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Chlamydiosis in Sheep: Immunological Examination And Pathomorphological Changes

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Abstract

The article provides information on the stability of the immune system and path morphological changes in chlamydia when using the formalin GOA vaccine against chlamydia in sheep.

Keywords: *Chlamydia, Immunoglobulin, Vaccine, Immunity, Immunophone, Antigen, Microorganism, Receptor, Serotype*

1. Introduction

Relevance of the research

Chlamydiosis in small and big horned animals is well documented to inflict significant economic harm to all farms in our nation. Investigations have shown that chlamydia is responsible for an average of 12 percent of abortions in animals. Up to 50% of miscarriages in farm animals are caused by chlamydia, claim I.I. Nosov and A.A. Volkova. The care of sick animals and the efforts to stop the sickness cost a lot of money.

According to the literature, the prevalence of chlamydia is 18-43.6 in the United States, 9-21.6 in Canada, 16-24.8 in the Netherlands, 18-57 in France, and 14-46.4 in England. In Australia and Israel, the prevalences are 6-26.2% and 3-34%, respectively.

Inflammation of the placenta, particularly the cotyledons, abortion in the second half of the cervix, the birth of weak lambs and calves (often young animals), and inflammation of the lungs (pneumonia) are all symptoms of the spreading, enzootic infection known as chlamydia.

Chlamydia abortus ovis, a member of the Chlamydiacea family and Chlamydiacea psittaci genus, is the responsible party. Chlamydia is a parasite that may grow up to 250–300 nm in size. They are bacteria with thick cell walls that carry DNA and RNA.

The chlamydia-causing agent has a complicated antigen structure with three antigenic centers that are unique to the genus, species, and serogroup. Its gender is lipopolysaccharide because, like gram-negative bacteria, it has a thermostable cell wall. The binding epitope, which establishes the specificity of the genus, comprises of a unique receptor found in carbohydrates and an oligosaccharide molecule made up of three monomers, which expresses antigenicity (G.A. Dmitriev et al., 1999). The particular location of cysteine-rich amino acids in the protein membrane of species-specific determinants varies between antigen serotypes.

The purpose of the research. Stability of the effect of the GOA formalin vaccine against sheep chlamydiosis on the immune system with IgM and IgG test kits developed by JV "UNIGEN" is the main criterion of our experience to determine the effectiveness of the vaccine.

2. Materials And Methods

Research was done at the VITI Regional Diagnostics, Microbiology, and Pathomorphology laboratories as well as in the livestock complex in the Dehkanabad District of the Kashkadarya Region called "Karakolchilik Shirkati named after M. Ibragimov."

The quantity of immunoglobulins and how they affected the infections affected the body's ability to fight against germs. Farm animals almost never have immunoglobulin-E or immunoglobulin-D identified (F.J. Bourne et al. 1978). The first step of immunological responses is marked by the appearance of IgM from macroglobulins. The primary immunoglobulin in blood serum is IgG, which comes in two varieties: IgG_1 and IgG_2. The primary cellular components of the body, in addition to immunoglobulins, are macrophages (monocytes), active T and V lymphocytes, and immunoglobulins, which enable the body's resistance against viruses and microbes (Green S.A., Albulov A.I., Ruban E.A., Green A.V.). The morphological and pathological status of the body's tissues and cells is negatively impacted by the antibiotics used to treat the disease. It is important to remember that polyclonal activation syndromes are what lead to false positive outcomes in such enzymatic and sequential processes. The creation of specific defensive protein enzymes against diverse foreign antigens entering the body is simultaneously stimulated by superantigens, which are present in the individual (ontogonistic) phase of the animal body, and V cells respond in a particular manner. In actuality, these activities manifest as a simultaneous, non-specific rise in the titer of antigens to several infections. According to literature sources, technical flaws in the production of the response as well as immunodeficiency circumstances may be to blame for false negative findings in the detection of antigens. A specific apparatus (colorimeter) was used to measure the color intensity of the liquid in the tablet wells after the reaction had halted, and a special gadget was utilized to compute the results. The control samples' optical densities were compared, and the analysis's findings underwent mathematical processing. It was determined that the sample contained more particular chlamydial antibodies the greater the optical density in this well.

Antigen was preadsorbed on the well walls of 96-well polystyrene tablets for IEA. The well of the tablet was filled with the blood serum that would be evaluated. In this instance, the homologous antibodies join after attaching to the previously adsorbed antigen. During washing, chlamydia antibodies that are not bound are eliminated. The well was subsequently filled with enzyme-labeled antibodies (chlamydia antibodies) directed to rabbit or other animal immunoglobulins. If any chlamydial antibodies are found in the blood serum under examination, they act as antigens at this point and mix with chlamydial antibodies found for the enzyme. After washing, a chromogenic (coloring) material was applied, allowing for the possibility of accounting for the reaction on the growth of staining in the wells. Since the number of chlamydia antibodies and the amount of enzyme are quantitatively identical, the intensity of staining is proportional to both. The optical density of the liquid in the wells was measured and compared to the optical density of the control sample to determine the antibody concentration per unit volume. Results were calculated using optical density units. For taking IEA findings, levels of normal indicators, and pathological indicators into consideration, each test system has its own indicators. Results of immunoenzyme analysis are based on them.

IEA was conducted using "Socorex" dispensers, ELx405 microplate cleaning apparatus, and ELx808 microplate automated analyzers. Using a computer and the Bio-Tek KC4TM software, the reaction's findings were electronically (and hence electronically) interpreted.

In an experiment, the serological and immunological responses in sheep that had received the vaccination were examined to determine the efficacy of the IgM and IgG test kits created by "UNIGEN" and "XEMA" LLC as preventative measures.

Ten sheep were administered two subcutaneous injections of the "emulsified vaccine against chlamydiosis" in experimental group I.

Only one dose of the "Chlamydiosis vaccine" was administered to the II comparable group of 10 heads.

Group III (10 heads) served as the control group, receiving no medication. Based on the farm veterinarian's anamnesis data and taking into consideration the fact that the lambs that miscarried and gave birth the previous year were not viable, the sheep chosen for the study were decided.

3. Results and Discussion

We studied immunoenzymatic analysis (IEA) based on the short-term detection of the reaction against the provoking antigen or the specific antibody formed against it. Although the IEA method allows differentiating between infected and vaccinated animals in the case of small horned animals vaccinated against chlamydia in the experiment, we used the serological (KBR) method in order to determine that it is easier, faster, and more convenient to diagnose compared to the immunological method (Table 1).

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	000	000	000	000	000	000	000	000
B	1,451	0,069	0,962	000	000	000	000	000	000	000	000	000
C	0,257	0,136	0,164	000	000	000	000	000	000	000	000	000
D	0,224	0,228	0,135	000	000	000	000	000	000	000	000	000
E	0,096	0,923	1,899	000	000	000	000	000	000	000	000	000
F	0,092	1,926	0,892	000	000	000	000	000	000	000	000	000
G	1,517	0,038	0,055	000	000	000	000	000	000	000	000	000
H	1,578	0,052	0,034	000	000	000	000	000	000	000	000	000

The samples in the first pair (A1 and V1) are the optical density standards for negative samples, while the following pair (S1 and D1) are for positive blood samples, as can be seen from the table's data. Blood samples from cells G1, H1, F2, and E3 were positive, whereas blood samples from cells E2, A3, and B3 were doubtful, according to the examination of 21 analyzed blood samples.

Based on the high specificity and sensitivity of "antigen-antibody" immunological responses, enzyme immunoassay is a laboratory experiment. IEA is made up of two distinct parts: immunological and enzymatic processes. The immunological response (bacteria and viral molecules) acted as an antigen and a binding site for antibodies.

Additionally, the outcomes of the immunological reaction may be observed and quantified thanks to the enzymatic reaction. In order to manage the epizootological situation and ascertain the general immunophone in the farm of "Karakolchilik shirkat named after M. Ibragimov" LLC, Kashkadarya area, Dehqonabad district, immunoenzymatic analysis (IEA, ELISA) reaction was utilized as an immunobiological approach. Prior to starting the reaction, precautions were taken to assure compliance with the rules and guidelines for biological safety in the laboratory.

Immunoenzymatic analysis (IEA or ELISA) for detection of IgG-specific antibodies against chlamydia in the blood sera of cattle and small horned animals. "Chlamydia IgG-IFA" IgG antibodies to the causative agent of chlamydia "Chlamydia IgG-IFA" developed in cooperation with the joint ventures "UNIGEN" and "XEMA" LLC. "Set of reagents for detection with" ("Nabor reagentov dlya immunofermentnogo vyyavleniya IgG antitel k vozbuditelyu chlamydiosis krupnogo i melkogo rogatogo skota") was performed using the test system (Figures 1, 2). and was performed following the general rules for performing the IEA reaction.

Figure 1. IgM-immunoglobulin

Figure 2. IgG-immunoglobulin

Table 2 Immunological analysis of chlamydiosis vaccine.

		Types of analysis

Groups	Number of animals	C-reactive protein (normally 0.1-0.3 mg/l)	IgM (normally 0.4-2.3 mg/l)	IgG (normally 7-16 mg/l)
Experimental group I	10	0,32±0,025	2,8±0,23	17,1±0,96
Experimental group II	10	0,287±0,015	2,04±0,143	16±1,144
Experimental group III	10	0,108±0,0058	0,286±0,022	7,3±0,46

In experimental group I, it was discovered that the level of C-reactive protein was 1.55 times greater than the average. It was discovered to be 1.13 times higher than the first comparison experimental group and at the standard level in the II comparative experiment group. It was discovered that experimental group I had a result that was 1.69 times higher than the norm and group II had a result that was 1.21 times higher when determining the course of the disease in a chronic condition according to the change of IgG. IgM and IgG levels were found to be particularly high in the first group, with just a slight difference from the II group and a significantly greater effect from the III group.

"M.Ibragimov" firm farm, Dehkanabad district, Kashkadarya area; and "Jizzakhlik" m.f.y., Jizzakh region. By using the enzyme-linked immunosorbent assay technique, IgM markers were found in 11 samples of citizen blood as well as aborted babies that were 4 months old. In these farms, the parenchymatous organs, caruncles, and cotyledons around the uterus of two sheep were histologically analyzed.

In collaboration with the staff of the local diagnostics and microbiology laboratories, pathological samples collected from farms were thoroughly examined pathologoanatomically in order to research the epizootic status of chlamydia illness in the areas of our country. The following pathological disorders were primarily seen in these animals when they were being examined.

During pathologoanatomical investigations, atelectasis, air buildup, and blood stagnation were seen in the lambs' lungs. It was discovered that the heart muscles were relaxed, the auricles were developing dotted and spotted hemorrhages, and the volume of blood in the heart chambers was extremely low. These alterations suggest heart failure, and other internal organs have also been impacted by this process. The liver's consistency has hardened, and various-sized abscesses and necrosis foci have appeared. The kidneys' surface has point hemorrhages, the spleen is loose, and there are swellings in certain places on its surface. There is a buildup of gas and catarrhal irritation, and the undigested milk in the rennet has transformed into a floating mass.

All of these animals' pathological samples were fixed after which they were embedded in paraffin, cut into histosections, and stained with hematoxylin-eosin. The following findings were found when the produced histological slices were viewed under a microscope.

Hyperemia of the interalveolar barriers, enlarged pulmonary artery walls, and polymorphic cellular infiltrates are also seen. As a result of these modifications, different pneumonias, or inflammations with tiny foci, foci, and inflammations related to foci, developed (Fig. 8).

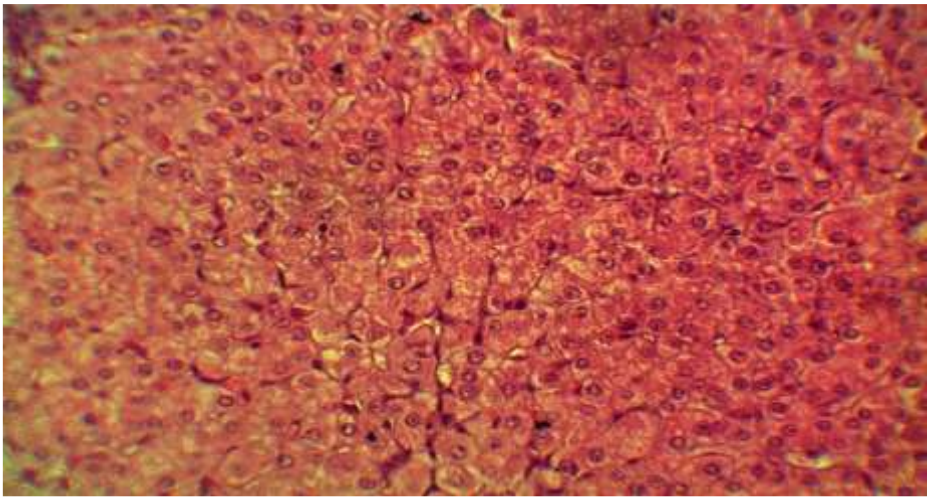

Figure 10. Hydropic dystrophy of hepatocytes.

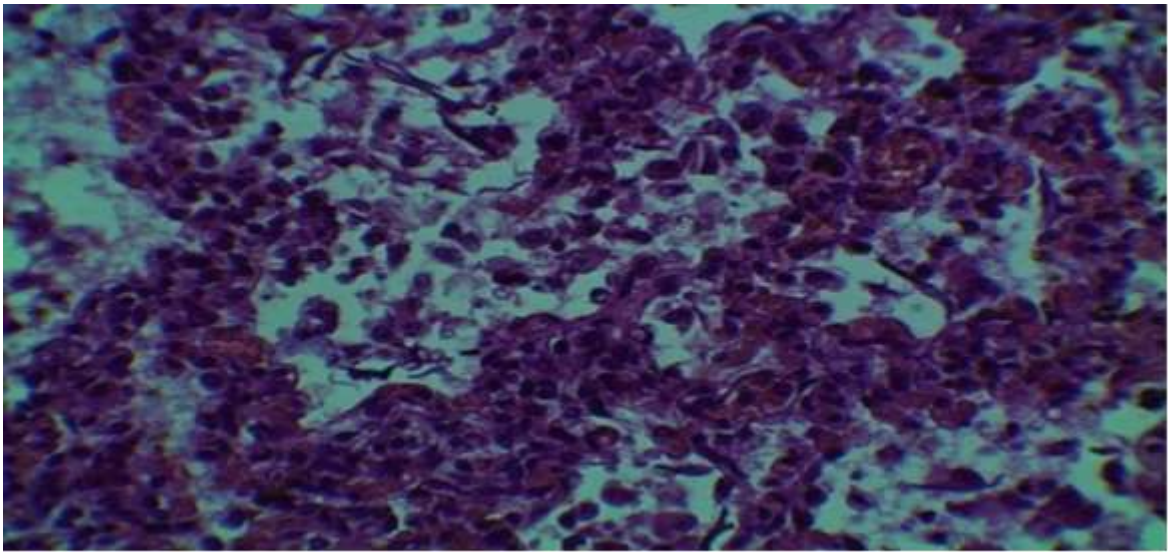

Figure 8. Hyperemia of the interalveolar barriers in the lungs and various cellular infiltration.

On the basis of responses of arteries and cells, the stroma, the connective tissue that forms the foundation of the heart, swelled. Different regions of the myocardium also developed collagen and elastic fibers, and the cardiomyocytes clearly underwent dystrophy (Fig. 9).

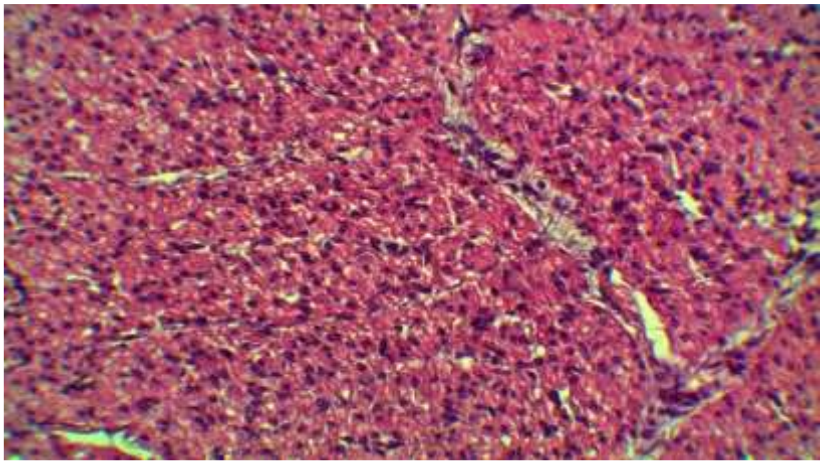

Figure 9. Heart stroma swelling and cardiomycetic dystrophy.

Due to the change in the permeability of the vessel walls, hepatocytes of the liver develop various degrees of hyaline droplet and watery (hydrophic) dystrophies. There are also inflammatory reactions in the tissues of the liver tract (Fig. 10).

An infectious inflammatory process is present in the liver if lympho-macrophage infiltrates with a few plasma cells start to develop.

As a result, tissue degradation, hemodynamic abnormalities, alterative processes, immunopathological processes, and systemic inflammation were all symptoms of small horned animal chlamydiosis.

4. Conclusion

It From February to May, Samarkand region animal farms tested positive for 8.2 percent chlamydia; Kashkadarya region farms tested positive for 6.9 percent chlamydia. Despite the excellent sensitivity and accuracy of the responses in both cases when diagnosing chlamydiosis by serological (KBR) and immunological (IFT) approaches, it was discovered that immunoenzymatic analysis was simple to perform. The chlamydiosis-causing agent's cultural, morphological, tinctorial, virulence, biochemical, and pathogenic properties were found in several cattle farms with a focus on breeding in the Kashkadarya and Samarkand areas. It was shown that oleandomycin is sensitive to doxilox, teliosin, oxacillin, and gentamicin are not sensitive, and erythromycin is less sensitive than these antibiotics in treating chlamydia.

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