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DYNAMICS OF IMMUNOLOGICAL PARAMETERS OF BLOOD SERUM OF CALVES IN THE TREATMENT OF KERATOCONJUNCTIVITIS USING THE DRUG LIGFOL

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Abstract

Ophthalmopathology in young cattle is one of the reasons for the decline in the growth and development of calves. The cause of keratoconjunctivitis is very often a different microflora. Economic losses in keratoconjunctivitis are formed due to a slowdown in the growth and development of young animals, a decrease in productivity, and a loss of live weight. 25-30% of recovered animals remain blind, the same number lose sight by 50%. Determination of the protein spectrum and immunoglobulins in the blood of calves is of great diagnostic and prognostic value, which reflects the degree of intensity of metabolic processes and the level of nonspecific resistance of the organism. Serum proteins are components of a dynamic circulating system and reflect the physiological and biochemical characteristics of the body as a whole. They take part in tissue nutrition, the formation of immunity in infections and invasions (γ-globulin is a fraction of serum globulin, which consists mainly of immunoglobulin antibodies), maintaining pH and osmotic pressure. In ophthalmic diseases, there is a violation of the ratio of plasma protein fractions (dysproteinemia).

Key words*: albumin, blood serum, globulins, keratoconjunctivitis, Ligfol.*

INTRODUCTION

Ophthalmic pathology in young cattle is one of the reasons for the decline in the growth and development of calves. The cause of conjunctival keratitis is very often different microflora (Bezruk, 2019).

Keratoconjunctivitis causes serious economic damage due to the high frequency of manifestation and widespread prevalence in dairy farming (Zagumennov, 2019). Economic losses in keratoconjunctivitis are formed due to a slowdown in the growth and development of young animals, a decrease in productivity, and a loss of live weight. 25-30% of recovered animals remain blind, the same amount lose sight by 50% (Zagumennov et al., 2019).

Determination of the protein spectrum and immunoglobulins in the blood of calves is of great diagnostic and prognostic value, which reflects the degree of intensity of metabolic processes and the level of nonspecific resistance of the organism. Serum proteins are components of a dynamic circulating system and reflect the physiological and biochemical characteristics of the body as a whole. They take part in tissue nutrition, the formation of immunity in infections and invasions (γ-globulin is a fraction of serum globulin, which consists mainly of immunoglobulin antibodies), maintaining pH and osmotic pressure (Ermolaev et al., 2015).

The purpose of our research is to study the dynamics of the immunological parameters of the blood serum of young cattle with keratoconjunctivitis, using various treatment regimens.

Recently, in the Russian Federation, the number of farms unfavorable for infectious keratoconjunctivitis (KCS) has increased, especially among the calves of the current year of birth and fattening animals. Bacterial pathogens are taken into account as a concomitant factor. But there is also a specific bacterial pathogen of KCS - *Moraxella bovis*. This pathogen is not rare (Kondrakhin et al., 2004).

Rather, it is poorly understood due to the lack of diagnostic schemes both in livestock enterprises and in diagnostic laboratories.

Moraxellosis is a highly contagious eye disease in cattle, characterized by conjunctival

hyperemia, lacrimation, photophobia, opacity and ulceration of the cornea, deformation of the eyeball, loss of vision of the affected eye of the animal (Figure 1).
The incubation period lasts from 2 to 18 days, depending on the season of the year and the external ambient temperature. The infection affects one or both eyes of the animal. During clinical examination of sick animals, swelling of the eyelids, conjunctivitis and lacrimation are given: at first, serous-mucous, and a little later, the outflow of purulent exudate.

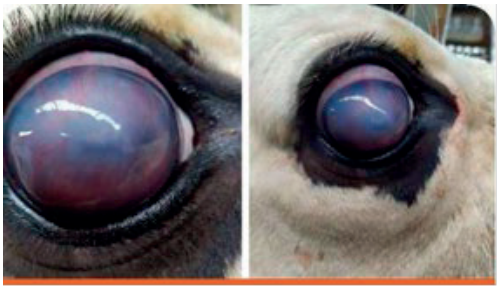

Figure 1. A characteristic symptom of initial stages of keratoconjunctivitis caused by *Moraxella* spp.

The eyesight of animals is weakened, they look for a dark and cool place. Due to the reduced consumption of feed and water, animals lose weight. Palpation reveals soreness of the eyelids, an increase in local temperature. After 24-72 hours, a milky-white cloudiness forms on the cornea, leading to loss of vision.
The causative agent of the disease *Moraxella bovis* belongs to the Neisseriaceae family. Short coccobacilli, located more often in pairs, can form a capsule. Optional aerobic. It grows on media in the form of small, rough, milky-white or yellowish colonies, slightly growing into the medium (Figures 2-3). They exhibit hemolytic properties of varying intensity, directly related to the degree of their pathogenicity. Oxidase- and catalase-positive (Daricheva et al., 2009).
The pathogenicity and virulence factors of moraxella are the surface structures of the bacterial cell - fimbria, which allow bacteria to attach to the epithelial cells of the cornea of the eye. Additionally, the causative agent releases endotoxins and hemolysins, which cause irreversible changes typical for keratoconjunctivitis. Moraxellosis is more often recorded in countries with warm climates and, accordingly, with a long grazing season. In the Russian Federation it is registered everywhere.
The predisposing factors of the disease are:
• crowded content in poorly ventilated rooms,
• grazing animals in hot, dry weather on pastures with high herbage,
• increasing the density of the population of flies,
• hypovitaminosis A.
The seasonality of the disease is seen. In the republic, it is more often registered from mid-summer to late autumn, in isolated cases - in the winter stall period. In a severe form, the disease manifests itself in farms where it was not previously registered.
The source of the disease is infected, sick or ill carriers of bacteria (within several months after recovery), which excrete the pathogen with exudate from the eyes and nose. On the body of mechanical carriers - *Moraxella* flies can survive up to three days. Calves are most susceptible to diseases. After recovering from the disease, animals develop immunity, which restrains repeated infections or causes a mild form of the disease (Kopenkin et al., 2008).
In the pathogenesis of the disease, infectious rhinotracheitis (IRT) often acts as a triggering mechanism. Solar ultraviolet light irritates the cornea of the eye, exacerbating the inflammatory process.

MATERIALS AND METHODS

The diagnosis of moraxellosis is made taking into account clinical data and laboratory research methods. Swabs from affected eyes can be sent to the laboratory on a transport medium.
For the differential diagnosis, viral infections are often taken into account rickettsiosis, mycoplasmosis, chlamydia, and thelaziosis.
Treatment.
One of the most important stages in the treatment of animals is the elimination of accompanying factors: flying insects (mice and flies), mechanical damage, trauma.
As modern means of fighting flies, the use of Larvenol GR and Kelion KE is relevant. Fly control significantly reduces the spread of infection.
As a specific prophylaxis, you can vaccinate animals. However, experience shows that the *Moraxella bovis* vaccine can reduce morbidity

but cannot completely prevent morbidity. Do not forget that the main method of prophylaxis is the quarantine of all newly admitted animals, which are subjected to mandatory clinical examination and diagnostic testing, vaccination according to indications.

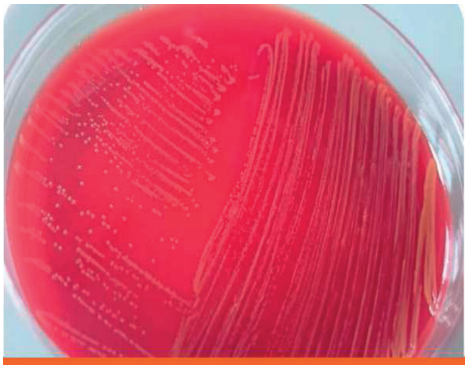

Figure 2. Growth of *Moraxella bovis* on blood agar

Treatment is carried out with broad-spectrum antibacterial agents (gentamicin, ceftiofur, cephalexin, levofloxacin, amoxicillin with clavulanic acid). Treatment is started as soon as the first signs of the disease are identified. An additional agent that alleviates the course of the disease is novocaine blockade. Animals should be placed in a shaded, isolated room. The use of vitamin A is shown.

Disinfection of premises should be carried out with Nanocide, the most effective agent, which includes both detergent components and active substances in the form of glutaraldehyde and quaternary ammonium compounds (QAC).

Animals are provided with free access to clean drinking water, since the secretion of the lacrimal glands provides effective washing of the eyes (Kopenkin et al., 2008).

Based on the analysis of the literature review, it can be concluded that today veterinary ophthalmology has a large number of veterinary medicines to combat infectious keratoconjunctivitis (Shcherbakova, 2013).

According to many scientists, there is a great need for complex treatment of ophthalmopathologies, in which it is necessary to take into account drugs and therapy intended for pathogenetic, symptomatic and etiological methods of treatment.

For the use of etiotropic therapy, much attention is paid to antimicrobial drugs, but these drugs have a large number of disadvantages: resistance of microorganisms, the presence of an antibiotic in milk and meat products, which significantly reduces the veterinary and sanitary quality of the products obtained and, as a result, causes a large number of undesirable consequences in humans with the use of such products (Stekolnikov et al., 2017).

Food allergies in animals often have the etiology of the use of low-quality products, because antibiotics are chemotherapy drugs and their presence in meat and dairy products is unacceptable (Shcherbakova, 2013).

The clinical picture is the most important criterion in the early diagnosis of viral keratitis. However, its inherent polymorphism makes it difficult timely diagnosis of this disease, however, it is possible to identify a group of common signs characteristic of various clinical forms of the disease: frequent connection of viral keratitis with a general infectious disease; the presence of concomitant herpetic eruptions on the skin of the face and the mucous membrane of the lips; neurotrophic nature of the lesion; neurological pain along the branches of the trigeminal nerve; a tendency to relapse.

In the agro-industrial development of the Ulyanovsk region in cattle, data on clinical and etiological signs have not been studied, differential diagnosis and prevention of keratoconjunctivitis is an urgent issue when conducting planned preventive medical examinations.

All the existing pharmaceutical and diagnostic arsenals for the treatment of animal ophthalmopathologies cannot reach the required level for a cost-effective method of these pathologies.

Therefore, the introduction, study and development of new comparative treatment methods available for mass use is of great relevance for veterinary medicine.

Ligfol is a safe new generation stress corrector adaptogen. The drug is of natural origin and is recognized as environmentally friendly. Ligfol contains humic substances obtained by hydrolysis of natural lignin, sodium pyrophosphate decahydrate, sodium chloride and pyrogen-free water.

The drug is a sterile dark brown liquid for intramuscular injection. Ligfol is packed in glass bottles from 1.0 to 100.0 ml. It is recommended to store it in a dry, dark place at a temperature of 10 to 25°C.

Pharmacotoxicological studies have proved that the drug is not toxic, does not exhibit mutagenic and embryotoxic effects.

Recently, more and more interest has been attracted by preparations of natural origin, characterized by harmlessness and environmental safety.

The main advantages of Ligfol are adaptogenic, antioxidant, antitumor and regenerative properties.

The existing effects of using the drug are directly related to its chemical structure, namely the presence of modified humic substances in its composition. The main products of lignin hydrolysis are the so-called humic substances (from the Latin humus - earth, soil).

The formation of humic substances in natural conditions occurs in the soil and peatlands in the process of enzymatic degradation of plant residues.

The study was carried out on the basis of LLC Megaferma - Oktyabrsky, Cherdaklinsky district, Ulyanovsk region. Four groups of Holstein calves were formed at the age of 5-6 months.

Each group consisted of eight heads with characteristic signs of keratoconjunctivitis. All animals had a similar constitution, weight and exercise, were kept in the same microclimatic conditions, their diet was the same. For each group of calves, a specific treatment regimen was determined.

A feature of our chosen treatment was the daily irrigation of the conjunctiva with a 0.5% Dioxidine solution and intramuscular administration of Ligfol in an amount of 5 ml (Table 1).

Table 1. Treatment regimens for calves

Room group	Amount heads	Dioxidin solution 0.5% + Ligfol 5 ml/m + "additional drug"	Status
1	8	Tetracycline ointment 10,000 units	Background/control
2	8	Levomycetin 0.25%, 2-3 drops	Experience
3	8	Ciprofloxacin 0.3%, 2-3 drops	Experience
4	8	Gentamicin sulfate 3%, 2-3 drops	Experience

In the blood serum, the following parameters were determined: Albumin globulins α, β, γ and immunoglobulins, A, M and G.

Blood for the study was taken from the jugular vein into disposable tubes with coagulation activator (SiO2).

The calves were treated daily for 10 days. Blood samples were taken for research on days 1, 3, 7, 10, and 14.

Calf blood serum investigated in a clinical laboratory, the interdepartmental center of veterinary medicine of the Ulyanovsk State Agrarian University using an acoustic, reagent-free, computerized analyzer of protein and protein fractions - AKBa-01- "BIOM®".

The data obtained by us were subjected to statistical processing in the computer program "Statistika 12".

RESULTS AND DISCUSSIONS

Results of the research are reflected in Table 2.

During the period of application of the immunomodulator Ligfol in the first experimental group, the albumin indicator on the third day decreased by 19.9%, in the second group it decreased by 11.72%, in the third group it decreased by 2.2%, and in the fourth group it increased by 1.6%.

On the 14th day of the experiment, it was found that in the first group the albumin level was reduced in all groups, in the first group by 17.8%, in the second group by 9.2%, in the third group by 26.9%, in the fourth group by 16.6%.

The cornea consists of 80% water, 18% definitive collagen of mesenchymal origin, as well as mucopolysaccharides, proteins (albumin, globulin), lipids, vitamins C, B2, etc.

A decrease in the level of albumin is one of the factors in the occurrence of keratitis.

Level α1-globulins on the 3rd day increased by 8%, in group 2 by 8.1%, in group 3 by 11%, in group 4 decreased by 10.8%.

On the 10th day, according to the experimental scheme, the use of the immunomodulatory drug was suspended; in the study of the modified serum on the 14th day of the experiment, it was revealed that in the first group the level α1-globulins decreased by 11%, in the second group by 8%, in the third group increased by 8%, in the fourth group decreased by 6%.

Table 2. Dynamics of immunological parameters of blood serum

Gr.	Day	Albumen	Globulins				Immunoglobulins		
			α1	α2	β	γ	A	M	G
1st group	1	46.17 ± 1.19	4.42 ± 0.22	7.17 ± 0.33	16.20 ± 0.61	20.23 ± 1.04	8.31 ± 0.63	1.19 ± 0.07	1.83 ± 0.02
	3	36.97 ± 3.63 *	4.78 ± 0.19	10.04 ± 2.41	7.94 ± 0.54 ***	22.01 ± 3.19	8.51 ± 1.18	1.50 ± 0.08 *	2.95 ± 0.28 **
	7	37.42 ± 2.25 **	4.34 ± 0.15	6.58 ± 0.60 *	10.30 ± 1.55 **	21.28 ± 1.70	9.58 ± 0.62	1.82 ± 0.07 ***	2.07 ± 0.14
	10	34.05 ± 1.40 ***	4.10 ± 0.20	6.84 ± 0.64	8.53 ± 0.67 ***	20.86 ± 2.38	8.12 ± 0.77	2.06 ± 0.02 ***	2.52 ± 0.31 *
	14	37.92 ± 1.41 ***	3.93 ± 0.27	7.94 ± 0.63	7.97 ± 0.41 ***	15.01 ± 2.34	11.42 ± 1.40	2.22 ± 0.01 ***	2.97 ± 0.16 ***
2nd group	1	39.67 ± 1.44	4.49 ± 0.15	6.78 ± 0.27	14.24 ± 0.56	19.56 ± 0.93	7.56 ± 0.25	1.24 ± 0.08	1.96 ± 0.01
	3	35.02 ± 1.16 *	4.85 ± 0.43	7.45 ± 0.48	9.86 ± 0.34 ***	27.67 ± 2.97 *	8.10 ± 0.53	1.51 ± 0.12	1.83 ± 0.05 ***
	7	35.83 ± 1.32	4.77 ± 0.08	8.55 ± 0.58	10.33 ± 0.95 **	24.82 ± 0.66 ***	10.79 ± 0.36 ***	1.85 ± 0.09 ***	2.45 ± 0.26 ***
	10	36.83 ± 1.59	4.12 ± 0.38	7.15 ± 0.50	10.13 ± 1.18 **	22.43 ± 1.16	10.41 ± 0.43 ***	2.13 ± 0.07 ***	1.92 ± 0.18
	14	36.00 ± 1.08	4.12 ± 0.21	6.41 ± 0.39	6.64 ± 0.64 ***	18.52 ± 2.26	8.11 ± 0.83	2.24 ± 0.04 ***	3.25 ± 0.34 **
3rd group	1	40.77 ± 2.08	4.45 ± 0.25	7.18 ± 0.35	14.74 ± 0.86	20.62 ± 1.12	8.07 ± 0.36	1.37 ± 0.06	1.98 ± 0.02
	3	39.84 ± 1.08	4.98 ± 0.57	6.87 ± 0.29	10.50 ± 1.35 *	19.28 ± 3.23	6.66 ± 0.60	1.60 ± 0.07 *	4.10 ± 0.99
	7	38.43 ± 2.26	4.37 ± 0.43	8.25 ± 0.50	10.12 ± 0.98 **	23.90 ± 1.16	8.91 ± 1.01	2.00 ± 0.04 ***	3.13 ± 0.20 ***
	10	40.56 ± 0.75	3.72 ± 0.26	6.10 ± 0.39	8.06 ± 1.24 ***	19.92 ± 1.93	7.00 ± 0.53	2.17 ± 0.03 ***	1.75 ± 0.20
	14	29.79 ± 3.79 *	4.84 ± 0.42	7.46 ± 0.48	9.64 ± 0.27 ***	25.99 ± 3.34	7.70 ± 0.66	2.42 ± 0.04 ***	3.88 ± 0.05 ***
4th group	1	44.28 ± 0.64	5.00 ± 0.18	7.84 ± 0.20	16.43 ± 0.42	22.36 ± 1.07	9.01 ± 0.30	1.43 ± 0.07	1.96 ± 0.07
	3	45.00 ± 1.74	4.46 ± 0.18	6.94 ± 0.38	15.87 ± 0.73	20.14 ± 1.02	8.07 ± 0.66	1.75 ± 0.08 *	3.92 ± 0.05 *
	7	35.00 ± 1.52 ***	4.06 ± 0.35 *	6.19 ± 0.45 **	9.27 ± 1.12 ***	19.92 ± 1.93	9.28 ± 0.47	2.03 ± 0.07 ***	3.18 ± 0.19
	10	35.32 ± 1.01 **	3.05 ± 0.56 **	6.01 ± 0.43 **	6.70 ± 1.63 ***	12.61 ± 1.73 ***	4.77 ± 0.61 ***	1.92 ± 0.18 *	2.66 ± 0.33
	14	36.90 ± 2.69 ***	4.67 ± 0.39	7.30 ± 0.22	9.74 ± 0.99 ***	23.16 ± 3.10	6.13 ± 0.58 ***	2.41 ± 0.05 ***	3.06 ± 0.26 **

*Note: the difference in values in comparison with the 1st day of the study: *** - p <0.001, ** - p <0.01, * - p <0.05*

The α1-globulin fraction includes acute-phase proteins: α1-antitrypsin (the main component of this fraction) - an inhibitor of many proteolytic enzymes - trypsin, chymotrypsin, plasmin, etc., as well as α1-acid glycoprotein (orosomucoid). It has a wide range of functions, in the area of inflammation it promotes fibrilogenesis.

Globulins include transport proteins: thyroxine-binding globulin, trancortin (functions - binding and transport of cortisol and thyroxine, respectively), α1-lipoprotein (function - participation in lipid transport).

When researching α2-globulins in the first group on the third day showed an increase of 40%, in the second experimental group increased by 9%, in the third group decreased by 4%, in the fourth group by 11%.

On the 14th day of the experiment compared with the 1st day of the experiment, the levelα2 globulins in the first group increased by 10.7%, in the second group decreased by 5.4%, in the third group increased by 3.89%, in the fourth group decreased by 6.8%.

The α2-globulin fraction predominantly includes acute-phase proteins - alpha2-macroglobulin, haptoglobin, ceruloplasmin. Alpha2-macroglobulin (the main component of the fraction) is involved in the development of infectious and inflammatory reactions.

In the study of β-globulins on the third day, a decrease was noted in all groups, in the first group by 50.9% ($p<0.001$,), in the second group 30.7% ($p<0.001$,), in the third group 28.7% ($p<0.05$), in the fourth group by 3.5%.

In the study of modified serum on day 14 compared to the first day, the following data were obtained: in the first group, a decrease in the level of β-globulins by 50.8%, in the second group - 53.3%, in the third group - 34.5% ($p<0.001$), in the fourth by 40.7% ($p<0.001$.)

In the study of γ-globulins on the third day, an increase of 8.7% was noted in the first group, an increase of 41.4% in the second group ($p<0.05$), in the third group there was a decrease of 6.4%, in the fourth group there was a decrease of 9.9%.

In the study of modified serum on day 14 compared with the first day, the following data were obtained: in the first group there was a decrease by 25.8%, in the second group a decrease by 5.3%, in the third group an increase by 26%, in the fourth group an increase of 3.5%.

In the study of immunoglobulin G on the third day, an increase of 61.2% ($p<0.01$), in the second group there was a decrease of 6.6% ($p<0.001$), in the third group there was an increase of 107%, in the fourth group by 100% ($p<0.05$).

In the study of modified serum on day 14 compared to the first day, the following data were obtained: an increase was noted in all groups, in the first group by 62.2% ($p<0.001$), in the second group 65.8% ($p<0.001$), in the third 95.9% ($p<0.001$), in the fourth 56.1% ($p<0.001$).

In the study of immunoglobulin A on the third day, an increase of 2.4% was noted in the first group, an increase of 7.14% in the second group, a decrease of 17.4% in the third group, and a decrease of 10.4% in the fourth group. In the study of modified serum on day 14 compared to the first day, the following data were obtained: in the first group, an increase of 37.4% was noted, in the second group, an increase of 7.2%, in the third, a decrease of 4.5%, in the fourth decrease by 31.9% ($p<0.001$).

In the study of immunoglobulin M on the third day, an increase was noted in all groups, in the first group by 26% ($p<0.05$), in the second group 21%, in the third group by 16% ($p<0.05$), in the fourth by 22% ($p<0.05$).

In the study of modified serum on day 14 compared with the first day, the following data were obtained: an increase was noted in all groups, in the first group by 86.5% ($p<0.001$), in the second group by 80.6% ($p<0.001$), in the third group by 76.6% ($p<0.001$), in the fourth group by 68.5% ($p<0.001$).

Immunoglobulin A protects the mucous membranes of the eye from pathogenic microorganisms, potential allergens and autoantigens.

By binding to antigens, it inhibits their adhesion to the surface of epithelial cells and prevents their penetration into the internal environment of the body.

IgA deficiency leads to repeated infections, autoimmune disorders, and allergies.

CONCLUSIONS

In ophthalmic diseases, there is a violation of the ratio of plasma protein fractions (dysproteinemia).

Dysproteinemias are observed more often than changes in the total amount of protein and, when observed in dynamics, can characterize the stage of the disease, its duration, and the effectiveness of the therapeutic measures.

The results of the study of immunoglobulins A, M, G in the blood serum of sick calves showed a slight increase in their concentrations, which indicates the activation of the humoral mechanisms of immunopathogenesis.

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TECHNOLOGIES OF ANIMAL HUSBANDRY