Volume 3, Issue 6, June – 2025 **ISSN (E):** 2938-379X

IMMUNOLOGICAL ANALYSIS OF CHLAMYDIASIS IN SHEEP AND PATHOMORPHOLOGY CHANGES IN THE FETAL

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Abstract
The results of an immunological analysis for chlamydia in pregnant women who suffered a miscarriage and had an abortion were found to be optically significant. A pathoanatomic examination of the internal organs of pregnant women who had an abortion at the age of 135-142 days showed that there were no histological data.

Keywords: Chlamydiosis, abot, fetus, Chlamydia abortus ovis, optical density, blood serum, internal organ, hematoxylin, eosin, dosator, chromogen, antitelo, immunoferment analysis, conjuncture, dystrophy, infiltration, myocardial, atelectasis.

Introduction

It is known that chlamydia of small and large horned animals causes significant economic damage in all farms in our republic.

Chlamydia is an enzootic, contagious infectious disease characterized by inflammation of the placenta, especially the cotyledons, and abortion in the 2nd half of gestation or the birth of

weak lambs and calves (young animals in general), as well as inflammation of the lungs (pneumonia).

According to the results of examinations, up to 12% of abortions among livestock are caused by chlamydia. Up to 50% of abortions in agricultural animals have been found as a result of chlamydia [1]. A large amount of money is spent on the treatment of sick animals and measures to combat the disease. According to some data, among small horned animals in the USA, 18-43.6; in Canada, 9-21.6; The prevalence of chlamydia in the Netherlands is 16-24.8; in France 18-57; in England 14-46.4; in Australia 6-26.2 and in Israel 3-34% [2].

The causative agent is Chlamydia abortus ovis, belonging to the Chlamydiacea family and the Chlamydiacea psittaci genus. Chlamydiae are intracellular parasites, measuring 250-300 nm. They are microorganisms with thick cell walls and contain DNA and RNA.

The causative agent of chlamydia has a complex antigenic structure, which contains 3 antigenic centers specific to the genus, species and serogroup. Its sex-dependence is due to the thermostability of the cell wall, like gram-negative bacteria, which is lipopolysaccharide [3].

Antigenicity is expressed in the presence of a specific receptor located on a carbohydrate, which determines the specificity of the species, and an oligosaccharide molecule consisting of 3 monomers. Antigenic serotypes differ in the specific localization of species-specific determinants of cysteine-rich amino acids in the protein membrane [4]. The body's fight against microbes is determined by the amount of immunoglobulins and their effect on pathogens. Immunoglobulin-E and immunoglobulin-D are practically not detected in farm animals [7]. Of the macroglobulins, IgM appears at the initial stage of immune reactions. IgG is the main immunoglobulin in blood serum, and there are two types of it, Ig-G1 and Ig-G2. In addition to immunoglobulins, the main cellular elements of the body are macrophages (monocytes), as well as activated T and B lymphocytes, which provide the body's resistance to microorganisms and viruses [5].

Antibiotics used in the treatment of diseases have a negative effect on the morphological and pathological state of the body's tissues and cells. It is necessary to take into account that polyclonal activation syndromes can be the cause of false positive results in such enzymatic and stepwise reactions.

At the same time, in the animal's body, during the individual (ontogonistic) period, special substances - superantigens - stimulate the production and response of B lymphocytes to specific protein enzymes that protect against various foreign antigens entering the body [8]. In practice, these processes are expressed in an uncharacteristic increase in antigen titers to many pathogens at the same time.

It is reported in the literature that false negative results in the detection of antigens may be due to immunodeficiency states, as well as technical errors in the formation of the reaction.

Purpose of the study. The main criteria of our experiment are the study of the results of the enzyme immunoassay reaction of sheep with a short period of time before birth and suspected of chlamydia, and the detection of pathomorphological changes in the internal organs of aborted fetuses at 135-142 days.

Object and methods of the study. The studies were conducted in the laboratories of Microbiology, Regional Diagnostics and Pathomorphology of the VITI and in production conditions at the specialized Karakul breeding farm "Turon" in the Mubarak district of the Kashkadarya region. In order to study the epizootic situation of chlamydia in the regions of our republic, pathological samples brought from farms were examined in collaboration with pathomorphology and microbiology laboratories. In a 136-day-old head of a sheep brought from the "Rokhat" farm in the Gallaorol district of the Jizzakh region, in a 4-month-old (141-day-old) aborted fetus belonging to the personal farm of "Yoldosh Suvonov" located in the Uzunsoy MFY of the same district, and in a small horned animal fetus (138-140-day-old) brought from the "Turon" Karakulchilkilik Naslchilik LLC in the Muborak district of the Kashkadarya region, external clinical signs of chlamydia were detected. In addition, blood sera from other aborted sheep from these farms were examined using an immunobiological method. After fixation of the aborted fetus, samples from the internal organs were subjected to histological processing and embedded in paraffin. Histochemists prepared histoplasms from the fixed sections on a slide microtome, stained with hematoxylin-eosin, and examined under a microscope.

Pathomorphological changes in the body of sheep infected with chlamydia were examined by dissecting the aborted fetus and using pathohistological and pathomorphological methods of parenchymal organs [9; p. 346].

Skin tissue samples, internal parenchymal organs (liver, lungs, spleen, lymph nodes, heart, and kidneys) taken from the fetus infected with chlamydia were examined by dissecting, and pathoanatomical changes in internal organs were examined by histological methods.

For histological examination of pathological materials (sections) taken from internal organs and tissues, a histopreparation was prepared using the paraffin method as follows (in 50-100 ml containers (dark glass bottles).

I. Fixation

1. The obtained pathological samples (sections) were stored in a 10-12% formalin solution for 24 hours;
2. They were stored in a 1:1 solution of 96° ethyl alcohol and formalin for 24 hours;
3. They were stored in Carnoy's liquid for 2-4 hours;
4. They were stored in 96°-100° alcohol for 12-24 hours.

II. Dehydration

To dehydrate the obtained pathological samples (sections), they were stored in a 96° alcohol solution for 24 hours;

1. The next day, they were also stored in a 96° alcohol solution for another 24 hours stored.

III. Paraffin embedding

1. Placed in a 1:1 solution of 96° alcohol and chloroform for 6-12 hours;
2. Stored in a pure chloroform solution for 6-12 hours. At the end of storage, the color of the sections was observed to lighten;
3. For uniform and better absorption of paraffin, the sections were placed in a 1:1 solution of melted paraffin and chloroform and left in a thermostat at a temperature of +35-40°C for 2-3 hours. Sometimes such solutions were stored in a solid state when not in use;

4. Then the sections were placed in melted paraffin stored in a thermostat at +54 - +55°C. In this case, the sections were first placed in portion I, i.e. in the melted paraffin in the first container for 1.5-2.5 hours, and then in portion II with the help of heated tweezers and kept for 0.5-1.5 hours The pieces were stored, paying attention to the size and thickness of the pieces;
5. The pieces were placed in a jar with glycerin on the bottom and heated to +60+70°C using a gas burner, and melted clean paraffin was poured over them until they were covered with a thickness of 0.5 cm. The pieces were placed at free distances from each other so that they could be easily separated;
6. The paraffin container with the pieces was cooled in a large container with cold water. In this case, the cooling of the paraffin was carried out from the bottom up, based on its melting;
7. When the paraffin solidified, it was cut from the edges, and since there were limited flow areas in the paraffin (which crumble and crumble when broken), a new portion of it was poured again;
8. Blocks were cut from the solidified paraffin, leaving a paraffin layer of at least 2 mm around the pieces was placed. Each piece was taken separately;
9. The obtained blocks were glued to the paraffin blocks with a heated spatula on the most part. Sections were taken from the blocks using a microtome, a micropreparation was prepared on a glass slide, stained with hematoxylin and eosin, and examined microscopically. As a result of microscopy, morphological changes that occurred in the internal organs of the aborted fetus were detected (Figure 1).

After the enzyme-linked immunosorbent assay was completed, the staining density of the liquid in the wells of the plate was measured using a special apparatus (colorimeter), and special equipment was used to calculate the results. The control samples were compared with their optical density, and the analysis results were mathematically processed. It was concluded that the higher the optical density in this well, the greater the amount of specific chlamydia antibodies in the sample.

For IFT, antigens were pre-adsorbed to the walls of the wells 96-well polystyrene plates were used. The serum to be tested was placed in the well of the plate. In this case, homologous antibodies bound to the pre-adsorbed antigen and bound. Unbound antibodies of chlamydia were washed out during the washing process. Then, a conjugate; enzyme-linked antibodies against rabbit or other animal immunoglobulins (anti-chlamydia antibodies) were added to the well. If detectable chlamydia antibodies are present in the serum to be tested, they act as antigens in this step and bind to the enzyme-linked chlamydia antibodies.

The chromogenic substance added after washing allowed to record the reaction by the development of staining in the wells. The intensity of staining is proportional to the amount of enzyme, therefore, chlamydia is quantitatively equivalent to the amount of antibodies.

When measuring the optical density of the liquid in the wells and comparing it with the control sample, the concentration of antibodies was calculated in units of volume. The calculation of the results in units of optical density was used. Each test system has its own indicators for recording the results of the ELISA, the levels of normal and pathological indicators. They are used as a basis for calculating the results of the immunoenzyme analysis.

When performing the ELISA, "Socorex" dispensers, an Elx405 microplate washing device, and an ELx808 microplate automatic analyzer were used. The interpretation of the results obtained during the reaction process was carried out electronically (on a computer) using the Bio-Tek KC4™ software.

In order to study the preventive effectiveness of the vaccine using IgM and IgG test kits prepared by "UNIGEN" and "XEMA" LLC, serological and immunological reactions in the body of sheep vaccinated with the vaccine and their natural disease were studied in 45 sheep divided into 3 groups.

15 sheep in the experimental group I were injected subcutaneously with the "Emulsified vaccine against chlamydia" 2 times.

15 sheep in the comparative group II were vaccinated with the "Anti-chlamydia vaccine" only once.

Group III (15 sheep) was a control group, and no drugs were used. The sheep allocated for the study were determined based on the anamnesis data of the farm veterinarian, taking into account the fact that the lambs that had aborted in the previous year and were not viable.

Results of the study:

We studied the enzyme-linked immunosorbent assay (ELISA) based on its ability to detect the antigen that triggers the reaction or the specific antibody produced against it in a relatively short time.

Although the ELISA method allows us to distinguish between infected and vaccinated animals in experimental small ruminants vaccinated against chlamydia, we used the serological (CBR) method to determine whether it is easier, faster and more convenient to diagnose than the immunological method (Table 1).

Optical density results Table 1

Optical Density Values

	1	2	3	4	5	6	7	8	9	10	11	12
A	**1,413**	**0,062**	**0,867**	**0,245**	**0,164**	**0,108**						
B	**1,451**	**0,069**	**0,962**	**1,520**	**0,135**	**0,228**						
C	**0,257**	**0,136**	**0,164**	**1,608**	**0,126**	**1,716**						
D	**0,224**	**0,228**	**0,135**	**1,518**	**0,164**	**1,129**						
E	**0,096**	**0,923**	**1,899**	**1,492**	**0,135**	**0,012**						
F	**0,092**	**1,926**	**0,892**	**1,441**	**1,899**	**1,652**						
G	**1,517**	**0,038**	**0,055**	**0,216**	**1,164**	**0,142**						
H	**1,578**	**0,052**	**0,034**	**1,517**	**0,135**	**0,081**						

The results of the table show that the samples in the first pair (A1 and B1) are optical density standards for negative samples, and the next pair are for positive blood samples (C1 and D1). According to the analysis of 45 blood samples tested, it was determined that the blood samples in wells G1, H1, F2, E3 were positive, while the blood samples in wells E2, A3, B3, F3 were suspicious.

Enzyme immunoassay is a laboratory study based on the high specificity and sensitivity of the "antigen-antibody" immunological reactions. IFT consists of 2 different components - immune and enzyme reactions. The immune reaction (microorganism and virus molecules) served as the binding of antigen and antibody.

The enzyme reaction made it possible to see and measure the results of the immunological reaction. In the farm of the "Turon" Karakulchilk breeding complex LLC, Mubarak district, Kashkadarya region, the enzyme-linked immunosorbent assay (ELISA) reaction was used as an immunobiological method to monitor the epizootological situation and determine the general immunophenotype. A total of 45 sheep vaccinated with an emulsified vaccine against chlamydia were isolated. When carrying out the reaction, first of all, measures were taken to ensure the rules of biological safety in the laboratory.

Enzyme-linked immunosorbent assay (ELISA) for the detection of IgG-specific antibodies to chlamydia in blood serum of cattle and small ruminants was performed using the test system "Set of reagents for the immunoenzymatic detection of IgG antibodies to the causative agent of chlamydia "Chlamydia IgG-IFA"" developed in collaboration with the Veterinary Research Institute and the joint ventures "UNIGEN" and "XEMA" LLC (Figures 1, 2). All ELISA reactions were performed in accordance with the instructions for use of the manufacturer of the test system kit and the general rules for performing the ELISA reaction.

The reactions were performed in parallel using the test kit "Set of reagents for the detection of antibodies to Chlamydia abortus bacteria by ELISA" (series 6, expiration date 2025.11) manufactured by JV TOO NIPTS "MVA GROUP" of the Republic of Kazakhstan. It was found that the results of the reactions in both cases were the same and the appropriate conclusions were drawn.

Figure 1. IgM-immunoglobulin

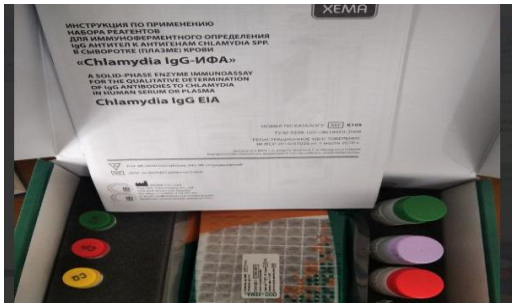

Figure 2. IgG-immunoglobulin

Table 2 Immunological analysis of chlamydiosis vaccine.

Groups	Number of animals	Type of analysis		
		C-reactive protein (normal 0.1-0.3 mg/l)	(normal 0.4-2.3 mg/l)	IgG (normal 7-16 mg/l)
I experimental group	15	0,32±0,025	2,8±0,23	17,1±0,96
II comparative group	15	0,287±0,015	2,04±0,143	16±1,144
III control group	15	0,108±0,0058	0,286±0,022	7,3±0,46

The level of C-reactive protein was found to be 1.55 times higher than the norm in experimental group I. In comparative experimental group II, it was at the norm, and in the first group it was found to be 1.13 times higher than in the comparative experimental group. When determining

the chronic course of the disease based on the change in IgG, it was found that experimental group I gave a result 1.69 times higher than the norm, and in group II - 1.21 times higher. In particular, these indicators were found to be high in IgM and IgG in the first group, with a slight difference compared to group II, and significantly higher than in group III.

During the tests conducted in the "Rohat" farm of Gallaorol district of Jizzakh region and in the "Jizzakhlik" municipal economic unit of Jizzakh region, IgM indicators were determined by the method of immunoenzymatic analysis in 43 blood samples and fetuses that were aborted at 130-140 days of age and belonging to citizens. In these farms, two sheep fetuses were pathologically examined, and the parenchymal organs and the caruncles and cotyledons around the uterus were histologically examined.

In order to study the epizootic situation of chlamydia in the regions of our republic, pathological samples brought from the farms were thoroughly pathologically examined in collaboration with employees of regional diagnostics and microbiology laboratories. During the examination of these animals, the following pathological conditions were mainly observed.

During pathological examinations, atelectasis, air accumulation, and blood stagnation were observed in the lungs of the lambs. It was found that the heart muscles were relaxed, punctate and spotty hemorrhages developed in the auricles, and a very small amount of blood was present in the heart chambers. These changes indicate heart failure, and this process also affected other internal organs. The liver consistency is dense, abscesses and necrosis of various sizes have developed. The spleen is loose and there are edemas in some places on its surface, and there are pinpoint hemorrhages on the surface of the kidneys. There are complete catarrhal inflammations around the spleen.

After fixation, pathological samples from all these fetuses were embedded in paraffin and histosms were prepared on a slide microtome and stained with hematoxylin-eosin. When the prepared histological sections were examined under a microscope, the following results were found.

The walls of the pulmonary vessels are thickened, hyperemia of the interalveolar barriers and polymorphic cellular infiltrates are observed. These changes, in turn, caused the development of various pneumonias, namely, small-focal, focal and focal-communicated inflammations (Fig. 3).

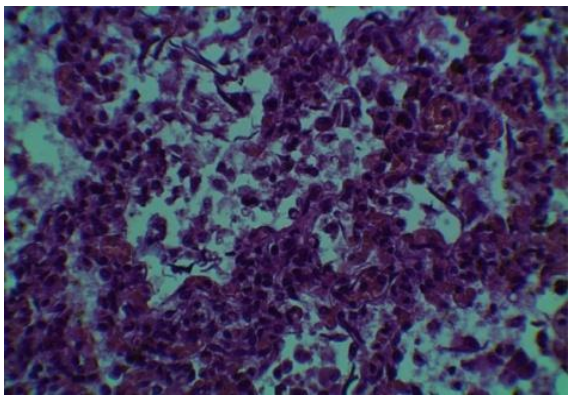

Figure 3. Hyperemia of interalveolar barriers in lungs and various cellular infiltration.

The connective tissue basis of the heart - the stroma - was swollen, and the formation of collagen and elastic fibers in various parts of the myocardium, as well as obvious dystrophy of cardiomyocytes, were shown on the basis of reactions of vessels and cells (Fig. 4).

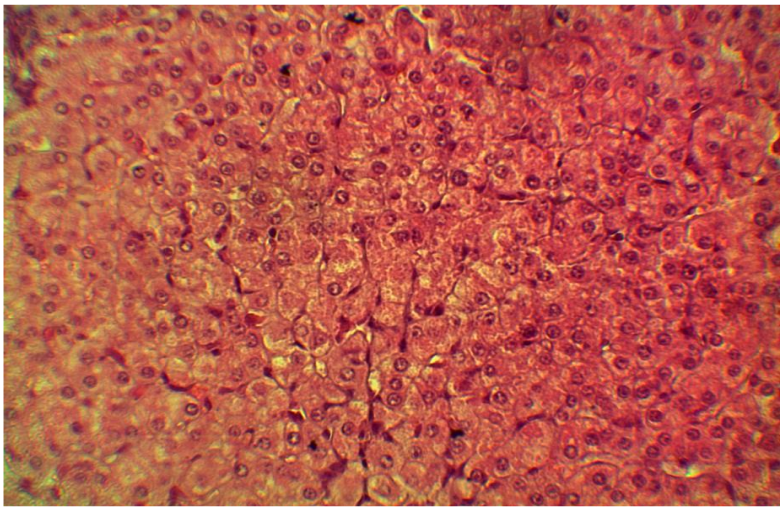

Figure 5. Hydropic dystrophy of hepatocytes.

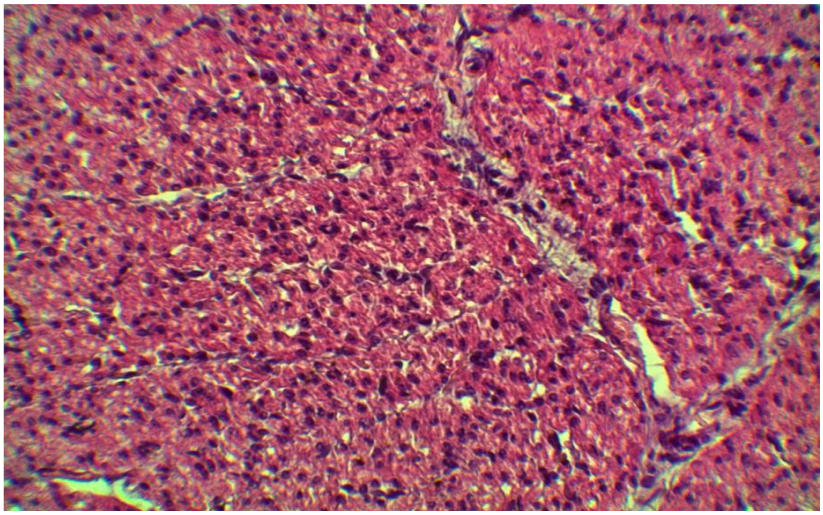

Figure 4. Cardiac stromal thickening and cardiomyocyte dystrophy.

Due to changes in the permeability of the vessel walls, hepatocytes of the liver develop various degrees of hyaline droplet and watery (hydrophic) dystrophies. There are also tissue inflammatory reactions in the liver tracts (Fig. 5).

The formation of lympho-macrophage infiltrates with a small number of plasma cells indicates the presence of an infectious inflammatory process in the liver.

Thus when chlamydiosis of small horned animals is immunologically diagnosed; manifestations in the form of tissue swelling, hemodynamic disorders, alterative, immunopathological processes and systemic inflammations were determined in the case of these farms.

Conclusions:

1. In the Gallaorol district of Jizzakh region, in the private farm "Rohat", located in the territory of the Uzunsoy municipal economic unit, in the farms of I. Egamov located in Sh. Rashidov district, in the farm of B. Avliyakulov and "XUMO" LLC of Gallaorol district, in the farm of Forish district, in the farm of "JIZZAX ORGANIK" LLC of Zamin district, out of 153 blood samples tested for the latent form of chlamydia (IgG), 39, or 25.49 percent, were positive and 105, or 68.63 percent, were negative. The total number of samples tested was 5.7 percent across the region.
2. Serological (CBR) and immunological (IFT) methods for diagnosing chlamydia were used, and the convenience of using enzyme-linked immunosorbent assay was found, despite the high sensitivity and specificity of the reactions in both cases.
3. The cultural morphological (in the light microscopic view of the mature morphological structure of chlamydia, it is spherical in shape with a diameter of up to 250-350 nm, bounded by a rigid cell wall and cytoplasmic membrane), tinctorial and pathogenicity (pathogenicity for white mice is from 10 to 40 days) characteristics of the causative agent of chlamydia detected in farms of Jizzakh and Kashkadarya regions were studied.
4. According to the sensitivity of chlamydiosis to antibiotics, it was found that roxilong-300 is sensitive to doxilox, teliosin, oxacillin, gentamicin are not sensitive and less sensitive to erythromycin.

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