Local oxidative responses of lacrimal fluid in horses in various types of keratopathies

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Abstract. Equine keratopathies are commonly occurring ophthalmic condition affecting the equine industry. In this article, was using clinical material, a correlation was revealed between the occurrence of corneal diseases and increased tear’s antioxidant activity. The occurrence of keratopathies flows with a multidirectional alteration in antioxidants tears, what determines oxidative stress with inflammatory in cornea tissue - result of cell apoptosis and necrosis of the epithelium and endothelium barriers is triggered, the occurrence of corneal defects with marginal vascularization. Research result expands can help understand of pathogenesis keratopathies and can be applied in the future for approaches for prophylaxis and therapy of inflammation corneal diseases.

1. Relevance
It is believed that the occurrence of cellular oxidation initiates local inflammatory what will cause corneal injury. Taking these features into account, we conducted a research of the antioxidant activity in tear samples of horses with various forms of keratopathies. A complex of mechanisms of etiopathogenesis plays an important role in the occurrence of keratopathies, so the diseases are manifested by clinical polymorphism. The causes of keratopathies are diverse, their course can be aggravated by the presence of concomitant diseases, age, stress factors, overwork, etc. [1,2,3]. The main pathogenetic link of keratopathies is a violation of the precorneal tear film, an ultra-thin structure that constantly retains a three-layer structure. Therefore, the study of the mechanisms that take place in the structures that form the tear film, especially in the tear that performs nutritional and protective functions in relation to the cornea (the water layer) is relevant. [4,5,6].

The purpose this work: to give a comparative assessment of the activity of an antioxidant agent in primary and secondary keratopathies in horses.

2. Materials and research methods
The studies involved 20 patients and 10 control animals, of which 10 horses with primary keratopathy and 10 horses with secondary keratopathy. To study the eyeball, a complex research method was used. The eyelids, conjunctiva, and cornea were examined. The survey spent using a Heine magnifier and a Schwabe slit lamp. The cornea was stained with 1% sodium fluorescein to detect defects, the tear failure was assessed using Schirmer test strips, and the presence of mucin deficiency was determined by the method lissamine green paint [7].

The level of antioxidant activity in tear samples was measured using the chemiluminescent method.
3. Research results and their discussion

Previous studies to identify risk factors for the onset and development of keratopathies in horses showed the presence of two groups of keratopathies (Table 1): primary and secondary. Primary (primary ulcerative keratitis, corneal abscess, keratolysis) - they are the result of various injuries, seeding of defects by microorganisms, proteolysis of cornea cells and collagen fibers. Secondary (secondary ulcerative keratitis, non-ulcer keratouveitis, bullous keratopathy, marginal vascular keratitis) - arising spontaneously due to the destruction of the endothelial-epithelial barrier of the cornea or against the background of chronic hypoxia of the corneal tissue, which develop due to a violation of interaction with the parts of the tear film.

Table 1. Classification of primary and secondary keratopathies in horses.

| Keratopathies in horses                      |
|---------------------------------------------|
| **Primary keratopathies**                   |
| Primary ulcerative keratitis                |
| Keratolysis                                 |
| Stromal abscess                             |
| **Secondary keratopathies**                 |
| Secondary (creeping) ulcerative keratitis   |
| Non-ulcer keratouveitis                     |
| Bullous keratopathy                         |
| Marginal vascular keratitis                 |

According to the purpose of the research, the horses were share on 3 groups: 1 - healthy horses (corneal transparency, specularity, and luster are preserved), 2 - horses with primary keratopathies (horses showed blepharospasm, conjunctival hyperemia and edema, abundant exudate, corneal ulcer or abscess, keratolysis), 3 - horses with secondary keratopathies (in horses there was a slight discharge of exudate from the conjunctival cavity, or corneal ulcer with uneven edges, changes in the transparency of the cornea that occurs during endothelial regeneration and stroma saturation with intraocular vascular fluid corneal infiltration).

Table 2. Data obtained in the study of the antioxidant activity of tears in horses with primary and secondary keratopathies.

| Group of horses        | Measurement on the first day of therapy (T) | Measurement on day 15 of therapy (T) | Measurement on day 30 of therapy (T) |
|------------------------|---------------------------------------------|--------------------------------------|--------------------------------------|
| 1 - control            | 130±27                                      | 127±13                               | 122±13                               |
| 2 - horses with primary keratopathies | 310±15                                      | 295±11                               | 210±9                                |
| 3 - horses with secondary keratopathies | 1378±97                                     | 1114±32                              | 607±21                               |

Based on this, sampling to assess the antioxidant activity of lacrimal fluid was performed three times on the first day of therapy, on the 15th day of therapy, and on the 30th day of therapy, taking into account the symptoms of the disease. The analysis of samples of 10 horses with the primary form of the course of keratopathies showed that the values of the indicator significantly differ from those in the control group and are 295 ± 11 on the 15th day of treatment and 210 ± 9 on the 30th day of treatment. It is noteworthy that the indicators of antioxidant activity do not reliably change even in cases of an extremely acute course, for example, with the development of a corneal abscess. Statistical analysis of the data obtained showed that the indicators of antioxidant activity of tears in horses of the third group
before therapy were 1378 ± 97, on the 15th day of therapy -1114 ± 32, on the 30th day of therapy 607 ± 21.

The degree of increase of tear’s antioxidant activity does not reflect the severity of the keratopathies. Thus, primary keratopathies, characterized by an acute course and significant inflammatory process in the cornea, proceeded with an increase the tear’s antioxidant activity by three times, and secondary keratopathies with subacute or chronic course were flowing with increase tear’s antioxidant activity 15 times on the first day of therapy. On the 15th day of therapy, there was a decrease in the tear’s antioxidant activity of in the second and third groups. On the 30th day of treatment, the signification of indicators of tear’s antioxidant activity in the group two were 210 ± 9, in the third group 607 ± 21. The drop in the signification of the tear’s antioxidant activity of tears in horses during the convalescence period suggests reducing the content of inflammatory components in the lacrimal fluid and restoring homeostasis of the anterior segment of the eye.

4. Conclusion
Currently, the tear’s antioxidant activity is one of the important indicators for characteristics of the pathological process. It is known that the induction of the pathological process is accompanied by the activation of free radical reactions of lipid peroxidation, denaturation of proteins and nucleic acids [8,9].

As a result of the study, it was found that with the development of inflammatory or degenerative processes in the cornea and conjunctiva, it is accompanied by an increase in the antioxidant activity of the tear, which is a protective reaction to damage to the corneal tissue. Changes in the antioxidant activity of tears were established in comparison with the control group; with the primary form of the flow, a threefold increase was revealed, with the secondary form of the flow, a fifteenfold increase. It is important that the degree of increase in the antioxidant activity of tears is not proportional to the severity
of the course of keratopathy. It has been shown that the determining risk factor for the occurrence of secondary forms of keratopathy is the change in the biochemical properties of the lacrimal fluid. The data obtained as a result of the study expands understanding of the pathogenesis of keratopathies and can be used to search for approaches to prevention and treatment.

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