



THE ROLE OF CHITOSAN IN THE PATHOMORPHOLOGY AND IMMUNOPROPHYLAXIS OF COLIBACILLOSIS OF CALVES

N.I. Navruzov

Junior researcher Veterinary Research Institute.

B.A. Elmurodov

Doctor of veterinary sciences, senior researcher
Veterinary Research Institute.

G. Kh. Mamadullaev

Doctor of veterinary sciences, senior researcher
Veterinary Research Institute

| Article history: | Abstract: |
|--|--|
| Received: April 11 th 2021 Accepted: May 1 st 2021 Published: May 31 th 2021 | The article presents the results of pathological anatomical and histological changes in the body of calves with colibacillosis under natural conditions, when the polyvalent GOA against colibacillosis, salmonellosis and pasteurellosis is used in combination with the formol vaccine and natural chitosan. |
| Keywords: Colibacillosis, immunoglobulin, agglutinabilation, chitosan, immunostimulant, vaccine, salmonellas, pasteurellosis, plasmocyte, lymphoblast, fibroblast, atelectasis, exudate, hemostasis, plasmorrhagia. | |

INTRODUCTION

Agriculture has a special place in our country economy and our state attaches great importance to this sector development. The development and increase the livestock efficiency, which is the main agriculture branch, depends on factors such as increase the number of stocks, increase productivity, get a healthy cubs, proper maintenance, protection from various infectious and invasive diseases. Infectious diseases of stocks are a major risk factor in livestock. Colibacillosis, which is especially common among cubs, causes great economic detriment. According to B.F. Bessarabov, E.S. Voronin (2007), colibacillosis was highlighted to be 13–50.8 % in cubs in the United States, 11–29 % in Canada, 6 % in the Netherlands, 58 % in France, 4 % in the UK, 6 % in Australia and 6–47 % in Israel.

Considering the dearth of veterinary drugs in private, subsidiary farms and the influx of foreign countries our research focuses on creating competitive biopreparations using local resources. As long as the biopreparations produced at the Veterinary research institute, it is possible to save the state currency spent on imports from out of the country.

Presently, the use of natural, organic, pure ecological products is important for the national economy. Especially, in agriculture, veterinary medicine spheres and pure natural ecological products are of paramount importance. Enrichment of veterinary drugs with natural environmentally friendly organic substances increases the natural resistance of animals and the immune system is stable.

Colibacillosis is an acute infectious disease of cubs, largely occurring at 2-7 days of life of newborn calves, piglets, lambs and chickens from the first day to 3-5 months and in fur animals at 1-5 days.

Colibacillosis is characterized by colitis, severe diarrhea, septicemia, and weakness symptoms.

It is natural that the animal's immune system is weakened, leading to frequent infections and invasive diseases (respiratory, gastrointestinal). In particular, this condition increases the economic damage when young calves are infected with colibacillosis. Antibiotics used in the treatment of this disease type adversely affect the body's immune system, morphopathological, morpho-functional state.

The ecologically pure drug chitosan has been widely studied in fauna, however its importance in the stocks, the effect degree, the changes in the immune system have not yet been studied.

Therefore, our study purpose is the research of colibacillosis immunoprophylaxis and pathomorphology in calves with the chitosan addition to the mixture "Polyvalent GOA formal vaccine against stock colibacillosis, salmonellosis and pasteurellosis" (0.03 ml of 4% chitosan succinate solution per 1 ml of vaccine).

RESEARCH OBJECT, MATERIALS AND METHODS.

The researches were conducted by VRI Microbiology laboratory, immunology laboratory of "Sangzor" diagnostic center in Samarkand, pathanatomy department of Samarkand medical institute and in the specialized complex for animal breeding "Siyob-Shavkat-Orzu".

The main criterion of our experience is the enrichment of vaccines against colibacillosis, pasteurellosis and salmonellosis with a natural, ecologically pure compound - "chitosan".

With the addition of chitosan succinate (4%) solution to the "Polyvalent GOA formal vaccine against pasteurellosis, colibacillosis and salmonellosis in farm animals" prepared by VRI Microbiology laboratory in order to study the effectiveness of prophylaxis in the experiment, immunological reactions, pathoanatomical and histological studies in the calves body vaccinated with chitosan and naturally infected calves were studied in 9 head calves divided into 3 groups.

3 head of calves in experimental group I were injected subcutaneously with a natural organic, environmentally friendly chitosan succinate (4% solution) added to the "Polyvalent GOA formal vaccine for colibacillosis, salmonellosis, and pasteurellosis in farm animals".

Experimental group II was vaccinated only with the "Polyvalent GOA formol vaccine against colibacillosis, salmonellosis, and pasteurellosis of stocks."

Group III was the control group and no biopreparations were applied to them.

RESEARCH RESULTS

The body's fight against germs and viruses is determined by the immunoglobulins activity. Immunoglobulins E and D are almost non-existent in the stocks body. (F.J. Bourne and etc. 1978).

IgM are macroglobulins that are formed in the early stages of immune reactions. IgG is the major immunoglobulin in the serum, of which there are two types, IgG1 and IgG2. In addition to immunoglobulins, the main cellular elements of the body are macrophages (monocytes), as well as vital T- and V-lymphocytes. The results of pre- and post-agglutination reactions and immunological (IgM and ImG) analysis before the vaccines introduction with "naturally activated" chitosan solution in calves during the study are presented in Table 1. Immunological and agglutination reaction analyzes were performed on serum obtained from calves in the experimental and control group on days 15, 30, and 90 of the experiment(**Table №1**).

Immunological analysis of chitosan succinate preparation and vaccine association.

| Groups | Animal number | Types of analysis | | | | |
|----------------------|---------------|----------------------------------|---------------------------------|---|-----------------------|----------------------|
| | | AR titer (before the experiment) | AR titer (after the experiment) | C reactive protein is normally 0.1-0.3 mg/l | IgM norm 0.4-2.3 mg/l | IgG norm 7 - 16 mg/l |
| I group experience | 1 | 1 : 50 | 1 : 1600 | 0,31 | 2,6 | 20 |
| | 2 | 1 : 50 | 1 : 800 | 0,34 | 2,5 | 16 |
| | 3 | 1 : 100 | 1 : 1600 | 0,36 | 2,8 | 18 |
| II comparative group | 4 | 1 : 50 | 1 : 400 | 0,3 | 2 | 17 |
| | 5 | 1 : 50 | 1 : 800 | 0,26 | 2,4 | 16 |
| | 6 | 1 : 100 | 1 : 400 | 0,24 | 2,2 | 17 |
| control group | 7 | 1 : 100 | 1 : 50 | 0,12 | 0,9 | 9 |
| | 8 | 1 : 50 | 1 : 100 | 0,16 | 1,2 | 12 |
| | 9 | 1 : 50 | 1 : 50 | 0,13 | 1,0 | 8 |

The table shows that the average antibody titer to colibacillosis pathogens in experimental group I calves was 1: 1333, in group II - 1: 533, in control group III, the titer was 1:67, the immunoglobulins level in the blood was 0.5 mg/l higher than normal in this group, normal in group II, calves in group III were 1.03 less than normal.

In particular, it was found that these indicators had higher levels of IgM and IgG in the first group, a slight difference compared to group II, and a much higher effect than in group III.

At the "Siyob-Shavkat-Orzu" farm, the internal organs of calves with natural colibacillosis were studied using pathological and histological methods.

Pathological changes in calves with colibacillosis were observed mainly in the presence of inflammation in the stomach and intestines. The causative agent of Escherichia coli is localized in stromal tissue, connective tissue layers, and perivascular spaces in the abdomen, intestines, lungs, and liver. The pathogen glomerulocyte accumulates and is located in the right tubules of the kidneys. An uneven distribution of the pathological process according to the course of the infectious process was revealed. The active agent is released from the blood, urine, manure, parenchymal organs, accompanied by inflammation of the gastrointestinal mucosa, digestive disorders, the degenerative processes development, the microflora penetration through the damaged wall, the blood introduction and lymph into other organs. The waste products of the microflora were absorbed from the gastrointestinal tract, causing the body poisoning. They, in turn, irritated the nerve nodes of the intestine and exacerbated the intestinal system contraction, leading to diarrhea. The body examination from the outside revealed the presence of the mucous membranes anemia. After an abortion, the skin surface is covered with manure. Examination of the internal organs revealed bleeding in the mucous membranes of the heart, lungs, kidneys, liver, spleen and intestines.

The spleen is slightly enlarged. Abomasum contains colostrum. Its mucous layer is swollen, hyperemic, covered with mucus. Gas bubbles in the intestine have an unpleasant odor, blood traces are observed. The mucous layer is swollen, covered with mucus, there are spotted and twisted hemorrhages. The mesenteric lymph nodes in the department are enlarged. In the description of pathological changes, bleeding indicates the septicotoxic nature of the infectious process. Changes in the internal organs continue depending on the exudative, productive type. In this case,

the spleen enlarges or remains at normal size. Growths appeared at the internal organs edges, they were slightly rounded, and the consistency became gelatinous. The capsule was smooth, bleeding was observed under it. The cross-sectional surface is dry, reddish-brown, covered with white streaks.

Histological examination revealed the following: Under the microscope, pulmonary alveoli, aerogematic barrier, and visceral pleura were seen. Alveoli vary in size and shape, with serous fluid accumulated in the cavity, some with fibrinous exudate. Most alveoli are in a state of distelectasis and atelectasis. There are also foci of emphysematous changes. Hemostasis in the lung tissue capillaries, around which the cells dystrophy was seen (Fig. 1). The aerogematic barrier is thickened. Infiltrated with dead cells during inflammation. Infiltrates are composed mainly of lymphocytes, plasmoblasts, plasmocytes, fibroblasts, fibrocytes, and macrophages.(Fig.2)

Fig .1

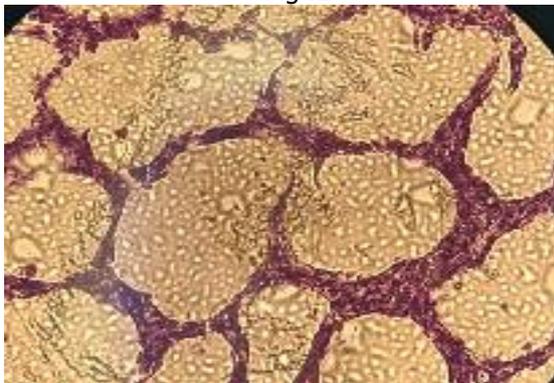
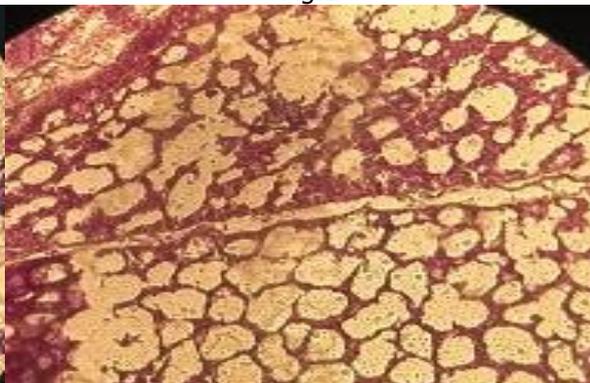


Fig.2



Microscopic cardiomyocytes were found to be symplastically located when the heart was stained with myocardial layer, hematoxylin, and eosin stain. Muscle tissue, a homogeneous fluid accumulated between myofibrils. The muscles are fibrous, disorganized in some places, hemostasis is observed in the capillaries (Fig. 3).



Under the microscope, fullness in the liver tissue veins, stasis in the sinusoidal capillaries is formed. The central vein integrity is preserved, around it appear edema, plasmorrhagia, dystrophic and necrotic changes in hepatocytes. Dystrophy and fatty dystrophies are detected in many hepatocytes. In the perivascular spaces, the cells appeared to be swollen, filled with plasma. Lymphohistiocytic, infiltrates (lymphocytes, macrophages, fibroblasts, fibrocytes) were detected in the periportal tract. Hemorrhages, hemosiderin cells (old blood transfusions) and cholestasis appear around the hepatocytes. (Figure 4.5)

Fig.4.

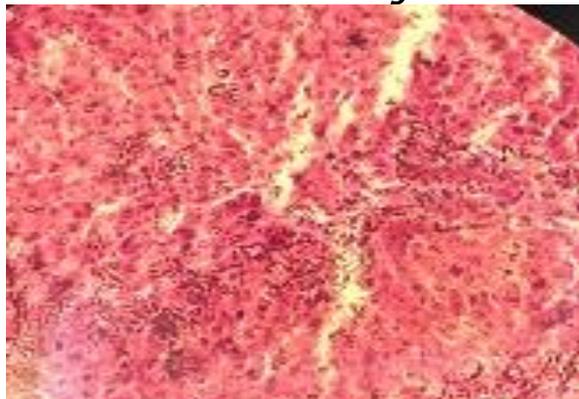
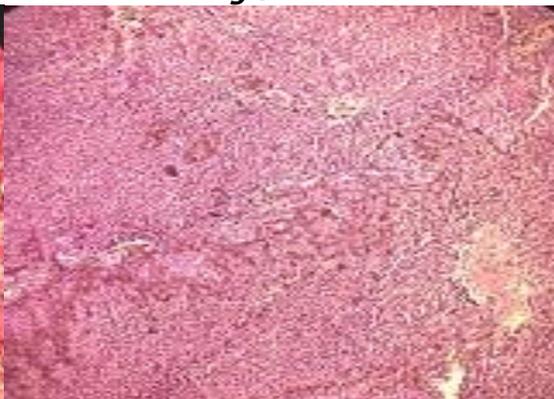


Fig.5.



CONCLUSION:

-Young calves are given a "Polyvalent GOA formal vaccine against colibacillosis, salmonellosis and pasteurellosis" high prophylactic effect when injected with a solution of natural organic, environmentally friendly, pure chitosan succinate (4%).

- specific typical pathoanatomical changes were detected in calves with colibacillosis under natural conditions.

-AR titer and the body's immune system stability were ensured when a mixture of GOA formal vaccine enriched with a natural chitosan solution was used to prevent colibacillosis, salmonellosis and pasteurellosis in calves.

-antibodies in group I calves vaccinated with the vaccine+chitosan were found to have a titer of 1: 1333, only in vaccinated group II with a titer of 1: 533.

-high immunogenicity was detected in the animals' body in the vaccine+chitosan vaccine group compared to the GOA formal vaccine.

REFERENCES:

1. Kean T, Roth S, Thanou M (2005). "Trimethylated chitosans as non-viral gene delivery vectors: cytotoxicity and transfection efficiency". *J Control Release* 103 (3): 643–53.
2. Kh.S. Salimov. *Epizootology*. Tashkent -2016. p.445-451.
3. Kudryashov A.A. *Infectious diseases of animals/A.A. Kudryashov. M.: Lan, 2007. – p.624.*
4. Kislenko, V.N. *Veterinary microbiology and immunology. V. 3. Private microbiology/V.N.Kislenko, N.M.Kolychev, O.S. Suvorina. -M.: Kolos, 2007. p. 215.*
5. B.A. Elmurodov, S.Kh. Abdalimov, I.D. Sheralieva. *Diseases of cubs. Samarkand 2016. "Zarafshan" publishing house. p.100-113.*
6. F. Ibodullaev. *Pathological anatomy of stocks. "Uzbekistan" publishing house. Tashkent -2000. p.288.*
7. www.web.sience.uz