

## ABSTRACT

**the dissertation work of Boranbaeva Karlygash Yerbolatovna on the topic “Development of real-time multiplex polymerase chain reaction to identify the causative agents of infectious keratoconjunctivitis (pinkeye) in cattle”, submitted for the degree of Doctor of Philosophy (Ph.D) in the educational program 8D09101 – “Veterinary Medicine”**

**Relevance of the research topic.** To date, many economic entities of the Republic of Kazakhstan import cattle from countries near and far abroad. At the same time, diseases that were not previously registered in our country are often found among animals, including Pink eye of cattle.

Infectious keratoconjunctivitis (Latin - *Keratoconjunctivitis*; English - Pink-eye (pink eye); moraxellosis, ocular epizootic, infectious keratitis, «New Forest Disease») is an infectious disease characterized by eye damage, manifested by lacrimation, hyperemia of conjunctival vessels, photophobia, clouding and ulceration of the cornea, deformation of the eyeball in the form of keratoglobus or keratoconus, partial or complete loss of vision.

Contagious eye diseases of animals cause significant economic damage to livestock farms due to premature culling of animals, loss of their breeding value, reduction in milk yield, increase in live body weight, costs of veterinary-sanitary and special veterinary measures. The highest incidence rate is observed in calves aged 1 to 6 months (50–70%). In the rearing and fattening groups, especially when livestock are located in areas with high population density, the disease is recorded in up to 30%. IBK among dairy herds ranges from 10-12%. In large livestock breeding complexes in foreign countries for raising beef cattle, the annual damage reaches from 150 to 230 million dollars.

On livestock farms, the disease is recorded year-round, but most frequently in the summer months, when cattle are on pasture and are susceptible to attack by insects that carry the pathogen.

The fight against moraxellosis in cattle, due to the expanding nosoarea of this disease in the territory of the Republic of Kazakhstan, is a very pressing problem in modern market conditions.

According to monitoring data carried out in the Republic of Kazakhstan in 2018-2020, moraxellosis was detected in many epizootic units among the Aberdeen-Agnus, Hereford, and Kazakh white-headed breeds.

In our country, despite the widespread prevalence of infectious keratoconjunctivitis, issues of diagnosis, therapy and prevention have not been studied. The reason for this is that moraxellosis in cattle in our country is a new, poorly studied disease, and diagnosis is carried out labor-intensive routine method - bacteriological research.

The leading role in the occurrence of infectious keratoconjunctivitis in cattle belongs to the hemolytic bacteria *Moraxella bovis*, *Moraxella bovoculi* and *Moraxella ovis*. Among the immunological tests in countries far and near abroad, RDP, ELISA, and genotyping are used. The development of real time PCR for the

diagnosis of infectious keratoconjunctivitis in cattle is of great scientific and practical importance, since timely identification of the pathogen is indisputable evidence of the presence of infection as a nosoform, which will entail the effective development of appropriate anti-epizootic measures. The production of a diagnostic drug can be established at biological industry enterprises.

Based on the above, the development of fast, specific and accurate diagnostics – real time PCR - is very relevant. In addition, along with the details of the disease and measures for its prevention, the use of therapeutic drugs that have an effective effect occupies an important place during treatment. Currently, due to the deterioration of the environmental situation, the problem of improving new herbal medicines that have not only symptomatic, but also effective pathogenetic effects in comparison with synthetic drugs is becoming urgent.

**Purpose of the dissertation research.** Improving measures to combat infectious keratoconjunctivitis of cattle.

**Research objectives.**

1. Select an epidemiological culture and determine the type of the causative agent of infectious keratoconjunctivitis of cattle on the territory of the Republic of Kazakhstan;

2. To develop a multiplex polymerase chain reaction (PCR) in real time to identify the causative agents of infectious keratoconjunctivitis in cattle;

3. Test the developed multiplex real time PCR test system in laboratory and production conditions;

4. Determine the comparative effectiveness of therapeutic measures used for infectious keratoconjunctivitis in cattle.

**Research methods.** Research works were carried out between 2021-2023 in the laboratory of bacteriology of KazSRVI LLP, the laboratory of Green Biotechnology and Cell Engineering of the Kazakh-Japanese Innovation Center and the scientific laboratory of the Department of Clinical Veterinary Medicine of the Kazakh National Agrarian Research University, the laboratory of the Faculty of Veterinary Medicine of the Selcuk University of the Republic of Turkey, production tests in epizootic units of Almaty, Zhetysu, Akmola, West Kazakhstan, Turkestan and Kostanay regions.

Isolation of epizootological culture and determination of the type of the causative agent of bovine IBK on the territory of the Republic of Kazakhstan was carried out by bacteriological examination of biomaterial obtained from clinically sick animals. The cultural, morphological, tinctorial and other biological properties of the isolated *Moraxella* cultures were studied in accordance with the “Guidelines for the diagnosis, treatment and specific prevention of infectious keratoconjunctivitis in cattle caused by the bacteria *Moraxella bovis* and *Moraxella bovoculi*”, approved on October 19, 2017. Ministry of Agriculture of Russia, according to the methods described by M.A. Sidorov “Identifier of zoopathogenic microorganisms” and “Brief determinant Bergeys bacteria”.

Biological material was obtained from pedigree cattle (Aberdeen-Angus, Hereford), local (Kazakh white-headed, outbred) livestock in economic entities of Almaty, Zhetysu, Akmola, West Kazakhstan, Turkestan, Kostanay regions. For

bacteriological studies, biological material was selected from the conjunctiva and mucous membrane of the nasal cavity, and blood serum used for serological tests (CFT, LCFT). Bacteriological studies of the isolated *Moraxella* cultures were carried out in comparison with the ATCC reference strains *Moraxella bovis* ATCC 17948TM and *Moraxella bovoculi* BAA 1259TM.

The therapeutic preparation - phytomia "KerKon" consists of a collection of medicinal plants: eyebright herb (*Euphrasia officinalis* L.), plantain leaves (*Plantago stepposa*), common thyme herb (creeping thyme - *Tymus serpyllum* L.), chamomile flowers (*Matricaria chamomilla* L.) based on lanolin.

To analyze genomes and search for specific primers, the SpeciesPrimer algorithm was used, which uses nucleotide sequences of complete genomes of bacterial strains as initial data. All primers and probes obtained during the analysis were checked for specificity and for the absence of dimers using the BLAST+, MFEprimer, MPprimer, and Mfold programs.

Testing of an experimental series of the "PCR-Moraxella-RV" reagent kit for the detection of DNA of *Moraxella bovoculi*, *Moraxella ovis* and *Moraxella bovis* using the multiplex PCR method in real time were carried out in accordance with ST LLP 071240018450-002-2023.

#### **Main provisions submitted for defense.**

1. Epizootic cultures of *M. bovis* and *M. bovoculi* were isolated. The cultural, morphological, tinctorial and biochemical properties of the isolated cultures corresponded to the ATCC reference strains *Moraxella bovis* ATCC 17948TM and *Moraxella bovoculi* BAA 1259TM. Type affiliation was confirmed by PCR.

2. A multiplex polymerase chain reaction (real time PCR) was developed in real time to detect three pathogens (*M. bovis*, *M. bovoculi* and *M. ovis*) of infectious bovine keratoconjunctivitis in one sample. The analytical sensitivity using a set of oligonucleotides in the real time PCR for the detection of *Moraxella ovis*, *Moraxella bovoculi* and *Moraxella bovis* DNA was 21 copies, which corresponded to 50 fg in the reaction. The specificity of the set of *Moraxella* oligonucleotides has been proven by testing closely related microorganisms and pathogens of some zoonoses (*Salmonella enterica*, *Streptococcus equi*, *Helicobacter pylori*, *Pasteurella equi*). The use of a set of oligonucleotides in a real time PCR reaction showed 100% specificity for the detection of *Moraxella* cultures.

3. Approbation tests of the developed test system were carried out in laboratory and production conditions. The specificity, sensitivity and shelf life of the test system are confirmed by approbation tests on the basis of the RSE "National Reference Center for Veterinary Medicine" of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan (Registration certificate No. RK-VP-2-5289-24). Production tests were carried out on 542 swabs from the affected eyes of cattle of economic entities of Akmola, Zhetysu, Turkestan, Kostanay regions. At the same time, 15 positive samples were identified from the Akmola region. In these 15 positive samples identified as *Moraxella bovoculi* (100%), *Moraxella ovis* was 33.3% and *Moraxella bovis* was 6.7%.

4. The medicinal herbal medicine “KerKon” has been developed. Therapeutic effectiveness was 86,7% with a treatment duration of 10-16 days. Phytomase does not have a local irritant effect on the conjunctiva.

**Description of the main results of the study.** For the first time in the Republic of Kazakhstan, a multiplex PCR real time has been developed to identify pathogens of infectious keratoconjunctivitis in cattle. The value of this test system is confirmed by its specificity, analytical activity and the absence of an analogue of a commercial kit in the Republic of Kazakhstan, near and far abroad.

Due to the existing potential danger of further spread of moraxellosis among local animal breeds, the developed set of “PCR-Moraxella-RT” reagents for detecting the DNA of *Moraxella bovoculli*, *Moraxella ovis* and *Moraxella bovis* using the multiplex PCR in real time is of great practical interest for farmers, scientists, veterinary specialists in the field of livestock farming, allowing us to predict and prevent the occurrence of keratoconjunctivitis in cattle, preventing huge economic damage to agriculture, and timely and effectively developing anti-epizootic measures.

For the first time, «KerKon» phyto-ointment was developed for the treatment of infectious keratoconjunctivitis in cattle, prepared on the basis of medicinal herbs.

**Justification of the novelty and importance of the results obtained.** For the first time in the Republic of Kazakhstan, optimal reaction parameters for conducting PCR were established, which included the selection of the optimal annealing temperature and components of the reaction mixture. The composition of herbal ointment based on medicinal plants has been optimized. Also a treatment mode for conjunctivitis using the therapeutic drug «KerKon» has been developed. The proposed diagnostic test and therapeutic drug will allow for the timely development of effective anti-epizootic measures.

The novelty of the work is protected by 3 documents of protection (No. 7332 of 08/12/2023, No. 8214 of 06/30/2023, No. 8587 of 04/05/2024), 1 recommendation was published (Rules for the implementation of veterinary and sanitary measures for the prevention and elimination of Pink eye in cattle).

**Compliance with areas of scientific development or government programs.** The dissertation work was completed in the period from 2021 to 2023 on the basis of the scientific and technical program “Develop and offer for production means and methods for diagnostics, disease prevention, therapy of sick animals and neutralization of anthrax foci in soil”, under the project “Develop and offer for production multiplex polymerase chain reaction (PCR) in the mode real time for identifying pathogens of infectious bovine keratoconjunctivitis” (BR10764975).

**Description of the doctoral student's contribution to the preparation of each publication.** Based on the results of scientific research, the doctoral student prepared and published 17 scientific papers under the guidance of scientific consultants, including 4 articles in journals of the Committee for Quality Assurance in Science and Higher Education, 3 articles in a journal included in the Scopus database (Research Journal of Pharmacy and Technology 2023; Vet World. 2023; International Journal of Veterinary Science, 2023), 1 article in a foreign publication included in Higher Attestation Commission (Russia), 5 articles were published in

materials of international conferences (Kazakhstan, Russia, Georgia), 1 recommendation, 3 documents of protection were also received.

**Scope and structure of the dissertation.** The dissertation work is presented on 132 pages of computer type setting. Its content consists of an introduction, a literature review, materials and research methods, research results, a discussion of the results, conclusions, practical recommendations, a list of references and an appendix. The dissertation is illustrated with 19 tables, 36 pictures. The list of references includes 156 sources, including 72 in a foreign language.