Use of bacteriophage cocktails for ulcerative keratitis in horses, clinical and ophthalmological justification

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Abstract. In the presented work, the authors assessed the possibility of using the drug from bacteriophage cocktails for the treatment of ulcerative keratitis in horses. The study was carried out on 30 horses (30 eyes), which were previously treated with antibiotics empirically, which resulted in the development of complications in the form of keratolysis and corneal abscess. On admission, horses were examined using a forehead magnifier and a portable slit lamp, and the cornea was stained with fluorescein to assess the diameter and depth of the corneal ulcer. For the treatment of horses with ulcerative keratitis and their complications, a bacteriophage was prescribed 3-5 times a day, depending on the severity of the disease. After using the bacteriophage drug, the authors noted a positive dynamics of the inflammatory process in a short period of time: vascularization and the formation of a granulation barrier with further restoration of the integrity of the cornea.

1. Relevance
Systemic changes in the field of therapeutic tactics of pathologies with the participation of bacterial pathogens dictate the need to introduce alternative targeted chemotherapeutic agents. Before the era of antibiotics, these were bacteriophages, the relevance of which is again increasing due to the growing antibiotic resistance [1]. Research in the field of phage prophylaxis and phage therapy of infectious and inflammatory diseases involving bacteria in animals received an additional impetus with the adoption of the Antibiotic Resistance Program at the government level [2]. Prospects for the use of bacteriophages are indicated in agriculture, veterinary medicine, and the food industry [3]. Thus, a successful experience of using cocktails of bacteriophages was obtained in postpartum endometritis in cows [4], salmonellosis of birds [5], studies are underway on the use of phages in mastitis [6, 7] and respiratory pathologies with the participation of our research group. In the concept of phage therapy as a method of antimicrobial action of primary use, attention is drawn to inflammatory pathologies of the organ of vision, in the etiology or pathogenesis of which bacteria play an essential role.

The microbial and fungal factors play a leading role in the occurrence and development of corneal diseases, since the characteristics of the course of the disease depend on the type of pathogen that caused the disease [8,9,10]. With traumatic damage to the cornea, there is a violation of the integrity, seeding with microflora, the development of acute purulent inflammation [11,12,13,14].

In the treatment of purulent processes in the anterior segment of the eyeball, it is important to adhere to the following principles: identification and elimination of factors that initiate inflammation,
reasonable use of antibiotics and prevention of complications, reduction of pain, assessment of the need to use keratoprotectors, control of the inflammation process [15, 16, 17, 18, 19, 20]. Currently, antibiotics are used in veterinary medicine to treat inflammation of the anterior segment of the eye, including the cornea, in some cases without determining the sensitivity of microflora. A common mistake is the systemic use of non-steroidal anti-inflammatory drugs; the use of keratoprotectors often helps to keep the infection in the cornea. Acute pain and prolonged inflammation lead to the development of local autoimmune reactions, accompanied by keratolysis or corneal perforation. The haphazard use of antibiotics and the rapid adaptation of microorganisms dictate the need to choose new drugs.

In this regard, the aim of the work was to include bacteriophage drugs in the treatment regimen for traumatic diseases of the cornea.

2. Materials and research methods
The material for the study was horses of various ages, breed and sex, in the amount of 30 heads (30 eyes) with a diagnosis of purulent ulcerative keratitis, corneal abscess, keratomalacia (table 1). Before starting treatment with the bacteriophage drug, all horses received antibiotic treatment for 3-4 weeks. As a rule, antibiotics were prescribed without taking into account the sensitivity of the microflora. The work was carried out at the Department of Biology and Pathology of Small Domestic, Laboratory and Exotic Animals of the Federal State Budgetary Educational Institution of Higher Education of Moscow State Academy of Medical Sciences, as well as at private stables in Moscow and the Moscow region.

| Complication          | Number of sick eyes abs. | Relative number of diseased eyes, % |
|-----------------------|--------------------------|-------------------------------------|
| Keratomalacia         | 5                        | 16                                  |
| Corneal abscess       | 7                        | 23                                  |
| Purulent corneal ulcer| 18                       | 61                                  |

Table 1. Corneal diseases in which a bacteriophage drug was used.

Devices for the study of the pathological process zone: Heine forehead magnifier, Heine slit lamp and study with the vital dye sodium fluorescein. Bacteriological examination of washings from the conjunctival cavity using universal, special, selective and differential diagnostic media.

3. Research results and their discussion
The microbial factor played an important role in the development of the infectious process of the primary form of keratopathy - the course depended on the type of pathogen that caused the disease. All horses admitted for treatment had an acute course: blepharospasm, hyperemia and edema of the conjunctiva, purulent or mucopurulent discharge from the conjunctival cavity, impaired corneal integrity, edema, superficial and deep vascularization (table 2).

At the time of admission for treatment, all horses received topical antibiotic treatment, usually without regard for the sensitivity of the microflora. Bacteriological examination of the contents of the conjunctival cavity revealed the growth of mixed microflora, which was represented by E. Coli, Staphylococcus aureus, Bact. spp., Streptococcus spp., Diplococcus spp., Pseudomonas aeruginosa (table 3).
### Table 2. Clinical criteria for pathological changes in the cornea in corneal injuries in horses.

| Changes (symptom)                                      | Number of sick eyes abs. | Relative number of diseased eyes, % |
|-------------------------------------------------------|--------------------------|---------------------------------|
| Blepharospasm                                         | 30                       | 100                              |
| Outflow from the conjunctival cavity:                  |                          |                                  |
| - mucopurulent                                        |                          |                                  |
| - purulent                                            | 10                       | 33                              |
| - 20                                                  | 66                       |                                  |
| Conjunctival edema and hyperemia                       | 30                       | 100                              |
| Corneal edema                                         | 30                       | 100                              |
| Damage depth:                                          |                          |                                  |
| - stratified squamous epithelium                       | 0                        |                                  |
| - stratified squamous epithelium and superficial layers of the stroma | 10                       | 33                              |
| - stratified squamous epithelium and deep layers of the stroma | 17                       | 56                              |
| - all layers of the cornea                            | 3                        | 10                              |
| Corneal color:                                         |                          |                                  |
| - yellow-green                                        | 11                       | 36                              |
| - grey                                                | 19                       | 63                              |
| Vascularization:                                       |                          |                                  |
| - superficial                                         | 23                       | 76                              |
| - mixed                                               | 7                        | 23                              |

### Table 3. Microflora isolated from the conjunctival cavity of diseased horses.

| Type of microflora                                |
|--------------------------------------------------|
| Staphylococcus aureus                             | +  |
| Pseudomonas aeruginosa                            | +  |
| Candida albicans                                  | +  |
| Intestinal bacteria                               | +  |
| E. coli                                          | +  |
| Staphylococcus epidermidis                        | +  |
| Diplococcus spp.                                  | +  |
| Citrobacter spp.                                  | +  |

According to the purpose of the work, for the treatment of corneal ulcers, we used a bacteriophage preparation (table 4), replacing them with antibiotics. The therapy was carried out according to the following scheme: preliminary cleansing of the conjunctival cavity with metrogil solution, then instillation of bacteriophage 1 drop 3-5 times a day, depending on the severity of the disease.

Three to five days later, a control examination of the diseased eye was carried out in order to record the dynamics of treatment: in all 30 horses, a decrease in the amount of secreted from the conjunctival
cavity, the disappearance of purulent exudate, a decrease in signs of inflammation of the conjunctiva and cornea were noted; on days 9-11 of treatment, the formation of a granulation barrier was noted; on days 21-29, the formation of granulomas. Further treatment was carried out in order to reduce the density and size of the scar tissue, using steroid anti-inflammatory drugs.

Table 4. Treatment scheme for corneal injuries using a bacteriophage drug.

| Drug name          | Multiplicity and duration of use |
|--------------------|----------------------------------|
| Metrogyl (metronidazole) | 2-3 times / day 21 days          |
| Bacteriophage      | 3-5 times / day 21 days          |

![Figure 1. Third day of bacteriophage treatment. Reduction of signs of inflammation of the cornea and conjunctiva.](image1)

![Figure 2. Tenth day of treatment. Superficial and deep vascularization, formation of granular barrier.](image2)

![Figure 3. Seventeenth day of treatment. The formation of granular barrier, superficial vascularization and pigmentation of the cornea.](image3)

![Figure 4. Twenty-ninth day of treatment. Granuloma and pigmentation of the cornea.](image4)
Figure 5. The eye before treatment bacteriophage. Trauma of the cornea, purulent conjunctivitis.

Figure 6. Fifth day of treatment. The formation of the granular barrier, superficial vascularization.

Figure 7. Nineteenth day of treatment. Granuloma.

Figure 8. Twenty-eighth day of treatment. The formation of leucoma.

Figure 9. Perforated ulcer of the cornea, prolapse of the iris, hyphema.

Figure 10. The fifth day of treatment. Reduction of inflammation of the eye, formation of a granular barrier.
4. Conclusion
In this work, we have assessed the possibility of using a bacteriophage drug (instead of antibiotics) to treat corneal injuries in horses, including those accompanied by complications. The use of a bacteriophage preparation made it possible to reduce the acute clinical signs of the anterior segment of the eyeball in a short time (3-5 days). On 9-11, the formation of a granulation barrier took place, on days 21-29 of the corneal scar-leukomas. The data obtained indicate that the use of a bacteriophage drug is promising for the treatment of corneal ulcers.

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