease is registered is up to 40 % and more [4, 5].

In 1950 Stamp et al. was the first to reveal chlamydial etiology of abortion in sheep. His findings allowed other researchers to diagnose the disease in sheep in Europe, USA, Asia, Africa and Australia. Abortions of chlamydial etiology in farm animals are often registered in many countries, including Kazakhstan and neighboring republics such as Uzbekistan, Kyrgyzstan and Tajikistan. Therefore, a study on the agricultural animals' chlamydiosis' agent is very important in the Republic of Kazakhstan.

**Materials and methods**

In our research we used continuous cell line M-Hella (epithelial cervix carcinoma) to obtain Chlamydia-containing cultural suspension. In a laminar box 20 ml of Chlamydia-containing cultural suspension from previous passage and 20 ml of Igla MEM medium was added in 250 ml mattresses containing 3- day M-Hella cell culture. Then, mattresses were placed in a thermostat at 37 °C and left for 1 hour for contacting. After one hour the medium was replaced by the growth medium with 10% of bovine serum. Mattresses with infected cell culture were incubated at 37$^0$C and cultured without medium exchange during 6-7 hours. At the end of the period after the cytopathic effect and the partial destruction of the monolayer mattresses containing cell culture were frozen and thawed. Furthermore subsequent passaging of Chl. psittaci isolates in M-Hella cell culture in vitro was conducted. Accumulation of Chlamydia-containing suspension was done during the passaging of Chlamydia. For purification and concentration of Chlamydia in Chlamydia-containing suspension we used the method of ultrafiltration in a cassette system Pellicon- 2 with a pore size of 300 kDa.

Electron microscopic studies on the detection of Chlamydia presence in the infected M-Hella cell culture were conducted. After cytopathic effect in M-Hella cell culture the cells were removed with a scraper and re-suspended in a small amount of medium. The obtained suspension was centrifuged at 1300 rpm/min for 10 minutes. The supernatant was discarded and the pellet was re-suspended in a solution of glutar aldehyde. After the material fixation we prepared semi-thin and ultrathin sections that were used for electron microscopy.

**Results**

During passaging of Chlamydia in M-Hella cell culture 4 liters

of Chlamydia-containing suspension was accumulated and frozen at -20 °C.

Electron microscopic studies of infected M-Hella cells were conducted to determine the presence of Chlamydia. Light-optical study of semi-thin sections revealed the presence of culture cells, occasionally connected with each other (Figure 1). The cells had a large eccentrically located nucleus. It had a round or slightly irregular shape with a thin rim of condensed chromatin and a large nucleolus. A distinctive feature of the histological structure of culture cells was the presence of numerous vacuoles, mainly located near the nucleus. In many vacuoles small dense structures stained positively in burgundy color were seen. Lipid inclusions were located in the cytoplasm of some cells.

Electron microscopic study showed that plasma membrane of most cells had numerous micro-villi. Individual cells were connected by tight cell-to-cell contacts. The nuclei were characterized by a high content of euchromatin. Heterochromatin was located pre-inconsequentially. In the cytoplasm there were numerous small mitochondria of oval and oblong form with electron dense matrix and light inter-crista spaces. Tubules of granular endoplasmic reticulum were rare. The cytoplasm was rich in free ribosomes. Also microfibrils and microtubules were seen. During our electron microscopic studies we found vegetative forms of Chlamydia which were large (about 1500 nanometers) reticular cells of net structure, surrounded by a thin cytoplasmic membrane (Figure 2).

Figure 1. Chlamydia infected cells. Semi-thin section. Stained with methylene blue, azure-2 and basic roszin. Magnification 1000x.

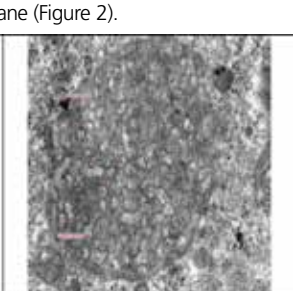

Figure 2. Chlamydia reticular body with the size 1462.78x1719.13 nm, net structure with thin cytoplasmic membrane. Electron microscopic magnification 9000x.

In numerous vacuoles, the so-called inclusion bodies we saw dense spherical structures (Figure 3), the large fibrillar (Figure 4), and mixed structures (Figure 5), which were regarded as the various stages of Chlamydia development: the transformation of elementary cells into the reticular cells, intermediate forms, and maturation of elementary bodies within the reticular bodies.

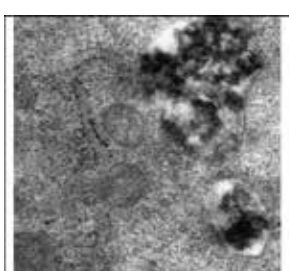

Figure 3. Inclusion body with dense rounded structures. Electron microscopy magnification 9000x.

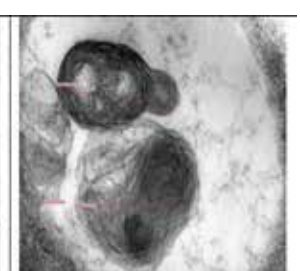

Figure 4. Inclusion body with fibrillar structures. Electron microscopy magnification 14000x.

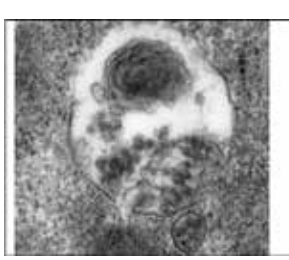

Figure 5. Inclusion body with rounded and fibrillar structures. Electron microscopy magnification 9000x.

For Chlamydia concentration and purification Chlamydia-containing suspension was 2-3 times frozen and thawed. Then we conducted ultrafiltration using a Pellicon- 2 system through a cartridge having a pore size of 300 kDa. The result was 1 liter concentrated Chlamydia-containing suspension.

**Conclusion and evaluation of research results**

Summarizing the data obtained during the implemented research we may say that applying the method of successive passaging the cultivation technology of field isolate of the local Chl. psittaci strain on a continuous M -Hella cell culture was worked out, Chlamydia-containing suspension was obtained, purified and concentrated. Electron microscopic study of Chlamydia-containing suspension was fulfilled.

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