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GROWING THEILERIA ANNULATA CELL CULTURE IN ARTIFICIAL ENVIRONMENTS

SH. A. Abdurasulov
C.b.s., Department of General Zootechnics and Veterinary Medicine,
Tashkent State Agrarian University

Abstract

Theileriosis is one of the blood-parasitic diseases of cattle and is considered the most dangerous among them. This disease occurs in all regions of our country and causes great economic damage to livestock. High mortality of animals from the disease (40-80%), i.e. abortions, reduced birth and milk yield, decreased weight and quality of meat of slaughtered animals, and costs of keeping and treating sick animals are determined.

As it was mentioned above, the daily milk production of dairy cows is 10-15 liters in the first 5-10 days, and after the disease, productivity decreases to 4-8 liters. After the animal recovers from the disease, only 70-75% of productivity is restored.

Breeding efforts to improve local livestock breeds in areas with theileriosis occur because high-yielding imported livestock are more susceptible to the disease.

In the last years of the last century, a number of scientists carried out a number of works on the growth of tissue cultures in artificial nutrient media for the reproduction of Theileria in cells. This method is promising, which, in turn, allows for a comprehensive study of the morphological and biological characteristics of the pathogen and the preparation of immunizing agents against theileriosis.

I. Tsur and S. Adler (1962) reported on the cultivation of Theileria annulata in monolayer cell culture, they isolated monolayer cell cultures from tissues infected with Theileria by washing with 0.25% trypsin solution. Cell cultures were kept for 3 months and it was found that the number of pomegranate bodies increased simultaneously with the increase in the number of cells in them.

L. Halliger (1965) grew three types of theileria (Theileria annulata, Theileria parva, Theileria lawrencei) in artificial media in a mixed culture of epithelial cells with lymph nodes, spleen and blood leukocyte cells of animals infected with theileriosis.

V. T. Zablotsky (1967) isolated parasites from lymph nodes and spleen cells from animals infected with theileriosis, in a mixed culture with chicken embryo cells, and grew them in artificial environments.

P. Khushmand et al. (1968) noted that strains with weak virulence are better grown than strains with high virulence. Highly virulent strains, on the other hand, almost do not grow in artificial environments.

Lymph nodes, spleen, or blood leukocytes from theilerized animals were used to obtain cultures of damaged cells. Parasites have been found to develop in the cytoplasm of lymphocytes, less often in cells such as macrophages. No parasites were detected in fibroblast-like cells. We investigated the possibility of growing Theileria annulata in cell cultures from different organs of calves that died during the incubation period, during the acute period of the disease, and during carriage of the parasites.

Material and Method

In order to obtain cell cultures infected with Theileria annulata schizonts, a strain with low virulence was used from theileriosis-free farms from all regions of our Republic. In the experiment, 6-10-month-old calves were infected by placing 10 male and 10 female hungry imago infected Hialomma anatolicum mites in special bags in the area near the female sac (regmonary lymph nodes).

In the acute period of the disease, in order to obtain cell cultures of severely damaged parenchymatous organs, liver and kidneys, in addition to lymph nodes and spleen, blood (pieces of spleen, kidneys and lymph nodes) were placed in Hanks' solution with antibiotics, and the crushed tissues of lymph nodes and spleen were washed with a nutrient change. Trypsinized, 2-3mm fragments of the target material were centrifuged in a refrigerated centrifuge at 3-5000 rpm for 10 minutes with the addition of Igla-99, RPMI nutrient media, after which the resulting pellet, trypsinized and washed lymphoid cells were added to 20% blood serum. and above-mentioned artificial nutrient media were suspended and poured into disposable sterile culture dishes. Kidney and liver tissues were trypsinized according to the generally accepted method, the cell concentration during cultivation was 600-700 thousand / ml. cultures were grown on day 4-6 at 37C, culture fluid was centrifuged at 2,000 rpm with suspended cells, supernatant was removed, pellet was resuspended in fresh Igla-99, or RPMI medium with 20% bovine serum. And the cell culture was incubated until a monolayer was formed in nutrient media specially prepared for the growth of Theileriosis schizonts.

Subsequent stages of subcultures were carried out on days 5-6 after treatment with a mixture of versen and trypsin solutions in a ratio of 9:1 using lymphoid cells and a cell monolayer in the culture fluid, the suspension was centrifuged at the same speed and method as mentioned above (at the same speed), a new Igla - 99, or resuspended in RPMI and 20% serum was added and transferred to other vials. Cytological analysis was performed on culture preparations grown in single-use 25 ml mats. Cell cultures grown in artificial media were stained by the Romanovsky-Giemsa method.

Research results. For experiments on culturing cells infected with Theileria annulata schizonts from calves. The material was obtained at different stages of the development of the pomegranate body - during the incubation and acute periods of the disease and during the transportation of parasites. We were unable to obtain cell cultures containing schizonts from the spleen and lymph nodes during the 8-10 day incubation period after ticks were attached to the body for feeding (injury). For no apparent reason, the cultures grew very slowly and the resulting monolayer contained fibroblast-like cells. We did not find cells containing Theileria in fingerprint smears and in preparations of growing cultures.

In most cases, cell culture was obtained during the acute phase of theileriosis. Contains schizonts. On the 7-8th day of cultivation, a cell monolayer was formed from the spleen and lymph node, and on the 4th day from the kidney, without changing the artificial nutrient media. Tissues - whole organ and fragmented sections, placed in Igla-99 or RPMI medium, treated with trypsin for different storage times. As mentioned, the viability of the cells in the tissue is divided into small pieces and placed in an artificial nutrient medium. Stored at 4C for 24 hours to 3 days. The initial trypsinized suspension was stored at 4C for up to 4 days, with monolayer formation

on day 7-8 after inoculation. The cell culture containing Theileria was kept in the laboratory for 10-12 months (observation period). In all cultures, spindle-shaped fibroblast-like cells with oval nuclei, round lymphoid cells of various sizes with pale cytoplasm and eccentrically located monocytoid nuclei, and well-defined light cytoplasm containing numerous theileria were observed in the first sections of all cultures. In the later stages, the number of lymphoid cells increased, and polymorphous nuclei of a fine structure with round, but clear nuclei of blue light were noted. A large number of mitoses were detected. Giant and multinucleated lymphoid cells were found.

In the third or fourth phase, the number of lymphoid cells increased and the transfer of the culture cell suspension to new mats was relatively easy, i.e. suspension cell culture was performed without centrifugation without the use of trypsin or versen mixtures, which led to the isolation of tissue culture free of fibroblast-like cells.

Starting from the sixth phase of the cell culture, polygonal cells of epithelioid type appeared in the culture, sometimes forming syncytium, in the rings of which many lymphoid cells are located. And schizonts were found in the cells.

With the growth of lymphoid cells, the nuclear chromatin structure became thinner, and their nuclei increased. sizes, sometimes occupied 1/3 of the core. Nuclei are polymorphic but have retained their round shape, and there are large vacuoles in the cytoplasm. A large number of binucleate and multinucleate cells appeared, some of which reached enormous sizes.

So. At the end of the incubation period and in the acute period of the disease, we were able to obtain in vitro lymphoid cells infected with Theileria annulata schizonts from the organs of experimentally infected calves and for long-term subcultivation. In a comparative study of the growth of lymphoid cells and the nature of their invasion, no significant differences were noted depending on the level of virulence.

In the acute period of the disease, with significant damage to parenchymatous organs, lymphoid cells containing schizonts in the liver and kidneys are successfully cultivated.

Tissue culture of Theileria annulata is used in immunization experiments and for diagnostics.

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