INTERNATIONAL JOURNAL ON ORANGE TECHNOLOGY

https://journals.researchparks.org/index.php/IJOT e-ISSN: 2615-8140 | p-ISSN: 2615-7071
Volume: 5 Issue: 4 | April 2023

Determination of Immunobiological Reaction in Sheep Chlamydiosis

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Abstract: The article reports on the stability of the immune system in the body when using the GOA formalin vaccine against sheep chlamydiosis.

Key words: Chlamydia, immunoglobulin, vaccine, immunity, immunophone, antigen, microorganism, receptor, serotype.

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, chlamydia prevalence rates for small horned animals were 18-43.6 in the USA, 9-21.6 in Canada, 16-24.8 in the Netherlands, 18-57 in France, 14-46.4 in England, 6-26.2 in Australia, and 3-34 percent in Israel.

Chlamydia is a contagious, enzootic infectious illness that causes inflammation of the lungs, abortion in the second part of the cervix, incompetent lambs and calves (usually in young animals), and inflammation of the placenta, especially the cotyledons. (pneumonia).

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 and includes a unique receptor found in carbohydrates, and an oligosaccharide molecule with three monomers are the two components that define 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.

Purpose of the research. The key criteria of our experiment to assess the efficacy of the vaccination is stability of the impact of the GOA formalin vaccine against sheep chlamydiosis on

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the immune system using IgM and IgG test kits created by JV "UNIGEN."

Research object and methods. Research was conducted in VITI's Microbiology, Regional Diagnostics, and Young Livestock Disease Research Laboratories as well as under production circumstances at the livestock complex in the Kashkadarya region's Dehqonabad district 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 in them. (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: Ig–G_1 and Ig–G_2. The primary cellular components of the body, in addition to immunoglobulins, are macrophages (monocytes), as well as active T and V lymphocytes, which guarantee the body's resistance against pathogens and viruses. (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 IFT. 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. Chlamydia antibodies were then introduced to the well along with enzyme-labeled antibodies against 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 staining intensity is inversely correlated with enzyme concentration, chlamydia antibody concentration is likewise inversely correlated with enzyme concentration. (they are quantitatively equivalent). The optical density of the liquid in the wells was measured and compared to the

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optical density of the control sample to determine the antibody concentration per unit volume. Results were calculated using optical density units. For taking IFT 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.

IFT 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 a test to determine the preventative efficacy of IgM and IgG test kits created by "UNIGEN" and "XEMA" LLC, serological and immunological responses in the body of sheep that had received the vaccination were examined.

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

Only one of the ten heads in the II comparative group received the "Chlamydiosis vaccine."

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.

Results. Based on the short-term detection of the response to the inciting antigen or the particular antibody generated against it, we investigated immunoenzymatic analysis (IFT). In spite of the fact that the serological (KBR) method can distinguish between infected and vaccinated animals in the case of small horned animals immunized against chlamydia in the experiment, we used it to find that it is simpler, quicker, and more practical to diagnose than the immunological method. (1 -table).

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

The samples in the first pair (A1 and V1) are the optical density standards for negative samples, while the samples in the next pair are for positive blood samples, as can be seen from the findings of the table. (S1 and D1). Blood samples from cells G1, H1, F2, and E3 were found to be positive in the examination of 21 examined blood samples, whereas blood samples from

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cells E2, A3, and B3 were determined to be suspicious.

Based on the high specificity and sensitivity of "antigen-antibody" immunological responses, enzyme immunoassay is a laboratory experiment. IFT 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 (IFT, 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.

Detecting IgG-specific antibodies against chlamydia in the blood sera of cattle and small horned animals using immunoenzymatic analysis (IFT or ELISA). Chlamydia IgG-IFA was created in collaboration with the Veterinary Institute of Veterinary Medicine and the joint ventures of "UNIGEN" and "XEMA" LLC. It is an IgG antibody against the chlamydia-causing agent. The test system was used to perform "Set of reagents for detection with" ("Nabor reagentov dlya immunofermentnogo vyyavleniya IgG antitel k vozbuditelyu chlamydiosis krupnogo i melkogo hornatogo skota"). (Figures 1, 2). and were carried out in accordance with the standard guidelines for carrying out the IFT response.

Figure 1 IgM- immunoglobulin

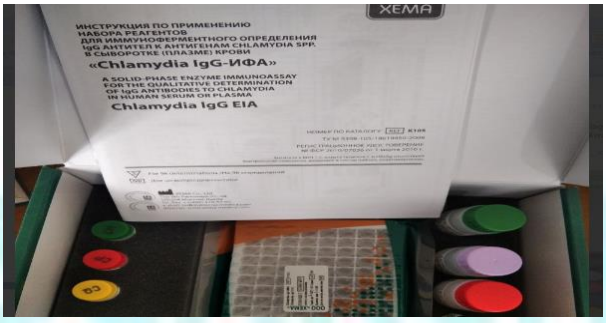

Figure 2 IgG- immunoglobulin

Table 2

Immunological analysis of chlamydiosis vaccine.

Groups	Number of animals	Types of analysis		
		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
Comparative group II	10	0,287±0,015	2,04±0,143	16±1,144
Control group III	10	0,108±0,0058	0,286±0,022	7,3±0,46

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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 greater than the first group II, which was in the comparative experimental group II at the standard level. It was discovered that experimental group I was 1.69 times higher than the norm than the control group, and group II was 1.21 times higher when assessing 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.

Conclusions:

1. From February to May, animal farms in the Samarkand area tested positive for 8.2 percent of chlamydia; farms in the Kashkadarya region tested positive for 6.9 percent of chlamydia.

2. 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.

3. The chlamydiosis-causing agent's cultural, morphological, tinctorial, virulence, biochemical, and pathogenic properties were detected in several cattle farms with a focus on breeding in the Kashkadarya and Samarkand areas.

4. Based on the sensitivity of chlamydia to antibiotics, it was shown that erythromycin is less sensitive than oleandomycin, doxilox, teliosin, oxacillin, and doxilox.

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