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## Welcome Address from the Editor-in-Chief

Dear Readers, Authors, and Colleagues,

Since 1994, S. Seifullin Kazakh Agrotechnical Research University (KATRU) has been publishing the scientific journal *Vestnik Nauki KATRU*, which has become one of Kazakhstan's recognized academic platforms in agricultural sciences and a respected venue for agrarian researchers.

In 2023, a new English-language journal was launched as a specialized offshoot — *Herald of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences*. The journal is included in the List of Level 2 scientific publications recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan. This status enables the journal to publish results of publicly funded research projects and programs, as well as scientific works submitted by applicants for the PhD degree and for the academic titles of Associate Professor and Professor (Full Professor) in veterinary sciences.

At the end of last year, the journal underwent a comprehensive rebranding aimed at strengthening its visibility and credibility, enhancing the quality and transparency of editorial workflows, aligning more closely with the requirements of international indexing systems (including Scopus and Web of Science), and attracting a broader range of high-quality submissions from Kazakhstan and beyond.

Beginning this year, the journal will be published under its new title: *Central Asian Journal of Veterinary Science*. As part of the rebranding, we have renewed the Editorial Board by engaging distinguished scholars from other Central Asian countries; developed a new visual identity (cover design, logo, website, article templates, and layout); and updated key editorial policies to reflect modern standards in scholarly publishing — including publication ethics, manuscript submission procedures, transparent peer review, conflict of interest disclosure, and policies on the responsible use of AI tools and plagiarism prevention. The journal's identifiers and metadata have also been updated to ensure consistency and discoverability across academic databases and library systems.

This first issue released under the new title marks an important milestone for our editorial team and for the broader veterinary research community of Central Asia. We view the journal as an open, rigorous, and internationally oriented platform for publishing impactful research in veterinary medicine, animal health, zoonoses, food safety, veterinary biotechnology, epidemiology, immunology, parasitology, and related fields within the One Health framework.

We sincerely invite researchers, clinicians, educators, and practitioners to contribute their best work to **Central Asian Journal of Veterinary Science**. We are committed to editorial integrity, fair and timely peer review, and continuous improvement of publication quality. Your submissions, citations, reviews, and feedback will shape the journal's reputation and help us build a strong scholarly community across the region and worldwide.

Thank you for your trust and support. We hope you find this issue informative and inspiring, and we look forward to welcoming you as authors, reviewers, and readers in the next chapters of the journal's development.

With kind regards,
**Aitbay Bulashev**
Editor-in-Chief, *Central Asian Journal of Veterinary Science*

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# *VETERINARY SCIENCES*

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## Antibiotic resistance and the safety of probiotic microorganisms

Kairtay Kh. Almagambetov[1], Zinigul S. Sarmurzina [2], Botagoz K. Mussabayeva [3]
Daniayl Sh. Zhakenov [1], Zhanar B. Tekebayeva [3]

[1]Biobank of industry microorganisms of the Republican collection of microorganisms Astana, Kazakhstan
[2]Laboratory of biotechnology of the Republican collection of microorganisms, Astana, Kazakhstan
[3]Laboratory of microbiology of the Republican collection of microorganisms, Astana, Kazakhstan

**Corresponding author:** Zinigul S. Sarmurzina: sarmurzina@list.ru
**Co-authors:** (1: KA) rcmkz@list.ru; (2: BM) aveabasum@mail.ru
(3: DZh) zhakenov.daniyal@mail.ru; (4: ZhT) j.tekebaeva@mail.ru
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**Abstract**

Background and Aim. Antibiotic resistance is a global threat to health. One of the current safety concerns regarding probiotic microorganisms is their antibiotic resistance, which is often associated with mobile genetic elements. There are risks of horizontal gene transfer of antibiotic resistance genes from commercial probiotic microorganisms - used in biologics, food, and feed additives - to pathogens affecting humans and domestic animals.

Materials and Methods. In this work, the criteria for assessing the safety of probiotic microorganisms, as set by researchers and national regulatory bodies, are summarised. The safety criteria set out in the most well-known QPS and GRAS systems are analysed in detail. The

literature on the antibiotic resistance of probiotic microorganisms was analysed from the following aspects: intrinsic and acquired resistance; phenotypic and genotypic profiles; species- and strain-level differences, and the role of the ecological niche.

Results. This study presents the results of an analysis of the safety and antibiotic resistance of probiotic microorganisms employed as biopreparations in public health measures, veterinary medicine, and the food industry. Safety assessment relies on a comprehensive approach, including the genetic characteristics of probiotic strains. The priority of full-genome sequencing and bioinformatic analysis is emphasised, as these enable a detailed review of the probiotic microorganism genome for the presence of genes determining the synthesis of virulence factors, antibiotic resistance, bioamines, and others.

Conclusion. The review emphasizes the importance of assessing the safety and antibiotic resistance of strains of lactic acid bacteria. Whole-genome sequencing of the strain is considered a priority.

**Keywords:** probiotic microorganisms; antibiotic resistance; whole-genome sequencing; safety.

### Introduction

In the last few decades, the improvement of the quality of life, development of all branches of both fundamental and applied sciences, and consumer demand for healthier products have become key factors in the development of new strategies in the formulation of food products, including the rediscovery of the beneficial roles of lactic acid bacteria (LAB) in improving food safety [1].

The aim of this review is to analyse and summarise the findings of recent research on the safety and antibiotic resistance of probiotic microorganisms. These organisms are widely used in human and

veterinary medicine as food and feed additives, as well as in the production of fermented products. The review summarises the results of 47 publications.

The majority of the reviewed studies highlight the relevance of molecular genetic methods in the safety assessment of probiotic microorganisms. Particular emphasis is placed on the importance of whole-genome sequencing, as it enables accurate identification of probiotic strains and the detection of genes associated with virulence, antibiotic resistance, biogenic amine production, and other traits of interest. The main safety criteria for probiotic strains include molecular genetic identification and a well-documented history of safe use, as supported by scientific research.

We analysed published articles on the antibiotic resistance of probiotic microorganisms using the following comparative aspects: intrinsic and acquired resistance, phenotypic and genotypic profiles, dependence on the ecological niche, and strain- or species-specific features. This approach enabled us to demonstrate that probiotic microorganisms carrying acquired antibiotic resistance genes pose a significant risk due to the possibility of horizontal gene transfer to pathogenic organisms. Accordingly, the review highlights the importance of analysing the genotype of probiotic strains for the presence of mobile antibiotic resistance genes. The analysis reveals significant differences in antimicrobial resistance profiles depending on the species or strain, as well as on the ecological niche - for example, isolates from the intestinal microbiota that are used in probiotics and starter cultures.

**Materials and Methods**

*Search Strategy and Selection Criteria*

A literature search was conducted using online databases, including Google Scholar, PubMed, Web of Science, and Scopus. The search criteria included articles related to the safety and antibiotic resistance of probiotic microorganisms. A total of 55 publications were selected for analysis.

The key search terms included phenotypic and genotypic profiles, strain/species specificity of antibiotic resistance, and the safety of probiotic microorganisms. Exclusion criteria involved the elimination of publications in which the study subjects were not probiotic microorganisms or in which antibiotic resistance and safety were investigated in other taxonomic groups.

*Article Review and Data Extraction*

The selected publications were analysed with a focus on extracting data related to phenotypic and genotypic methods used to assess antibiotic resistance in probiotic microorganisms. Particular attention was given to information regarding the presence of mobile genetic elements, such as plasmids, transposons, and others elements, carrying antibiotic resistance genes.

When analysing publications on the safety of probiotic microorganisms, particular attention was paid to molecular genetic methods for species and strain identification, with a primary focus on whole-genome sequencing. Bioinformatic analysis of the sequenced genome enables the detection of mobile antibiotic resistance genes, virulence factors, genes involved in the production of pro-inflammatory biogenic amines, and other undesirable traits.

The results of the analysis concerning the safety criteria and antibiotic resistance of probiotic microorganisms are presented in this article.

**Results and Discussion**

1. The safety of probiotic microorganisms

Probiotic microorganisms - primarily *Lactobacillus, Lactococcus,* lactic acid *Streptococcus, Leuconostoc, Bacillus species*, and yeasts - are widely used as biologics. They have a long history of safe use, and many species are included in the QPS (Qualified Presumption of Safety) and GRAS (Generally Recognized As Safe) lists [2].

Regulatory frameworks for assessing the safety of probiotic microorganisms used in fermented food products, as well as in food and feed additives, have been developed in the USA, the European Union, Russia, China, Australia, Brazil, South Korea, Canada, India, Thailand, and other countries. Overall, the structure of national regulatory policies concerning the safety assessment of probiotic microorganisms is similar across different countries.

The most widely recognized systems are the QPS framework, used by the European Food Safety Authority (EFSA), and the GRAS designation, regulated by the United States Food and Drug

Administration. The fundamental criteria in both systems include molecular genetic identification of the strain, inclusion of the species or strain in the QPS or GRAS lists, a documented history of safe use, and the absence of virulence traits. The criteria also consider that probiotic microorganisms in food and feed additives and fermented products can be a reservoir of mobile antibiotic resistance genes that can be horizontally transferred to other microorganisms, including pathogens.

A summary of the probiotic safety testing paradigm is presented in Figure 1 [3, 4].

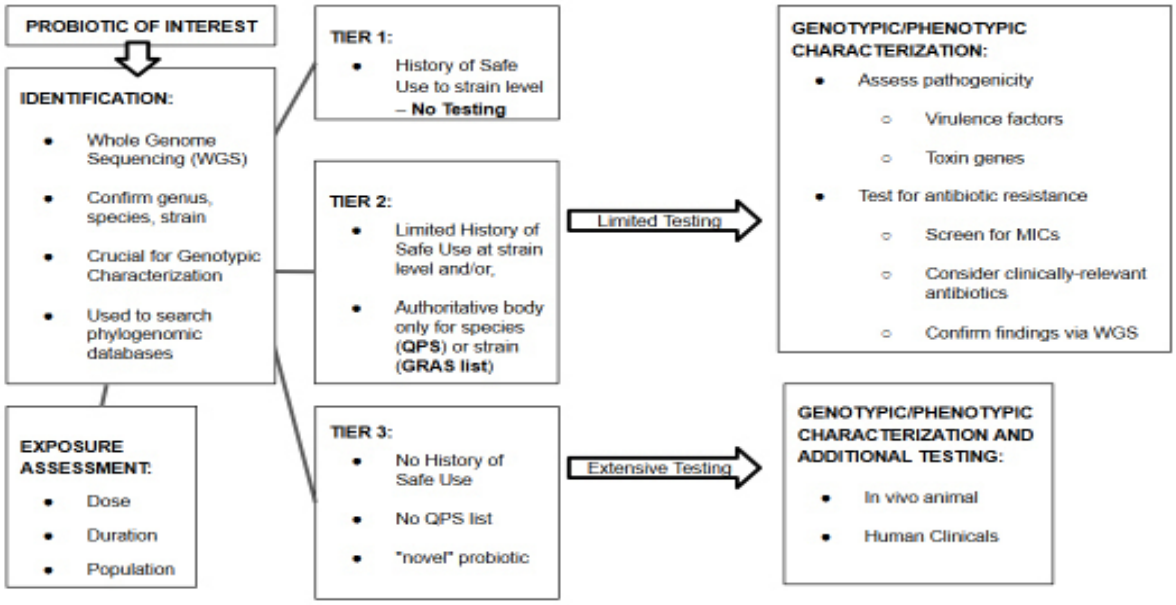


Figure 1. A proposed testing tier to guide data needs and studies for assessing probiotic safety

The safety assessment of probiotic microorganisms has significantly improved due to the increasing availability of whole-genome sequencing. This method is preferred because it enables a comparative analysis of detected undesirable genes - such as those responsible for virulence and antibiotic resistance - against genome databases of well - characterized probiotic strains/species listed in QPS or GRAS and used in commercial formulations.

The core internationally accepted safety criteria for probiotic microorganisms include molecular genetic identification of the strain/species and a well-documented history of safe use in probiotics, food and feed additives, and fermented products. The history of safe use must be substantiated by published scientific and methodological evidence.

If the strain's usage history is unclear or there are insufficient data, and the identified strain/species is not listed in GRAS or QPS, additional safety assessments are required, including in vivo studies using laboratory animals. Virulence and toxicity testing of probiotic microorganisms in animal models is essential during the development of new biologics, food products, or feed additives [3].

Our review of numerous published studies investigating the oral administration of probiotic microorganisms to laboratory animals did not reveal any evidence of virulent or toxigenic effects, regardless of the dose or duration of exposure. This calls into question the reliability of oral administration of live probiotic microorganisms to laboratory animals as a valid model for assessing human safety [4]. The classical method of oral administration of probiotic microorganisms in order to determine their virulent toxicity does not allow for the detection of certain negative characteristics of these microorganisms (hemolytic activity, production of pro-inflammatory bioamines, etc. Given the growing accessibility of whole - genome sequencing, there is increasing justification for its broad application as an *in vitro* tool for detecting toxicity - associated genes in probiotic strains.

Whole-genome sequencing is relevant not only for assessing the safety of probiotic strains but also for evaluating starter cultures [5]. A high-quality annotated genome sequence enables the identification of genes associated with antibiotic resistance, toxicity, as well as genes involved in the synthesis of exopolysaccharides and flavour-active peptides [6].

The necessity of investigating the safety of probiotic microorganisms arises from certain undesirable biological properties they may possess. For example, lactobacilli are capable of producing hydrolytic enzymes such as glycosidases, arylamidase proteases, and adhesive protein that bind to fibrinogen, collagen, and fibronectin, which can induce bacterial translocation within the host organism. Haemolysin, a protein toxin, has been described in strains of *Lacticaseibacillus rhamnosus (L. rhamnosus), Lacticaseibacillus zeae, Lacticaseibacillus saniviri* and et al. [7]. Moreover, lactic acid bacteria are known to produce decarboxylase enzymes that catalyse the synthesis of histamine, bradykinin, and other pro-inflammatory bioamines from amino acids [8]. Lactobacilli also have the ability to form biofilms and demonstrate enhanced adhesion to vascular endothelium, an ability attributed to the expression of genes responsible for exopolysaccharide and pili synthesis [9].

Given the growing use of probiotic microorganisms in human and veterinary medicine, as well as in food and feed additives, and the development of novel fermented products, ensuring the quality and safety assessment of these products is becoming increasingly important [6].

In Kazakhstan, the expanding development of probiotic and starter cultures for the fermentation of mare's milk and camel's milk [10, 11] underscores the relevance of using whole-genome sequencing as a core method for evaluating their safety.

2. Antibiotic resistance of probiotic microorganisms

*1) Natural and acquired resistance*

Resistance of probiotic microorganisms to antibiotics is categorised into natural and acquired resistance. Natural resistance can vary in antibiotic resistance profiles depending on the taxonomic affiliation and ecological niche of the organism. Natural resistance is typically stable and is not characterised by the possibility of horizontal transfer of resistance genes to other groups of microorganisms in the community. Natural resistance to antibiotics, determined by chromosomal genes, is considered an advantage of probiotic microorganisms, especially when used in conjunction with antimicrobials. Most lactobacilli show natural resistance to aminoglycosides (gentamicin, kanamycin, neomycin, and streptomycin), vancomycin, ciprofloxacin, and trimethoprim [12].

The natural resistance of lactic acid bacteria (LAB) to the glycopeptide antibiotic vancomycin, which disrupts peptidoglycan synthesis in the cells of Gram-positive bacteria, including penicillinase- and methicillin-resistant pathogens, is well known.

Resistance is due to the ability of LAB to alter the metabolic pathway of peptide synthesis by changing d-Ala-d-alanine to D-Ala–D-Lac or D-Ala–D-Ser, which reduce the affinity of vancomycin for the peptide precursor, thereby preventing vancomycin from binding to the microbial cell [13].

Phenotypic analysis of the resistance of 182 typical strains of lactobacilli isolated from different sources (human and animal intestines, inoculum, and fermented foods) to 16 antibiotics revealed the highest resistance to trimethoprim (152 of 182 strains, 84%), vancomycin (141 of 182, 77%), and kanamycin (111 of 181, 61%). These type strains were obtained from American, Belgian, Spanish, German, Japanese, Korean, and Spanish microbial collections. Genes encoding penicillin - binding proteins (PBP) and d -alanine -d -alanine ligase (Ddl), which determine resistance to vancomycin, have been identified in the genomes of lactobacilli [14].

Among the 15 tested strains of lactobacillus species (*L. plantarum, L. acidophilus, and L. pentosus*), *L. plantarum* strains were characterised by high antibiotic resistance [15]. The stable natural resistance of *L. plantarum* to vancomycin has been confirmed in recent studies [16].

A chromosomal mutation in a strain of *L. rhamnosus* was characterised by a specific mutation of the 23S rRNA gene, leading to a decrease in the affinity of erythromycin for microbial ribosomal proteins and thereby increasing the resistance to this macrolide antibiotic [17].

The antibiotic resistance profiles of 33 strains of lactobacilli isolated from fermented milk collected from various regions of China were studied. Antibiotic resistance was analysed by standard phenotypic methods and PCR analysis using gene-specific primers. Of 33 strains, 19 were resistant to vancomycin, 10 to ciprofloxacin, and one to tetracycline due to the presence of van(X), van(E), gyr(A), and tet(M) genes, respectively [18].

The resistance of lactobacilli to aminoglycoside antibiotics such as gentamicin, kanamycin, streptomycin, and neomycin has been described [19]. Resistance to aminoglycoside antibiotics has

been attributed to the absence of cytochrome-mediated electron transport, a process necessary for the antibiotic to enter the microbial cell [20].

The known resistance of lactobacilli to fluoroquinolones (nalidixic acid, norfloxacin, moxifloxacin, gatifloxacin, and ciprofloxacin) is also due to natural resistance [21]. A study of lactobacilli isolates from commercial probiotic preparations and dietary supplements demonstrated resistance to amikacin, ciprofloxacin, and norfloxacin [22].

A review of published studies reporting antibiotic resistance in probiotic lactobacilli has shown that intrinsic resistance is more commonly observed against antimicrobial agents that inhibit the synthesis of microbial cell wall components [23].

Acquired antibiotic resistance, unlike intrinsic resistance associated with chromosomal determinants, is often mediated by mobile genetic elements (MGEs) such as plasmids and transposons. The localisation of antibiotic resistance genes on plasmids or transposons significantly increases the risk of horizontal gene transfer between different groups of microorganisms. Such horizontal transfer is most frequently facilitated by conjugative plasmids [24].

Although many probiotic microorganisms are recognised as being safe (GRAS) or included in the Qualified Presumption of Safety (QPS) list, molecular genetic studies have revealed that antibiotic resistance in probiotics is often mediated by MGEs. Lactic acid bacteria found in probiotics, fermented products, foods, and feed additives that carry mobile antibiotic resistance genes may transfer these genes to members of the intestinal microbiota after ingestion and colonisation of the gastrointestinal tract [25].

The active role of conjugative plasmids in the horizontal transfer of tetracycline resistance genes from *Lactiplantibacillus plantarum* to *Enterococcus faecalis* cells has been demonstrated in in vitro studies. The genes determining resistance to tetracycline - tet(M), tet(S), tet(W), tet(O), and tet(Q) - are known as the most frequently acquired resistance genes in lactobacilli [26]. At the same

time, an association of tet(M) with transposons was identified in Lactobacillus [14]. The mechanisms of action of the tet gene group include active efflux, ribosomal protection, and enzymatic modification of the antibiotic.

In a study conducted with another strain of *Lactiplantibacillus plantarum* that exhibited both phenotypic and genotypic resistance to tetracycline, no horizontal gene transfer was observed when the bacteria were co-cultivated with *Staphylococcus aureus, Listeria monocytogenes, Acinetobacter baumannii, Citrobacter freundii,* and *Escherichia coli*. The authors attributed the negative result to the fact that the tetracycline resistance genes in this Lactiplantibacillus plantarum strain were not associated with mobile genetic elements (MGEs) [27].

Probiotic microorganisms are often characterised by multiple antibiotic resistance. Thus, PCR analysis using specific primers for lactic acid bacteria strains from probiotic tablets has revealed the presence of genes involved in resistance to erythromycin, rifampicin, trimethoprim, chloramphenicol, quinupristin, vancomycin, and streptomycin. Multiple antibiotic resistance increases the risk of potential association with MGEs; therefore, studies aimed at determining the localisation of these genes are necessary to confirm or exclude their association with MGEs [28].

Experiments were conducted to evaluate the horizontal transfer of tetracycline resistance genes from lactobacilli to *Enterococcus faecalis, E. hirae, and Listeria* monocytogenes under different co-cultivation conditions: on nutrient media, in the intestines of laboratory animals, and as part of a fermented product. Regardless of the co - cultivation conditions, horizontal gene transfer occurred predominantly via conjugation [29-30]. A similar result was obtained in an earlier study when the tetracycline resistance gene tet (M), located on the transposon Tn916 in L. paracasei, was transferred to *E. faecalis* when they were co - cultured together [31].

An analytical review of 25 research studies containing the results of antibiotic resistance research demonstrated that nine reported positive findings on the horizontal transfer of antibiotic resistance genes from lactobacilli to pathogenic bacteria. Attention is drawn to the fact that horizontal transfer of tet resistance genes into lactobacilli cells increases the risk of the spread of antibiotic resistance, particularly among the elderly and newborns [23].

At the same time, studies on the antibiotic resistance of commercial probiotic microorganisms based on whole-genome sequencing have shown a low potential for horizontal gene transfer of antibiotic resistance determinants [32].

Strains of lactobacilli have also been described that contain mobile genetic elements (MGEs) - such as plasmids, transposons, and others - which encode the synthesis of acetyltransferases, nucleotidyltransferases, and phosphotransferases capable of neutralising the antimicrobial activity of aminoglycosides [33].

*2) Phenotypic and genotypic profiles of antibiotic resistance.* Probiotic microorganisms have been studied for resistance to the most clinically relevant antibiotics using both phenotypic and genotypic methods. For phenotypic assessment of antibiotic resistance in probiotic microorganisms, the E-test, the disk diffusion method, and the broth microdilution method to determine the minimum inhibitory concentration (MIC) of the antibiotic are employed. Among genotypic methods, PCR genotyping using gene-specific primers is the most frequently approach, and whole-genome sequencing is also considered an effective tactic.

Identifying antibiotic resistance by phenotypic methods does not determine the presence of resistance-determining genes within the microbial cell or their possible localisation in mobile genetic elements (MGEs). Conversely, even when phenotypic resistance is absent, the strain may harbor antibiotic resistance genes within MGEs [34]. Thus, the presence of antibiotic resistance genes in a microbial cell does not necessarily imply functionality, as they may be rendered inactive due to stop codons or mutations such as insertions or deletions. Therefore, it is recommended to combine phenotypic and genotypic analyses when assessing antibiotic resistance.

The following results of a comparative study on the phenotypic and genotypic resistance of probiotic microorganisms to antimicrobial agents are particularly illustrative. The researchers compared the phenotypic and genotypic resistance profiles of 182 *Lactobacillus* strains to 16 antibiotics belonging to the most important classes of antimicrobials used in human and veterinary medicine.

Among 79 *Lactobacillus* strains that exhibited phenotypic resistance to chloramphenicol, only 20 possessed the corresponding genetic determinants. Conversely, among 82 strains that showed phenotypic susceptibility to this antibiotic, 59 carried antibiotic resistance genes. Similarly discrepancies between phenotypic and genotypic antibiotic resistance were observed for tetracycline and erythromycin: resistance genes were detected in only 12.7% and 10.3%, respectively, of phenotypically resistant strains. Overall, among the 182 *Lactobacillus* strains examined, phenotypic resistance was correlated with the presence of corresponding resistance genes in 67% of cases.

The same study reported a high correlation between phenotypic and genotypic resistance of probiotic microorganisms to vancomycin. The gene encoding the type F Ddl enzyme involved in peptidoglycan modification and vancomycin insusceptibility was identified in 99% of the vancomycin-resistant strains. The study also showed that the type strain Lactobacillus thailandensis DSM 22698T was phenotypically resistant to all 16 tested antibiotics. In contrast, the strains Lactobacillus sanfranciscensis LMG 16002T and *Lactobacillus* pobuzihii NBRC 103219^T were susceptible to these antibiotics, including vancomycin [14].

Differences between phenotypic and genotypic antibiotic resistance profiles are also reflected in the results of the following study. Sixty-five strains of lactic acid bacteria (47 collection strains and 18 isolated from fermented products), including 57 strains of *Lactobacillus* (*Lactiplantibacillus plantarum* – 26, *L. fermentum* – 7, among others), were tested for susceptibility to eight antibiotics. Nearly all strains (98.5%) were resistant to ampicillin, and 96.9% wereresistant to chloramphenicol. Resistance to aminoglycosides (37%) and tetracycline (26.2%) were the most frequent phenotypes. Among the phenotypically tetracycline-resistant strains, heterofermentative lactobacilli (64%) and pediococci (100%) predominated; however, no tet genes, which confer resistance to this antibiotic, were detected in any of the strains. In *Lacticaseibacillus rhamnosus* and *L. johnsonii* strains that were phenotypically resistant to chloramphenicol, the cat gene that encodes acetyltransferase responsible for chloramphenicol resistance was also not identified [35].

An earlier study described the antibiotic resistance of 45 lactic acid bacteria strains (40 starter and 5 probiotic strains) belonging to the genera *Lactobacillus, Streptococcus, Lactococcus, Pediococcus,* and *Leuconostoc*. Antibiotic resistance was assessed using the E-test and PCR analysis with specific primers. The frequency of resistance to erythromycin, chloramphenicol, and tetracycline was low (less than 7%). In contrast, resistance to aminoglycosides (gentamicin and streptomycin) and ciprofloxacin

was high (over 70%). Fifteen strains of the 45 studied contained the chloramphenicol resistance gene cat; however, all strains were phenotypically sensitive to the antibiotic. Based on PCR analysis, the authors suggested that the cat gene was not expressed in these strains [36].

Susceptibility testing was performed on 101 *Lactobacillus strains* (10 *L. acidophilus*, 31 L. amylovorus, 7 *L. crispatus*, 7 *L. gallinarum*, 26 *L. gasseri*, and 20 *L. johnsonii*) to 13 antibiotics (ampicillin, chloramphenicol, erythromycin, gentamicin, linezolid, and quinupristin/dalfopristin, among others). The analysis employed the phenotypic broth microdilution method and PCR analysis with specific primers. Phenotypic analysis revealed resistance to ampicillin, chloramphenicol, clindamycin, erythromycin, quinupristin/dalfopristin, streptomycin, and tetracycline. At the same time, corresponding resistance genes were detected in most of the resistant Lactobacillus strains, including *tetB, tetM*, and *tetO* genes associated with tetracycline resistance [37].

*3) Antibiotic resistance depends on the ecological niche of probiotic microorganisms*

The main ecological niches of probiotic microorganisms are the intestinal tract and fermented food products. Phenotypic and genotypic antibiotic resistance have been identified among *Lactobacillus* strains isolated from both niches [38]. *Lactobacillus* isolates obtained from fermented food products were susceptible to quinolones ciprofloxacin and gatifloxacin whereas strains isolated from the intestinal tract exhibited resistance to these antibiotics. Researchers attribute this difference to the fact that intestinal isolates, unlike those from food sources, are more frequently exposed to antibiotics used for therapeutic purposes [39].

A total of 122 strains of *Lactobacillus* isolated from homemade and commercial fermented products (yogurt and pickles), as well as 37 strains from the intestines of healthy individuals residing in Sichuan Province, China, were analyzed for antibiotic resistance. Phenotypic resistance to 17 clinically significant antimicrobial agents was assessed using the microdilution method with determination of the minimum inhibitory concentration. Antibiotic resistance was more frequently detected among intestinal isolates, whereas resistance was observed significantly less often among isolates obtained from fermented food products. This can also be explained by the fact that intestinal *Lactobacillus* strains are more frequently exposed to therapeutic antibiotics [40].

In another study, whole-genome sequencing of 401 strains of lactic acid bacteria isolated from starter cultures, probiotics, and the human gut revealed the presence of antibiotic resistance genes (ARGs) within mobile genetic elements (MGEs). The majority of ARGs (75.5%) were detected in plasmids and integrative and conjugative elements (ICEs). ARGs conferring resistance to tetracyclines, macrolides, and aminoglycosides were the most frequently identified. A comparative analysis of ARGs across the three ecological niches showed that they were most commonly found in intestinal isolates, less frequently in probiotic strains, and least often in starter cultures. According to the authors, intestinal isolates pose a significantly higher risk for horizontal transfer of ARGs via MGEs than starter or probiotic strains. Nevertheless, antibiotic-resistant strains included in probiotic formulations also require careful monitoring and analysis for the presence of MGEs capable of horizontal gene transfer within the gut microbiota [32].

*4) Antibiotic resistance depends on the species/strain of probiotic microorganisms* Phenotypic resistance of lactobacilli isolated from 20 patients with compromised immune status to 19 antibiotics (vancomycin, benzylpenicillin, amoxicillin, streptomycin, erythromycin, azithromycin, tetracycline, and chloramphenicol, among others) was studied using the microdilution method with the determination of minimum inhibitory concentrations (MICs). Differences in the susceptibility of lactobacilli to antibiotics were noted depending on their species affiliation. Thus, the minimum inhibitory concentrations of vancomycin, amoxicillin, and benzylpenicillin for *Lactiplantibacillus plantarum* and *Lactobacillus paracasei subsp. paracasei* were several times higher than those of *Lactobacillus acidophilus* [41].

In earlier studies, during phenotypic evaluation of antibiotic resistance in other Lactobacillus strains, high minimum inhibitory concentration (MIC) values were detected for erythromycin and tetracycline, but not for aminoglycosides [42]. Differential susceptibility among various *Lactobacillus* strains was also demonstrated with respect to macrolides. One study revealed a high level of resistance of Lactobacillus strains to macrolides [43]. In contrast, an earlier study found that the tested *Lactobacillus* strains were highly susceptible to macrolides (39). Interestingly, the overwhelming majority of the studied *L.delbrueckii*

strains were susceptible to low concentrations of vancomycin. Concentrations below 1 μg/mL inhibited the growth of 92% of the investigated strains, a result that contradicts the commonly held view that lactobacilli are characterized by intrinsic resistance to this glycopeptide antibiotic [17].

A recently conducted bioinformatic analysis of resistance genes in 583 *Lactiplantibacillus plantarum* strains to macrolides, fluoroquinolones, carbapenems, and aminoglycosides based on the CARD, ARGANNOT, and ResFinder databases revealed that resistance gene profiles varied among strains. These differences concerned genes encoding enzymes that inactivate carbapenems and aminoglycosides, as well as genes activating efflux pumps responsible for the extrusion of aminoglycosides, fluoroquinolones, and macrolides from the cell. The researchers associated the involvement of specific genes in different strains with their expression activity and functional state [44].

**Conclusion**

Analysis of the research findings revealed significant discrepancies between the phenotypic and genotypic profiles of antibiotic resistance in probiotic microorganisms. The correlation between phenotypic and genotypic antibiotic resistance profiles ranges from high to low, depending on the nature and mechanism of action of the antibiotic, as well as on the species/strain and the ecological niche of the species.

However, there was no consistent pattern in the susceptibility of probiotic microorganisms to antibiotics associated with species/strain or ecological niche. Indeed, different studies employed various methods for phenotypic assessment of antibiotic susceptibility (e.g., minimum inhibitory concentration, the E-test, and disk diffusion assays). These methodological differences may partly explain the observed variation in the quantitative assessment of resistance levels in probiotic strains. Nevertheless, this is not the most critical factor. Unlike the fundamentally important approach of whole-genome sequencing, the need for standardized methods of phenotypic evaluation of antibiotic resistance is of comparatively lesser significance.

Many species of probiotic microorganisms with a long history of use are considered safe and are included in GRAS, QPS, and other international and national safety databases. However, when selecting or comparing a studied strain of a probiotic microorganism, it is often necessary to conduct a comprehensive analysis, and this is further complicated by the frequently low quality of the available reference databases used for comparison [45].

Our analysis of the literature revealed a consensus concerning the necessity of comprehensive safety and antibiotic resistance assessment of probiotic strains, regardless of their source of isolation (the intestinal tract, fermented products, or probiotic formulations). Strains isolated from the intestinal environment pose a greater risk in terms of harboring mobile genetic elements (MGEs) conferring antibiotic resistance, compared to those derived from fermented products. Lactic acid bacteria used in the development of probiotics are predominantly selected from the human gut, while starter cultures are typically sourced from fermented dairy products. Therefore, starter cultures carry a lower risk of containing and horizontally transferring antibiotic resistance genes [46]. At the same time, one of the most rational and accessible approaches to minimizing the risk of horizontal transfer of antibiotic resistance MGEs by probiotic microorganisms may be the selection of strains from traditional fermented dairy products.

There is a lack of research related to the horizontal transfer of antibiotic resistance genes from probiotic microorganisms to pathogens. Even when mobile genetic elements (MGEs) associated with antibiotic resistance are identified through whole-genome sequencing, there remains the question of the functionality of the detected genes.

The general consensus among authors of the reviewed publications regarding the increasing availability and promise of whole-genome sequencing (WGS) in assessing the safety and antibiotic resistance of probiotic microorganisms is further supported by the conclusions in individual studies emphasizing the necessity of analyzing the functionality and expression of resistance-determining genes. This refers to the application of metagenomic, transcriptomic, and proteomic approaches. For example, the commonly accepted notion of the intrinsic resistance of lactobacilli to vancomycin is challenged by findings indicating their sensitivity to this glycopeptide antibiotic [17]. Naturally, such

results underscore the importance of conducting transcriptomic studies and metagenomic analysis to assess the potential for horizontal transfer of mobile antibiotic resistance genes [47].

The increasingly widespread use of probiotic microorganisms in medicine and veterinary practice, as well as in food and feed additives, highlights the urgency of ensuring the quality and safety of probiotics.

**Authors' Contribution**

KA: designed and drafted the manuscript. ZS: conducted and supervised the study. BM: conducted and translater the study. DZh: conducted the study and drafted the manuscript. ZhT: conducted the study and analysis of references. All authors have read, reviewed, and approved the final manuscript.

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# Persistence of infectious peste des petits ruminants virus in clinical materials and environmental matrices

Lespek B. Kutumbetov, Balzhan Sh. Myrzakhmetova, Gulzhan A. Zhapparova
Ayan M. Tuyakov, Talshyngul M. Tlenchiyeva, Karina B. Bissenbayeva
Kuandyk D. Zhugunissov

LLP «Research Institute for Biological Safety Problems», Gvardeyskiy, Kazakhstan

**Corresponding authors:** Balzhan Sh. Myrzakhmetova: b.myrzakhmetova@biosafety.kz
**Co-authors:** (1: LK) lespek.k@gmail.com; (2: GZh) g.zhapparova@biosafety.kz
(3: AT) a.tuyakov@biosafety.kz; (4: TT) t.tlenchiyeva@biosafety.kz
(5: KB) k.bissenbayeva@biosafety.kz; (6: K.Zh.) k.Zhugunisov@biosafety.kz
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**Abstract**

Background and Aim. Peste des petits ruminants (PPR) is a major transboundary infection causing substantial economic losses in sheep and goat farming. For epizootic surveillance and laboratory diagnostics, data on the persistence of peste des petits ruminants virus (PPRV) in clinical material and environmental matrices are essential, yet remain limited. This study aimed to quantitatively assess the persistence of infectious PPRV in clinical material and selected environmental objects and to determine the effect of key physicochemical factors on the loss of infectivity.

Materials and Methods. Infectious virus was recovered from organs/tissues of deceased animals, excretions/secretions from diseased animals, and residual drinking water after storage under controlled conditions. Virus infectivity was assessed after isolation in a susceptible primary lamb kidney cell culture and quantified by titration ($TCID_{50}$). Stability was evaluated under different temperature regimes, desiccation, extreme pH values, freezing (−10 °C and −50 °C), and lyophilization under vacuum and non-vacuum packaging.

Results. Under isolator conditions (14-16 °C, ~80% relative humidity), infectious virus was generally recoverable from carcass material and secretions mainly within the first 24-48 hours and was not recoverable after 48 hours. Freezing markedly prolonged infectivity: virus-containing material retained infectious activity for 6.5 ± 0.5 months at −10 °C and up to 24 months at −50 °C (observation period). Lyophilized material stored under vacuum at −10 °C remained infectious for at least 18 months with a substantial reduction in titre, whereas non-vacuum storage resulted in loss of infectivity within 3-5 days. In model matrices, infectivity declined rapidly at positive temperatures, during drying, and at extreme pH values; heating to 80 °C/boiling caused immediate inactivation.

Conclusion. PPRV infectivity in clinical material and environmental matrices is strongly determined by temperature, pH, and desiccation, while freezing and properly packaged lyophilization provide prolonged preservation. The results support practical recommendations for sampling, storage, and transport of material for PPR laboratory diagnostics.

**Keywords:** environmental stability; freezing; infectivity persistence; lyophilization; peste des petits ruminants virus (PPRV).

**Introduction**

The growth of international trade, seasonal migrations, and cross-border movements of animals substantially increases the risk of introduction and dissemination of zoonotic and anthropozoonotic pathogens, posing a threat to food security and the resilience of agriculture [1]. Among the critically important threats to small ruminants is peste des petits ruminants (PPR), a highly contagious disease of sheep, goats, and several wild ruminants caused by a Morbillivirus of the family Paramyxoviridae (PPRV) [2-5]. Clinically, PPR ranges from mild subclinical forms to severe generalized disease with high mortality in susceptible populations [6, 7, 8]. In response to the economic and epizootic burden of PPR, international organisations such as WOAH (World Organisation for Animal Health) and FAO (Food and Agriculture Organization of the United Nations) have established a disease eradication programme, underscoring the need for applied research aimed at improving epizootic surveillance, diagnostics, and control measures [5, 7, 9, 10].

For practical epizootic surveillance and laboratory diagnostics, data on the persistence of PPRV in the external environment and in clinical material are of particular importance. In the literature, information on PPRV stability in real biological matrices (cadaveric tissues, excretions, water, feed, surfaces) and on the significance of fomite transmission under field conditions remains fragmentary. Available evidence indicates rapid inactivation by heating and desiccation, whereas lyophilized or frozen materials can retain infectivity for substantially longer periods, with direct practical implications for sampling, storage, and the logistics of transporting material to the laboratory [1, 11-15]. Clarifying virus persistence in typical environmental objects and clinical matrices is necessary both for the correct interpretation of laboratory findings and for planning anti-epizootic measures.

Some issues remain the subject of scientific debate. Controversial points include the true epidemiological significance of fomite transmission under typical field conditions [13, 14], as well as the possibility of extrapolating data on the stability of vaccine strains to the behaviour of field isolates [12, 16]. Resolving these controversies requires comparable experimental approaches, studies of virus persistence in representative matrices, and standardized laboratory procedures for determining infectivity.

Until 2014, cases of PPR in Kazakhstan were not officially recorded, and the disease was traditionally considered exotic for the region. In 2014, epizootic outbreaks were described in the Zhambyl, Kyzylorda, and Almaty regions, with a clinical picture typical of PPR [17]; however, these events were not formally reflected in state veterinary reports. Nevertheless, the country as a whole retains the status recognized by WOAH as free from PPR [18]. In a region characterised by intensive agricultural trade links and cross-border movements, the practical importance of data on PPRV persistence is evident: a lack of systematic epizootic surveillance and limited capacity for sample preservation under field conditions may lead to cryptic circulation and delays in response following virus introduction [19-21].

On this basis, the aim of the present study was to quantitatively assess the persistence of infectious PPRV in clinical material and environmental objects, and to determine the influence of key physicochemical factors on the loss of infectivity. The study investigated virus stability in organs and tissues of deceased animals, in excretions and secretions from diseased animals, and in water, as well as the effects of temperature, desiccation, extreme pH values, freezing, and lyophilization on the preservation of infectivity. The resulting data are intended to substantiate practical recommendations for sampling, storage, and transport of material and to improve the effectiveness of epizootic surveillance and laboratory diagnostics for PPR.

**Materials and Methods**

***Ethical approval***

All animals used in this study were owned by the Research Institute for Biological Safety Problems (RIBSP), Committee of Science, Ministry of Education and Science of the Republic of Kazakhstan. No client-owned or privately owned animals were involved; therefore, written informed consent from animal owners was not required. All experimental procedures involving animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the European Commission Recommendation 2007/526/EC (18 June 2007), and the WHO Guidelines for Ethics Committees Reviewing Biomedical Research

(Geneva, 2000). The study protocol was reviewed and approved by the Local Committee on Biological Ethics of the Research Institute for Biological Safety Problems (Protocol No. 1 dated 14 July 2023).

Virus: Peste des petits ruminants virus (PPRV) strain Nigeria/76/1 was used in this study and was provided by the Microorganism Collection Laboratory of the LLP "Research Institute for Biological Safety Problems" (Gvardeyskiy, Kazakhstan). The isolate was maintained in the institute's virus collection and, prior to use, was passaged in primary lamb kidney (PLK) cell culture; viral material was aliquoted and stored at −70 °C until the experiments. The specificity of PPRV detection was confirmed by real-time RT-PCR for PPRV RNA using the genesig Standard Kit for PPRV (Primerdesign Ltd, UK), as well as by a virus neutralization test.

*Animals, sampling, and storage of specimens for assessment of persistence:* To assess the persistence of infectious PPRV, organs and tissues of dead animals (lung, intestine, lymph nodes, spleen), excretions and secretions (nasal secretions, saliva, faeces), and residual drinking water from troughs after use by diseased animals were examined. Material was obtained from infected animals of three groups: Cameroonian goat kids (n=4), local goat kids (n=4), and saiga antelopes (n=4). Sampling was performed immediately after animal death (carcass material) or during clinical disease (excretions/secretions, water).

Samples were stored in the isolator at 14-16 °C and relative humidity of approximately 80% and were tested at 0, 6, 12, 24, and 48 h.

*Virus isolation and cell cultures:* For virological isolation of PPRV from the tested matrices, primary lamb kidney (PLK) cell culture was used, prepared from animals aged 30-45 days and cultured in PSP medium supplemented with 10% fetal bovine serum (FBS; Gibco, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL). Monolayers were inoculated with the tested material, incubated, and assessed daily for cytopathic effect (CPE). In the absence of CPE, one blind passage was performed in PLK culture. Samples with absent CPE, as well as samples in which the infectious titre could not be determined, were additionally examined by reverse transcription real-time PCR (real-time RT-PCR) using the genesig Standard Kit for PPRV (Primerdesign Ltd, UK). Virus detection was recorded on the basis of recovery after blind passage and/or detection of PPRV RNA by real-time RT-PCR.

After pronounced CPE developed, virus-containing material was harvested after one freeze–thaw cycle, centrifuged at 2,000 × g for 10 min, aliquoted, and stored at -70 °C.

Identification of the isolated virus was performed using RT-PCR (QIAGEN OneStep RT-PCR Kit, QIAGEN, Hilden, Germany) and a virus neutralization test.

*Determination of infectious titre:* Infectious activity was determined by titration in 96-well plates (8 replicates per dilution) in parallel in PLK, Vero, and chicken fibroblast cultures. $TCID_{50}$ values were calculated by the Reed–Muench method. If infectious activity after primary isolation was insufficient for titre determination, additional passages were performed in PLK culture, followed by titration.

Freezing and lyophilization: In the laboratory, virus stability was studied in tissue suspensions, excretions/secretions, and culture fluid at −10 °C and −50 °C, as well as in lyophilized samples. Lyophilized material was placed into ampoules that were either sealed under vacuum or left unsealed; after storage, infectivity was assessed by virological isolation in PLK cell culture followed by determination of infectious activity.

*Effect of temperature, pH, and desiccation:* To assess virus stability, three matrices were used: culture fluid containing 5% fetal serum, drinking water (pH 6.8-7.2), and pelleted feed moistened with a virus-containing suspension. Experiments were conducted under four temperature conditions: 4 °C, 18-20 °C, 37 °C, and 80 °C. The effect of pH was evaluated in Hanks' solution at fixed preset pH values of 7.0, 6.1, 3.0, and 8.0 at 18-20 °C. To model desiccation, the virus suspension was placed onto the bottom of a Petri dish and maintained at 18-20 °C until complete drying. The dried residue was subsequently resuspended in culture medium and examined for the presence of infectious virus.

*Statistical analysis:* Infectious titres are presented as mean ± SD log10 TCID50. Quantitative data, where applicable, were analysed by ANOVA followed by Sidak's multiple comparisons test using GraphPad Prism v10.6.0 (GraphPad Software, USA). A p-value $< 0.05$ was considered statistically significant. Qualitative virus recovery/non-recovery results were interpreted descriptively.

**Results and Discussion**

*Stability of PPRV in excretions and secretions from diseased animals and in organs and tissues of deceased animals.*

Recovery of infectious virus from carcass material, excretions, and secretions – general observations.

When investigating organs and tissues of animals that died of PPR, as well as excretions and secretions from diseased animals, infectious virus could generally be isolated during the first 48 h of material storage in the isolator. Samples were stored under the conditions described in the Materials and Methods section (14-16 °C, ~80% relative humidity). The duration of detectable infectivity correlated with the level of infectious activity: at higher infectious activity the virus remained infectious for a longer period.

*Stability in tissues and secretions in different species*

In samples from Cameroonian kids, the infectious activity of the virus determined after isolation on PLK was 3.19 ± 0.8-5.08 ± 0.2 $\log_{10}$ $TCID_{50}$/g; infectious virus in organs/tissues and in secretions persisted for 24-48 h. In carcasses and secretions of saiga antelopes, infectious virus could be isolated up to 24 h after death. In cases where infectious activity determined after isolation was low (≤ 1.0 $\log_{10}$ $TCID_{50}$/g; local kids), infectious virus could not be isolated from carcass organs after ≥6 h, whereas in excretions and secretions (samples with activity ~1.87 ± 0.5 $\log_{10}$ $TCID_{50}$/g) it sometimes persisted up to 24 h. Residual water in drinking troughs after use by diseased animals retained infectious virus within 12-24 h, although successful isolation from water was rare.

The results of studies of carcass organs, excretions and secretions from diseased animals, and residual water in drinking troughs after storage in the isolator are presented in Table 1. Table 1 shows that PPRV in these objects was progressively inactivated, and after 48 h infectious virus was not isolated (i.e., it was below the detection limit of the applied virus isolation scheme).

*Effect of storage temperature (freezing)*

Freezing of samples substantially increased the duration of virus infectivity. Thus, virus-containing material with an infectious activity of 4.08 ± 0.3 $\log_{10}$ $TCID_{50}$/g, when stored at −10 °C, retained infectious activity for an average of 6.5 ± 0.5 months, whereas at −50 °C activity was preserved for up to 24 months (observation period).

*Effect of lyophilization and packaging conditions*

Lyophilized virus in ampoules with an infectious activity of 4.58 ± 0.5 $\log_{10}$ $TCID_{50}$/g, when stored under vacuum at −10 °C, retained infectivity for at least 18 months, although an average reduction in infectious activity of 3.16 ± 0.3 $\log_{10}$ was noted. When the storage period was extended to 30 months in such ampoules, infectious virus was not isolated. In contrast, storage of the lyophilizate without vacuum led to a sharp loss of activity, and infectious virus was not isolated within 3-5 days.

*Virus stability under the influence of various physicochemical factors*

The conducted series of experiments demonstrated that physicochemical factors – temperature, medium pH, and desiccation in the presence of air – substantially determine the persistence of cultivable PPRV. The dynamics of loss of infectivity largely depend on the matrix (culture fluid, water, pelleted feed) and storage conditions; in several series, a near log-linear decline in infectious activity was generally observed with increasing temperature and with pH deviations from neutrality. Quantitative results for key experimental series are given below (see Figures 1-3).

*Effect of temperature*

A culture suspension (initial infectious activity 5.25 ± 0.14 $\log_{10}$ $TCID_{50}$/mL) stored at 4 °C for two months exhibited a decrease in infectious activity of 1.75 ± 0.25 $\log_{10}$ $TCID_{50}$/mL. At room temperature (18-20 °C) the same batch lost a comparable amount of activity over 10-15 days and had become culture-negative by day 35 (Figure 1).

Table 1. Persistence of PPRV in carcasses, excreta, and secretions from infected animals under isolation conditions

| Examined material | Presence and titer of the virus over storage period (hours) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cameroon goat | | | | | Saiga | | | | | Local goat | | | | |
| | Initial titer | 6 | 12 | 24 | 48 | Initial titer | 6 | 12 | 24 | 48 | Initial titer | 6 | 12 | 24 | 48 |
| Respiratory organs | 4.58±1.1 | 4.1±0.9 | n/i | 2.31±0.6 | - | 2.23±0.7 | 1.0±0.2 | + | - | - | - | - | - | - | - |
| Digestive organs | 5.08±0.2 | 4.4±0.6 | n/i | 3.25±0.5 | + | 1.75±0.5 | + | - | - | - | + | - | - | - | - |
| Lymph nodes | 1.75±0.4 | 1.25±0.2 | n/i | - | - | 3.31±0.7 | 2.0±0.5 | 1.25±0.3 | + | - | + | - | - | - | - |
| Spleen | 0.75±0.2 | + | n/i | - | - | 3.22±0.9 | 2.5±0.3 | 1.75±0.4 | + | - | - | - | - | - | - |
| Feces | 3.19±0.8 | n/i | n/i | 1.5±0.4 | - | 2.71±0.4 | n/i | n/i | + | - | 1.87±0.5 | n/i | n/i | + | - |
| Nasal mucus and saliva | 3.62±0.7 | n/i | n/i | + | - | 3.46±0.8 | n/i | n/i | + | - | 1.20±0.3 | n/i | n/i | - | - |
| Drinking water | + | n/i | + | + | - | + | + | - | - | - | n/i | n/i | n/i | n/i | n/i |

*Note: "+" – qualitative recovery of infectious virus after blind passage in PLK culture (titer not determined);*
*"–" – no recovery of infectious virus after blind passages;*
*"n/i" – not investigated.*

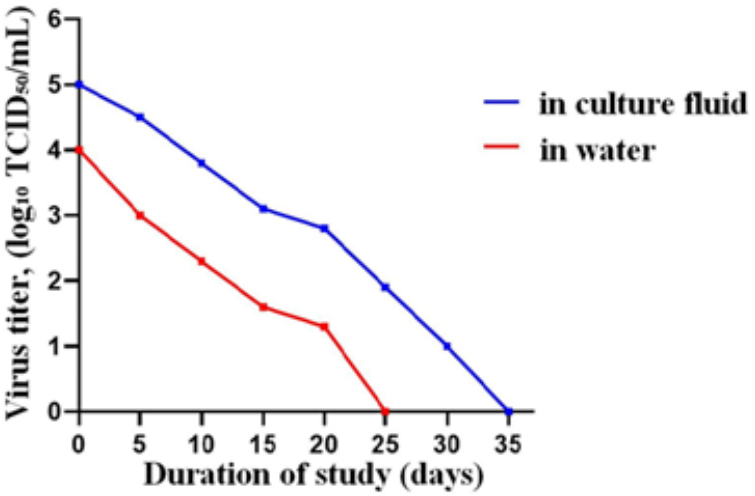


Figure 1. Changes in virus titre at a temperature of 18-20 °C

In the culture suspension maintained at 37 °C, cultivable infectivity was detectable only for 4-5 days (Figure 2).

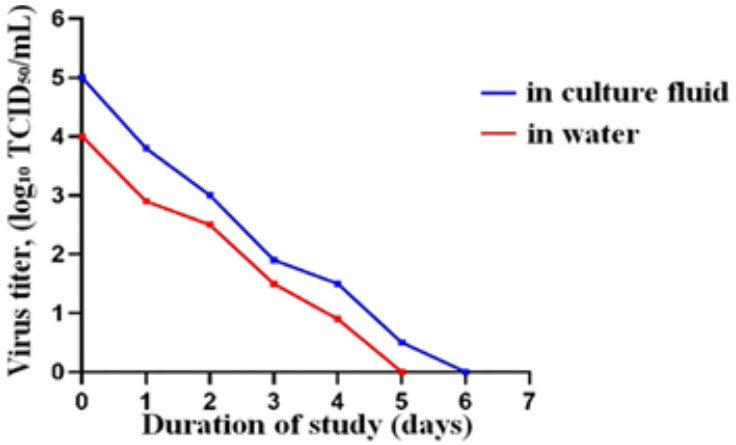


Figure 2. Changes in virus titre at a temperature of 37 °C

Heating to 80 °C and boiling resulted in immediate (culture-detectable) inactivation of the virus.

*Matrix: water and pelleted feed*

Persistence parameters in drinking water generally corresponded to the results obtained in culture fluid. Water with an initial infectious activity of 4.93 ± 0.40 $\log_{10}$ $TCID_{50}$/mL retained cultivable infectivity up to 25 days at 18-20 °C and 3-5 days at 37 °C. In moistened pelleted feed (initial infectious activity of 2.91 ± 0.50 $\log_{10}$ $TCID_{50}$/g) the virus remained cultivable for approximately 3 days at room temperature and ~1 day at 37 °C. Thus, an organic, adsorptive matrix (feed) accelerates loss of infectivity compared with aqueous media.

*Effect of pH and desiccation*

pH exerted the most pronounced influence on persistence. In Hanks' solution at neutral pH (7.0 ± 0.05) an initial infectious activity of ≈5.0 ± 0.3 $\log_{10}$ $TCID_{50}$/mL decreased by 0.75 ± 0.20 $\log_{10}$ over 4-5 days at 18-20 °C. At pH ≈6.15 the decline over the same interval amounted to ≈1.91 ± 0.30 $\log_{10}$, whereas at pH ≈3.0 infectious virus was not isolated within 24 h (Figure 3).

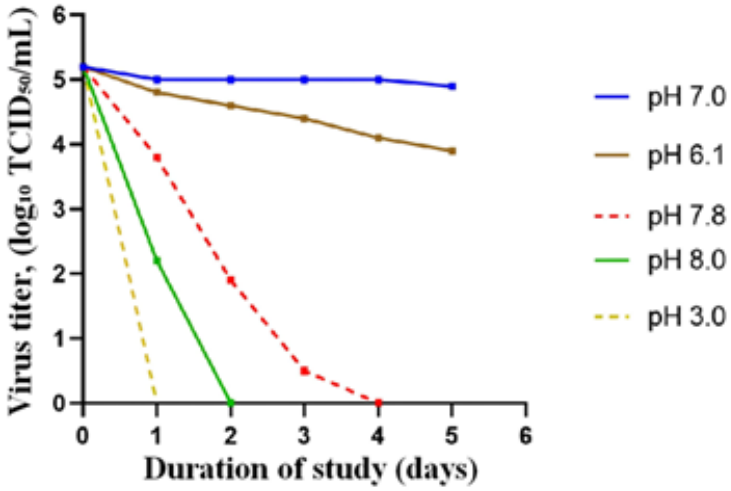


Figure 3. Changes in virus titre at different pH levels of the medium

Alkaline conditions (pH 8.0) led to rapid loss of cultivable infectivity within 1-2 days. The desiccation model showed that in dried droplets of water and culture fluid, infectious virus was not isolated after 24 h; therefore, drying in the presence of air is a critical factor that sharply reduces persistence times.

Stability at subzero temperatures and lyophilization

Frozen material and lyophilized samples stored at low temperatures demonstrated prolonged stability of infectivity. Lyophilization combined with vacuum packaging and storage at low temperatures provides the greatest preservation of the infectious potential of the material.

Interpretation of Results

The persistence of infectious PPRV in clinical material and in the environment appears to be governed by the initial viral load and abiotic factors, primarily temperature, pH, and exposure to drying. Rapid loss of cultivable infectivity at positive temperatures and during desiccation is consistent with the general properties of enveloped viruses, including morbilliviruses, where disruption of the lipid envelope and instability under non-neutral pH conditions contribute to inactivation [22-25]. Conversely, cooling, deep freezing, and lyophilization markedly prolong infectivity, highlighting the critical role of low-temperature storage and appropriate packaging in preserving sample viability for laboratory diagnostics [1, 11, 12, 26, 27, 28].

These results emphasize an important practical distinction: under warm and dry conditions, the potential for fomite-mediated transmission is expected to decrease rapidly over time, whereas in cooled or frozen materials infectious virus may persist substantially longer, particularly when the initial titre is high and the pH remains close to neutral. Benchmarks reported in national guidance documents [29] describing the lability of PPRV outside the host and relative stability within a defined pH range are broadly consistent with the overall patterns observed in our experiments.

At the same time, survival of PPRV under field conditions remains insufficiently characterised. Factors such as matrix composition, fluctuations in temperature and humidity, sunlight/UV exposure, and adsorption to different surfaces may substantially alter persistence. Therefore, further research is required to quantify survival and transmission risks via water, feed, and various surfaces under representative farm conditions, and to refine practical recommendations for sampling, storage, and transport [13, 14].

**Conclusions**

Infectious PPRV in clinical material and environmental matrices showed limited persistence at positive temperatures and under conditions promoting desiccation. Under isolator storage conditions (14-16 °C, ~80% relative humidity), infectious virus was generally recoverable from organs/tissues, excretions/secretions and residual drinking water mainly within the first 24-48 h, with detectability associated with the level of infectivity.

Freezing markedly prolonged preservation of infectivity in virus-containing material: at −10 °C infectious activity was maintained for an average of 6.5 ± 0.5 months, whereas at −50 °C infectivity was preserved for up to 24 months within the observation period.

Lyophilization and packaging conditions critically affected virus survival. Lyophilized material stored under vacuum at −10 °C retained infectivity for at least 18 months, although a substantial reduction in infectious activity was observed; extension of storage to 30 months resulted in loss of recoverable infectious virus. Storage of lyophilizate without vacuum led to rapid loss of infectivity within 3-5 days.

In controlled model matrices, temperature, pH and desiccation were key determinants of PPRV stability. Heating to 80 °C and boiling caused immediate inactivation; extreme acidic conditions (pH ≈3.0) resulted in loss of recoverable infectivity within 24 h, and alkaline conditions led to rapid inactivation within 1-2 days. Drying in the presence of air eliminated recoverable infectious virus in dried droplets of water and culture fluid after 24 h.

These findings apply to PPRV strain Nigeria/76/1 under the controlled experimental conditions used in this study. Accordingly, the practical implications should be interpreted as guidance for sampling, storage, and transport under comparable conditions rather than as direct estimates of persistence for all field isolates under natural farm conditions.

**Author Contributions**

Conceptualization, LK; Data curation, BM, and LK; Formal analysis, LK; Methodology, LK and BM; Investigation, GZ, TT, KB, AT, and KZ.; Writing – original draft preparation, LK and BM; Writing – review and editing, BM and LK. All authors have read and agreed to the published version of the manuscript.

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**UDC 619:614.31:631.576.2(574) (045)** **Research article**

# Sanitary assessment of nuts in the markets of the Republic of Kazakhstan

Laura T. Auteleyeva[1], Balgabay S. Maikanov[1], Aigul Sh. Sharipbayeva[2]
Ayana S. Smagulova[3], Zhazira Zh. Yermuhametova[4]

[1]S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan
[2]Kazakh Academy of Nutrition, Almaty Kazakhstan
[3]L.N. Gumilyov Eurasian National University, Astana, Kazakhstan
[4]National Accreditation Center of the Republic of Kazakhstan, Astana, Kazakhstan

**Corresponding author:** Laura T. Auteleyeva: l.auteleeva@kazatu.edu.kz
**Co-authors:** (1: MB) b.maykanov@kazatu.edu.kz; (2: ShA) aigul-69.69@mail.ru
(3: SA) smagulovaayana28@gmail.com; (4: YZh) z.ermuhametova@nca.kz
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**Abstract**

Background and Aim. Nuts are a vital component of the human diet; however, they are highly susceptible to contamination by mycotoxins. This study aimed to conduct a comprehensive sanitary assessment of various domestic and imported nuts sold in Kazakhstan's markets, specifically focusing on the quantification of Aflatoxin B1 (AFB1) to evaluate potential carcinogenic risks to consumers.

Materials and Methods. Samples of walnuts, peanuts, almonds, hazelnuts, and pistachios were collected from major wholesale and retail markets in Almaty, Taraz, Shymkent, Astana, and the Turkestan and Kyzylorda regions. The assessment employed a multidisciplinary approach, including organoleptic, physicochemical, and instrumental analyses (High-Performance Liquid Chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA) to detect and quantify AFB1 contamination.

Results. Aflatoxin B1 was detected in all analyzed samples. The highest contamination levels were identified in samples from Shymkent, ranging from 0,039 to 0,045 mg/kg. These concentrations significantly exceed the maximum permissible limit (MPL) of 0,005 mg/kg established by the Customs Union and European Union food safety regulations.

Conclusion. The findings indicate that current levels of AFB1 contamination in nuts sold in Kazakhstan pose a notable public health concern. The results highlight the urgent need for systematic monitoring of mycotoxins and the implementation of more robust national food safety control protocols to ensure consumer protection.

**Keywords:** quality; safety; contamination; aflatoxin; nut.

## Introduction

Aflatoxins are among the most potent mutagenic and carcinogenic substances known (JECFA, 1999), affecting all vertebrates, including humans. According to the Food and Agriculture Organization of the United Nations, mycotoxins contaminate a quarter of the world's harvest [1, 2, 3].

Four mycotoxins are commonly found in plant products: aflatoxins B1, B2, G1, and G2 (JECFA, 2002). *Aspergillus, Fusarium,* and *Penicillium* are the three main fungal genera that produce these compounds [4]. Aflatoxins are highly toxic and contaminate a wide range of food products, including corn, peanuts, dried fruits, and meat- and milk-based products [5, 6]. Aflatoxin-producing Aspergillus species include A. flavus, A. nomius, A. parasiticus, and A. astellatus [7].

Mycotoxins are resistant to technological processes, such as cooking, frying, baking, distillation, and fermentation. These fungal secondary metabolites contaminate products of both animal products

(beef, pork, poultry, lamb, fish, game meat, and milk) and plant products (cereals, processed cereals, vegetables, and nuts) origin. They can damage crops and cause various mycotoxicoses. The presence of mold in food increases the impact of mycotoxins on humans and animals. AFB1 is widely found in food and is considered a public health problem worldwide because of its carcinogenicity. Therefore, AFB1 concentrations should be monitored [8].

The World Health Organization classifies aflatoxins as teratogenic, mutagenic, carcinogenic, and invisible poisons. Contamination can occur at any stage of food production, from harvest to storage. Of all the mycotoxins affecting food and feed, aflatoxin B1 (AFB1) causes the most harm to human and animal health [9].

Temperature interacts with water activity to affect the regulatory gene ratio, which is directly proportional to AFB1 production. In particular, this interaction affects AFB1 production by *Aspergillus flavus* [10].

This study aimed to provide a scientific sanitary assessment of various nuts sold in Kazakhstan's markets. The following tasks were performed: 1) monitoring the production of domestic and imported nuts; 2) evaluating the quality and safety of various nut samples.

**Materials and Methods**

A total of 440 samples were collected, comprising 155 domestic and 285 imported products. Nut samples were collected from large markets in Almaty (Sayakhat, Optovka, and Altyn Orda), Taraz (Auyl Bereke), and Shymkent (Aina and Kyrgy). In the Turkestan region, samples were collected from the following locations: the Kalen farm, Sarapkhana rural district, Turbat rural district, Baidibek Ata farm, and Akqum rural district. The Qakpak rural district included the Tegen, Tazabek, Aldan, and Dauey Ata farms. In the Tülkibas district, Zhambyl village, Zhambyl LLP, and the Sansyzbay farm were included. In the Saryagash district, the sampling sites included Silk Alley Winery LLP, Saryagash Zher Syy farm, Saryagash Zher Syy LLP, and the Eski Bazaar market in Kyzylorda. Additionally, in Astana, samples were collected from Artem, Asem, Alem, Eurasia, Shapagat, and the major supplier Sarvinos-S LLP.

The organoleptic examination of the nuts assessed their appearance, shell color, size, surface texture, color, quality, taste, and aroma. The physicochemical assessment included the percentage of nuts damaged by pests, the percentage of underdeveloped and broken nuts, the presence of live pests and their larvae, and the moisture content. The following technical specifications were applied: walnuts GOST 32874-2014); unshelled pistachios GOST 31788-2012; peanuts GOST 31784-2012; cashew kernels GOST 31855-2012; almond kernels GOST 32857-2014. The mass fraction of moisture was determined by sequential drying at 130 ± 2 °C for 40 min in a drying oven [11, 12, 13, 14, 15].

The aflatoxin B1 (AFB1) content of the nuts was quantified using high-performance liquid chromatography in accordance with GOST 30711-2001 [16]. The HPLC equipment was from Waters Corporation (USA) in the accredited laboratory of LLP "Nutritest" of Kazakh Academy of Nutrition (Almaty, Kazakhstan). Immunoenzymatic analysis was performed using a RIDA ABSORBANCE 96 microplate spectrophotometer and RIDASOFT® Win.NET software (Germany). Safety measures were observed when working with methanol (certificate numbers AC 568/1, AC 568/2, and AC 567/1). Calibration was performed using ready-to-use standard solutions included in the kit: 0, 1, 5, 10, 20 and 50 μg/l.

To control method specificity, control samples of nuts free from aflatoxin B1 were used and analyzed in each assay batch (n=3-4 per batch). The optical density of control groups corresponded to the zero standard (0 μg/l), confirming the absence of non-specific binding and adequacy of sample preparation.

**Results and Discussion**

Several large nut farms operate in the Almaty region. For instance, Integration-Turgen LLP, located in the Enbekshikazakh district, covers 20 ha. This farm grows hazelnuts, which are exported to Italy for use in Nutella products. The trees are young (2-3 years old) and yield up to 10-15 kg per tree. The farm also sells walnut seedlings and is working on cultivating an Italian hazelnut variety, which is larger and has light red skin. Manshuk Farm recently began production on 40 ha. Many nut farms in Kazakhstan have been unproductive in recent years because of spring frosts and the lack of subsidies from the Kazakhstani Ministry of Agriculture. Thus, we obtained nut samples from large markets in Almaty

(Sayakhat, Optovka and Altyn Orda) which sell mainly imported nuts and nuts grown in the Turkestan region (shelled and unshelled walnuts, shelled and unshelled peanuts, and almonds).

The organoleptic characteristics of the nuts sampled from the Almaty and Zhambyl regions met the requirements for fresh nuts, except for the white coating on the shell walnuts from Taraz. No shell defects, broken nuts, mechanical damage or mold were detected in the nut samples from the Almay and Zhambyl regions. The number of dry or underdeveloped kernels was within the permitted limit (2% for walnuts). The presence of dust and dry pericarp was minimal and did not exceed the permissible limits. No live pests were found.

All nut batches met the organoleptic quality indicators corresponding to the premium and first grades; these indicators were the appearance, shell quality, nut size by maximum transverse diameter, nut surface, kernel yield, kernel separability from the shell kernel color and quality, kernel taste and smell, presence of foreign impurities, nut shells, kernel moisture content, presence of nuts with dried skin, presence of nuts damaged by pests, rancid nuts, underdeveloped nuts and presence of live pests (insects or their larvae) inside the nut. Nut plantations in the Zhambyl region are mainly of a small scale and located in private households. Most of the nut harvest from these plantations is for personal use, with the surplus being sold at the Auyl Bereke market in Taraz.

The concentration of AFB1in nuts from the Almaty region ranged from 0,0017 ± 0,001 to 0,0038 ± 0,0034 mg/kg and did not exceed the maximum residue concentration (MRL) (Table 1). However, excessive concentrations of AFB1 were found in some walnut samples sold in markets in Taraz (Zhambyl region). Concentrations in other samples ranged from 0,001 ± 0,00012 to 0,0003 ± 0,0013 mg/kg.

Table 1. The average concentration of aflatoxin B1 in nuts from different regions of Kazakhstan (MRL 0.005 mg/kg)

| Number of samples | Type of nuts | Place of sampling, city | The average concentration |
|---|---|---|---|
| 5 | Walnuts in shell | Shymkent Market ‘Aina’ | 0,045 ± 0,0004 |
| 5 | Hazelnuts | Shymkent Market ‘Aina’ | 0,002 ± 0,0002 |
| 4 | Walnuts in shell | Lengir | 0,006 ± 0,0002 |
| 5 | Walnuts in shell | Tulkibas | 0,013 ± 0,0018 |
| 5 | Walnuts in shell | Sairam | 0,0158 ± 0,0018 |
| 4 | Walnuts in shell | «Saryagash Zher syy» | 0,001 ± 0,0018 |
| 7 | Almonds | Sairam | 0,001 ± 0,0001 |
| 7 | Hazelnuts | Sairam | 0,002 ± 0,0001 |
| 4 | Walnuts in shell | «Baidibek ata» | 0,002 ± 0,0014 |
| 7 | Almonds | Lengir | 0,001 ± 0,0001 |
| 7 | Hazelnuts | Lengir | 0,002 ± 0,0002 |
| 7 | Walnuts in shell | Kyzylorda | 0,012 ± 0,0001 |
| 7 | Walnuts in shell | Shymkent | 0,039 ± 0,003 |
| 7 | Peanuts | Shymkent | 0,002 ± 0,0001 |
| 5 | Walnuts in shell | Sairam | 0,01581 ± 0,0013 |
| 4 | Walnuts in shell | Tülkibas | 0,0247 ± 0,0002 |
| 4 | Walnuts in shell | Lengir | 0,004 ± 0,0012 |
| 11 | Peanuts | Turkestan | 0,045 ± 0,00045 |
| 7 | Peanuts | Kyzylorda | 0,001 ± 0,0041 |
| 5 | Walnuts in shell | Taraz | 0,001±0,00012 |
| 5 | Walnuts | Taraz | 0,02714±0,0027 |
| 4 | Walnuts in shell | Taraz | 0,0003±0,0013 |
| 7 | Walnut | Taraz | 0,0145±0,046 |

Continuation of Table 1

| | | | |
|---|---|---|---|
| 5 | Walnut in shell | Almaty | 0,0038±0,0034 |
| 7 | Almond | Almaty | 0,0017±0,001 |
| 4 | Walnuts in shell | Taraz | 0,001±0,00012 |
| 6 | Walnut | Taraz | 0,02714±0,0027 |

The Turkestan region has favorable conditions for nut cultivation. Nuts are mainly produced by private farms. The largest nut farms in the region are located in the Kazygurst district: the Kalen peasant farm in the Sarapkhana rural district (6 ha) and the Baidibek Ata peasant farm in the Turbat rural district (2 ha), which has 100 nut trees (almonds and walnuts). Approximately 3 t of nuts are harvested annually. These are sourced from Lengir, Saryagash, Zher syiy, Silk alley Winery LLP (Saryagash district), JSC 'Zhambyl', the Sansyzbay farm (Tülkibas district), Kalen farm, Sarybekovtar farm, Baidibek Ata farm (Sarapkhana rural district), Tazabek farm, Aldan farm, Dauey Ata farm (Qakpak village), Integration LLP (Turgen, Tülkibas, Sairam and Zhanatalap districts) and Kyrgy and Aina markets (Figure 1).

Figure 1. Walnut tree at the «Baidibek Ata»

There are several peasant farms in the rural district of Qakpak: Tegen (2.73 ha), Tazabek (2 ha), Aldan (3 ha) and Dauey Ata (2.6 ha). The Zhambyl agricultural enterprise of Zhambyl LLP (30 ha) and the Sansyzbay farm (6 ha) are located in the Tülkibas district.

Silk Alley Winery LLP (60 ha) and the Saryagash Zher Siyy peasant farm (100 ha) are located in the Saryagash district (see Table 2). Almonds, hazelnuts, walnuts (280 bushes) and pistachios are grown there. The entire harvest of Saryagash Zher Syy LLP is sold in the Turkestan and Zhambyl regions and exported to Kyrgyzstan (Figures 2 and 3).

Figure 2. Hazelnut seedlings Saryagash Zher Syy LLP

Figure 3. Almond tree Silk Alley Winery ZHS

Table 2. Nut farming enterprises in the Turkestan region

| Name | Location | Area, hectares | Type |
|---|---|---|---|
| Saryagash District | | | |
| «Saryagash Zher Syy» | Saryagash District, Zhemisti Village | 100 | almonds, hazelnuts, walnuts, pistachios |
| | | | |
| «Silk Alley Winery» ZhShS | Kurkeles rural district, Enkes village | 60,0 | walnuts, almonds, hazelnuts, pistachios |
| Tülkibas District | | | |
| «Zhambyl» ZhS | Zhambyl rural district | 30,0 | walnuts |
| «Sansyzbay» | Zhambyl Rural District | 6,0 | walnuts |
| Kazygurt District | | | |

Continuation of Table 2

| | | | |
|---|---|---|---|
| «Kalen» | Saraphana Village | 6 | walnuts |
| Turbat rural district | | | |
| Sarybekovtar | Ondiris, s.Akkum | | walnuts, almonds |
| «Baidibek ata» | Ondiris, s.Enbek | 2 | walnuts, almonds |
| Rural district Kapkapak | | | |
| «Tazabek» p/s | Kakpak Village | 2 | walnuts |
| «Aldan» | Kakpak Village | 3 | walnuts |
| «Dauei ata» | Kakpak Village | 2,6 | walnuts |

The moisture content of the nuts from the two regions studied averaged between 6,08% ± 0,001 and 8,2% ± 0,003.

Analysis of import data revealed that China was the largest source of nut imports between 2021 and 2024, accounting for 58,294.6 t (74.6% of the total volume of imports). The main types of imported nuts were walnuts (43,482.3 t), almonds (11,688 t), macadamia nuts (1,596 t), cashew (344.5 t), pistachios (119.7 t), chestnuts (0,2 t), and pecans (1,063.9 t) (Figure 4).

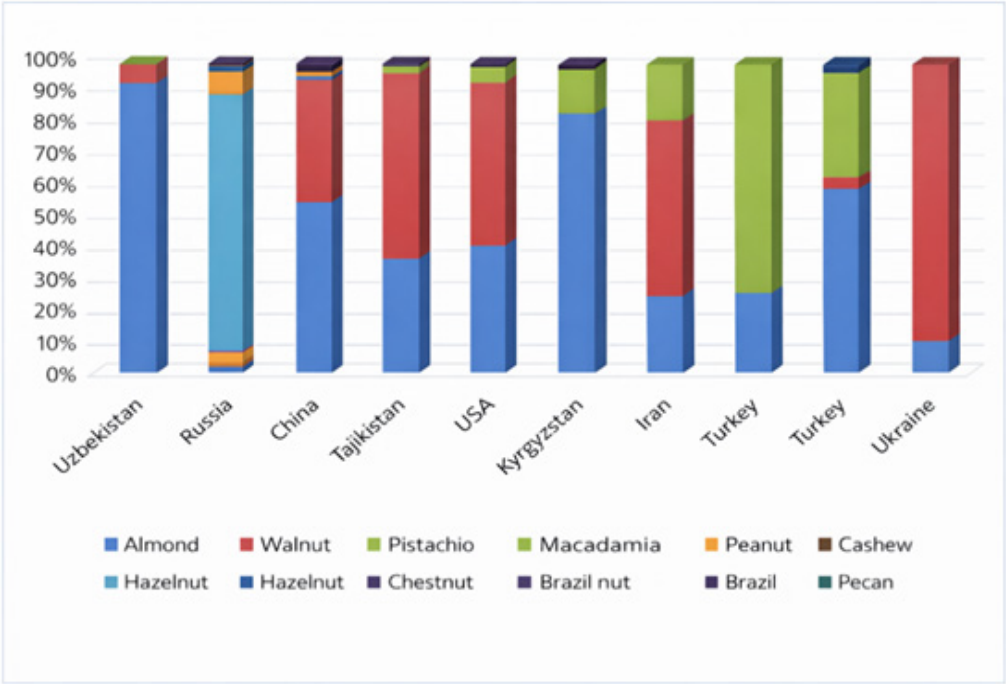


Figure 4. Imports of various nuts to the Republic of Kazakhstan, 2021-2022

The volume of nut imports from the United States was 15,702.9 t (13,712 t of almonds, 1,985.3 t of pistachios, and 4,9 t of pecans), while 2,126.2 t were imported from Russia (1,693.8 t of peanuts).

According to official statistics, the main nut importers are Kyrgyzstan (125.8 t, including 1.3 t of almonds and 5 t of walnuts) and Russia (64.5 t). However, our monitoring data differs from these figures.

From January to July 2023, the largest volume of cashews (1,561.7 t) came from Vietnam (1,427.8 t, or 91,5%), while the largest volume of almonds (10,370 t) came from the United States (60%). China was the leading exporter of walnuts (91,36%, 6,966 t) and pistachios (2,647.6 t).

Uzbekistan is one of the world's leading importers of walnuts (741,9 t) and almonds (274.9 t). Iran is a major importer of pistachios (4,858 t), followed by Turkey (1,137.8 t), which imported 594.8 t of almonds and 454.1 t pistachios in 2023. Other major importers include Tajikistan (953.9 t), Ukraine (438.2 t) and Kyrgyzstan (342.3 t).

It was revealed that out of the 53 nut samples from China, 35.8% exceeded the maximum permissible level of AFB1. Furthermore, walnuts, peanuts, and almonds exceeded the permissible values for total aflatoxins, with concentrations ranging from 0,0014 to 0,6 mg/kg (Table 3).

Table 3. Contamination of imported nuts with aflatoxin AFB1

| Country | Number of samples | Positive | %, positive samples | Average AFB1 concentration |
|---|---|---|---|---|
| China | 53 | 19 | 35,8 | 0,004 mg/kg |
| Uzbekistan | 43 | 11 | 25,5 | 0,009 mg/kg |
| Iran | 45 | 8 | 17 | 0,01 mg/kg |
| Russia | 24 | 5 | 20,8 | 0,01 mg/kg |
| Turkey | 16 | 4 | 25 | 0,02 mg/kg |
| India | 21 | 4 | 19 | 0,015 mg/kg |
| Vietnam | 17 | 3 | 17,6 | 0,006 mg/kg |

Of the 43 nut samples from Uzbekistan, 25,5% exceeded the maximum permissible level of AFB1. Furthermore, peanuts (0-0.6 mg/kg), walnuts (0-0,55 mg/kg), pistachios (0-0,07 mg/kg), hazelnuts (0-0,26 mg/kg), and almonds (0-0,045 mg.kg) exceeded the permissible values for total aflatoxins. The average concentration was 0,009 mg/kg.

The average maximum concentration of AFB1 in nuts from Russia was 0,01 mg/kg, detected in pecans and walnuts (0-0,009 mg/kg) and hazelnuts (0-0,045 mg/kg). Of the 16 samples imported from Turkey and analyzed for AFB1, 25% exceeded the limit (walnuts, 0-0,003 mg/kg; pistachios, 0-0,045 mg/kg). The average concentration of AFB1 in nut samples from India was 0,015 mg/kg (19%). Among nut samples from Vietnam, three out of 17 (17,6%) exceeded the maximum permissible level of the mycotoxin.

According to organoleptic indicators, all imported nuts were classified as premium and first grade. However, some exceptions were noted: dry, underdeveloped, or damaged shelled walnuts (8,00%) and moldy (1,00%) samples from China; kernels with mechanical damage (20,00%); moldy samples (2,00%); several shelled walnut samples from Chile (20,00%); dry, underdeveloped and damaged hazelnut kernels from Russia (8,00%); shelled walnuts and almonds from Uzbekistan (10,00%)

The domestic nut market mainly comprises imports, which account for more than 70% of the total volume. Large-scale nut cultivation in Kazakhstan began only in 2016-2017, so there is still a lack of experience, specialists, and domestic scientific developments in this area. However, domestic production remains insufficient to meet market demand, leading to continued walnut imports from China, the world's largest producer.

Overall, the results demonstrate that aflatoxin contamination in nuts sold on the domestic and international markets exceeded the maximum permissible levels. Therefore, food safety regulatory authorities should take immediate measures to address this issue, which has serious health implications.

Sampling nuts in markets and reviewing the documents of a major supplier, Sarvinoz-S LLP, revealed that nuts produced in China (almonds, walnuts, cashews, and peanuts), Vietnam (shelled cashew and peanuts), India (peanuts), Azerbaijan (hazelnuts), and Brazil (peanuts) exceeded the maximum permissible levels of aflatoxins.

A quarantine phytosanitary control certificate is issued when units are imported in accordance with the quarantine rules for protecting the territory of the Republic of Kazakhstan. To grant this certificate, samples for analysis and examination (storage pests) are taken by state plant quarantine inspectors. Unfortunately, there is inadequate control and verification of quality compliance certificates.

The research illustrates that nuts imported from various countries (China, Iran, and Turkey, among others) exceed the aflatoxin limits for imports into the European Union (EU). The EU has one of the highest food safety standards in the world, largely due to its robust legislation, ensuring the safety of the food and feed sold there. On the other hand, the food safety standards in Kazakhstan and the CIS countries need to be harmonized with the Codex alimentarius because only then will it be possible to

assess the safety of the nuts consumed in the region. Aflatoxin contamination has often caused concern in international trade, as evidenced by notifications published by the EU (RASFF Annual report, 2016) on peanuts exceeding the maximum permissible level of AFB1. Given the increased awareness of the serious health risks associated with aflatoxins, the EU has established strict rules according to which products sold for human consumption cannot exceed 2 and 4 μg/kg of AFB1 and total aflatoxin, respectively (EC 1881/2006). Codex Alimentarius has set a maximum residue limit (MRL) for AFB1 at 15 μg/kg (CODEX STAN 193-1995), while the Food Safety Standards Authority of India has set it at 10 μg/kg for ready-to-eat nuts, 0,004 mg/kg according to EU Commission Regulation No. 1881-2006, and 15 μg/kg for nuts for further processing.

According to data from the Rapid Alert System for Food and Feed (RASFF), most notifications in the EU regarding agricultural products that do not meet requirements, followed by a prevention of entry into the domestic market or withdrawal, concern nuts. Nuts and nut products have consistently been ranked first in this rating over the past four years. The main reasons for non-compliance are pesticides (47,7%), mycotoxins (35,0%), and pathogenic microorganisms (9,6%). Aflatoxins in nuts are the most common cause for concern reported by countries such as Turkey, China, the United States, Argentina, Egypt, and Iran.

**Conclusion**

This study provides the first comprehensive assessment of mycotoxin contamination in nuts commercialized in Kazakhstan. Three key findings emerge from the analysis. First, despite favourable organoleptic characteristics classifying all Kazakh walnut samples as top grade according to GOST standards, AFB1 contamination was detected across all regional sampling sites. Second, contamination levels in walnuts from Shymkent (0,039-0,045 mg/kg) substantially exceeded the MPL of 0,005 mg/kg established by the Customs Union and EU regulations, posing potential food safety risks. Third, Kazakh walnuts demonstrated lower AFB1 levels compared to imported nuts from China, Uzbekistan, and Russia, where 17,6-35,8% of samples violated safety standards.

The findings carry significant implications for public health and trade regulation. The discrepancy between organoleptic quality and mycotoxin content highlights the inadequacy of visual inspection alone for food safety assurance in nut production and import control. The pronounced geographic variation within the Turkestan region suggests localized factors – possibly pre-harvest conditions, drying practices, or storage parameters – warranting targeted intervention.

Practical recommendations:

1. Test imported nuts, issue certificates of conformity, and develop a national online database modeled after RASFF to identify non-compliant nut importers.
2. Eliminate the fragmented approach to nut safety.
3. Conduct mandatory moisture content analysis of imported nuts at Veterinary and Sanitary Examination laboratories in the markets in Astana.
4. Maintain regulated temperature controls and prevent high humidity during storage and commercial distribution.

**Authors' Contributions**

LA, BM, ASh and AS: participated in the collection of domestic and imported nut samples in various regions of Kazakhstan and conducted the organoleptic analysis; LA, AS and ZhY: performed the physicochemical characterization to determine the quality and moisture content of the products in accordance with regulations; LA, ASh and AS: determined the content of aflatoxin B1 using HPLC and ELISA.

LA, BM, ASh, AS and ZhY: monitored the market, performed statistical data processing, and prepared a literature review and the final draft of the article; LA, BM, ASh, AS and ZhY: conducted the final revision and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the article.

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# Rhodococcal antigen: Evaluation of the antigenic unit using horse blood serum in the prolonged complement fixation test

Gulnaz D. Ilgekbayeva, Makpal Z. Zanilabdin, Bauyrzhan. K. Otarbayev
Yerlan Dutbayev, Kanat A. Orynkhanov, Bayan A. Valieva

Kazakh National Agrarian Research University, Almaty, Kazakhstan

**Corresponding author:** Gulnaz D. Ilgekbayeva: gulnaz66@mail.ru
**Co-authors:** (1: MZ) m.zanilabdin@mail.ru; (2: BO) bauken_68@mail.ru
(3: YD) yerlan.dutbayev@kaznaru.edu.kz; (4: KO) k_orynkhanov@mail.ru
(5: BV) v.ba.yan@mail.ru
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**Abstract**

Background and Aim. *Rhodococcus equi* pneumonia occurs endemically on some farms, whereas on others it occurs sporadically or is not detected at all. On endemic farms, the prevalence and severity of the disease may also vary seasonally. This study aimed to assess the seroprevalence of R. equi infection in horses from selected regions of Kazakhstan.

Materials and Methods. A serological study was conducted to assess the prevalence of *Rhodococcus equi* infection in horses. A total of 260 serum samples collected from four administrative regions (Astana city, Erementau district, Shieli district, and Zhanakorgan district) were examined using the prolonged complement fixation test (PCFT). Statistical analysis included descriptive statistics, distribution assessment, and one-way analysis of variance.

Results. The overall seroprevalence was 19.6%. The highest seroprevalence was observed in the Erementau district (41.8%) and Astana city (36.0%), whereas the lowest was recorded in the Shieli district (12.0%), with an intermediate value in the Zhanakorgan district (17.1%). No statistically significant differences in mean seroprevalence were detected between districts ($p = 0.231$). However, analysis at the individual farm level revealed a highly heterogeneous, clustered distribution of the pathogen. The distribution of seropositivity was markedly right-skewed, with a median of 15%, and 25% of farms were completely free of infection. At the same time, localized outbreaks with infection rates of up to 66.7% were detected. The antibody titers were predominantly at the 1:10 level (56.9%), which, together with the focal distribution pattern, suggests endemic circulation of the pathogen.

Conclusion. The findings indicate that epidemiological surveillance should be shifted from territorial to farm-level monitoring in order to ensure more targeted detection and control of infection foci.

**Keywords:** clustered distribution; endemic circulation; epizootology; long-term complement fixation test; *Rhodococcus equi*; seroprevalence.

## Introduction

*Rhodococci* are aerobic, gram-positive, pleomorphic, non-motile bacteria commonly found in soil and capable of proliferating on simple nutrients provided by herbivore manure. They also grow well in the intestines of grazing animals. *Rhodococcus equi* is the most frequently isolated species of the genus *Rhodococcus* and is recognized as an important veterinary pathogen. It causes bronchopneumonia,

particularly in foals [1]. *Rhodococcus equi* was first isolated from foals with bronchopneumonia in 1923 [2]. Currently, the genus *Rhodococcus* is distinguished from closely related acid-fast or partially acid-fast genera, such as Gordonia, Nocardia, and Mycobacterium [3].

*Rhodococcus* equi is a ubiquitous bacterium. The genus *Rhodococcus* is closely related to the genera *Mycobacterium* and *Corynebacterium*. These bacteria are aerobic, gram-positive coccobacilli capable of invading macrophages. *Rhodococcus equi* is widely recognized as the causative agent of purulent bronchopneumonia in foals, also known as rhodococcosis. The disease primarily affects foals during the first 3 months of life. Due to high morbidity, mortality, and treatment costs, the disease has a significant economic impact on horse breeding worldwide. However, our understanding of this disease remains limited, and many aspects remain unclear [4-9].

*Rhodococcus equi* is a soil organism that is widely distributed in the environment and colonizes the intestinal tract of many herbivores [1, 10, 11]. Virulent strains expressing 15-17-kDa antigens have frequently been isolated from horses and soil on horse-breeding farms [12-14], and human transmission from soil or animals has been documented [15-17].

*Rhodococcus equi* pneumonia occurs endemically on some farms, whereas on others it occurs sporadically or is absent on others. Prevalence and severity may vary seasonally [18, 19]. In Australia, 1%-10% of Thoroughbred foals are diagnosed with *R. equi* pneumonia annually, with mortality rates historically reaching 61%. Early diagnosis and improved antimicrobial therapy have contributed to reduced mortality [20].

In many endemically infected farms, morbidity reaches 20%, while mortality ranges from 5% to 100% [21, 22].

Regardless of the diagnostic methodology, *R. equi* pneumonia is often primarily a clinical diagnosis supported by laboratory tests. Early detection and treatment can significantly improve prognosis.

This study obtained a *rhodococcal* antigen for the diagnosis of *rhodococcosis* in horses and evaluated its performance in the prolonged complement fixation test.

**Materials and Methods**

Preparation of the Seed Culture A lyophilized culture of *Rhodococcus equi* was used as the inoculum source for antigen production.

Sterile physiological saline was added to an agar slant containing the culture to prepare the inoculum. After the microbial mass was completely dissolved, 10-fold serial dilutions were prepared ($10^{-7}$-$10^{-9}$) in sterile saline (4.5 cm3 per tube). From the last two dilutions, 0.1 $cm^3$ aliquots were inoculated onto 3-5 Petri dishes containing tryptone soy agar (TSA). The agar plates were pre-dried in an incubator at 37-38 °C for 24 h. The microbial suspension was evenly spread and incubated at 37 °C-38 °C for 48 h. Colonies were examined for purity and morphological characteristics. Typical colonies were subcultured in test tubes containing nutrient medium. The culture grown for 48 h at 37 °C was used as the working seed culture. It was stored on agar slants at 2-4 °C for no longer than 5 weeks.

Inoculation of *R. equi* suspension into flasks. The 48-h-old culture of the strain was washed off with physiological saline to obtain a suspension containing 2-5 × $10^9$ microbial cells per 1.0 cm3, according to the optical turbidity standard. This suspension was used to inoculate flasks containing TSA. The inoculated flasks were moistened with 4.0-5.0 cm3 of the suspension and incubated for 3 days at 37 °C. After 24 h, the agar surface in the flasks was re-moistened to enhance growth. After a further 48 h, the purity and typical growth characteristics of the grown culture were visually assessed.

Antigen preparation. The culture was washed off using sterile 0.5% phenolized physiological saline (pH 7.0-7.2) at a volume of 25-30 $cm^3$ per flask. The resulting suspension, at an approximate concentration of 20-30 × 109 microbial bodies per 1.0 $cm^3$ (turbidity standard), was filtered through a double layer of gauze into bottles and heated in a water bath at +70 °C for 60 min.

The heated suspension was stored in a refrigerator at 2-4 °C for 7-10 days after cooling. In parallel, it was tested for purity and sterility by microscopy and culture.

Sterility testing. From five antigen vials, inoculations were performed using a sterile glass pipette as follows: 0.2-0.3 cm3 onto meat-peptone agar and Sabouraud agar in tubes, and 0.5-1.0 $cm^3$ onto meat-peptone broth and meat-peptone liver broth under a layer of petroleum jelly oil in vials.

The inoculated media were incubated for 10 days at 37 °C-38 °C; Sabouraud agar was incubated at 20-24 °C. All cultures were required to remain sterile throughout the observation period.

Antigen control in the prolonged complement fixation test (PCFT).

Titration of the indicator system. Reaction components (blood serum, antigen, and complement) were used at a volume of 0.2 $cm^3$ each, whereas the indicator system was used at the working dose determined by titration (Table 1).

Table 1. Determination of the indicator system titer in PCFT

| Reaction components | Tube No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Negative blood serum, 1:5 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Rhodococcosis antigen, 1:200 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Complement at working dilution | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Refrigerator at 2-6 °C for 16-18 h | | | | | | | | | | |
| Indicator system | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1.0 |
| Water bath at 37 °C for 20 min | | | | | | | | | | |
| Result | CH | CH | CH | CH | CH | CH | PH | PH | PH | NH |
| *Note: CH - complete hemolysis; PH - partial hemolysis; NH - no hemolysis* | | | | | | | | | | |

To perform the titration, 10 tubes were placed in a rack. Negative blood serum diluted to 1:5 was added and inactivated in a water bath at 62-64 °C, after which the *rhodococcosis* antigen at the working titer (antigen unit) and complement at the working titer were added at 0.2 $cm^3$ each. The tubes were thoroughly mixed and incubated at 2-4 °C for 16-18 h. The rack was maintained at room temperature for 20 min. The indicator system (2% sheep erythrocyte suspension and a triple dose of hemolysin in equal volumes) was then added in increments from 0.1 to 1.0 $cm^3$ at 0.1 $cm^3$ intervals, followed by incubation in a water bath at 37 °C for 20 min. The working titer was defined as the close one interval below that which yielded complete erythrocyte hemolysis.

Determination of antigen activity in the PCFT. The antigen was diluted in physiological saline (pH 7.0-7.2) to 1:100, 1:150, 1:200, 1:250, 1:300, and 1:350 for chessboard titration. Positive anti - *R. equi* serum was preliminarily dispensed into tubes at dilutions of 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1,280 in a volume of 0.2 $cm^3$ and inactivated in a water bath at 62-64 °C for 30 min. For each dilution, 0.2 $cm^3$ of antigen and complement (with a 50% increase over the working titer) were added in a volume of 0.2 $cm^3$ each, and the tubes were incubated in a refrigerator at +2 to +4 °C for 16-18 h (Table 2).

Table 2. Scheme for performing the main PCFT experiment

| Reaction components | Tubes for each dilution | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Test blood serum | 0,04 | 0,04 | 0,02 |
| Physiological saline | 0,16 | 0,16 | 0,18 |
| Water bath (62-64 °C), 30 min | | | |
| Rhodococcosis antigen | - | 0,2 | 0,2 |
| Physiological saline | 0,2 | - | - |
| Complement at the working dilution | 0,2 | 0,2 | 0,2 |
| Refrigeration at 2-6 °C for 16-18 h | | | |
| Indicator system | 0,4 | 0,4 | 0,4 |
| Water bath at 37 °C for 20 min | | | |

Anticomplementary activity was assessed in series containing all antigen dilutions and positive serum along with physiological saline (Table 3).

Table 3. Antigen titer determination using the chessboard method in the PCFT

| Positive serum | Test antigen | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1:100 | 1:150 | 1:200 | 1:250 | 1:300 | 1:350 | PS |
| 1:5 | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | - |
| 1:10 | 4+ | 4+ | 4+ | 4+ | 4+ | 2+ | - |
| 1:20 | 4+ | 4+ | 4+ | 4+ | 4+ | - | - |
| 1:40 | 4+ | 4+ | 4+ | 4+ | 3+ | - | - |
| 1:80 | 4+ | 4+ | 2+ | - | - | - | - |
| 1:160 | 4+ | 2+ | - | - | - | - | - |
| 1:320 | 4+ | 2+ | - | - | - | - | - |
| 1:640 | 4+ | 2+ | - | - | - | - | - |
| 1:1280 | 2+ | - | - | - | - | - | - |
| PS | 4+ | 3+ | - | - | - | - | - |
| *Note: PS. - physiological saline* | | | | | | | |

Reaction outcomes were recorded using crosses based on the degree of erythrocyte hemolysis.

The working antigen titer was defined as the dilution that produced a high antibody titer with positive serum and did not inhibit hemolysis in the physiological saline control (i.e., did not exhibit anticomplementary activity). As shown in Table 3, the dilutions of 1:200-1:300 met these criteria, yielding the highest antibody titer of 1:40. The 1:300 dilution was taken as 1 antigen unit (AU), the 1:250 dilution corresponded to 1.25 AU, and the 1:200 dilution corresponded to 1.5 AU. Thus, the working titer of the rhodococcosis antigen was selected as an AU within the range of 1.0-1.5.

Evaluation of the rhodococcosis antigen under field (production) conditions. Serological testing of equine sera for anti – *R.equi* antibodies. Blood samples (5-8 mL) were collected aseptically from the jugular vein of horses into sterile tubes. Serum was obtained from clotted blood by centrifugation (3,000 rpm for 10 min) and stored at −20 °C until use in serological assays.

Data were analyzed using RStudio software and the nonparametric Kruskal-Wallis test at a significance level of $p < 0.05$ [23, 24, 25].

**Results and Discussion**

Table 4 presents the results of testing equine blood sera for the presence of antibodies to *R. equi* using the PCFT.

Table 4. Results of equine blood serum testing using PCFT for antibodies against *R. equi*

| No. | District/area | Number of samples | PCFT results | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Positive (antibody titer) | | | | | Negative |
| | | | 1:5 | 1:10 | 1:20 | 1:40 | Total positive | |
| 1 | Astana city | 25 | 1 | 8 | 0 | 0 | 9 | 16 |
| 2 | Erementau District | 55 | 3 | 15 | 3 | 2 | 23 | 22 |
| | “Nurali” farm | 55 | 3 | 15 | 3 | 2 | 23 | 22 |
| 3 | Shieli District | 100 | 3 | 3 | 4 | 2 | 12 | 88 |
| | Kurbanbekov N. | 20 | 0 | 1 | 1 | 0 | 2 | 18 |
| | “Aliyev” farm | 20 | 1 | 0 | 1 | 1 | 3 | 17 |
| | “Kozhakhmet” (IE) | 20 | 0 | 1 | 0 | 1 | 2 | 18 |
| | “Kanagat” farm | 20 | 1 | 1 | 1 | 0 | 3 | 17 |

Continuation of Table 4

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | “Bibi” farm | 20 | 1 | 0 | 1 | 0 | 2 | 18 |
| 4 | Zhanakorgan District | 80 | 3 | 6 | 5 | 2 | 16 | 64 |
| 1 | “Qosqozha” farm | 6 | 1 | 1 | 1 | 1 | 4 | 2 |
| | “Damu” farm | 4 | 0 | 1 | 0 | 0 | 1 | 3 |
| 2 | “Aidarhan” farm | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| | “Barys-4” farm | 4 | 0 | 0 | 0 | 0 | 0 | 4 |
| | “Miras” farm | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| | Nurmaganbek E. | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3 | “Nur-Abyl” farm | 10 | 0 | 1 | 1 | 0 | 2 | 8 |
| 4 | Alimbetov A. | 10 | 1 | 1 | 1 | 0 | 3 | 7 |
| 5 | “Damir-S” farm | 10 | 0 | 1 | 1 | 1 | 3 | 7 |
| 6 | “Aksarai” farm | 10 | 0 | 0 | 0 | 0 | 0 | 10 |
| 7 | “Kobeldes” farm | 10 | 1 | 1 | 1 | 0 | 3 | 7 |
| 8 | “Nur” farm | 10 | 0 | 0 | 0 | 0 | 0 | 10 |
| | Total | 260 | 9 | 29 | 8 | 5 | 51 | 209 |

Serological testing of 260 equine serum samples by PCFT revealed that antibodies to *Rhodococcus equi* were detected in 51 cases, yielding an overall seroprevalence of 19.6%. The district-level seroprevalence is presented in Table 5.

Table 5. Seroprevalence of *R. equi* by district

| District/area | Number of samples | Positive | Seroprevalence, % |
|---|---|---|---|
| Astana city | 25 | 9 | 36,0 |
| Erementau District | 55 | 23 | 41,8 |
| Shieli District | 100 | 12 | 12,0 |
| Zhanakorgan District | 80 | 16 | 20,0 |
| | | P-value | 0.2311 |

Table 5 summarizes the mean seroprevalence values in the four administrative units under investigation. The highest seroprevalence was recorded in the Erementau District (41.82%), whereas the lowest was observed in the Shieli District (12.00%). The result of a one-way analysis of variance, expressed as $p = 0.2311$, exceeds the commonly accepted threshold for statistical significance ($\alpha = 0.05$), indicating that there were no statistically significant differences among the mean seroprevalence levels in the compared groups at the time of the study. Therefore, the observed variation may be attributed to random intra-sample variability. The distribution of antibody titers among the 51 samples that tested positive for *rhodococcosis* is shown in Table 6 and Figure 1.

Table 6. Distribution of the antibody titers

| Titer | Number of samples | Proportion, % |
|---|---|---|
| 1:5 | 9 | 17,6 |
| 1:10 | 29 | 56,9 |
| 1:20 | 8 | 15,7 |
| 1:40 | 5 | 9,8 |

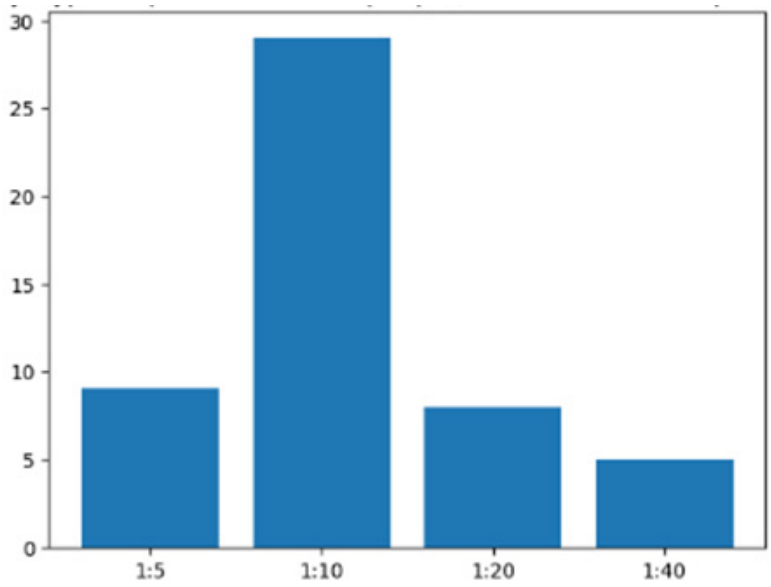
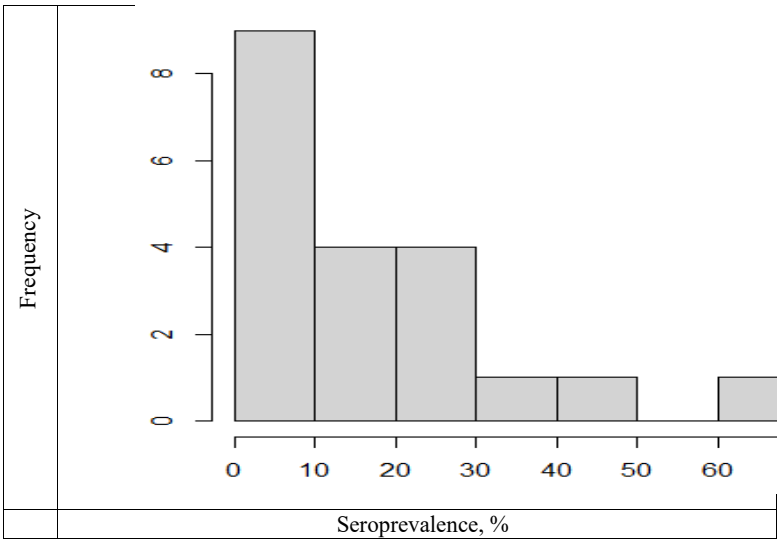



Figure 1. Distribution of R. equi antibody titers among positive samples (n = 51)

Within the structure of positive reactions, the 1:10 titer predominated (56.9%), whereas high titers (1:20-1:40) accounted for 25.5%, indicating pathogen circulation and active epizootic foci.

*R. equi* infection in horses occurs endemically on some breeding farms and sporadically on others, and it remains unrecognized on most farms [26]. Despite these epidemiological differences, immunological evidence of infection develops in most horses [27, 28], although higher antibody levels are most commonly observed on farms where the disease is endemic. Extrapolating from these epidemiological characteristics, as well as from in vitro studies of macrophage – *R. equi* interactions [29], it appears that young foals may overcome infection when exposed to low numbers of *R. equi*; however, intense or continuous exposure predisposes them to clinical disease, particularly in the absence of antibodies or fully competent cell-mediated immune mechanisms. A progressive increase in environmental contamination with *R. equi* has been observed on horse-breeding farms [30], which is associated with the duration of farm use for housing horses (presumably reflecting horse density and manure disposal practices, summer temperatures, soil type, and whether the farm is used for raising foals).

A statistical summary of the seroprevalence distribution across farms demonstrates pronounced right-skewness: the minimum value and the first quartile are both 0%, indicating a substantial proportion of unaffected farms; the median is 15%, reflecting a moderate typical level; and the arithmetic mean (17.97%) exceeds the median, suggesting the influence of a limited number of high values. This result is further supported by a third quartile of 30% and a maximum of 66.67%. This distribution highlights the heterogeneous nature of the epizootic process, with the formation of localized high-intensity foci against an overall favorable situation in most surveyed sites (Figure 2).




| Min. | 1st Qu. | Median | Mean | 3rd Qu. | Max. |
|---|---|---|---|---|---|
| 0.00 | 0.00 | 15.00 | 17.97 | 30.00 | 66.67 |

Figure 2. Overall distribution of seroprevalence values in the districts under study

Epizootiological assessment. The predominance of an antibody titer of 1:10 suggests previous exposure or latent infection. High titers (≥1:20) indicate active circulation of the pathogen on multiple farms.

The presence of seropositive animals in all districts indicates a wide distribution of R. equi. The predominance of low and moderate titers is characteristic of an endemic focus with continuous pathogen circulation. The detection of a 1:40 titer necessitates epizootiological control and monitoring of young stock.

**Conclusions**

Based on the serological survey, infection caused by R. equi is widespread in the studied region but statistically homogeneous at the administrative district level, as evidenced by the absence of significant inter-district differences ($p = 0.231$). The markedly heterogeneous, clustered distribution of the pathogen at the individual farm level is the key epizootiological feature. This is manifested by the formation of local foci with high seroprevalence (up to 66.67%) against a background of predominantly unaffected or minimally affected populations (median 15%, first quartile 0%). This pattern, together with the predominance of the 1:10 titer among positive results and a substantial proportion of high titers (≥1:20), is typical of the pathogen's sustained endemic circulation. Under such conditions, the primary risks and control measures should be focused on specific affected farms rather than based on a territorial approach.

**Authors' Contribution**

GI and MZ: conceived and designed the study, performed a comprehensive literature search, analyzed the collected data, and prepared the manuscript. MZ, KO, and BV: participated in sample collection, serum separation, and storage. GI, BO, and BV: prepared reaction components and participated in performing the assays, recording the data, and interpreting the results. ED: performed the biostatistical analysis of the obtained results. All authors have read, reviewed, and approved the final version of the manuscript.

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# Outbreak prediction methods and measures to control the spread of camelpox in Kazakhstan

Yekaterina O. Ostapchuk [1,2], Andrey V. Zhigailov[1], Yuliya V. Perfilyeva[1]
Anna S. Nizkorodova[1], Aida M. Abdybekova[3], Seidigapbar M. Mamadaliyev[1]

[1]Almaty Branch of the National Center for Biotechnology, Almaty, Kazakhstan
[2]ECO-Consulting LLC, Almaty, Kazakhstan
[3]Kazakh Scientific Research Veterinary Institute, Almaty, Kazakhstan

**Corresponding author:** Yekaterina O. Ostapchuk: katyostapchuk@gmail.com
**Co-authors:** (1: AZh) andrzhig@gmail.com, (2: YP) perfilyevayulya@gmail.com
(3: AN) cool.niz@yandex.ru, (4: AA) aida_abdybekova@mail.ru
(5: SM) mamadaliyev.s@bk.ru
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**Abstract**

Background and Aim. *Camelpox* is a highly contagious orthopoxviral disease of camelids causing fever, lymphadenopathy, skin lesions, abortions, and high mortality in young animals. It is endemic across Africa, the Middle East, and Asia and has been repeatedly reported in Kazakhstan, most recently in 2019-2020 in Mangystau and Atyrau oblasts. With the camel population on the rise, the risk of rapid disease spread from new foci is rising. This study aimed to summarize current evidence on *camelpox* in Kazakhstan and to propose practical approaches for assessing the epizootiological situation, forecasting outbreaks, and implementing veterinary control measures based on surveillance data and a risk analysis conducted in 2021-2022.

Materials and Methods. A targeted review of publications indexed in PubMed/Medline and Google Scholar, WOAH resources, national statistics, and selected media reports was performed (coverage up to November 22, 2025). Evidence was synthesized to define key risk indicators for introduction and spread, establish principles for surveillance design (including minimum sample size estimation and sampling across epizootiological units), and formulate recommendations for diagnostics and interventions. Findings from the authors' 2021-2022 cross-sectional survey in western and southern Kazakhstan were incorporated.

Results. Historical outbreak data indicate periodic recurrence in western regions. In the 2021-2022 survey (486 camels from 63 herds across seven regions), antibodies to CMLV were detected in 10.9% of unvaccinated and 73.6% of vaccinated animals; all unvaccinated seropositive cases were confined to Atyrau oblast. Viral nucleic acid was detected in 1.1% of unvaccinated seropositive animals, and phylogenetic analysis confirmed CMLV. A three-zone framework for Kazakhstan was proposed to guide risk-based surveillance and vaccination strategies.

Conclusion. Continuous risk-based surveillance integrating serology and molecular testing, coupled with targeted vaccination and strengthened movement control in high-risk areas, is essential to prevent introduction, limit spread, and support potential regional eradication of *camelpox* in Kazakhstan.

**Keywords:** *Camelpox virus*; Kazakhstan; outbreak forecasting; veterinary measures.

**Introduction**

*Camelpox* is a highly contagious disease of tylopods caused by the camelpox virus (*Camelpox* virus, CMLV; genus *Orthopoxvirus*, family *Poxviridae*). Clinically, camelpox presents with systemic and dermatological signs, including fever, lymph node enlargement, and progressive skin lesions that may appear as nodules, papules, or generalized rash. Reproductive disorders such as abortion can occur, and mortality is particularly high among juvenile animals [1].

CMLV is a member of the genus *Orthopoxvirus* and possesses a large linear double-stranded DNA genome characterized by terminal hairpin loop structures. The viral genome is approximately 205.7 kb in length and encodes more than 200 predicted genes. Structurally, the virion consists of a nucleoprotein core surrounded by a complex, multilayered envelope composed of lipoproteins with embedded surface projections. Replication of CMLV occurs entirely within the cytoplasm of infected cells, where virus-specific inclusion bodies are formed. Despite their environmental stability, virions are susceptible to commonly used disinfectants and detergent-based agents.

A distinguishing biological property of *orthopoxviruses* is their capacity to induce hemagglutination. Besides CMLV, this genus comprises several other species of medical and veterinary relevance, including cowpox virus (CPXV), variola virus (VARV), vaccinia virus (VACV), monkeypox virus (MPXV), raccoonpox virus (RCNPV), vole poxvirus (VPXV), skunkpox virus (SKPXV), and ectromelia virus (ECTV) [2]. From a phylogenetic perspective, CMLV exhibits the closest genetic relationship to variola virus, the etiological agent of smallpox [2]. In addition to genomic similarity, these viruses share comparable biological properties and aspects of pathogenesis, which has attracted particular scientific interest. Therefore, the mutational processes affecting the CMLV warrant close attention from the scientific community, as it is considered a potential zoonotic infectious agent. Although traditionally regarded as a disease of camelids, accumulating evidence indicates that CMLV possesses zoonotic potential. Human infections have been documented in recent years, typically presenting with mild to moderate clinical symptoms such as febrile episodes, cutaneous lesions, and gastrointestinal disturbances [3, 4]. In view of this capacity to infect humans, CMLV is categorized as a Group 2 biological risk agent. From a veterinary standpoint, *camelpox* is recognized internationally as a disease of regulatory importance and is listed among notifiable animal diseases maintained by the World Organisation for Animal Health (WOAH). Due to reduced productivity in infected adult animals, high mortality rates among young stock, and abortions in female camels, *camelpox* causes significant economic losses in countries and regions with developed camel husbandry. Large outbreaks of *camelpox* typically exhibit a cyclical pattern, recurring in the same territories at intervals of 10-25 years [1, 5].

*Camelpox* remains enzootic across large parts of the Middle East, Africa, and Asia. In Kazakhstan, the infection has historically been concentrated in the western regions of the country. Recurrent outbreaks were documented in Mangystau and Atyrau oblasts in the 1930s, again during 1942-1943, and later between 1965 and 1969 [6], with another episode reported in 1996 [5]. In 2012, increased mortality among camels was recorded in the Mangystau region, with clinical manifestations resembling *camelpox*; however, laboratory investigations did not confirm the presence of CMLV [7]. More recently, during 2019-2020, confirmed cases of camelpox were registered in the rural districts of Tazhen and Syngyrlau (Mangystau region), affecting at least 75 animals [5]. Camels in Kazakhstan are mainly imported from Turkmenistan [8], where outbreaks of *camelpox* occur regularly. The most recent outbreak in Turkmenistan was reported in 2018, during which, in addition to infected camels, at least five cases of human infection were documented [9].

Since the most recent outbreak in 2020, the camel population in Kazakhstan has been increasing annually, thereby elevating the risk of rapid disease spread in the event of a new outbreak. Unfortunately, studies on the prevalence of *camelpox* in Kazakhstan are largely lacking, with the exception of the Mangystau and Atyrau regions, despite the widespread distribution of camel breeding in other parts of the country [10].

As a result of a cross-sectional study conducted by us in 2021-2022, the circulation of CMLV in the Atyrau region was demonstrated. Serological surveillance, which analyzed serum samples from 486 camels originating from 63 herds across seven regions of western and southern Kazakhstan, revealed the presence of antibodies against CMLV in 10.9% of unvaccinated camels and 73.6% of vaccinated camels. All seropositive unvaccinated animals were identified exclusively in the Atyrau region. In

addition, CMLV RNA was detected in three (1.1%) unvaccinated seropositive animals from the Atyrau region. Phylogenetic analysis of one sequenced PCR-positive sample confirmed that the detected strain belonged to CMLV [11]. These findings highlight the importance of continuous surveillance of this infection and the implementation of effective control measures in regions at increased risk.

However, the lack of a clear understanding of the epizootiological situation regarding *camelpox* in the country limits the effective application of control measures, such as mass vaccination of camels in regions at high risk of infection. In the absence of a unified national disease control system, vaccination is carried out privately by farmers, often resulting in only partial herd coverage. The effectiveness of such privately administered vaccination remains unknown. Incomplete and ineffective vaccination facilitates the spread of infection within herds in the event of virus introduction from neighboring countries. Eradication of the infection under such conditions would require substantial financial and labor resources.

**Materials and Methods**

To prepare the literature review and to develop approaches for assessing the epizootiological situation and forecasting outbreaks, as well as to formulate recommendations for veterinary interventions and the camelpox control in Kazakhstan, we analyzed scientific publications indexed in the Medline (PubMed) and Google Scholar databases, data from official sources of the World Organisation for Animal Health (WOAH) and the Bureau of National Statistics of the Republic of Kazakhstan, as well as reports from news agencies. The literature search was conducted using the following keywords: *"camelpox"* and/or "camelpox transmission routes" and/or "risks of camelpox spread." In total, 42 data sources were identified from scientific databases and 3 from other sources. Following a thematic analysis of the literature across three main research areas and after the exclusion of sources containing duplicate data, the final review included 17 publications from scientific databases, 2 sources containing statistical data, 2 reports from news agencies, and 2 publications authored by the present study's authors addressing the distribution and risk assessment of *camelpox* spread in the Republic of Kazakhstan. The literature search was limited to publications available up to November 22, 2025.

**Results and Discussion**

*Transmission mechanisms, clinical signs, and disease course*

The primary route of virus transmission is direct contact. Virus dissemination occurs primarily through direct contact with infected animals. Pathogen shedding into the environment takes place via mucosal secretions and lesion exudates containing high viral loads. In addition, tissues and fluids associated with abortion represent a significant source of infection. Transmission typically follows the penetration of the virus through damaged skin or mucous membranes, with increased susceptibility observed in animals experiencing epithelial injury or nutritional deficiencies [2]. The presence of CMLV genetic material has also been reported in *Hyalomma dromedarii*, a principal ectoparasite of camels, suggesting a potential auxiliary role of arthropods in virus maintenance and mechanical spread [12]. The involvement of other competent arthropod vectors, such as blood-feeding flies, cannot be excluded. However, if vector-borne transmission occurs, it does not appear to play a primary role in virus spread [2].

The incubation period ranges from three to fifteen days and is shorter in young animals than in adults. The disease may present in acute (most commonly in newborn and young animals), subacute, or chronic forms and is often latent, with the latter being more frequently observed in pregnant camels. The most characteristic manifestation of *camelpox* is the cutaneous form with a subacute disease course, characterized by mucous discharge, edema, and rash, which subsequently progresses to gray-colored papules and pustules. In some animals, corneal opacity (leukoma) develops. Infected newborn camel calves often die. In cases of systemic infection, diarrhea and anorexia may be observed. Although *camelpox* rarely results in fatal outcomes in adult animals (mortality rates in adults range from 5% to 28%, whereas in young animals they range from 25% to 100%), death may occur due to secondary infections arising from post-infection immunosuppression, as well as sepsis [13].

At present, there is no approved specific antiviral therapy for camelpox. Although experimental studies have demonstrated that certain antiviral compounds can inhibit viral replication under laboratory conditions, these approaches have not translated into routine clinical application [14]. Consequently,

prevention remains the cornerstone of disease control. Immunization is the principal strategy used to reduce morbidity and limit virus circulation. Both live attenuated and inactivated camelpox vaccines are available and have been implemented in different countries. In addition, vaccinia virus–based preparations have demonstrated cross-protective efficacy against CMLV infection [13]. In terms of protective efficacy, attenuated vaccines generally induce stronger and more durable immunity than inactivated formulations. Protection following administration of inactivated vaccines is typically short-term and requires annual revaccination, whereas live attenuated vaccines may provide immunity lasting several years. Nevertheless, their use in areas officially free of *camelpox* should be approached cautiously. Circulation of vaccine-derived strains within naïve camel populations may occur and can be associated with undesirable effects, including temporary reductions in productivity such as decreased milk yield and slower weight gain.

*Methods, principles, and procedures for forecasting camelpox outbreaks*

To analyze the risks of introduction and spread of camelpox, as well as to assess the level of potential economic damage associated with the dissemination of this pathogen among tylopods, it is essential to evaluate the following factors:

1) the history of *camelpox* outbreaks in the target territory;
2) the density and total population size of animals susceptible to CMLV (camels) in the given region;
3) the mode of pathogen transmission and, where vectors are involved, an assessment of their distribution within the region;
4) the availability of preventive measures aimed at minimizing the risks associated with disease spread (e.g., vaccines), the extent of their implementation in practice (including vaccine accessibility for farms), and the availability of therapeutic options;
5) the genotype of the pathogen, which determines the severity of clinical manifestations, mortality rates, and the level of infectivity;
6) the current status of the epizootiological process of *camelpox* in the given region (or country), including the average level of seroprevalence in herds;
7) the risk of disease introduction from endemic regions or neighboring countries and an assessment of the potential rate of spread, taking into account the geographical characteristics of regions (i.e., suitability or unsuitability for camel breeding);
8) the capacity for disease eradication in endemic areas, including the presence or absence of legislative frameworks for disease control and eradication.

Analysis of the history of *camelpox* outbreaks. A thorough analysis of data on previous *camelpox* outbreaks within the country is of critical importance, as well as an examination of media reports describing outbreaks of infectious diseases of unspecified etiology with clinical manifestations similar to camelpox. If a *camelpox* outbreak has previously occurred in a given region of the country, there is a high probability that a new outbreak will occur in the same region.

Animals susceptible to infection. All tylopods (camels, llamas, guanacos, and vicunas) are susceptible to CMLV infection. At the same time, only in the Old World does this infection, affecting dromedary and Bactrian camels (and their hybrids), remain widespread and exert a significant economic impact. The virus infects both dromedary and Bactrian camels with equal efficiency.

Mode of pathogen transmission. The primary route of virus transmission is direct contact. Infected animals shed the virus into the environment by producing and dispersing virus-containing exudates (gray mucus). Aborted materials are also infectious. Camels most commonly become infected when the virus enters the body through the skin and mucous membranes, particularly when their integrity is compromised or in cases of vitamin deficiency [2]. It should be noted that CMLV has been detected in the main ectoparasites of camels, the ticks *Hyalomma dromedarii* [12]. The presence of other competent arthropod vectors, such as blood-feeding flies, also cannot be excluded. However, even if vector-borne transmission exists, it does not play a primary role in virus spread [2].

Preventive control measures. Currently, therapeutic options for *camelpox* remain limited, as no specific antiviral treatment has been approved for routine veterinary use. Although experimental studies have demonstrated inhibition of viral replication by certain antiviral compounds, these findings have not led to standardized treatment protocols in field conditions [14]. Therefore, disease management relies

predominantly on preventive measures. Vaccination constitutes the main tool for controlling *camelpox*. Both live attenuated and inactivated vaccines have been developed and applied in various endemic settings. In addition, vaccinia virus–based preparations have shown cross-protective capacity against CMLV infection [13]. Comparative data indicate that live attenuated vaccines generally induce a more robust and durable immune response than inactivated formulations. Protection following inactivated vaccination is relatively short-term and typically necessitates repeated annual administration, whereas attenuated vaccines may confer immunity lasting for more than a year. However, the implementation of live vaccines in regions officially free from camelpox requires careful risk assessment. The unintended circulation of vaccine-derived strains in naïve camel populations may occur and has been associated with adverse production outcomes, including transient reductions in milk yield and slower weight gain.

Genotype of the infectious agent. Poxviruses are characterized by a very high degree of genomic stability (their genome consists of double-stranded DNA with covalently closed ends forming looped structures, and complex DNA repair systems operate within viral particles). Consequently, genetic diversity within this group is relatively limited. At the same time, certain loci allow differentiation between different poxvirus species. During prolonged virus passaging, the nucleotide loci of the ATI, L1R, and ORF-185 genes undergo changes that make them resemble the corresponding loci of vaccinia virus (VACV) [15, 16]. Thus, sequencing of amplicons derived from these CMLV gene loci enables discrimination between vaccine strains used for animal vaccination in a given region and field (non-vaccine) virus strains. Moreover, some CMLV strains may differ substantially in the severity of clinical manifestations and mortality rates.

Actual status of the epizootiological process. Regardless of the results of modeling the risk of introduction and spread of infection in a given territory, without consideration of actual data on the epizootшological process, the level of confidence in risk assessment outcomes cannot be regarded as high.

Among the key factual indicators used to assess the risk of *camelpox* is the level of seroprevalence of antibodies to CMLV in herds where vaccination has not been carried out. An overall seroprevalence of antibodies to *camelpox* exceeding 25%, together with the detection of viral DNA in the blood of at least one animal within a herd, indicates a high risk of an outbreak.

Risk of infection introduction. If a region is non-endemic for the infection, one of the most important indicators in risk assessment is the likelihood of virus introduction from regions of the country that are endemic for the disease or from other countries. To assess these risks, it is necessary, first, to analyze the epizootiological situation of *camelpox* in neighboring regions and to evaluate the proportion of livestock imported into the region from countries or areas endemic for the infection, as well as to determine the actual number of camels imported into the region. To assess the rate of infection spread in non-endemic territories, knowledge of the density of animals susceptible to CMLV alone is insufficient. It is essential to consider geographical barriers to virus dissemination, including the presence of mountain ranges, large rivers and lakes, and deserts. With regard to camelpox, climatic characteristics of the studied regions are of particular importance. Areas occupied by deserts and semi-deserts are the most suitable for camel distribution and, consequently, for the spread of the disease.

Assessment of the potential for eradication of the infection in regions endemic for the disease. If a region is determined to be endemic for *camelpox*, spontaneous elimination of the infection is highly unlikely. The disease tends to have a protracted course and persist for long periods within affected territories. Poxviruses in general are characterized by exceptionally high stability; in a dried state, they can remain viable for many years. Eradication of poxvirus infections from a given region requires the implementation of stringent national and transboundary disease control programs. An important indicator in this context is the degree of concentration of camel populations within production systems. If the majority of camels are kept by private owners in small-scale farms and household holdings, programs aimed at the control and eradication of camelpox cannot be considered effective.

*Principles for conducting surveillance studies in regions of the country with a high level of risk*

Surveillance is usually conducted in regions at the highest risk of infection. The minimum (critical) sample size required for annual surveillance studies of *camelpox* is determined using the following formula [17]:

$$\text{Sample size } (n) = N * [Z2 * p * (1-p)/e2] / [N - 1 + Z2 * p * (1-p)/e2] \quad (1)$$

where: N – camel population size in the surveillance area;
Z – critical value of the normal distribution at the required confidence level;
p – expected prevalence level, %;
e – acceptable margin of error.

As of November 13, 2025, according to official data from the Bureau of National Statistics of the Republic of Kazakhstan [18], the country's camel population totaled 297,569 animals. Since large-scale camelpox surveillance studies have not previously been conducted nationwide, the level of antibody seroprevalence was assumed to be 50%, as recommended in [17]. For epidemiological studies, a confidence interval of 95% is used in the vast majority of cases; therefore, this value is recommended for calculations, corresponding to a Z value of 1.96 [1, 17]. The acceptable margin of error is typically set at 5% in such calculations [13, 14]. Thus, for groups ranging from five to forty animals, the minimum required sample size was calculated to be 385 animals per year. As a rule, the number of animals included in surveillance exceeds the critical sample size by at least 10%, since a proportion of samples may be unsuitable for analysis (e.g., due to hemolysis of serum or coagulation of blood).

The established number of samples collected for surveillance purposes should be distributed across sampling sites, with sample collection carried out in at least ten different locations or epizootiological units (EUs). It is desirable that several districts within each region covered by the surveillance program be included. Within a given location (or EU), the selection of animals for surveillance should be random, provided that animals do not exhibit clinical signs attributable to camelpox. It is important that animals of both sexes and different age groups be included among those selected for monitoring. If animals displaying clinical signs suggestive of camelpox are identified, these animals should be additionally sampled for laboratory analysis.

Samples collected from live animals for laboratory testing include whole peripheral blood, serum, nasal swabs, and, if skin papules are present, swabs from papular lesions.

*Methods for detecting indicators of CMLV circulation*

Accurate diagnosis of *camelpox* requires careful differentiation from other infectious and non-infectious conditions presenting with similar clinical signs. Differential diagnosis should include necrobacillosis, foot-and-mouth disease, dermatophytosis and other fungal dermatoses, mange, contagious ecthyma, papillomatosis, brucellosis, as well as inflammatory skin reactions caused by arthropod bites.

A combination of serological and molecular approaches is employed for laboratory confirmation. Commonly applied serological assays include hemagglutination inhibition tests, virus neutralization assays, and enzyme-linked immunosorbent assays (ELISA). These are complemented by direct detection techniques, such as virus isolation in cell culture and polymerase chain reaction (PCR)-based methods targeting specific viral genes [13].

Serological interpretation, however, requires consideration of cross-reactivity within the genus *Orthopoxvirus*, as antibodies generated against one *orthopoxvirus* species may react with others. Despite this serological overlap, only CMLV is known to produce typical clinical disease in camels [14]. In contrast, *parapoxviruse*s and papillomaviruses infecting camelids do not exhibit serological cross-reactivity with CMLV, allowing reliable differentiation using antibody-based assays.

Additional molecular confirmation may be necessary in regions where other *orthopoxviruses* could be encountered. Although CPXV does not usually cause clinical disease in tylopods, experimental data indicate that replication in camels is possible; therefore, differentiation between CPXV and CMLV should be performed using PCR amplification and sequencing of specific genomic loci. Furthermore, in areas where vaccinia virus–based vaccines may have been used, diagnostic protocols must also exclude VACV to avoid misinterpretation of laboratory findings.

Serological assays, particularly ELISA and virus neutralization tests, are generally reliable tools for assessing exposure in unvaccinated camel populations. However, their interpretative value declines substantially in settings where vaccination has been implemented. This limitation is especially

pronounced following the use of live attenuated vaccines, as vaccine-induced antibodies are serologically indistinguishable from those generated after natural infection. Consequently, in vaccinated herds, serology alone cannot be considered a definitive indicator of active virus circulation within a region.

In the case of conventional PCR, the World Organisation for Animal Health (WOAH) recommends the use of the ATIP (A-type inclusion protein) locus for the detection of CMLV [14]. For virus detection by quantitative real-time PCR, primers targeting the HA gene developed by Pfeffer et al. [19] or the C18L gene locus [20] are recommended.

If vaccination of camels with a live attenuated vaccine is carried out in areas included in the surveillance zone, it is necessary to be able to differentiate mesogenic vaccine strains of CMLV from field virus strains at the molecular level.

*Camelpox control measures*

According to the WOAH requirements for *camelpox* [13], in the event that circulation of CMLV strains is detected in territories officially free from camelpox, it is necessary to strengthen surveillance programs in regions at risk. Such programs should include visual detection of clinical signs of the disease in camels as indicator animals, serological testing of serum samples, PCR-based analysis of nucleic acids isolated from skin swabs and peripheral blood, as well as vaccination.

Vaccination remains the principal measure for both prevention and control of camelpox. Two vaccine categories are currently in use: live attenuated and inactivated formulations. Attenuated vaccines are generally associated with prolonged immunity; however, animals immunized at an early age (before 6-9 months) require subsequent revaccination to ensure sustained protection. In contrast, inactivated vaccines induce shorter-term immunity and therefore necessitate annual administration.

Effective disease management also depends on timely laboratory confirmation. The implementation of rapid molecular diagnostic tools to verify suspected clinical cases is essential for early detection, containment, and interruption of virus transmission. Importantly, camelpox is host-specific and affects only camelids, with no known wildlife reservoir. Combined with the availability of reliable diagnostic assays and effective vaccines, this biological characteristic supports the view that *camelpox* fulfills the principal criteria for potential eradication.

From a sanitary perspective, CMLV demonstrates susceptibility to commonly used disinfectants. It can be readily inactivated by standard physical methods, including autoclaving, brief ultraviolet exposure, and boiling for at least 10 minutes. These measures may be practically implemented at the farm level to reduce environmental contamination. Control strategies should additionally include prompt isolation of clinically affected animals and immunization of susceptible stock using either established cowpox-based vaccines or specifically developed camelpox vaccines.

In order to prevent the occurrence of *camelpox* outbreaks and to ensure effective implementation of preventive measures, it is proposed to conditionally divide the territory of the Republic of Kazakhstan into three zones. These zones were defined based on our previously conducted cross-sectional study [11] and an analysis of the risks of *camelpox* emergence and spread in Kazakhstan [21]:

Zone 1 – *camelpox-affected* zone, where the infection is present in a latent form (Atyrau and Mangystau regions). In this zone, mass vaccination of camels is recommended, mandatory livestock surveillance should be implemented, and stricter control should be applied to animals that may enter the country from neighboring Turkmenistan.

Zone 2 – *camelpox-free* zone with a high risk of disease emergence and spread (West Kazakhstan, Kyzylorda, Turkestan, Zhambyl, and Almaty regions). In Zone 2, continuous serological as well as molecular genetic surveillance for camelpox is recommended. Vaccination of livestock should be carried out using inactivated rather than live attenuated vaccines until animals with clinical signs of infection are detected in these territories or until the virus or its nucleic acids are identified (by virus neutralization assay or PCR followed by DNA sequencing).

Zone 3 – *camelpox-free* zone with a low risk of infection emergence (North Kazakhstan, East Kazakhstan, Akmola, Kostanay, Pavlodar, and Karaganda regions). In this zone, animals transported from the other two zones should be carefully examined for clinical signs of camelpox. At present, an increasing number of farms in the central and northern regions of the country are purchasing camels; therefore, it is crucial to prevent the introduction of infection into these areas (Figure 1).

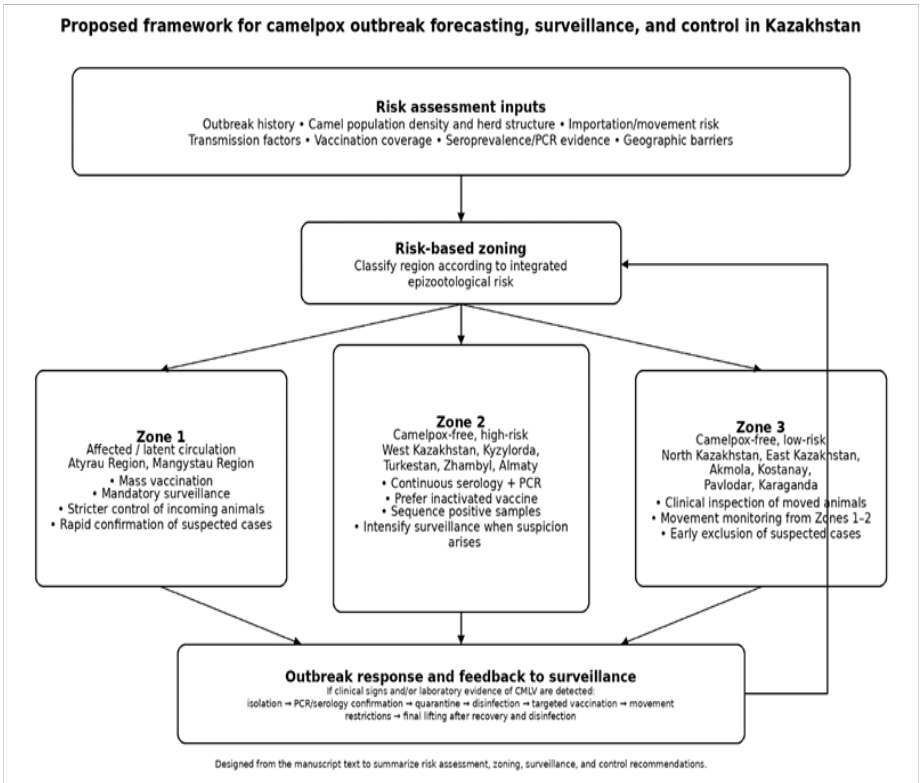


Figure 1. Proposed framework for *camelpox* outbreak forecasting, surveillance, and control in Kazakhstan. The scheme summarizes the key components of the proposed approach, including risk assessment inputs, risk-based zoning of the country, surveillance priorities for each zone, and the sequence of response measures to be implemented when clinical suspicion or laboratory evidence of *camelpox* virus circulation is detected

*Procedure for implementing veterinary measures in cases of camelpox*

This section outlines measures aimed at preventing the introduction and spread of the CMLV.

General recommendations:

- regular vaccination of camels, particularly young animals;
- in endemic areas, public awareness of risk factors is necessary to reduce the impact of the disease;
- camel herders should be informed about the zoonotic significance of *camelpox*. Routine epidemiological surveillance of the disease is required;
- validation of a simple, rapid, inexpensive, and accurate diagnostic method suitable for field use is necessary;
- further investigation of the zoonotic aspects of this disease is strongly recommended.

Measures for the prevention of *camelpox*:

To prevent the occurrence of *camelpox* and limit its spread, farm managers, organizations and institutions, veterinary specialists, as well as camel owners are required to:

- prevent the introduction (importation) into farms, holdings, subdivisions, and settlements of camels, as well as feed and equipment, from premises affected by *camelpox*;
- keep all newly introduced animals in isolation for a period of 30 days;
- maintain pastures, watering sites, and livestock facilities in proper veterinary and sanitary condition at all times;
- carry out systematic veterinary monitoring of animal health status;
- prohibit individuals from handling animals for a period of 14 days after vaccination against smallpox;

- vaccinate the entire camel population in farms and settlements located in zones threatened by *camelpox* using vaccines available on the market, in accordance with the manufacturer's instructions for camel immunization.

*Camelpox* can be controlled or prevented through vaccination. To date, four *camelpox* vaccines have been developed and evaluated (in addition to the domestic vaccine based on strain KM-40). All of them are derived from CMLV and include the Jouf-78 strain, the VD47/25 strain, Ducapox 298/89, and the CMLV-T8 strain. The Jouf-78 strain is an attenuated CMLV strain obtained through 80 serial passages in cell culture and has been shown to confer complete protection against CMLV infection. . According to field studies, a single vaccine dose in the range of 103 to 104 $TCID_{50}$ provides full protection. The attenuated VD47/25 strain, also passaged 80 times in cell culture, was evaluated in experiments conducted in Mauritania. This strain was found to be harmless to camels when administered subcutaneously at a dose of 104.7 $TCID_{50}$ and to fully protect camels against lethal CMLV infection. In the United Arab Emirates, a modified live CMLV vaccine obtained by passaging the CaPV298-2 strain in Vero cells has been used.

The vaccine known as Ducapox (DUbai CAmelPOX) is produced by Highveld Biological in South Africa. It was used for field vaccination shortly before the onset of a major *camelpox* outbreak in Dubai in 1993-1994. Among 2,000 vaccinated camels, disease developed in seven animals; however, it is unknown whether these animals had been infected prior to vaccination or whether these cases represented true vaccine failures. In addition, protection was shown to last for six years in two animals. Vaccine efficacy was also demonstrated in New World camelids against lethal CMLV infection. In Morocco, a vaccine containing inactivated CMLV (strain T8) combined with an adjuvant is produced and distributed by Biopharma. The T8 strain was isolated from scab material during an outbreak in Morocco in 1984. The vaccine has been shown to be safe for both young and adult camels and to induce neutralizing antibodies; however, effective protection requires a second injection administered one month later.

Due to the immaturity of the immune system, vaccination is generally recommended for camels aged at least 6 months, and booster vaccination may be required for young calves. In animals younger than 6 months, antiviral agents are used to prevent the spread of the disease.

Measures for detecting camelpox in animals. Although a presumptive diagnosis of camelpox may be established based on characteristic clinical manifestations, reliance on clinical presentation alone is insufficient. Cutaneous lesions caused by CMLV may resemble those observed in other viral infections of camelids, including contagious ecthyma (parapoxvirus infection) and papillomatosis, and may even be confused with non-infectious dermatological reactions such as insect bites. For this reason, laboratory confirmation is strongly recommended. For etiological verification, samples obtained directly from lesions such as skin crusts, nodules, or biopsy material are considered the most informative. A range of complementary laboratory techniques is available for specific identification of CMLV, including transmission electron microscopy (TEM), virus isolation in embryonated eggs or cell culture, polymerase chain reaction (PCR), immunohistochemical detection of viral antigens, and serological assays targeting neutralizing antibodies.

Among these methods, TEM provides rapid confirmation through visualization of the characteristic brick-shaped morphology typical of *orthopoxviruses* in lesion material. This morphological feature allows differentiation from *parapoxviruses*, which display an ovoid structure and represent the principal differential diagnosis (camel orf). However, mixed infections cannot be excluded, as both viral particles may be observed concurrently in the same specimen.

Immunohistochemistry represents an accessible alternative for laboratories without electron microscopy facilities. Detection of *camelpox* antigens in scabs or tissue sections enables confirmation of infection, and the use of paraffin-embedded material allows long-term storage and retrospective epidemiological analysis.

Biological isolation methods further support diagnosis. CMLV is capable of replicating on the chorioallantoic membrane of embryonated chicken eggs, producing characteristic lesions within several days. In cell culture systems, the virus induces a typical cytopathic effect, and infected cells demonstrate intracytoplasmic eosinophilic inclusion bodies consistent with poxvirus replication following hematoxylin and eosin staining.

The presence of viral nucleic acid can be confirmed by PCR, and different CMLV strains can be identified using restriction fragment length polymorphism (RFLP) analysis of viral DNA.

A wide range of serological tests is also available for the detection of *camelpox*, including virus neutralization assays and ELISA. However, given that all *orthopoxviruses* exhibit immunological cross-reactivity to varying degrees, immunodiagnostic methods are of limited value, with the possible exception of demonstrating neutralizing antibodies.

Measures for disease eradication.

Rapid molecular confirmation of suspected cases is a key prerequisite for timely containment of *camelpox* outbreaks. The availability of sensitive PCR-based assays enables early detection of virus circulation and supports targeted response measures, which are essential for effective control and long-term elimination strategies.

From an epidemiological perspective, *camelpox* possesses characteristics that favor eradication. The virus is host-specific and infects only camelids, with no recognized wildlife reservoir contributing to its maintenance in nature. When combined with the existence of reliable diagnostic tools and effective vaccines capable of interrupting transmission, these biological features position *camelpox* among diseases that meet the fundamental criteria for potential eradication. Environmental control measures further strengthen this prospect. CMLV is susceptible to a range of standard disinfectants and can be readily inactivated by conventional physical methods, including autoclaving, brief ultraviolet irradiation, and boiling for at least 10 minutes. Such procedures can be implemented at the farm level to reduce viral persistence in contaminated environments. In practice, outbreak management relies on rapid isolation of affected animals coupled with ring or mass vaccination, an approach conceptually similar to strategies successfully employed during smallpox eradication in humans.

The biological similarity between CMLV and variola virus, particularly their dependence on a single host species, further supports the theoretical feasibility of elimination through coordinated surveillance, vaccination, and quarantine measures. Historical evidence underscores this possibility: in the early 1990s, Higgins and co-workers reported successful interruption of an outbreak following immunization of camels with a human smallpox vaccine. However, due to concerns that smallpox virus might be inadvertently transmitted from recently vaccinated camels to unvaccinated humans, domestic animals, or wildlife, research efforts have focused on the development of attenuated *camelpox* vaccines that are capable of infecting camels only.

Significant progress has been achieved in the development of attenuated *camelpox* vaccines through serial passaging in cell culture systems. In the United Arab Emirates, repeated passaging of a field isolate in camel skin–derived cell lines (approximately 80 passages) resulted in a markedly attenuated strain subsequently commercialized as Ducapox®, which demonstrated high safety in young animals. In Saudi Arabia, a tissue culture adapted strain led to the development of Orthovac®, a vaccine that has shown both safety and protective efficacy under field conditions. Additional national products have been generated in Mauritania using attenuation approaches and in Morocco through formalin inactivation of the virus. An important practical advantage of these poxvirus vaccines is their relative thermostability compared with products requiring strict cold-chain maintenance, which facilitates deployment in desert and semi-desert regions where *camelpox* is endemic.

Global blanket vaccination is not a prerequisite for elimination of the disease. Instead, a targeted containment strategy may be more appropriate. The "ring vaccination" model – successfully applied during the final stages of smallpox eradication relies on rapid case identification, followed by immunization of all epidemiologically linked animals and intensified surveillance to interrupt transmission chains. In the context of *camelpox*, implementation of such an approach would require robust laboratory confirmation of suspected cases, ideally using multiple complementary diagnostic techniques to distinguish CMLV infection from clinically similar conditions, particularly contagious ecthyma caused by *parapoxvirus*. When *camelpox* occurs on a farm, quarantine restrictions are imposed, prohibiting the introduction and removal of animals from the quarantine area, the export of wool and products of animal slaughter, and requiring treatment of animals as well as the implementation of comprehensive sanitary and hygienic measures aimed at preventing disease spread. Restrictions are lifted no earlier than 20 days after recovery of the last affected camel and completion of final disinfection of the farm premises.

The diagnosis of *camelpox* is established on the basis of analysis of clinical and epizootiological data, pathological (post-mortem) findings, and the results of laboratory investigations. The initial stage of *camelpox* must be differentiated from contagious pustular dermatitis of camels (ecthyma) and foot-and-mouth disease.

1. Upon confirmation of *camelpox* in camels, the veterinary specialist responsible for the farm (settlement) shall immediately notify the district chief veterinary officer and, jointly with the farm management and authorized authorities:

- immediately isolate diseased and suspected camels;
- prohibit any movement of animal groups, restrict access by unauthorized persons, and ban the introduction or removal of working livestock, dogs, and other animals that may act as mechanical carriers of the CMLV;
- take measures to identify, localize, and eliminate the source of infection.

2. Upon receipt of notification of *camelpox* occurrence, the district chief veterinary officer shall urgently organize an epizootiological investigation of the affected area to identify the infection focus and implement measures for its rapid containment; the necessary documentation shall be submitted to the authorized authorities for the imposition of quarantine.

3. The executive body of the authorized authority, based on the submission of the district chief veterinary officer, shall issue a decision to impose quarantine on *camelpox*-affected territories. The boundaries of the affected area and the threatened zone shall be defined, key disease eradication measures specified, timelines for their implementation established, and responsible persons designated.

Under quarantine conditions, the following activities are prohibited:

a) introduction and importation of camels into affected settlements, as well as removal and exportation of camels from them;

b) collection of camel hides, wool, and down in affected settlements and exportation of previously collected hides, wool, and down from these areas;

c) regrouping of camels within a farm (with the exception of removing diseased animals to isolation facilities), as well as grazing, watering, and housing of diseased camels together with healthy animals of any species;

d) access of persons not involved in the care of animals from affected groups to premises and other locations where these animals are kept;

e) trade in animals and animal products, as well as the organization of exhibitions, fairs, markets, and other events involving the gathering of animals within the quarantined area;

f) removal of feed (hay, straw, etc.) that has come into contact with camels affected by *camelpox.* Such feed shall be used on-site (within the farm) for animals not susceptible to *camelpox* or for camels that have recovered from the disease and have been immunized against this infection;

g) use of camel milk and products derived from it in an untreated form. Milk obtained from camels on quarantined farms must be disinfected on-site by pasteurization at 85 °C for 30 minutes or by boiling for 5 minutes, followed by use within the farm;

h) passage of private, passenger, freight, and other vehicles through the *camelpox* outbreak area. In such cases, alternative routes to the destination must be designated.

5. In the affected settlement, a veterinary inspection and inventory of all camels are carried out, and owners are informed of the rules for animal management during the quarantine period.

6. Camels affected by *camelpox* are isolated and treated, while clinically healthy animals are vaccinated with available vaccines in accordance with the instructions for their use against *camelpox*. In addition to symptomatic treatment, young animals are administered serum or blood from convalescent camels obtained from clinically healthy animals 20-40 days after recovery.

7. Sanitary assessment and use of meat and other products obtained from the slaughter of camels affected by or suspected of *camelpox* are carried out in accordance with the current regulations for veterinary inspection of slaughter animals and veterinary and sanitary examination of meat and meat products.

8. Wool and hides obtained from camels during the period when the farm is affected by *camelpox* are disinfected in accordance with the current guidelines for disinfection of raw materials of animal origin.

9. Carcasses of camels that have died with clinical signs of *camelpox* are destroyed. Removal of hides and use of wool from such carcasses are prohibited.

10. In the outbreak area, livestock premises, equipment, harness items, and other locations associated with the presence of camels affected by *camelpox* are disinfected every five days throughout the quarantine period until final disinfection is carried out. Manure is disinfected using the biothermal method.

11. Quarantine restrictions are lifted 20 days after the complete recovery, death, or slaughter of the last camel affected by *camelpox* in the given settlement.

*Conditions for lifting quarantine measures and exit strategy*

Before lifting quarantine restrictions:

- final disinfection is carried out in accordance with the current instructions for veterinary disinfection, disinvasion, disinsection, and deratization;

- the district chief veterinary officer, together with the head of the farm (enterprise), verifies the implementation of veterinary and sanitary measures and prepares an official report authorizing the lifting of quarantine. This report specifies: when and in which territory *camelpox* was established, the source of infection, the number of camels affected, dead, and culled, the dates of vaccination and the number of vaccinated animals, the nature of the disease course, the date of slaughter, death, or recovery of the last affected animal, the date on which final measures for CMLV elimination were carried out in the affected area, as well as the measures to be implemented after quarantine removal.

**Conclusion**

Based on the research conducted by us, the results of the assessment of the epizootiological situation, and the analysis of the risks of *camelpox* spread in Kazakhstan during 2021-2022 [11, 22], we developed approaches for evaluating the epizootiological situation and forecasting outbreaks, as well as recommendations for implementing veterinary measures and controlling *camelpox* in Kazakhstan. Application of the recommendations described in this article will ensure the effective implementation of veterinary and control measures aimed at preventing the introduction of CMLV into the country, limiting the spread of infection to non-endemic territories, and eradicating the disease from endemic regions.

**Author contributions**

YO: original draft; AZ, YP: investigation, methodology, original draft; AN: investigation; AA: funding acquisition; SM: reviewing and editing.

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**UDC 578:578.4:578.42** **Review article**

# The role of Culicoides vectors in the transmission of the bluetongue virus in Kazakhstan and adjacent regions

Akezhan A. Isakhan, Orazbek N. Serikbayov, Nurgul K. Orazymbetova
Madina Zh. Kaukarbayeva, Zhumagali K. Koshemetov

«Research Institute of Biological Safety Problems» LLP, National Holding «QazBioPharm»
Gvardeyskiy, Kazakhstan

**Corresponding author:** Orazbek N. Serikbayov: o.orazbek@biosafety.kz
**Co-authors:** (1: OS) o.orazbek@biosafety.kz; (2: NO) n.orazymbetova@biosafety.kz
(3: MK) m.kaukarbayeva@biosafety.kz; (4: ZhK) zh.koshemetov@biosafety.kz
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**Abstract**

The bluetongue virus (BTV) is a transmissible pathogen whose circulation in natural and economic systems is determined by the triad "susceptible hosts – competent *Culicoides* woodlice – seasonal and climatic conditions". The non-contact nature of transmission (mainly through Culicoides bites) means that the risk of outbreaks is determined not so much by the "density of animal–animal contacts" as by the intensity of host-vector contacts and the timing of host viremia with vector activity.

Epizootologically cattle are often the key "hidden" link: infection in cattle is usually asymptomatic, but viremia can be relatively long-lasting, which increases the likelihood of infection by woodlice and maintenance of transmission when co-grazing with sheep (clinically the most vulnerable).

Complementary evidence of BTV circulation has been published for Kazakhstan: a 1997-1998 serological examination revealed widespread seropositivity in cattle/sheep/goats (~21-25%), which was interpreted as endemic in the absence of clinical recognition, and a 2021 review of the model risk assessment showed pronounced seasonality of potential transmission (spring-summer, peak in July) and a spatial gradient risk assessment, molecular research 2022-2024. The detection of BTV RNA in animals and in some *Culicoides* species in the southern regions was confirmed, while typing indicates the presence of at least separate genetic lines (BTV-9 "western topotype" was reported). Together, this justifies the transition from "serological confirmation of contact" to risk-based continuous surveillance with simultaneous monitoring of hosts and vectors.

**Keywords:** bluetongue; virus; blood-sucking woodlouse of the genus *Culicoides*; serotype; vector.

## Introduction

Bluetongue is a non-contagious transmissible viral disease of ruminants, mainly sheep, whose clinical manifestations are associated with systemic damage to the microcirculatory vessels and impaired tissue blood supply [1].

The causative agent of the disease is a virus belonging to the genus *Orbivirus* of the family Reoviridae. *Orbiviruses* are non-enveloped virions composed of a three-layered icosahedral capsid and containing a segmented genome. The bluetongue virus genome consists of ten segments of double-stranded RNA (S1-S10), which encode seven structural proteins (VP1-VP7) that form the architecture of the virion, as well as at least four non-structural proteins (NS1-NS4) involved in virus replication and interaction with the host cell [2].

BTV is one of the transboundary animal diseases that pose a significant threat to livestock farming due to its ability to spread rapidly across geographical areas and infect large populations of susceptible hosts

[3, 4]. The main feature of bluetongue epizootiology is the vector transmission route, which is carried out through blood-sucking midges of the genus *Culicoides*, allowing the virus to spread independently of direct contact between animals and to cross state and natural borders [4,5].

The transboundary significance of bluetongue is exacerbated by international trade in animals and animal products, as well as by changes in climatic factors that contribute to the expansion of the range and seasonal activity of vectors [5]. Outbreaks of the disease have been repeatedly recorded in regions previously considered non-endemic, highlighting the virus's ability to re-establish itself and circulate sustainably in new epizootic conditions [3].

Both domestic and wild cloven-hoofed animals are susceptible to BTV, although the degree of susceptibility and epizootiological role of different species vary. Sheep are considered to be the most susceptible, as the infection often takes a clinically pronounced form in them and serves as an epizootological indicator of the pathogen's circulation [6].

The incidence rate among sheep can reach 100%, and the mortality rate can reach 50%, which determines their key role in the epizootic process. Cattle, goats, and camels usually carry the infection in a subclinical or inapparent form and act as reservoirs for the virus, contributing to its preservation and spread in the epizootic system. It has been established that the virus is capable of persisting in the body of animals for a long time in the presence of specific antibodies. Cases of intrauterine infection are of particular epizootological significance, as the birth of dead or non-viable embryos may occur long after the mother's body has been infected [6].

In this regard, bluetongue is included in the list of priority diseases of the World Organisation for Animal Health (WOAH), and its control requires coordinated international measures, including epizootic surveillance, vector monitoring, restrictions on animal movements, and the application of preventive strategies [4, 5]. For countries with long borders and developed pastoral livestock farming, including Kazakhstan, bluetongue is of particular epizootic significance as a potential factor in cross-border risks [5].

Currently, the status of BTV in Kazakhstan shows active circulation with increasing seroprevalence, while in neighboring Central Asian countries, data are limited or outdated. The main risk factor is the presence of *Culicoides* vectors throughout the region [7].

The purpose of this review article is to analyze the role of *Culicoides* vectors in the transmission of bluetongue virus in Kazakhstan and neighboring regions based on current data on their species composition, biological and ecological characteristics, as well as factors affecting the circulation of the pathogen, with an assessment of the epizootological situation and current approaches to the control and prevention of infection.

**Materials and Methods**

This review article was conducted using a systematic analysis of scientific literature and available epizootological data on bluetongue virus circulation and Culicoides vectors in Kazakhstan and adjacent regions.

Scientific publications indexed in international databases, including Web of Science, Scopus, and PubMed, as well as reports from international organizations such as the World Organisation for Animal Health (WOAH), were analyzed. In addition, regional studies and epizootological monitoring reports related to bluetongue virus circulation in Central Asia were included in the analysis.

The selection of literature sources was based on their relevance to the epidemiology of bluetongue virus, vector ecology, host susceptibility, and environmental factors influencing virus transmission. Publications from the last decades with particular emphasis on recent studies were prioritized. The collected information was systematized and analyzed to identify key patterns in the circulation of bluetongue virus, the role of Culicoides vectors, and the environmental and biological factors affecting the epizootic process in Kazakhstan and neighboring regions.

**Results and Discussion**

*Virus hosts and the epizootic role of populations*

BTV belongs to the genus Orbivirus of the family Reoviridae and causes a transmissible non-contagious infection in domestic and wild ruminants, in which the pathogen is not transmitted through

normal direct contact between susceptible hosts [8, 9]. The BTV genome consists of ten segments of double-stranded RNA, and the virion is characterized by a double-layer capsid structure that plays a key role in the formation of the virus's antigenic properties. In particular, the internal capsid protein VP7 determines serogroup specificity, while the outer protein VP2 is the main determinant of the serotype-specific immune response and is used in serotype identification of the virus [8, 9].

From a biological point of view, BTV is a strictly vector-dependent pathogen transmitted by blood-sucking midges of the genus *Culicoides*. Carriers become infected when they feed on viremic animals, after which the virus undergoes an exogenous replication cycle in the insect's body. According to data from the World Organisation for Animal Health, the replication period required to achieve infectivity lasts an average of 6-8 days, after which infected midges remain capable of transmitting the virus throughout their lives [8]. This feature ensures the effective maintenance of virus circulation in natural and agricultural ecosystems.

The epizootological significance of various species of ruminants is determined not only by their susceptibility to infection, but also by the nature of the clinical course of the disease. These parameters do not always coincide: for example, cattle are often infected subclinically, but are capable of maintaining viremia for a long time and serving as an important reservoir of infection, ensuring the infection of vector populations and the further spread of the virus [8, 9, 10].

For vertebrate hosts, the difference between infectious viremia and persistent viremia is of fundamental importance. Infectious viremia is characterized by the presence of viable virus in the blood capable of infecting *Culicoides* midges during blood feeding, whereas viremia reflects only the detection of the viral genome by PCR and can persist for much longer without necessarily retaining transmissibility [10, 11]. Experimental studies have shown that the duration of viremia infectious to vectors is limited in time and depends on the host species, despite the longer detection of viral RNA in the blood of infected animals [10].

Table 1. Epizootological parameters of bluetongue virus infection in major domestic and wild ruminant species [8-16]

| Host / Population | Susceptibility to Infection | Clinical Manifestation (Typical) | Duration of Infectious Viremia (Approx.) |
|---|---|---|---|
| Sheep | High | High; severe forms and mortality possible in susceptible breeds | 11-54 days (depending on serotype/conditions and method) |
| Cattle | High | Often asymptomatic; clinical signs possible with certain serotypes | Usually <60 days; in datasets up to ~63 days; in some experiments up to 49 days with proven infectiousness to Culicoides |
| Goats | High | Often asymptomatic or mild; clinical disease possible | 27-54 days (experimental data for European breeds) |
| Saiga (wild populations) | Not specified (limited direct evidence of infection) | Not specified | Not specified |
| Roe deer (Capreolus capreolus) | Susceptibility confirmed by serological and molecular findings in Europe | Often subclinical; clinical signs variable | Not specified |
| Red deer (Cervus elaphus) | High (experimentally confirmed) | Often without obvious clinical signs; prolonged viremia possible | Not specified (for “infectious” viremia, Xeno diagnostic/vector-competent data required) |

Mechanically prolonged viremia in ruminants is associated with the interaction of the virus with erythrocytes, which ensures long-term circulation of the virus in the bloodstream without active replication in blood cells. Unlike vertebrate hosts, the infection is persistent in carriers, and after the end of the exogenous incubation period, ticks remain infectious throughout their lives [10]. This feature forms a stable system for maintaining the virus in natural and agricultural ecosystems.

The epizootiological role of different ruminant species varies significantly depending on their clinical susceptibility and ability to maintain viremia. In mixed herds (sheep, goats, and cattle), a unique epizootiological "bridge" is formed, ensuring the circulation of the virus. Sheep, as a rule, show pronounced clinical symptoms and serve as an indicator of virus circulation, while cattle and, probably, goats often carry the infection subclinically but are capable of maintaining longer viremia, which is infectious for carriers [8, 12]. As a result, subclinically infected animals play a key role in maintaining the epizootic process and creating a prolonged period of risk of virus transmission.

The practical significance of this feature is that the absence of clinical signs of disease in cattle cannot be considered an indicator of the epizootic well-being of the farm. On the contrary, asymptomatic infected animals can serve as a reservoir for the virus and ensure its hidden circulation, including the possibility of unnoticed introduction and persistence of infection in the carrier population [9, 10].

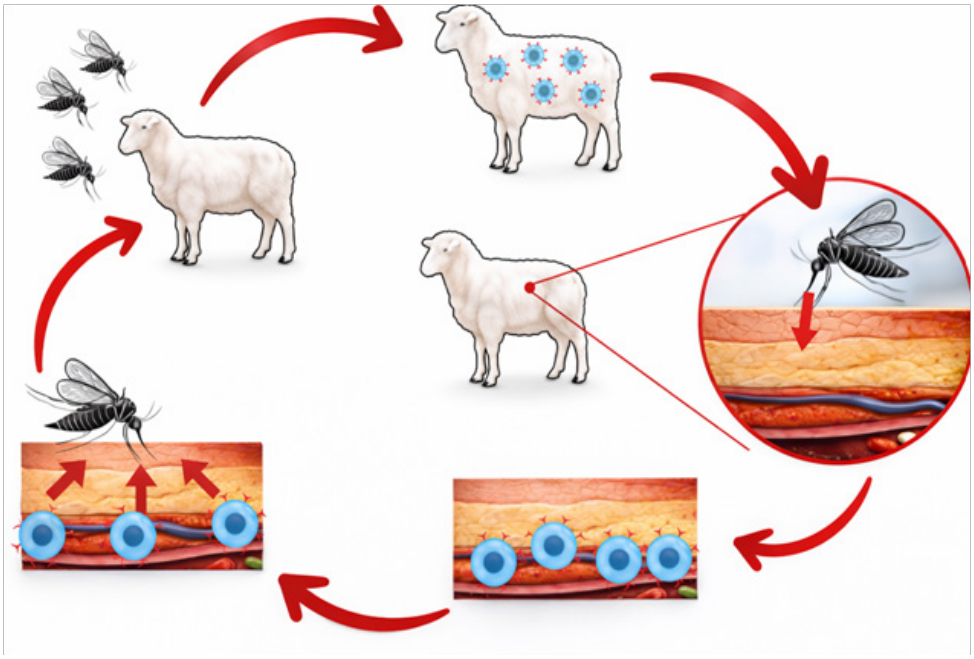


Figure 1. Diagram of transmissible transmission of BTV involving *Culicoides* midges and susceptible hosts (sheep)

The role of migratory wild animals in the spread of BTV is limited by a number of biological and environmental conditions. For effective transmission of the virus, a viremic host and active competent vectors must be present simultaneously along the migration route. Serological studies of saiga antelope (Saiga tatarica) in Kazakhstan have not detected antibodies to BTV, indicating no evidence of sustained virus circulation in populations of this species and making its role as a reservoir host unlikely under current conditions [17, 18]. However, the occasional involvement of wild ungulates in the circulation of the virus cannot be completely ruled out, especially in the presence of local epizootic foci.

Experimental and field studies conducted in Europe demonstrate the ambiguous role of wild ruminants in maintaining bluetongue virus circulation. In particular, red deer (Cervus elaphus) have been shown in experimental conditions to be capable of prolonged viremia, persisting for up to 98-112 days after infection, which could theoretically contribute to interaction with vector populations [15]. However, the results of long-term monitoring of natural populations in France did not confirm the role of red deer as a reservoir host for the virus, indicating the limited epizootological significance of this species in natural conditions [16].

Overall, the available data indicate that wild ruminants cannot be considered a proven reservoir of the bluetongue virus, but they can serve as epizootic indicators of virus circulation. Studies conducted in various regions of Europe have shown that monitoring the serological status of wild ruminants can be used for early detection of virus circulation and assessment of the spatio-temporal dynamics of infection [19, 20]. In this regard, wild ruminants in Kazakhstan should be considered primarily as potential "sentinel" species and subjects of epizootological monitoring, rather than as a confirmed reservoir of the virus.

*The role of vectors as a key link in the epizootic process*

The spread of the bluetongue virus is determined by the presence and activity of biological vectors, whose numbers and epizootic significance depend on a complex of micro- and macroclimatic factors, including temperature, humidity, wind speed, light intensity, and habitat characteristics [21, 22]. These parameters influence the growth rate, survival, feeding intensity, and spatial distribution of vector populations, determining the temporal and geographical boundaries of virus circulation.

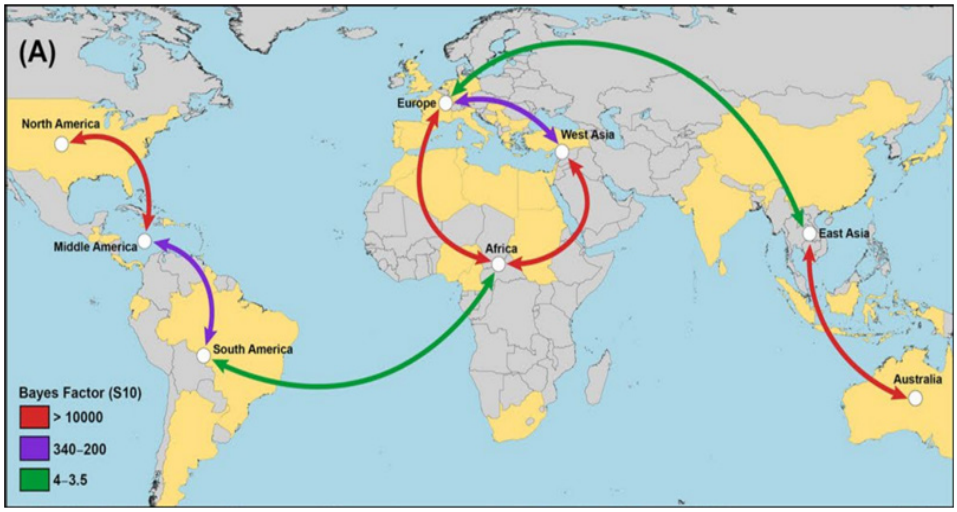
The bluetongue virus is transmitted mainly by blood-sucking midges of the genus *Culicoides,* which are the main biological vectors of the infection [6]. Currently, about 1,500 species of the genus *Culicoides* have been described, but the epizootological significance has been confirmed for at least 50 species, which is about 3% of their total diversity and reflects the expansion of understanding of the vector potential of this group [23]. These insects act not only as mechanical vectors but also as biological intermediate hosts, since the virus is capable of replicating in their bodies for a long time, ensuring subsequent transmission to susceptible vertebrate hosts [6].

The seasonal dynamics of *Culicoides* midge populations is a key factor determining the formation of the so-called "epizootological window" for bluetongue virus transmission. A model risk analysis for Kazakhstan showed pronounced seasonality of conditions favorable for virus transmission. According to forecasts, conditions for BTV transmission are absent during the winter period (approximately from October to March), while in April a low level of risk appears in the south of the country, which gradually expands geographically and reaches its maximum in July. Subsequently, there is a decrease in risk in September and its almost complete disappearance by October, reflecting the seasonal activity of vector populations and the climatic determinants of their numbers [13].

For most species, optimal conditions for flight and activity are observed at temperatures between +7 and +17 °C, while lower temperatures significantly limit their activity, especially at night [21, 22]. In addition, high light intensity can partially or completely suppress the activity of midges, while increased wind reduces the intensity of attacks on hosts, limiting the effectiveness of virus transmission. The characteristics of the habitat, including the type of ecosystem, vegetation index, soil cover properties, and degree of salinity, also have a significant impact, creating favorable or unfavorable conditions for maintaining stable vector populations [21, 22]. Thus, the epizootological role of vertebrate hosts is directly related to the phenology of herds and seasonal characteristics of animal husbandry, since spring and summer animal movements, the formation of herds, and the use of pastures coincide with the period of maximum risk of virus transmission.

The geographical spread of the bluetongue virus is closely linked to the habitat of various species of midges of the genus *Culicoides*, which are widespread in tropical and temperate climatic zones. The expansion of the range of vectors contributes to the emergence of new epizootic foci and changes in the spatial structure of virus circulation [24, 25].

Phylogeographic analysis of the bluetongue virus confirmed the existence of numerous intercontinental routes of spread, with Africa considered to be the key center of virus dispersion. It has been established that the most significant routes include the spread of the virus from Africa to Europe, Asia, and Australia, which is associated with a combination of factors, including the movement of infected animals, the spread of *Culicoides* vectors, and anthropogenic activity [26].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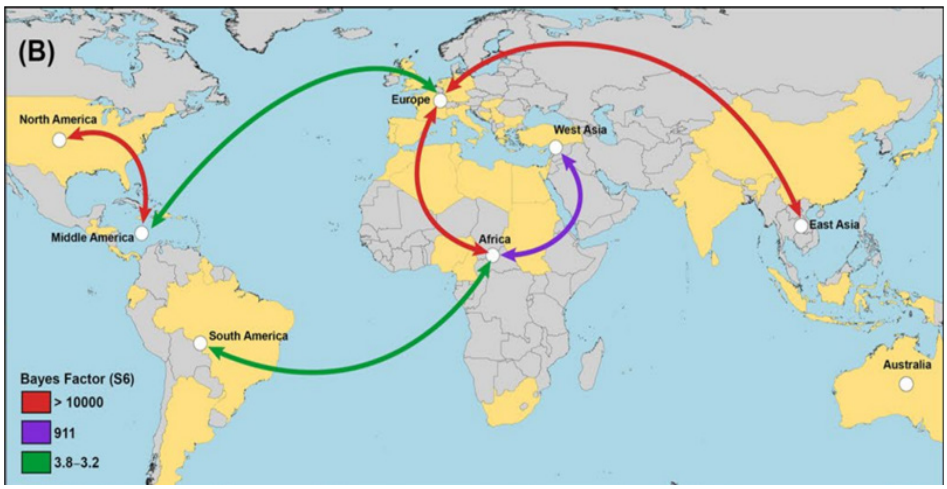
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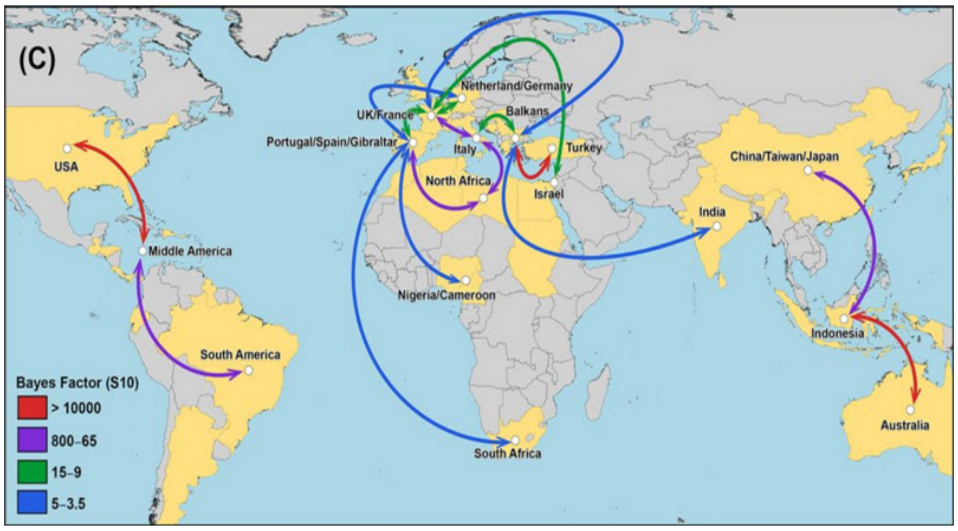
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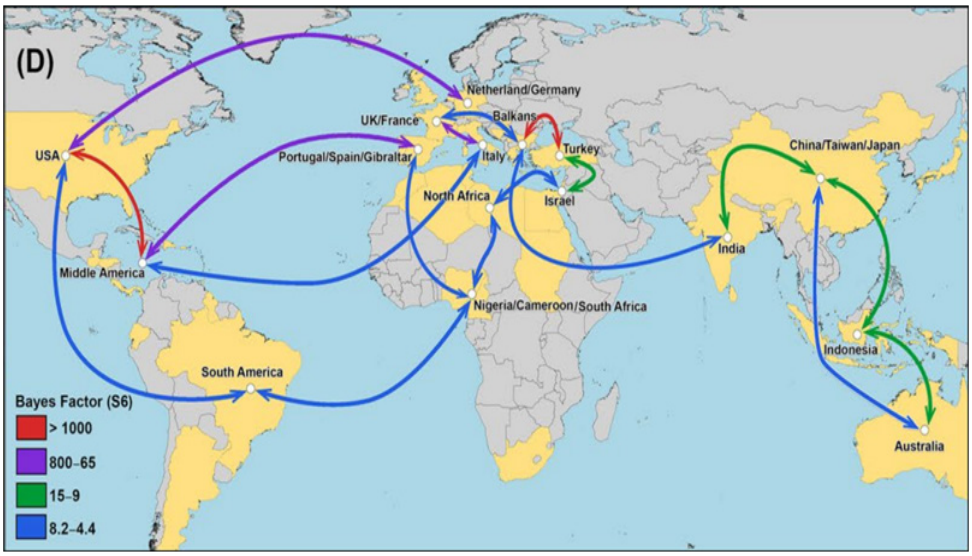



Figure 2. Phylogeographic reconstruction of the global distribution of the bluetongue virus based on genetic data analysis [26]

Thus, *Culicoides* midges are a key link in the epizootic process, ensuring biological transmission of the virus between susceptible hosts. Their population dynamics, ecological adaptation, and dependence on climatic factors determine the spatio-temporal patterns of bluetongue virus circulation and shape the epizootological risk of infection emergence and spread.

The presence of antibodies to BTV in farm animals in Kazakhstan indicates the natural circulation of the pathogen in susceptible host populations in the absence of vaccination. Serological studies conducted in Central Kazakhstan in 1996-1998 revealed antibodies to BTV in 23.2% of the animals examined, confirming the circulation of the virus in the country during that period and indicating its persistent presence in the region [17].

The detection of antibodies in various farms in Central Kazakhstan indicates the focal nature of the infection and confirms the possibility of the formation of natural and economic epizootic foci. Additional serological data obtained from farm animals also confirm the circulation of the virus in the absence of vaccination, indicating natural mechanisms for maintaining the infection, probably associated with the presence of competent vectors of the genus *Culicoides* [27].

Recent studies confirm the continued circulation of the bluetongue virus in Kazakhstan. Specifically, between 2018 and 2020, antibodies to BTV were detected in 3.8% of the agricultural animals examined, and viral RNA was detected in 0.7%, mainly in sheep, which indicates the continuing epizootic process and the activity of natural focal transmission mechanisms [28].

Genetic analysis of the identified isolates showed that they belong to the western topotype of serotype BTV-9, which indicates the stable presence of this genetic variant of the virus in the region and confirms its circulation in local populations of susceptible hosts [28].

The persistence of bluetongue virus circulation in Kazakhstan is directly linked to the presence and seasonal activity of *Culicoides* vectors. Epizootic risk modeling has shown that virus transmission in Kazakhstan is highly seasonal and occurs mainly from April to September, with the highest risk in July, which corresponds to the period of maximum activity of midge populations [13]. During the winter period, from October to March, the basic reproduction number ($R_0$) remains below the threshold level required for sustained transmission of the infection, making the occurrence of epizootic outbreaks unlikely [13].

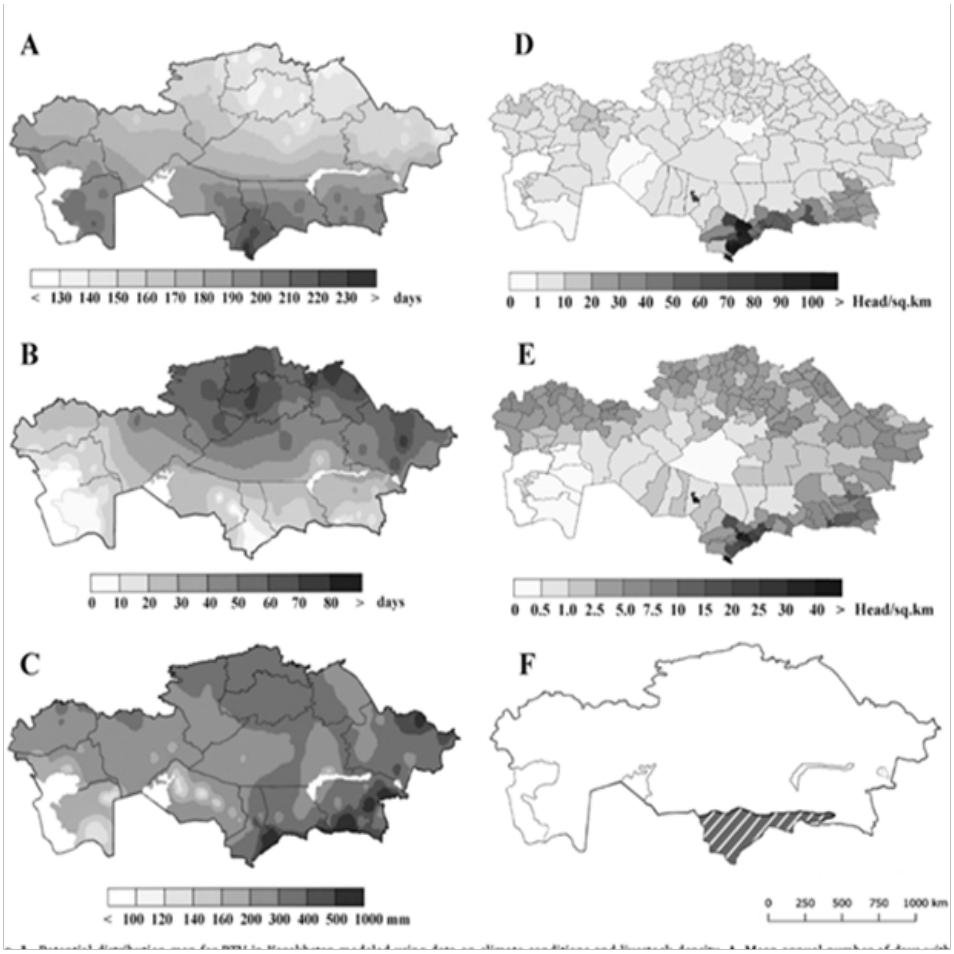
At the same time, the greatest potential for the spread of infection is observed in the northern and northeastern regions of the country, which indicates the influence of climatic and environmental factors on the spatial distribution of epizootological risk.

A key element in maintaining the circulation of the virus is the population of midges of the genus *Culicoides*, which are widespread in Kazakhstan. Entomological studies confirm the presence of several

species of midges capable of transmitting the bluetongue virus, which creates the necessary conditions for the formation of stable natural foci of infection [30]. The presence of susceptible vertebrate hosts and competent vectors forms a functional epizootic system that ensures the preservation of the virus in the ecosystem and its periodic activation during favorable seasons.

The potential spread of BTV in Kazakhstan is closely linked to climatic conditions and the density of susceptible animals, which determine both the ability to maintain populations of *Culicoides* vectors and the intensity of virus transmission. Spatial risk modeling has shown that the most favorable conditions for virus circulation are found in regions with a sufficient number of warm days, moderate temperatures, and adequate precipitation, as well as a high density of small and large livestock. These factors create environmental conditions that promote the maintenance of stable vector populations and increase the likelihood of virus transmission between susceptible hosts [30].

Phylogeographic analysis of the bluetongue virus confirmed the existence of numerous intercontinental routes of spread, with Africa considered to be the key center of virus dispersion (Figure 2). The most significant routes have been identified as the spread of the virus from Africa to Europe, Asia, and Australia, which is associated with a combination of factors, including the movement of infected animals, the spread of *Culicoides* vectors, and human activity.




A – Days with temperatures >10 °C (vector activity period)
B – Days with temperatures <20 °C (temperature restrictions on transmission)
C – Average annual precipitation (environmental conditions for Culicoides)
D – Small ruminant density
E – Density of cattle
F – Potential spread of BTV based on climate and animal density
Figure 3. Main intercontinental routes of bluetongue virus spread identified by phylogeographic analysis [13]

Modern epizootological studies confirm the active circulation of the bluetongue virus in the southern regions of Kazakhstan. Thus, in the period 2022-2024, antibodies to BTV were detected in 27.4% of the animals examined, and viral RNA in 8.9%, while the virus was successfully isolated in cell culture, confirming the presence of an active epizootic process and the circulation of infectious strains of the virus in the population of susceptible hosts [30]. The isolation of the virus in cell culture is direct confirmation of the presence of a viable pathogen and indicates the continued functioning of the natural focal system of infection transmission.

Analysis of climatic and epizootological parameters has identified key factors determining the risk of BTV spread. These include the number of days with temperatures above +10 °C, corresponding to the period of vector activity, and the number of days with temperatures below +20 °C, reflecting the temperature limitations for effective virus transmission. The average annual precipitation, which determines the habitat conditions for *Culicoides* midges, is also of significant importance, as is the density of susceptible animal populations, including small and large ruminants, which serve as a source of the virus for vectors. These factors together form a spatial model of the potential spread of bluetongue virus and determine regions of increased epizootic risk [30].

The data obtained confirm that the circulation of the bluetongue virus in Kazakhstan is determined by the interaction of climatic factors, the density of susceptible animals, and the presence of competent vectors of the genus *Culicoides*, forming a stable epizootological system capable of sustaining the virus in natural and agricultural ecosystems [29].

The data obtained confirm that the circulation of the bluetongue virus is a complex epizootological process in which biological vectors, primarily midges of the genus *Culicoides*, play a key role. Despite the availability of data on the possible involvement of other arthropods in virus transmission, including mosquitoes of the *Culex* and *Aedes* genera, the sheep louse *Melophagus* ovinus, as well as ixodid ticks (*Dermacentor dagistanicus, Rhipicephalus kochi, Rhipicephalus bursa*), from which the virus has been isolated in natural foci, their epizootiological role remains secondary to that of Culicoides. The ability of ixodid ticks to transmit the virus transovarially and transphasically, as well as their long survival time, potentially contributes to the persistence of the pathogen in natural conditions, but the available data do not confirm their leading role in the formation of the epizootic process [31-33]. The main and most effective vector of the bluetongue virus remains midges of the genus *Culicoides*, which ensure biological transmission of the virus between susceptible vertebrate hosts. These insects are capable of spreading over considerable distances by air currents reaching hundreds of kilometers, which explains the rapid geographical expansion of the infection area and the emergence of new epizootic foci [34]. The spatial distribution of the virus is directly related to the ecology and distribution of vector populations, as confirmed by studies demonstrating a close relationship between the range of *Culicoides* and the circulation of the bluetongue virus [35].

The ecological characteristics of individual *Culicoides* species significantly influence their epizootological significance. For example, Culicoides *imicola* mainly inhabits open and well-lit areas, while representatives of the Obsoletus complex prefer shaded areas with dense vegetation, which determines their involvement in virus circulation in various natural and climatic conditions [36]. Field and molecular studies have confirmed the involvement of certain species of midges in virus transmission: in particular, viral RNA was detected in 15.00% of *Culicoides* imicola individuals and in 8.14% of *Culicoides oxystoma* individuals, confirming their role as biological vectors of BTV [37].

Wild ruminants can also participate in the epizootic process, acting as reservoir or indicator hosts. The virus is transmitted between wild and domestic animals via common vectors of the genus *Culicoides,* which contributes to the preservation of the virus in natural foci and its periodic introduction into livestock populations [35, 38].

Current epizootological data confirm the continued spread of bluetongue virus in various regions of Europe, which is associated with the activity of *Culicoides* vectors. In particular, the re-registration of the disease in Austria in 2024-2025 with the detection of serotypes BTV-3, BTV-4, and BTV-8, as well as the confirmation of infection in cattle in Ireland in 2026, indicate the continued circulation of the virus and the cross-border spread of the infection [39].

These data confirm the virus's ability to persist and spread in new regions when favorable climatic conditions and competent vectors are present.

Regional studies in Central Asia also confirm the active circulation of the bluetongue virus. In particular, serological testing of sheep in Kyrgyzstan revealed antibodies to the virus in 36.94% of animals, indicating widespread infection and the presence of conditions conducive to the functioning of a natural focal transmission system involving *Culicoides* vectors [7].

For Uzbekistan, Tajikistan, and Turkmenistan, within the scope of this review, comparable peer-reviewed information on the current circulation of BTV and vector composition in available sources has been identified as unspecified; Therefore, regional risk should be assessed through animal movement and the presence of *Culicoides* and seasonality based on climatic analogues, as well as through targeted surveillance in border areas [13].

Given the similarity of climatic conditions, geographical proximity, and the presence of susceptible animal populations, similar mechanisms of virus circulation may also be characteristic of Kazakhstan.

Given its climate, the presence of susceptible animal populations, and the distribution of *Culicoides* vectors, Kazakhstan is a region with potentially favorable conditions for bluetongue virus circulation. The presence of serological and molecular evidence of virus circulation, as well as environmental conditions conducive to the existence of vectors, indicate the functioning of a natural focal system of infection transmission. Under these conditions, *Culicoides* populations are a key element in the epizootic process, ensuring the maintenance and spread of the virus among susceptible hosts and creating an epizootic risk of disease outbreaks.

**Conclusion**

The analysis of available scientific data demonstrates that the circulation of bluetongue virus in Kazakhstan is determined by a complex interaction between susceptible hosts, competent Culicoides vectors, and environmental factors. Among these components, biting midges of the genus Culicoides represent the key biological link that ensures virus transmission between vertebrate hosts.

The presence of serological and molecular evidence of bluetongue virus circulation in livestock, together with favorable climatic conditions for vector development, indicates the existence of a natural epizootic system capable of maintaining the pathogen in the region. Seasonal vector activity, animal density, and environmental conditions play a decisive role in shaping the spatial and temporal patterns of virus transmission.

Given the transboundary nature of bluetongue and the presence of competent vectors in Kazakhstan and neighboring regions, continuous epizootological surveillance is required. Integrated monitoring of both susceptible animal populations and Culicoides vectors is essential for early detection of virus circulation and for the implementation of effective preventive and control measures.

Further studies should focus on the detailed investigation of vector species composition, ecological dynamics of Culicoides populations, and molecular characterization of circulating bluetongue virus strains in Central Asia.

**Author Contributions**

AI and OS: conceptualized and designed the study; NO and MK: performed literature review and data analysis; ZK: wrote the original draft; all authors reviewed and approved the final manuscript.

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**UDC 579.62** **Research article**

# Evaluation of the potential antagonist's potential use to regulate the growth and toxin formation of the fungus Fusarium graminearum

Svetlana A. Semenova, Albert K. Galiullin, Renat A. Volkov, Yulia V. Krasovskaya, Aigul Y. Shaeva, Elvira A. Magdeeva

Kazan State Agrarian University, Kazan, Russian Federation

**Corresponding author:** Svetlana A. Semenova: lanochka-vet@mail.ru
**Co-authors:** (1: AG) albert-954@mail.ru; (2: RV) renv@ro.ru
(3: YK) ucheb_ot_kgavm@mail.ru; (4: ASh) a-shaeva@mail.ru; (5: EM) magdeeva.e@yandex.ru
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**Abstract**

Background and Aim. Fungi of the genus *Fusarium* are the most frequently isolated pathogens of major agricultural crops. Due to their high pathogenicity and toxigenicity, they cause significant economic losses. Fungi of the genus *Fusarium* have the ability to synthesize numerous toxic metabolites, including deokinivalenol. zearalenone et al. Toxins enter the human and animal food chains, causing serious human and animal diseases. The aim of this study was to evaluate the possibility of using a potential antagonist to regulate the growth and toxin formation of the fungus *Fusarium graminearum*.

Materials and Methods. *Fusarium graminearum, a producer of fusariotoxin deoxynivalenol*, and *Trichoderma Tr2 isolate*, previously isolated from the soil of the Republic of Tatarstan were used in this study. The effect of in vitro interactions between *Trichoderma Tr2* and *F. graminearum* on the growth rate of fungal mycelium was evaluated. And the effect of the in vitro interaction between *Trichoderma Tr2* and *F. graminearum* on the amount of F. graminearum and deoxynivalenol production after 10 days of incubation at 25 °C on rice grain.

Results. *Trichoderma Tr2* significantly ($p < 0.05$) reduced the growth of *F. graminearum* mycelium on potato agar by 74.3% compared to the control variant. Grain inoculation with *Trichoderma Tr2* isolate significantly ($p < 0.05$) reduced the amount of *F. graminearum* on rice grains after 10 days of incubation, reducing the number of CFU fungi by 44% compared to control vials. A similar pattern was found in the analysis of mycotoxin. The detectable level of deoxynivalenol was also significantly reduced ($p < 0.05$) when grain was treated with *Trichoderma Tr2 isolate*, which reduced toxin accumulation by 68.7% compared to control vials, both the final concentration of deoxynivalenol and the concentration of deoxynivalenol per CFU by 44.1%.

Conclusion. The results of this study suggest that the soil fungus *Trichoderma Tr2* isolate may be of biotechnological interest. In future studies, it will be possible to determine effective levels of application in the field, test on other species and strains, and develop an optimal strategy for using the future product.

**Keywords:** antagonism; deoxynivalenol; mycotoxin; *Fusarium; Trichoderma*.

## Introduction

Microscopic fungi and their toxic metabolites significantly reduce the quality of food and feed and pose a serious threat to food safety. Mycotoxins cause biochemical, physiological, and pathological changes in living organisms and have a toxic effect even at low concentrations [1, 2]. Fungi of the genus Fusarium are the most frequently isolated pathogens of important agricultural crops [3]. They are commonly found in soil, plant debris, and various organic substrates, where they live as saprophytes, feeding on dead organic matter. Due to their high pathogenicity and toxigenicity, they cause significant

economic damage. Fungi of the genus *Fusarium* have the ability to synthesize numerous toxic metabolites, including deokinivalenol, zearalenone et others. Toxins enter the human and animal food chains, causing severe human and animal diseases [4, 5, 6].

New solutions aimed at reducing the entry of mycotoxins into the food chain are relevant. The results of studies show that most *Fusarium mycotoxins* are stable and persist at the stage of food processing [7, 8]. This highlights that limiting the presence of toxic fungi in agricultural products is extremely important, and in response to this challenge, there is a growing interest in biological methods for controlling fungal contamination and controlling toxin formation. The literature reports the use of the phenomenon of antagonism for various applications [9, 10, 11].

The aim of this study was to evaluate the possibility of using a potential antagonist to regulate the growth and toxin formation of the fungus *Fusarium graminearum*.

**Materials and Methods**

This study used *Fusarium graminearum*, *a producer of fusariotoxin deoxynivalenol*, and *Trichoderma Tr2 isolate,* which previously demonstrated antagonistic properties to *F. graminearum* [12]. It was obtained from soil samples collected from various regions of the Republic of Tatarstan.

To prepare the mushroom inoculum, *Trichoderma Tr2* isolate was grown for 7 days at 25 °C in test tubes on Chapek-Dox agar. To obtain spore suspensions, the grown colonies were washed with 10.0 $cm^3$ of peptone water, filtered through sterile gauze to remove mycelium, and the spore concentration was adjusted to 106 CFU /cm3 by serial dilution. *F. graminearum* was grown on potato agar at 25 °C for 7 days, and Petri dishes were used to remove agar plugs (5 mm) from the edge of growing colonies with a sterile sampler. To obtain spore suspensions, the grown colonies were also washed with 10.0 $cm^3$ of peptone water, filtered through sterile gauze to remove mycelium, and the spore concentration was adjusted to 106 CFU /cm3 by serial dilution.

To assess the effect of *Trichoderma Tr2* isolate on the growth rate of *F. graminearum mycelium*, a modified method was used [13]. Aliquots (1.0 $cm^3$) of mushroom suspensions were mixed with 19.0 ml of molten potato agar, homogenized, and poured onto Petri dishes (final concentration 106 cells/$cm^3$). After solidification, agar plugs with *F. graminearum mycelium* were placed in the center of the cup. Control cups consisted of cups with sterile peptone broth (1.0 cm3) mixed with potato agar (19 $cm^3$) and inoculated in the center with *F. graminearum* agar blocks. Experimental and control plates were incubated for 7 days at 25 °C in three repetitions. The diameter of the fungal colony (mm) was estimated daily by measuring two radii located at right angles to each other using a caliper. The relative decrease in mycelium growth was calculated by the formula:

Inhibition (%) = [(CD-TD)/CD] × 100 where CD is the control diameter and TD is the antagonism diameter.

Biological control was performed on rice as follows: 25 g of autoclaved rice grains were added to glass vials. 1 $cm^3$ of *F. graminearum* spore suspensions ($10^6$ CFU / cm33) and 1$cm^3$ of *Trichoderma Tr2* isolate suspensions (106 CFU /cm3) were sprayed, and incubated in three repetitions at 25 °C for a period of 10 days. Control vials were inoculated with 1 cm3 of fungal suspension (106 CFU /$cm^3$) and 1$cm^3$ of peptone broth instead of *Trichoderma Tr2* isolate suspension. At the end of incubation, mycotoxin deoxynivalenol was quantified according to the method described in [14].

The obtained experimental data were processed by the generally accepted method of variational statistics using the student confidence criterion using special programs.

**Results and Discussion**

Table 1 presents the results of the in vitro interaction between *Trichoderma Tr2* and *F. graminearum* in terms of mycelial growth rate.

Table 1. Effect of in vitro interaction between *Trichoderma Tr2* and *F. graminearum* on mycelial growth rate.

| | Colony diameter, cm | Mycelium growth rate, cm / • day | Mycelium growth reduction, % |
|---|---|---|---|
| Control F. graminearum | 7.4 ± 0.32 | 0.93 ± 0.08 | 00.00 |
| Experiment F. graminearum + Trichoderma Tr2 | 1,9,9 ± 0,26* | 0,1717 ± 0,04* | 74,3,3 ± 8,6,6 |
| Note: * p ≤ 0.05 | | | |

The results presented in Table 1 demonstrate a pronounced antagonistic interaction between *Trichoderma Tr2* isolate and *Fusarium graminearum* under in vitro conditions. In the control variant, where *F. graminearum* was cultivated alone, the colony diameter reached 7.4 ± 0.32 cm, with a mycelial growth rate of 0.93 ± 0.08 cm/day, indicating the typical growth dynamics of this species on potato agar. In contrast, the presence of the antagonist significantly suppressed the development of the pathogen. When *Trichoderma Tr2* isolate was introduced into the growth medium, the colony diameter of *F. graminearum* decreased to 1.9 ± 0.26 cm, while the mycelial growth rate declined to 0.17 ± 0.04 cm/day. These changes correspond to a 74.3% reduction in mycelial growth, which was statistically significant ($p \leq 0.05$). Such a strong inhibitory effect suggests that the *Trichoderma Tr2* isolate possesses pronounced antagonistic properties toward *F. graminearum*. The mechanism of inhibition may be associated with several well-known biological control strategies characteristic of Trichoderma species, including competition for nutrients and space, secretion of antifungal metabolites, production of cell wall–degrading enzymes, and direct mycoparasitism. Numerous studies have demonstrated that species of the genus Trichoderma are capable of producing hydrolytic enzymes such as chitinases, β-1,3-glucanases, and proteases, which contribute to the degradation of fungal cell walls and suppression of pathogenic fungi. Therefore, the obtained results confirm that the *Trichoderma Tr2* isolate exhibits significant antagonistic activity against *F. graminearum,* which makes it a promising candidate for further studies on biological control of Fusarium infections.

The effect of *Trichoderma Tr2* on the quantity of *F. graminearum* and deoxynivalenol production is presented in Table 2.

Table 2. Effect of in vitro interaction between *Trichoderma Tr2* and *F. graminearum* on fungal growth and deoxynivalenol production after 10 days of incubation at 25 °C

| | CFU F. graminearum 106 / $cm^3$ | Deoxynivalenol content, mcg • * g of rice | Decrease in deoxynivalenol production, % deoxynivalenol | Content, mcg /• CFU | Decrease in deoxynivalenol production, % |
|---|---|---|---|---|---|
| Control | 7.5 ± 2.3 | 243.4 ± 26.7 | 00.0 | 32.4 | 00.0 |
| Experiment *F. graminearum + Trichoderma Tr2* | 4,2 ± 1,1* | 76,2 ± 15,9* | 68,7 | 18,1 | 44,1,1 |
| Note: * p ≤ 0.05 | | | | | |

The results shown in Table 2 indicate that the antagonistic interaction between Trichoderma Tr2 and F. graminearum affects not only the growth of the pathogen but also its ability to produce the mycotoxin deoxynivalenol (DON).

In the control samples inoculated with *F. graminearum* only, the fungal population reached 7.5 ± 2.3 × $10^6$ CFU/$cm^3$, while the concentration of deoxynivalenol accumulated in rice grain reached 243.4 ± 26.7 µg/g. These values confirm the high toxigenic potential of the pathogen under favorable growth conditions. However, when the *Trichoderma Tr2* isolate was introduced together with the pathogen, a

significant reduction in fungal growth and toxin formation was observed. The number of viable fungal cells decreased to $4.2 \pm 1.1 \times 10^6$ CFU/cm³, representing a 44% reduction compared with the control. At the same time, the accumulation of deoxynivalenol decreased markedly to 76.2 ± 15.9 µg/g, corresponding to a 68.7% reduction in toxin production. Moreover, the toxin production calculated per fungal unit (µg DON/CFU) also decreased by 44.1%, indicating that the antagonist not only limits the growth of F. graminearum but also suppresses its toxigenic activity. This observation suggests that the *Trichoderma Tr2* isolate may interfere with the metabolic pathways responsible for mycotoxin biosynthesis or create environmental conditions unfavorable for toxin production. These findings highlight the dual protective effect of the *Trichoderma Tr2* isolate: inhibition of pathogen proliferation and suppression of mycotoxin accumulation. Such characteristics are particularly important for the development of biological strategies aimed at improving the safety of agricultural products and reducing the risk of mycotoxin contamination in the food and feed chains.

**Conclusion**

The present study evaluated the antagonistic potential of the soil fungus T*richoderma Tr2* isolate against the phytopathogenic fungus *Fusarium graminearum*, a well-known producer of the mycotoxin deoxynivalenol. The results obtained under laboratory conditions clearly demonstrate that the Trichoderma Tr2 isolate exhibits strong antifungal activity and significantly suppresses both the growth of the pathogen and its ability to produce mycotoxins.

The in vitro experiments showed that the presence of *Trichoderma Tr2* reduced the growth rate of *F. graminearum* mycelium by more than 70%, indicating a pronounced antagonistic interaction between these microorganisms. In addition, the biological control experiment performed on rice grain confirmed that the antagonist effectively limited fungal development and significantly decreased the accumulation of deoxynivalenol. The observed reduction in toxin production suggests that the *Trichoderma Tr2* isolate may influence the metabolic activity of the pathogen, which is particularly important from the perspective of food and feed safety.

Species of the genus *Trichoderma* are widely recognized as effective biological control agents due to their ability to colonize soil, compete with phytopathogenic fungi, and produce various antifungal metabolites and hydrolytic enzymes. Chemical and pharmacological studies of secondary metabolites of various *Trichoderma* species have shown that these fungi produce a number of compounds potentially applicable in medicine, biotechnology, and agriculture [15]. These properties allow them to suppress plant pathogens through several mechanisms, including competition for ecological niches, secretion of inhibitory compounds, and direct mycoparasitism. Therefore, the antagonistic activity demonstrated by the *Trichoderma Tr2* isolate in this study is consistent with the well-known biological characteristics of this genus.

From a practical standpoint, the results obtained indicate that the *Trichoderma Tr2* isolate may represent a promising biological agent for the control of Fusarium infections and the prevention of mycotoxin contamination in agricultural products. The use of biological control agents such as *Trichoderma* may provide an environmentally friendly alternative to chemical fungicides, contributing to sustainable agricultural practices and improved food safety.

However, further research is required to fully assess the practical applicability of this isolate. Future studies should focus on determining optimal application rates, evaluating the effectiveness of the *Trichoderma Tr2* isolate under field conditions, investigating its activity against different Fusarium species and strains, and assessing its stability and safety in agricultural ecosystems. In addition, molecular and biochemical studies could help clarify the mechanisms underlying the antagonistic activity observed in this study.

In conclusion, the results obtained demonstrate that the soil fungus *Trichoderma Tr2* isolate possesses significant antagonistic activity against *Fusarium graminearum* and is capable of reducing both fungal growth and deoxynivalenol production. These findings suggest that this isolate may have considerable biotechnological potential and could serve as a promising candidate for the development of biological preparations aimed at controlling *Fusarium* contamination in agricultural systems.

**Authors' Contributions**

SS and AG: developed the research concept; ASH and EM: research; SS and EM: comprehensive literature search, analyzed the collected data and prepared a draft of the manuscript; RV and YK: completed the final editing and proofreading of the manuscript; All authors read, reviewed, and approved the final version of the manuscript.

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**UDC 595.421** **Research article**

# First report of *Hyalomma marginatum* (Arachnida, Ixodidae) in *Saiga tatarica* from Ural antelope population: Western Kazakhstan

Rano S. Sattarova[1], Zaure Z. Sayakova[1], Kuandyk M. Shynybayev[1], Flura A. Bakiyeva[1]
Aigerim K. Khamzina[2], Zhanna U. Katuova[3]

[1]Kazakh Scientific Research Veterinary Institute, Almaty, Kazakhstan
[2]Kazakh National Agrarian Research University, Almaty, Kazakhstan
[3]Ural Anti-Plague Station, Committee for Sanitary and Epidemiological Control, Ministry of Health, Republic of Kazakhstan, Zhimpity Anti-Plague Department, Uralsk, Kazakhstan

**Corresponding author:** Aigerim K. Khamzina: aigerim.khamzina55@gmail.com
**Coauthors:** (1: RS) ranosaitomarovna@gmail.com; (2: ZS) sayakovazz@gmail.com
(3: KS) shynybaev.k@mail.ru; (4: FB) fluarachka-78@mail.ru; (5: ZhK) katuova@mail.ru
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**Abstract**

Background and Aim. Ixodid ticks (Acari, Ixodidae) are among the most important groups of arachnids globally, significantly impacting human and veterinary practice. Five genera of ticks of the Ixodidae family have been registered in Kazakhstan. Ticks of the genus *Hyalomma* are particularly important from epizootiological and epidemiological perspectives. Bites from infected ticks can transmit pathogens of bacterial and viral diseases.

Materials and Methods. The research was conducted in the Bokeyorda State Nature Reserve, located in the Bokeyorda, Zhanibek, Kaztalov, and Zhanakala districts of the West Kazakhstan region of Kazakhstan, under a permit from the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan. Trapping for parasite carriage studies was conducted using the Coral method without harming animal health. To avoid stress, each captured animal was examined as quickly as possible. One or two ticks were removed safely, after which the animals were immediately released. The collected samples were placed in sterile laboratory Eppendorf tubes and stored at 2-4 °C. Tick species identification was performed under a trinocular stereoscopic microscope using identification tables.

Results. During the study, *Hyalomma marginatum* ticks, a major vector of the Crimean-Congo hemorrhagic fever virus (CCHF), were discovered for the first time in representatives of the Ural saiga population (*Saiga tatarica*) in Western Kazakhstan. This species have not previously been recorded on saiga in Kazakhstan. Morphological identification allowed us to clarify the characteristic diagnostic features of the discovered specimens, distinguishing them from other species of the genus *Hyalomma*.

Conclusion. The detection of H. marginatum in saigas is of epizootiological significance, as this tick species actively parasitizes farm animals, creating conditions for the circulation and possible transmission of zoonotic pathogens between wild and domestic animals. Saiga migrations between Kazakhstan and Russia contribute to the expansion of the range of *H. marginatum* and likely increase the risk of tick-borne infections in the steppe ecosystems of the Urals.

**Keywords:** ectoparasites; *Hyalomma marginatum;* transmissible diseases; *Saiga tatarica.*

**Introduction**

Ticks are vectors of numerous infectious diseases, including some highly pathogenic ones. Bites from infected ticks can transmit bacterial pathogens (tick-borne borreliosis, Lyme disease), relapsing fever, tularemia, and babesiosis), rickettsial infections (spotted fever, Q fever, ehrlichiosis, and anaplasmosis), and viral diseases (tick-borne encephalitis, colitis, Crimean-Congo hemorrhagic fever, etc.).

Ixodid ticks (Acari, Ixodidae) are one of the most important groups of arachnids globally, significantly impacting human and veterinary practice. They are highly resilient to adverse environmental conditions, capable of overwintering, and surviving habitat flooding for up to two weeks. In a state of starvation, a female can survive for up to three years and may lay up to 5,000 eggs, transmitting pathogens to subsequent generations transovarially, which is why biotopes infested with parasites are dangerous foci of infection for many years [1, 2]. In Kazakhstan, 5 genera of ticks in the family Ixodidae are recorded. Ticks of the genus *Hyalomma* are of particular epizootiological and epidemiological significance. Some species of ticks of this genus are the main carriers of pathogens of diseases such as human granulocytic anaplasmosis (HGA), sheep anaplasmosis (*Anaplasma ovis*), theileriosis of horses, small and large cattle (*Theileria annulata, T equi, T. orientalis, T. ovis*), babesiosis of cattle and horses (*Babesia occultans, B. caballi*), ehrlichia [3, 4]. Studies conducted in the Balkhash district of the Almaty region and in the adjacent territory of northwestern China have shown the presence of *B. caballi* in *H. asiaticum* [5, 6]. Tacheng tick virus 2 (TcTV-2) was detected in *H. scupense* from the East Kazakhstan and Almaty regions, in *H. asiaticum* from the Almaty region, and *H. anatolicum* from the Zhambyl region [7]. In the southern region of the republic, DNA of four pathogens (LSDV (lumpy skin disease virus), *Coxiella burnetii, Theileria annulata,* and *Babesia caballi*) were detected in *Hyalomma asiaticum, H. scupense,* and *H. anatolicum* by PCR. Infection with LSDV was detected in *Hyalomma asiaticum* ticks collected from cattle in the West Kazakhstan region. *Coxiella burnetti* (the causative agent of Q fever) was detected in Hyalomma anatolicum ticks in the Zhambyl region. *Theileria annulata* and *Babesia caballi* were detected in *Hyalomma* scupense ticks from cattle in the Turkestan region [8].

Ticks of the genus *Hyalomma* play an essential role in the transmission of the CCHF pathogen, serving as the primary vectors in natural foci in Kazakhstan. Phylogenetic analysis based on partial S and L segments of the viral genome revealed that Kazakhstani isolates grouped into the Asia-1 and Asia-2 genotypes and shared high sequence similarity with regional strains from Uzbekistan, Turkmenistan, and China, confirming cross-border circulation of the virus. These results provide additional molecular evidence for localized Crimean hemorrhagic fever virus activity in ticks associated with livestock in southern Kazakhstan, thereby expanding knowledge of the virus's geographic distribution and genetic diversity [9]. In the context of climate change and human economic activity over the last decade, significant changes in the ranges of certain tick species in Kazakhstan have been documented [10, 11, 12].

**Materials and Methods**

The research was conducted at the Bokeyorda State Nature Reserve, located in the Bokeyorda, Zhanibek, Kaztalov, and Zhanakala districts of the West Kazakhstan region of Kazakhstan, in accordance with permission from the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan No. 27-02-19/5707-KLKHZHM dated July 30, 2025. Parasite carriage studies were conducted using the Coral method (Figure 1A), which does not harm the animals [13]. Using this method, saigas were forced into a large mesh enclosure, and the gates were closed once the enclosure was full. Samples were collected from the animals using standard acarological methods [14]. To avoid stress, each captured animal was examined as quickly as possible. Any 1-2 ticks found were removed using specialized tweezers, while observing all safety precautions. The animals were then immediately released. No tick counts were performed on the animals.

Collected samples were placed in sterile laboratory Eppendorf tubes and stored at 2-4 °C. A logistics company with the required temperature control was used to deliver the samples to the laboratory. Tick species identification was performed at the Almaty branch of the National Center for Biotechnology using a trinocular stereoscopic microscope and identification tables [15-17].

**Results and Discussion**

Since 2006, a Memorandum of Understanding on the Conservation and Sustainable Use of the *Saiga tatarica* has been in effect between Kazakhstan, Russia, Uzbekistan, and Turkmenistan. The document was signed under the Convention on Migratory Species of Wild Fauna [24]. The Ural (Volga-Ural) saiga population inhabits the border of Russia (Volgograd, Astrakhan, and Orenburg regions) and West Kazakhstan, primarily in the steppe regions between the Volga and Ural Rivers. This population migrates between the two countries, especially in the summer, when saigas migrate north to Russia in search of cooler climates and more abundant pastures. In winter, they return to Kazakhstan. The migratory saiga herd shares an epizootiological unit with domestic livestock, increasing the likelihood of re-infection with contagious diseases.

In Kazakhstan, the Ural saiga population inhabits the West Kazakhstan region and Atyrau regions, which border the Russian Federation [25]. The shared use of pastures and the sharing of a single epizootiological unit of *Saiga tatarica* with domestic animals contribute to the intensification of pathogen circulation and, consequently, an increased risk of reinfection. The spread of pathogens causing pasteurellosis, helminthiasis, echinococcosis, and coenurosis has been documented among saigas [26-28]. High levels of theileriosis are known among the Ural, Betpak-dala, and Ustyurt saiga populations, with ticks being the primary vectors of the pathogens [29].

In July 2025, during metagenomic sampling, 2 ticks were found in the sub-tail region of one of the studied saigas (Figure 1B).

A B

A - Fixation of a saiga using the Coral method;
B - Ticks found in the sub-tail region of a saiga (by Sattarova R.)

Figure 1. Capture and study of saiga in the West Kazakhstan region

Upon species identification, it was determined that both specimens were males and belonged to the same species, *Hyalomma marginatum* (Figure 2).

A B

A – T op view; B- B ottom view

Figure 2. *Hyalomma marginatum*, taken from a saiga examined in the Bokeyorda State Nature Reserve (by Sayakova Z.)

Unlike other *Hyalomma* species found in Kazakhstan, the mites we discovered lack a distinct parma. In contrast to *Hyalomma asiaticum*, the cervical grooves are superficial, not reaching the midpoint of the conscutum. Unlike *H. scupense*, the posteromedian groove is narrow and superficial, not reaching the parma. The marginal grooves are long, almost reaching the eyes. The caudal area is clearly defined; large puncturation is relatively sparse, located mainly on the caudal area, lateral fields, and the anterior part of the central field. Small or medium puncturation is evenly scattered throughout the conscutum. The overall coloration is dark, ranging from black to brown. The leg segments exhibit rings of whitish enamel pigment at the articulations. The dorsal process of the peritreme is long, not sharply separated from the main part. The perforated part of the dorsal process of the peritreme is wide, straightened, with a bend at the apex (Figure 3).

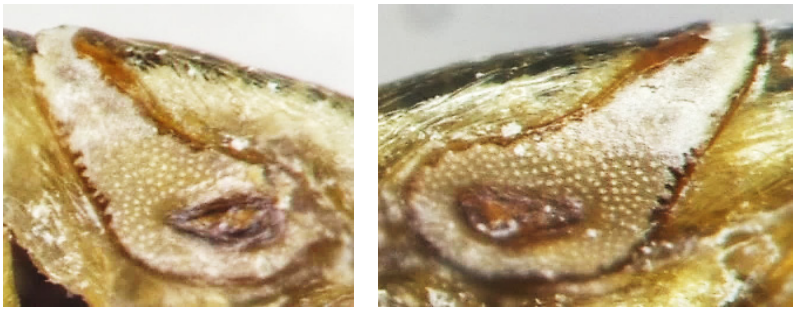

Figure 3. Peritremes of a male *Hyalomma marginatum* taken from a saiga in the Bokeyorda State Nature Reserve (by Sayakova Z.)

Currently, 15 *Hyalomma* species are known to transmit the CCHF virus [18, 19], of which nine species are known in Kazakhstan [17].

The Republic of Kazakhstan borders Russia, China, Kyrgyzstan, Uzbekistan, and Turkmenistan, and therefore, the potential for expansion of the nasal area for transboundary infectious diseases spread by blood-borne ticks cannot be ruled out. Transboundary diseases are those of exceptional relevance to the economies, trade, and/or food security of many countries, capable of widespread interstate spread on an epidemic scale, and the control of which, up to and including eradication, requires the cooperation of several countries [19]. The *Hyalomma marginatum* tick is a vector of such diseases. In southern Russia, this parasite remains the primary vector of the CCHF virus in endemic regions. The high abundance of the species, combined with the constant detection of the pathogen, underscores its central role in the ecology of tick-borne diseases in the country. Sporadic findings outside its primary range indicate the potential for further population expansion. In southern Russia, *Hyalomma marginatum* accounts for nearly 50% of ticks collected from domestic animals, and studies have confirmed the presence of pathogens in this species [19]. Previously, ticks of the genus *Hyalomma* were reported from saiga in Kazakhstan; *Hyalomma scupense* was identified in the Volga-Ural population of the antelope [20].

*Hyalomma marginatum primarily* inhabits steppes, deserts, and semi-deserts characterized by low to moderate humidity and long, hot, and dry summers [21].

The primary hosts of adult ticks are cattle, small ruminants, camels, horses, and lagomorphs. The pre-imaginal stage feeds primarily on birds. This is the first time we have discovered this species on the saiga. In Kazakhstan, this species is found primarily in the northwest of the country, along the Russian border (West Kazakhstan and Atyrau regions) [22]. Recently, an eastward expansion of this species has been observed. Previously, *H. marginatum* was regularly recorded in the Bokey-Orda and Zhanibek districts of the West Kazakhstan region. Since May 2012, ticks have been reported in the Uralsk region since 2017, in the Baiterek, Taskala, and Burli districts, and since 2019, in the Syrym and Karatobe districts [23]. In the Aktobe region, *H. marginatum* was first discovered by us in April 2023. During an inspection of cattle in the Uil district, 50 ticks (29 females and 21 males) were collected.

In the territory bordering Kazakhstan, *Hyalomma marginatum* is known in the Astrakhan, Saratov, and Volgograd regions [30-33] and coincides with the migration area of the Ural saiga population [25].

**Conclusion**

The present study documents, for the first time, the occurrence of *Hyalomma marginatum* ticks on the Ural population of *Saiga tatarica*. This observation suggests that shared pastures may facilitate parasite exchange between domestic animals and wildlife. Given the extensive migratory movements of saigas, these animals are likely to contribute to the dispersal and possible range expansion of *H. marginatum* within Kazakhstan.

**Authors' contributions**

SR, SZ, SK and BF: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript; AK, KZh RS: conducted final editing and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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# Sampling, transportation and preservation of fecal and guts samples from the saiga population for 16S rRNA sequencing

Aigerim R. Kozhayeva[1], Rano S. Sattarova[2], Zamir A. Abdreshev[3]
Shynggys D. Orkara[4], Artur R. Khairushev[5], Aigerim K. Khamzina[4]

[1]Shakarim University, Semey, Kazakhstan
[2]Kazakh Scientific Research Veterinary Institute, Almaty, Kazakhstan
[3]Bokey Orda State Nature Reserve, Oral, Kazakhstan
[4]Kazakh National Agrarian Research University, Almaty, Kazakhstan
[5]West Kazakhstan Agrarian-Technical University named after Zhangir Khan, Oral, Kazakhstan

**Corresponding author:** Aigerim K. Khamzina: aigerim.khamzina55@gmail.com
**Coauthors:** (1: AK) aigerim.kozhayeva@mail.ru; (2: RS) ranosaitomarovna@gmail.com
(3: ZA) zake-8788@mail.ru; (4: ShO) orkarashynggys@gmail.com
(5: AKh) hairushev-97@mail.ru
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**Abstract**

Background and Aim. The collection, transport, and preservation of fecal and intestinal samples from wild animals are critical steps in microbiome research, particularly in studies of the saiga antelope microbiome using 16S rRNA sequencing to investigate complex host–microbial interactions

The aim of this study was to compare four methods for the transport and preservation of saiga fecal and intestinal samples in order to evaluate their effectiveness in maintaining microbial DNA integrity for subsequent 16S rRNA sequencing.

Materials and Methods. The research was conducted in the Bokeyorda State Nature Reserve, located in the West Kazakhstan region of Kazakhstan, with permission from the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan. The animals were captured for study without harm to their health. Biological material in the form of feces from live saigas was collected using a non-invasive method into sterile test tubes. The autopsy of dead saigas was performed in clean field conditions using sterile instruments. All samples were immediately frozen in liquid nitrogen. Additionally, they were cooled to 4 °C, stored at room temperature, and preserved with RNAlater for subsequent analysis.

Results. A total of 37 samples were collected from two dead female saigas from different sections of the gastrointestinal tract, as well as fresh fecal samples and rectal swabs obtained from two live females and one male. Upon arrival at the laboratory, all samples were stored at −80 °C until the start of laboratory studies. The study demonstrated that sample storage conditions significantly affect the preservation of the saiga faecal microbiota structure. Samples stored at −80 °C showed the greatest stability in both alpha diversity and taxonomic composition of microbial communities. These findings are particularly relevant for microbiota research and RNA sequencing in remote and inaccessible regions of the saiga habitat.

Conclusion. The results suggest that in field conditions where strict cold-chain maintenance is not possible, particularly when sampling from wild and remote saiga populations, the use of liquid nitrogen or RNAlater provides an effective and practical alternative to rapid freezing.

**Keywords:** 16S rRNA sequencing; bacteria; microbiome; feces; Saiga tatarica tatarica.

**Introduction**

Transportation and preservation of fecal and gut samples, along with subsequent DNA extraction for rRNA sequencing, are critical steps in studying the complex interactions between host organisms and their microbiomes, particularly in wildlife populations such as the saiga. The quality of DNA obtained from fecal samples is a determining factor in the accuracy of genetic analyses aimed at characterizing these microbiomes and studying their functional dynamics. Notably, factors such as the preservation method, the time elapsed before processing, and the extraction protocol can significantly affect DNA integrity and yield.

Effective preservation of fecal samples is essential to prevent RNA degradation caused by ribonuclease (RNase) activity. For instance, *Reck* et al. emphasized that certain preservation methods yield better results by minimizing genomic DNA contamination while facilitating high-quality RNA extraction [1]. In the present study, the authors used immediate stabilization of fecal samples in RNA preservation solution (RNAlater) immediately after collection, and subsequently stored and transported samples under refrigerated conditions until laboratory processing. Optimized preservation methodologies significantly reduced contamination levels, which is crucial when dealing with the often complex mixtures found in fecal materials.

Another study highlighted the importance of appropriate storage conditions, which can significantly affect RNA integrity and the composition of the microbial community in fecal samples [2]. The authors compared the transport of fecal samples in different buffers (including SM buffer and DNA/RNA Shield) at room temperature, +4 °C, and freezing to assess the preservation of viral particles and nucleic acids [2]. The comparative analysis across different preservation methods showed that optimally preserved samples can lead to enhanced sequencing results, underlining the need for meticulous transport and storage practices [3]. Notably, methods such as RNA stabilization reagents can create a suitable environment that minimizes RNA degradation.

The extraction process must be conducted in a manner that preserves the integrity of the nucleic acids. The various methods for RNA extraction from fecal samples exhibit a broad spectrum of efficacy, particularly in low-biomass samples typical of wildlife studies [4]. Furthermore, the use of efficient extraction kits is paramount; for example, results from Schwochow et al. demonstrated that specific commercial RNA extraction protocols could substantially improve RNA yield and functionality in non-human species [4].

Furthermore, *Cieślik* et al. indicated the need for robust extraction protocols capable of handling degraded DNA, often resulting from inadequate preservation methods or outdated sample-handling practices [5]. The impact of these conditions on DNA quality has also been noted in studies showing that specific solutions, such as guanidine hydrochloride or other chaotropic agents, were deemed critical for preventing RNase-mediated degradation during extraction [6].

The application of rRNA sequencing (16S rRNA-seq) technology amplifies the potential of well-preserved, properly extracted DNA. It allows for comprehensive profiling of gut microbiota functionality, an essential aspect of understanding wildlife health and disease resistance mechanisms [3, 7]. Insights from 16S rRNA-seq can significantly inform conservation strategies and wildlife management practices that hinge on the biological well-being of species such as the saiga.

The successful transportation, preservation, and extraction of DNA from fecal and gut samples significantly influence the outcomes of genetic analyses performed on wildlife populations. A systematic approach that emphasizes rigorous preservation techniques and validated extraction protocols is essential to the integrity of 16S rRNA-seq-derived data.

The objective of this study was sample and compare four methods of transporting and preserving fecal and intestinal content samples collected from a saiga population: (1) immediate freezing in liquid nitrogen in the field; (2) storage at ambient temperature without preservative; (3) cooling at 4 °C; (4) stabilization with RNAlater under ambient field conditions, in order to determine their effectiveness in preserving microbial DNA integrity for subsequent 16S ribosomal RNA sequencing analysis.

**Materials and Methods**

Field sampling was conducted on October 25, 2025, in the Bokeyorda State Nature Reserve (West Kazakhstan Region), within the range of the Ural saiga population (Saiga tatarica tatarica). Laboratory

analyses were performed at Kazakh National Agrarian Research University and continued until the end of December 2025. The sampling area is illustrated in Figure 1.

All procedures involving animals were conducted in accordance with the principles established by the International Animal Ethics Committee and/or the relevant institutional animal ethics committee, as well as in compliance with local laws and regulations, and were approved under Protocol No. 2 dated December 19, 2024, by the Kazakh Scientific Research Veterinary Institute.

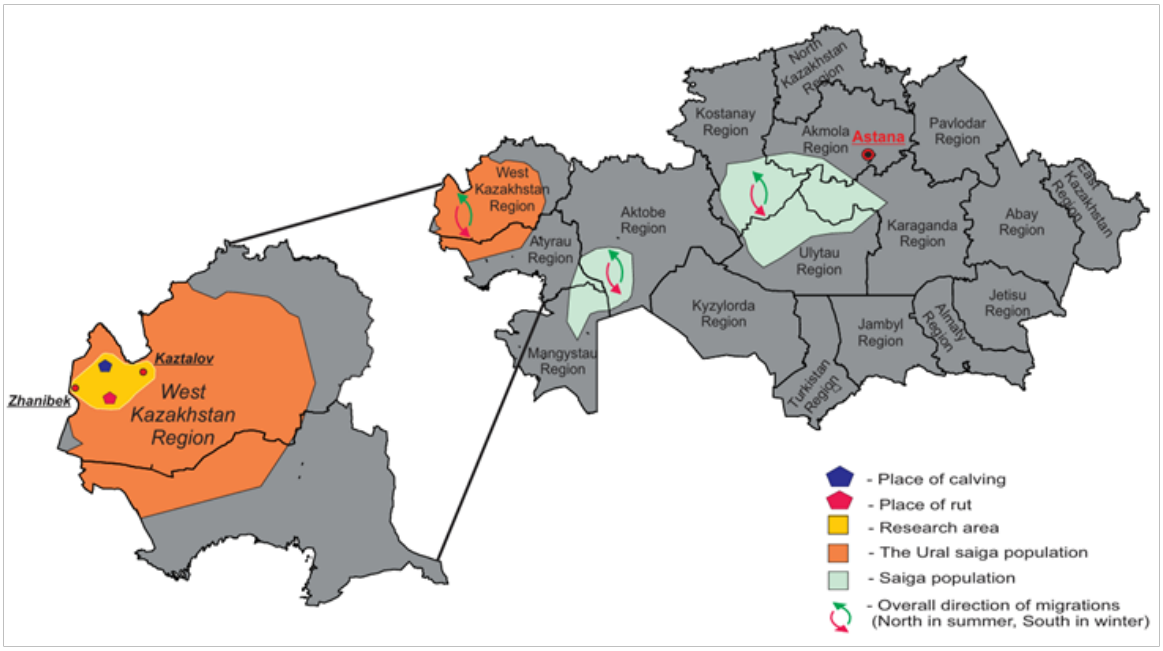


Figure 1. Map of Western Kazakhstan showing the location where saigas from the Ural population

Biological material was collected from live saigas using a non-invasive approach involving sterile rectal swabs. The swab was inserted 2-3 cm into the animal's rectum and then gently rotated to obtain fecal material. Additionally, 1-2 g of fresh feces were collected and placed in sterile test tubes. All samples were immediately frozen in a liquid nitrogen container in the field, while three additional preservation approaches were used for comparison: storage at ambient temperature without preservative, cooling at 4 °C, and stabilization with RNAlater under ambient field conditions.

Dead saigas were autopsied in the field using sterile instruments. Fragments of the small and large intestines were collected separately and opened with sterile scissors. The intestinal contents (2-5 g) were transferred to sterile test tubes, labeled, and immediately placed in liquid nitrogen to prevent nucleic acid degradation.

All samples were delivered to the laboratory within one day after collection. The following samples were included in the study:

- Sample No. 1 («Route 1»)% ostmortem samples taken from a female saiga antelope killed in a traffic accident. A total of 10 samples were obtained from various parts of the gastrointestinal tract (small intestine, large intestine, stomach, rectum).
- Sample No. 2 («Route 2»)% Postmortem samples taken from another female saiga that died in a traffic accident. Similarly, 9 samples were obtained from various parts of the gastrointestinal tract.
- Sample No. 3:Fresh fecal samples and rectal swabs collected from a live male saiga; 5 samples in total.Sample No. 4: Fresh fecal samples and rectal swabs collected from a live female saiga; 4 samples in total.

**Results and Discussion**

Upon arrival at the laboratory, all samples were stored at −80 °C until laboratory testing began. This temperature regime was considered optimal for preserving the structure of microbial communities and DNA integrity.

The study accounted for modern approaches to preserving fecal microbiota for sequencing. Rapid freezing in liquid nitrogen was considered the «gold standard» for sample storage. Additionally, the effect of alternative stabilization strategies was analyzed, including cooling at 4 °C, storage at ambient temperature, and the use of preservative buffers (RNAlater).

The effect of storage conditions on the microbial community composition was assessed by amplicon sequencing of the 16S rRNA gene after 72 hours of storage. DNA extraction was performed using standard molecular biology protocols recommended for fecal microbiota analysis.

The study demonstrated that sample storage conditions significantly influence the preservation of saiga fecal microbiota structure. Samples stored at −80 °C showed the greatest stability in both alpha diversity and taxonomic composition of microbial communities.

Cooling the samples to 4 °C for 72 hours did not result in statistically significant changes in the diversity or relative abundance of dominant bacterial taxa compared with control samples stored at −80 °C. At the same time, samples stored at ambient temperature without preservatives showed pronounced shifts in the microbial community composition.

The data obtained indicate that when it is impossible to maintain the cold chain in the field, especially when working with wild saiga populations, the use of RNAlater can be considered an effective alternative to rapid freezing. This is particularly important for microbiota research and RNA sequencing in remote and inaccessible regions of the saiga's range.

The integrity of fecal microbiota is crucial for microbiome studies, especially in understanding microbial diversity and community composition. This discussion evaluates modern approaches to preserving fecal microbiota, focusing on the gold standard of rapid freezing at −80 °C compared with alternative strategies such as refrigeration at 4 °C, ambient-temperature storage, and the use of preservative buffers such as RNAlater.

Rapid freezing at −80 °C has been established as the benchmark for preserving microbial communities, as it minimizes metabolic activity and reduces the risk of community shifts due to environmental exposure. Studies highlight that samples stored under these conditions maintain microbial diversity and taxonomic composition effectively over extended periods. Specifically, storage at −80 °C showed superior stability in both alpha diversity metrics and taxonomic structures, affirming its status as a best practice in microbiota preservation [8, 9].

Conversely, cooling samples to 4 °C has shown that while some aspects of microbial composition remain stable, significant shifts can occur over time, indicating that 4 °C may be an acceptable short-term alternative but is not optimal for long-term preservation [10, 11, 12]. Nevertheless, storage at ambient temperatures resulted in notable shifts in microbial community structures within just 48 to 72 hours, underscoring the risks associated with inadequate preservation methods [13, 14].

The introduction of preservative buffers has emerged as a potential solution when immediate freezing is unfeasible [15]. RNAlater has been noted to affect microbial profiles, emphasizing that not all preservative solutions perform equally well [16]. This variability is critical to note, particularly in field studies where maintaining the cold chain is often logistically challenging [17].

For field studies, particularly those involving wildlife samples like those from saiga populations, effective preservation methods are crucial given the often-remote collection locations [14, 17]. The results indicate that while −80 °C is ideal, RNAlater provides a viable alternative when immediate freezing is not feasible, offering a practical option that helps retain the microbial integrity needed for subsequent 16S rRNA sequencing and downstream analysis [18].

While rapid freezing remains the gold standard for preserving fecal microbiota for sequencing, the use of specific preservative buffers, particularly RNAlater, may serve as effective alternatives in field conditions where maintaining the cold chain is impractical. Continuous evaluation of storage protocols and their impacts on microbial community profiles is essential for advancing microbiome research methodologies.

**Conclusion**

The study showed that the conditions of transportation and storage of fecal and intestinal samples from saiga antelopes (*Saiga tatarica tatarica*) have a key impact on the preservation of microbial community

structure and DNA integrity, which, in turn, determines the reliability of subsequent molecular genetic analyses, including 16S rRNA sequencing.

It has been established that rapid freezing of samples in liquid nitrogen is the most reliable and stable method for preserving fecal microbiota, ensuring minimal changes in alpha diversity and taxonomic composition of microorganisms. This storage approach is considered the "gold standard" for studying the microbiota of wild animals.

It has been revealed that short-term cooling of samples to 4 °C (up to 72 hours) does not result in statistically significant changes in microbial community structure and can be used as an acceptable short-term alternative in field conditions. In contrast, storing samples at ambient temperature without preservatives results in pronounced shifts in the microbiota, significantly reducing the quality of the data obtained.

The results indicate that when strict adherence to the cold chain is not possible in the field, especially when working with wild and remote saiga populations, the use of RNAlater is recommended, and can be considered an effective and practical alternative to rapid freezing. This is relevant for further research on the microbiota, the functional activity of microbial communities, and mechanisms of saiga antelope resistance to infectious and environmental stressors, as well as for the development of scientifically sound approaches to monitoring population status and conservation. This study presents the first comparative evaluation of field-based fecal sample preservation methods for Saiga antelope, focusing on maintaining microbial DNA quality suitable for 16S rRNA sequencing under remote wildlife sampling conditions.

**Authors' contributions**

AK, RS, ShO, and AKh: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript; AK, ZA, RS: conducted final editing and proofreading of the manuscript. All authors read, reviewed, and approved the final version of the manuscript.

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# Species composition and distribution of animal ectoparasites in Kyrgyzstan

Nurzina S. Atabekova, Bekbolsun K. Aknazarov, Elmurat A. Jetigenov
Ularbek M. Suerkulov

I. Skryabin Kyrgyz National Agrarian University, Bishkek, Kyrgyzstan

**Corresponding author:** Bekbolsun K. Aknazarov: aknazarov-61@mail.ru
**Coauthors:** (1: NA) atbn.7@mail.ru; (2: EJ) agetigen@mail.ru
(3: US) suerkulovularbek@gmail.com
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**Abstract**

Background and Aim. Ectoparasites represent a significant threat to animal health and may act as vectors of infectious diseases. This study aimed to determine the species composition and geographical distribution of animal ectoparasites in Kyrgyzstan.

Materials and Methods. Ectoparasites were collected from livestock, companion animals, and birds across seven regions of Kyrgyzstan (Chuy, Talas, Issyk-Kul, Jalal-Abad, Osh, Batken, and Naryn) and the city of Bishkek during 2021-2022. Sampling included cattle, goats, sheep, horses, poultry, dogs, and cats. Collected specimens were identified using standard morphological methods.

Results. A total of 5,267 ectoparasite specimens were collected, which demonstrated substantial diversity across the study areas. The tick species *Rhipicephalus turanicus* was widely distributed in most regions, except for Issyk-Kul and Talas. Several species exhibited restricted geographical distribution. Specifically, *Haemaphysalis sulcata* and *Hyalomma anatolicum* were detected exclusively in the Chuy Valley, *Dermacentor ushakovae* in the Issyk-Kul region, *Hyalomma asiaticum* in Jalal-Abad region, and *Dermacentor niveus* and *Dermacentor ushakovae* in the Naryn region. Host specificity was observed among several ectoparasite species.

Conclusion. The study identified 12 tick species of the family *Ixodidae*, 2 species of the family *Argasidae*, and 2 species of blood-sucking insects of the family *Hippoboscidae*. The findings highlight the diversity and regional specificity of ectoparasite fauna in Kyrgyzstan and provide a basis for further epidemiological surveillance and control strategies.

**Keywords:** animals; blood-sucking; species composition; ticks.

## Introduction

Currently, ongoing changes in agricultural practices, together with climate change, are contributing to shifts in the habitats and distribution of ticks. These alterations are associated with the emergence of new natural foci of hemoparasitic diseases. This trend has been documented in numerous studies [1-3]. For instance, according to *Gubeydullina* et al., a comparative analysis of two observation periods (1966-1969 and 2007-2008) in the forests of the Volga Right Bank area of the Ulyanovsk Region demonstrated a significant reduction in the distribution range of Ixodes ricinus, accompanied by an increase in the abundance of Dermacentor reticulatus [1]. Similarly, Marques et al. reported that climate change is one of the most critical global challenges influencing the geographical distribution of vectors and pathogens, thereby increasing the risk of the spread of hemoparasitic diseases [2].

By the end of the twentieth century, the fauna of ixodid ticks in Kyrgyzstan included 42 taxa of seven genera: *Ixodes, Haemaphysalis, Anomalohimalaja, Dermacentor, Rhipicephalus, Hyalomma, Boophilus* [3]. Subendemic tick species that were registered for the first time in Kyrgyzstan are presented in the

works of domestic and Russian scientists, they include: Ixodes eldaricus (*N.A. Filippova* ,1974) [4]; Dermacentor ushakovae (*N.A. Filippova*, *I.V. Panova*,1987) [5]. *E.A. Bardzimashvili* (1990) registered the spread of new species of ixodid ticks for the fauna of the republic, such as *Ixodes kaizei, Ixodes arboricola, Ixodes lividus, Ixodes caledonicus, Ixodes semenovi* [6].

Research on the ixodid tick fauna of Northern Kyrgyzstan, including the Chuy Valley and the Issyk-Kul Basin, has shown that its current biodiversity comprises 27 species, 15 of which are ectoparasites of domestic and farm animals [7]. According to *Fedorova* [8], significant climatic and socio-economic changes have taken place both nationally and globally over recent decades.

These changes have inevitably affected the composition of ixodid tick faunal complexes, since ticks are temporary ectoparasites that spend most of their life cycle in the external environment. These changes have led to the expansion of the geographical range of certain arthropod species, such as *Rhipicephalus turanicus;* while changes in agricultural practices have resulted in the disappearance or contraction of the ranges of several previously abundant tick species, including *Haemaphysalis sulcata, H. punctata,* and others. In all natural zones, the species composition of dominant and subdominant ticks has changed in recent years. Currently, *R. turanicus* is the dominant species in the Chuy Valley, having displaced the formerly abundant *H. concinna*. In the Issyk-Kul Basin, *Dermacentor ushakovae* predominates, whereas in the high-mountain region of the Terskey Ala-Too, D. pavlovskyi is the dominant species.

Ixodid ticks are of major epidemiological and epizootiological importance as reservoirs and vectors of zoonotic pathogens and as essential components of natural foci of infectious diseases. According to *Fedorova* [7], the tick-borne infections of greatest medical and veterinary importance include tick-borne encephalitis, Lyme disease, Omsk hemorrhagic fever, Q fever, tularemia, anaplasmosis, and piroplasmosis in farm animals.

According to *Jane E. Sykes* [9], *Ixodes ricinus* and *Ixodes persulcatus* are presumed vectors of *Anaplasma phagocytophilum*, whereas *Rhipicephalus sanguineus* is considered the principal vector of *Anaplasma platys*. The author further notes that Ixodes persulcatus and *Dermacentor silvarum* serve as important vectors in Asia and Russia, although other *Ixodes* spp. may also be involved in transmission.

All *Babesia* species are transmitted by ticks with a relatively narrow host range. The primary arthropod vectors include *Ixodes ricinus*; however, in certain regions, species of the genus Rhipicephalus act as the main vectors, particularly for *Babesia bigemina* and *Babesia bovis* [10]. It has also been reported that *Rhipicephalus sanguineus, Dermacentor* spp., and *Haemaphysalis ellipticum* are capable of transmitting *Babesia canis*, whereas *Babesia gibsoni* is transmitted by *Haemaphysalis bispinosa* and *Haemaphysalis longicornis* [11].

Canine vector-borne diseases (CVBDs) represent a significant global health concern in dogs and may also pose zoonotic risks, particularly in developing countries where scientific data remain limited. In one study conducted in Tamil Nadu (southern India), blood and tick samples were collected from stray dogs to assess the prevalence of CVBD pathogens (*Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Hepatozoon* spp., filarioids, and *Leishmania* spp.). Of the 230 examined dogs, 229 (99.6%) were infested with ticks (mean intensity of 5.65), with *Rhipicephalus sanguineus sensu lato* and *Rhipicephalus haemaphysaloides* morphologically identified in 98.3% and 1.7% of infested animals, respectively [11]. In addition, fleas are among the most common ectoparasites of dogs and cats. Beyond causing discomfort to animals (and their owners), fleas may induce allergic dermatitis and act as vectors of pathogens and endoparasites [12].

The distribution, abundance, and vector competence of parasitiform ticks in Kyrgyzstan have not been systematically investigated over the past 30 years. During this period, substantial changes in environmental conditions and habitat structure have likely influenced the distribution of ixodid ticks, which, in turn, may have led to shifts in the species composition of pathogens responsible for vector-borne diseases. In this regard, the present study provides data on the distribution and species composition of ectoparasites in Kyrgyzstan.

**Materials and Methods**

The aim of this study was to investigate the species distribution of ectoparasites among farm animals, companion animals, and poultry in Kyrgyzstan. Accordingly, the objective was to assess the distribution and species composition of ectoparasites across the different regions of the country.

Ectoparasites were collected from farm animals, domestic poultry, dogs, and cats. The specimens were removed directly from the animals and preserved in 96% ethanol. Species identification was carried out at the Laboratory of Microbiology and Molecular Biology, Faculty of Veterinary Medicine, Kyrgyz National Agrarian University named after K.I. Skryabin. The collected ticks were identified using the Atlas of Animal Blood Parasites and Ixodid Ticks by V.F. Kapustin [13].

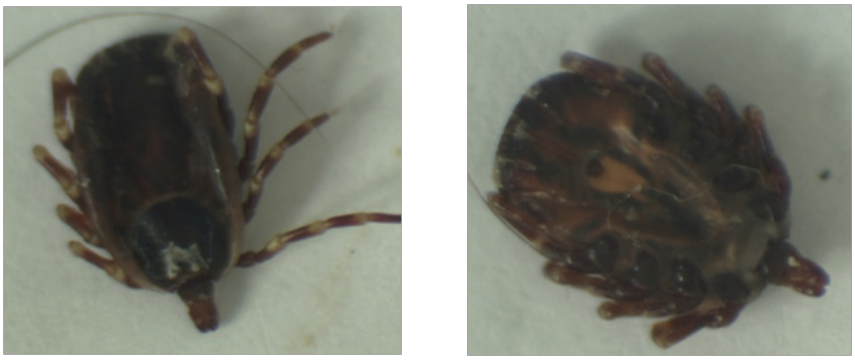
In addition, in collaboration with the Division of Vectors and Parasitic Diseases of the Republic of Korea, a molecular genetic study was performed to confirm the species identity of ixodid ticks within the framework of the research project entitled *Epidemiological Surveillance of Vector-Borne and Parasitic Diseases in Kyrgyzstan.* Microscopic examination was conducted using a stereomicroscope equipped with a VisiCam 16 Plus camera.

**Results and Discussion**

The principal hematophagous ectoparasites in Kyrgyzstan include ticks, horse flies, louse flies, fleas, and certain mosquito species. In the present study, particular attention was given to ticks, which appear to predominate in the republic in terms of abundance compared with other blood-feeding ectoparasites. Most ectoparasites are known to serve as carriers and disseminators of numerous infectious and parasitic diseases.

During the study, the species composition of ectoparasites, their abundance, and their distribution range were determined. In total, 5,267 ectoparasite specimens were collected during 2021-2022 from various animal species, including cattle, sheep, goats, horses, chickens, dogs, and cats. The tick species collected from animals are presented in Figures 1-9.

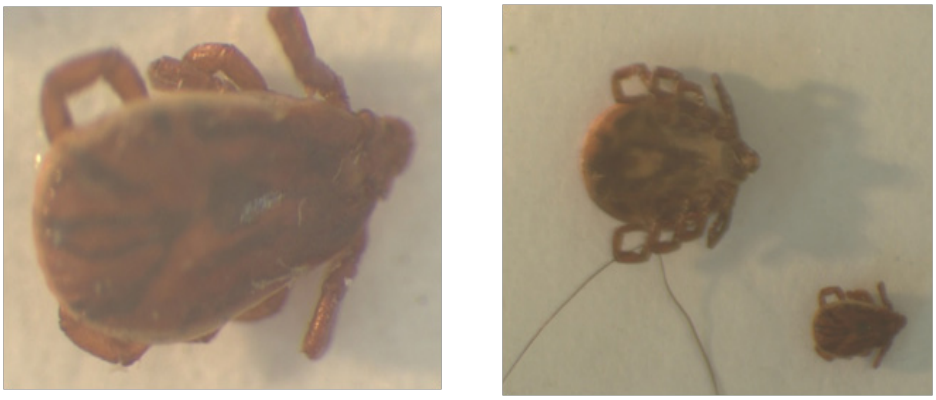
*Ixodes ricinus* specimens were collected in the village of Zher-Kazar, Alamudun District, Chuy Region.



*Ixodes ricinus – dorsally* *Ixodes ricinus – ventrally*

Figure 1. *Ixodes ricinus*

*Ixodes persulcatus* specimens were collected from sheep in the village of Lebedinovka, Alamudun District.



*Ixodes persulcatus – dorsally* *Ixodes persulcatus – ventrally*

Figure 2. *Ixodes persulcatus*

*Boophilus calcaratus* specimens were collected from sheep in the village of Kara-Zhigach, Alamudun District. The ticks were located predominantly in the head region. On average, 5 to 16 tick specimens were recorded per animal. Nymphs at the third developmental stage were the most frequently encountered.

*Boophilus calcaratus* nymph stage III – dorsally

*Boophulus calcaratus* nymph stage III – ventrally

Figure 3. *Boophilus calcaratus*, third-stage nymph

*Hyalomma scupense* specimens were collected from sheep and dogs in the village of Kashka-Suu (Ala-Archa Gorge area, Alamudun District).

*Hyalomma scupense – dorsally*

*Hyalomma scupense – ventrally*

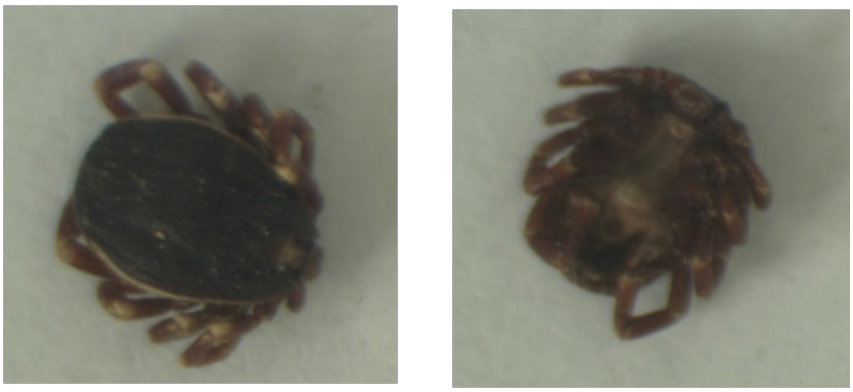
Figure 4. *Hyalomma scupense*

Ticks of the species *Dermacentor marginatus* were collected from sheep and cattle in the Tokmak region, in the village of Ak-Beshim.

*Dermacentor marginatus – dorsally*

*Dermacentor marginatus* – ventrally

Figure 5. *Dermacentor marginatus*

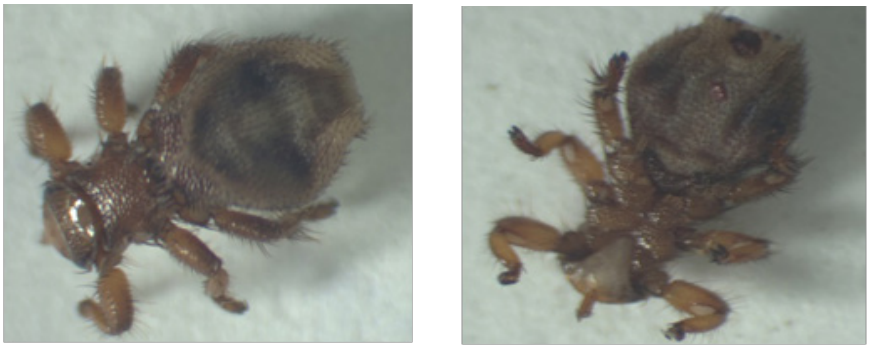


*Rhipicephalus bursa* – nymph, dorsally

*Rhipicephalus bursa* – nymph, ventrally

Figure 6. *Rhipicephalus bursa*

Louse flies of the species *Melophagus ovinus* were detected in large numbers in sheep in the village of Orto-Sai, Alamudun District. Approximately 10-15 insects were found on each sheep.

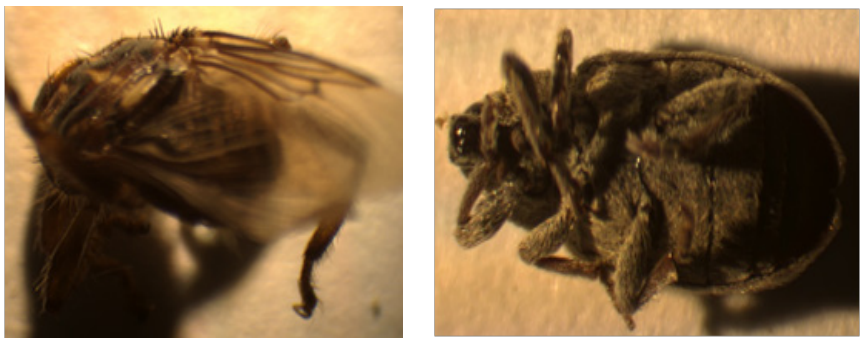


*Melophagus ovinus* – dorsally

*Melophagus ovinus* – ventrally

Figure 7. Melophagus ovinus

Among horses in the village of Orto-Sai, Alamudun District, winged blood-feeding flies of the species *Hippobosca equina* were also identified. On horses, these insects were distributed over the entire body, predominantly in the tail region. Up to 20-25 specimens were recorded per animal.



*Hippobosca equina* – dorsally

*Hippobosca equina* – ventrally

Figure 8. *Hippobosca equina*

At a veterinary clinic in Bishkek, two dogs of different breeds infested with ticks of the species *Haemaphysalis punctata* were examined. The ticks were located predominantly in the head region.

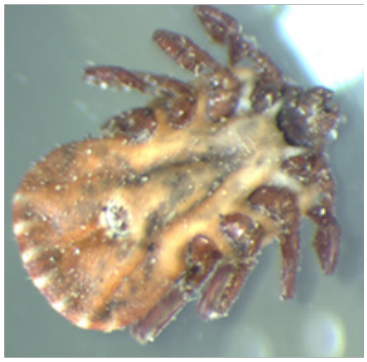


Figure 9. *Haemaphisalis punctate*

The conducted studies demonstrated the diversity of ectoparasite populations in the investigated territories, as summarized in Table 1.

Table 1. Distribution of ectoparasites across the regions of the Kyrgyz Republic

| No | Regions | Animal ectoparasites |
|---|---|---|
| 1 | Bishkek | *Rhipicephalus turanicus, R.sanguineus, Haemaphysalis punctate, Ctenocephalides canis* |
| 2 | Chuy Region | *R.turanicus, H. punctata, H.sulcata, D.marginatus, Hl.marginatum, Hl.scupense, Hl.anatolicum, Boophilus annulatus, A. Lahorensis Argas persicus Melophagus ovinus Hippobosca equina* |
| 3 | Talas Region | *H. punctata, Hl.marginatum, Boophilus annulatus, A. Lahorensis Argas persicus Melophagus ovinus* |
| 4 | Issyk-KulRegion | *H. punctata, Dermacentor marginatus, D. ushakovae A. Lahorensis Melophagus ovinus* |
| 5 | Jalal-Abad Region | *Rhipicephalus turanicus, Hyalomma scupense, Hl. marginatum, Hl.asiaticum, A. lahorensis Argas persicus Melophagus ovinus* |

Continuation of Table 1

| | | |
|---|---|---|
| 6 | Osh Region | *R.turanicus,*<br>*Hl.marginatum,*<br>*A. Lahorensis*<br>*Argas persicus*<br>*Melophagus ovinus* |
| 7 | Batken Region | *Rhipicephalus turanicus*<br>*Hyalomma marginatum,*<br>*Alveonasus lahorensis,*<br>*Melophagus ovinus* |
| 8 | Naryn Region | *R.turanicus,*<br>*H. punctata,*<br>*D.niveus,*<br>*D. ushakovae,*<br>*D.marginatus*<br>*Alveonasus lahorensis*<br>*Melophagus ovinus* |

As a result of the investigation of ectoparasites collected from domestic and farm animals during 2021-2022 in seven regions of the Kyrgyz Republic and the city of Bishkek, 12 species of ticks belonging to the family *Ixodidae*, two species of the family *Argasidae* (*A. lahorensis* and *Argas persicus*, highlighted in blue in the table), and two species of blood-feeding insects of the family *Hippoboscidae* (*Hippobosca equina* and *Melophagus* ovinus, highlighted in yellow in the table) were identified.

As shown in Table 1, ixodid ticks are distributed throughout all regions of the republic. According to our findings, *Rhipicephalus turanicus* occurs in all regions except the Issyk-Kul and Talas Regions. The two argasid species and representatives of the family *Hippoboscidae* were recorded across the republic. In the northern part of the country, *Dermacentor marginatus* and *Hyalomma marginatum* were frequently recorded, whereas in the southern regions, *Hyalomma marginatum* was encountered more commonly.

Over the two-year period of ectoparasite collection, several species were found only rarely and showed clear geographical restriction to specific territories. Thus, *Haemaphysalis sulcata* and *Hyalomma anatolicum* were detected only in the Chuy Valley, whereas *Dermacentor ushakovae* was recorded only in the Issyk-Kul Basin. In the Jalal-Abad Region, *Hyalomma asiaticum* was identified, while in the Naryn Region, two species restricted to this area were recorded, namely *Dermacentor niveus* and *D. ushakovae*.

The present study also demonstrated that ectoparasites exhibit a certain degree of host specificity with respect to the animals they parasitize. Table 2 presents the parasite species identified and the corresponding animal species on which they were found.

Table 2. List of ectoparasites and their hosts

| No | Ectoparasites | Hosts |
|---|---|---|
| 1 | *Rhipicephalusturanicus* | dogs, cats, cattle, sheep, goats, and horses |
| 2 | *Rhipicephalus sanguineus* | dogs |
| 3 | *Haemaphysalispunctata* | cattle, sheep, horses |
| 4 | *Haemaphysalis sulcata* | cattle, sheep |
| 5 | *Dermacentormarginatus* | cattle, sheep, horses |
| 6 | *Dermacentorniveus* | dogs, cattle, sheep |
| 7 | *Dermacentorushakovae* | cattle, sheep |
| 8 | *Hyalommamarginatum* | dogs, cattle, sheep, horses |
| 9 | *Hyalomma scupense* | dogs, cattle, sheep, horses |

Continuation of Table 2

| | | |
|---|---|---|
| 10 | *Hyalomma asiaticum* | cattle |
| 11 | *Hyalomma anatolicum* | cattle |
| 12 | *Boophilusannulatus* | cattle, horses |
| 13 | *Alveonasus lahorensis* | sheep, cattle |
| 14 | *Argas persicus* | hens |
| 15 | *Melophagusovinus* | sheep |
| 16 | *Hippobosca equina* | horses |
| 17 | *Ctenocephalides canis* | dogs |

According to the material obtained, *Rhipicephalus turanicus* exhibited the broadest host range, being recorded on six host species. A relatively wide host range was also observed for *Haemaphysalis punctata, Hyalomma marginatum*, and *Hyalomma scupense*, each of which was found on four host species. In contrast, *Rhipicephalus sanguineus, Hyalomma asiaticum, Hyalomma anatolicum, Argas persicus, Melophagus ovinus*, and *Hippobosca equina* were identified as monoxenous species. Fleas collected from captured stray dogs belonged to the family Pulicidae and were identified as *Ctenocephalides canis.*

To investigate the prevalence of ectoparasites within a natural focus, parasite collection was carried out during the period of their seasonal activity at selected sites in the Chuy Valley, as presented in Table 3.

Table 3. Summary data on the abundance of ticks collected from animals in the designated study area of the Chuy Valley during the period from May to November

| Ticks and blood-sucking insects | Animal species | Number of ticks collected | | | |
|---|---|---|---|---|---|
| | | larvae | nymphs | adults | total |
| Ixodids: | Cattle | - | 6 | 92 | 98 |
| | Sheep | - | 43 | 188 | 231 |
| | Horses | - | 4 | 39 | 43 |
| | Hens | 9 | 11 | 20 | 40 |
| | Dogs | 2 | 6 | 31 | 39 |
| Argasids: | Cattle | | 8 | 24 | 32 |
| | Sheep | | 12 | 164 | 176 |
| | Horses | | 5 | 24 | 29 |
| | Hens | 3 | 9 | 13 | 25 |
| | Dogs | | 6 | 11 | 17 |
| Gamasoids: | Hens | 11 | 29 | 276 | 316 |
| Melophagus ovinus, Hippobosca equina | Sheep | - | - | 238 | 238 |
| | Horses | - | - | 67 | 67 |
| | All species | 25 | 139 | 1187 | 1351 |

The prevalence of infestation of animals with hematophagous ectoparasites in the study area was 14.3% (657/4593 × 100). A high number of ectoparasites was detected in sheep, goats, and poultry. Ixodid and argasid ticks were identified in small ruminants, with 231 and 176 specimens recorded, respectively. The extent of infestation with ixodid ticks in these animals was 34.75%, whereas the extent of infestation with argasid ticks was 12.05%. The relatively low prevalence of argasid tick infestation may be explained by the fact that during the observation period (September-November), animals were predominantly kept outdoors rather than in enclosed premises, where these ectoparasites are typically localized.

A large number of gamasid mites was collected from chickens, amounting to 316 specimens; however, the extent of infestation in poultry was 11.86%. A considerable number of louse flies was also recorded in sheep, with 238 specimens collected and an extent of infestation of 10.93%.

The results of the analysis of tick infestation in animals are presented in Table 4.

Table 4. Infestation of animals with ticks and blood-sucking insects in the study area of the Chuy Valley

| Ticks and blood-sucking insects | Animal species | Number of animals examined | | Total number of ticks collected | Prevalence of infestation % | Abundance index | Mean intensity of infestation |
|---|---|---|---|---|---|---|---|
| | | Total | Number of infested animals | | | | |
| Ixodids | Cattle | 110 | 43 | 98 | 39.1 | 0.89 | 2.28 |
| | Sheep | 751 | 261 | 231 | 34.8 | 0.3 | 0.89 |
| | Horses | 139 | 13 | 41 | 9.4 | 0.3 | 3.15 |
| | Hens | 677 | 14 | 40 | 2.1 | 0.06 | 2.86 |
| | Dogs | 118 | 24 | 39 | 20.3 | 0.3 | 1.6 |
| | Total | 1795 | 355 | 449 | 19.8 | 0.25 | 1.36 |
| Argasids | Cattle | 185 | 9 | 32 | 4.8 | 0.17 | 3.6 |
| | Sheep | 846 | 102 | 186 | 12,1 | 0.22 | 1.2 |
| | Horses | 151 | 8 | 29 | 5.3 | 0.19 | 3.6 |
| | Hens | 13 | 5 | 25 | 38.5 | 1.9 | 5 |
| | Dogs | 2 | 2 | 17 | | | |
| Gamasoids | Hens | 700 | 83 | 316 | 11.8 | 0.45 | 3.8 |
| Blood-sucking flies | Sheep | 750 | 82 | 238 | 10.9 | 0.32 | 2.9 |
| | Horses | 151 | 11 | 67 | 7.3 | 0.44 | 6.1 |

As shown in Table 4, cattle exhibited a high prevalence of infestation with ixodid ticks (39.1%). At the same time, the abundance index of ectoparasites among the examined animals was 0.89, while the mean intensity of tick infestation was 2.28. The intensity of infestation reached up to 23 tick specimens per animal. A relatively high prevalence of ixodid tick infestation was also observed in small ruminants; however, both the abundance index (0.3) and mean intensity (0.89) were considerably lower than those recorded in cattle. Horses also showed a high mean intensity of infestation with ixodid ticks (3.15), reaching up to 13 specimens per animal. In addition, a high mean intensity of ixodid tick infestation was recorded in poultry (2.86).

Infestation with argasid ticks was comparatively high in poultry, with a prevalence of 38.5%, an abundance index of 1.9, and a mean intensity of infestation of 5. Among small ruminants, the prevalence of infestation with these ticks was 12.1%, while the abundance index was 0.22 and the mean intensity was 1.2. Cattle and horses showed almost similar levels of infestation with argasid ticks: prevalence was 4.8% and 5.3%, respectively; the abundance index was 0.17 and 0.19, respectively; and the mean intensity was 3.6 in both animal species.

A high level of infestation with gamasid mites was recorded in poultry, with a prevalence of 11.8%, an abundance index of 0.45, and a mean intensity of 3.8.

Blood-sucking flies were identified in small ruminants and horses. In small ruminants, the prevalence of infestation with these parasites was 10.9%, the abundance index was 0.32, and the mean intensity of infestation was 2.9. In horses, the prevalence was 7.3%, the abundance index was 0.44, and the mean intensity was 6.1. In some cases, more than 20 specimens of blood-sucking flies were detected on a single animal.

Figure 10 illustrates the percentage of infestation with ixodid ticks in different animal species.

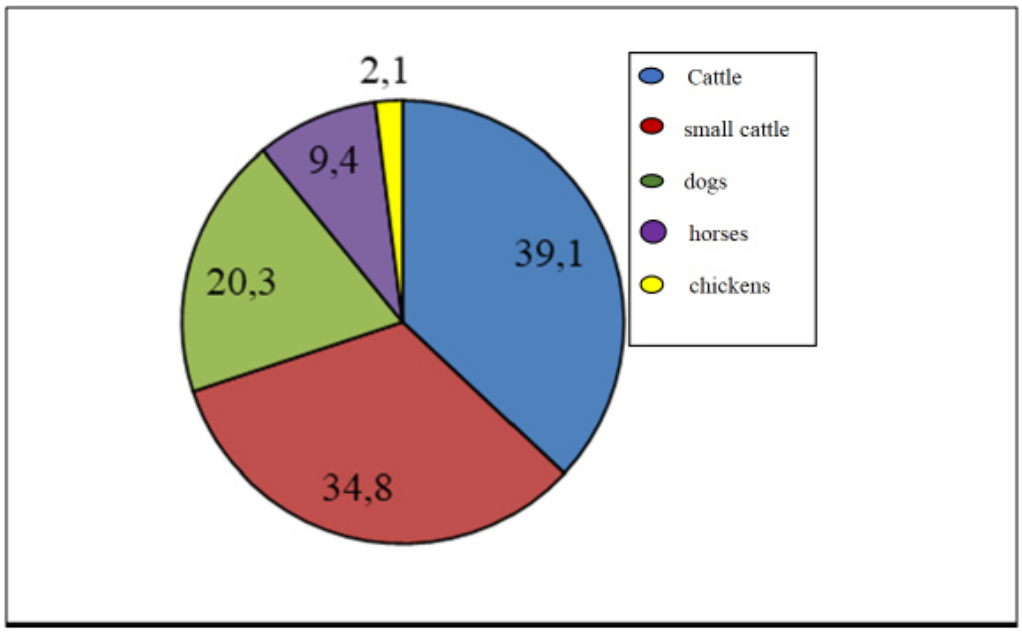



Figure 10. Prevalence of ixodid tick infestation in different animal species

The chart clearly illustrates the prevalence of ixodid tick infestation among the examined animals. The highest prevalence was observed in cattle (39.1%) and small ruminants (34.8%). In dogs, the prevalence of infestation was 20.3%. By contrast, horses and chickens showed relatively low levels of infestation with ixodid ticks, at 9.4% and 2.1%, respectively.

For visual comparison of argasid tick infestation in different animal species, the corresponding chart is presented in Figure 11.

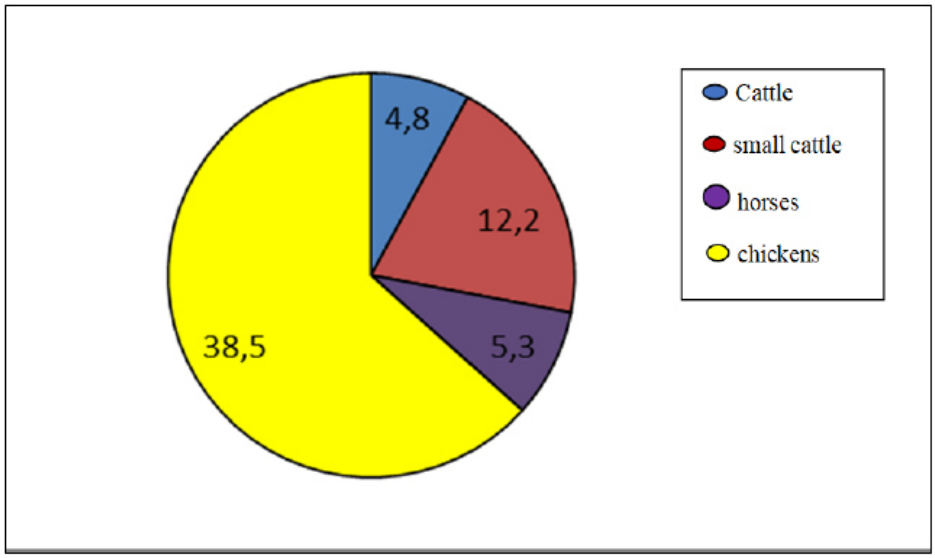



Figure 11. Diagram of the prevalence of argasid tick infestation in different animal species

The diagram of prevalence of argasid tick infestation shows that the highest proportion of infested animals was recorded in poultry (38.5%), which is apparently associated with the permanent housing of chickens in poultry shelters and the seasonal activity of argasid parasites. Considerably lower prevalence values were observed in small ruminants (12.2%), horses (5.3%), and cattle (4.8%). The relatively low infestation with argasid ticks may be explained by the fact that tick collection was carried out from late September, when the first specimens began to appear on animals, until November, when animals were still predominantly kept outdoors rather than in enclosed premises where these parasites are usually localized.

As demonstrated by the present study, ectoparasites are widely distributed in Kyrgyzstan and are represented by a diverse range of taxa, including ticks, horseflies, louse flies, fleas, and mosquitoes. Each group of these blood-feeding arthropods occupies its own ecological niche and poses a different epidemiological threat. Among them, ticks represent the greatest concern, with ixodid ticks being the principal vectors of transmissible infections. As a result of the investigations conducted in the republic, 12 species of ticks belonging to the family *Ixodidae*, two species of the family *Argasidae*, and two species of blood-feeding insects of the family *Hippoboscidae* were identified.

Certain ectoparasite species were found to be widely distributed in Kyrgyzstan and were recorded more frequently than others among animal hosts. The dominant species among ixodid ticks were *Rhipicephalus turanicus, Haemaphysalis punctata, Hyalomma marginatum,* and *Dermacentor marginatus.* Among argasid ticks, *Argas persicus* was the dominant species, whereas *Melophagus ovinus* predominated among blood-sucking insects.

According to our findings, *Rhipicephalus turanicus* was distributed throughout all regions except the Issyk-Kul and Talas Regions. The two argasid species and members of the family *Hippoboscidae* were recorded across the republic. In the northern part of the country, *Dermacentor marginatus* and *Hyalomma marginatum* were frequently encountered, whereas in the southern Over the two-year period of ectoparasite collection, several species were encountered only rarely and exhibited a clear association with specific territories, suggesting local endemism. Thus, *Haemaphysalis sulcata* and *Hyalomma anatolicum* were detected only in the Chuy Valley, whereas *Dermacentor ushakovae* was found only in the Issyk-Kul Basin. In the Jalal-Abad Region, *Hyalomma asiaticum* was identified, while in the Naryn Region two species restricted to this area were recorded, namely *Dermacentor niveus* and *D. ushakovae*.

The present study demonstrated that ectoparasites show a certain degree of host specificity with respect to the animals they parasitize. According to the material obtained, *Rhipicephalus turanicus* exhibited the broadest host range, having been recorded on six host species. A relatively broad host range was also observed for *Haemaphysalis punctata, Hyalomma marginatum,* and *Hyalomma scupense*, each of which was found on four host species. In contrast, *Rhipicephalus sanguineus, Hyalomma asiaticum, Hyalomma anatolicum, Argas persicus, Melophagus ovinus,* and *Hippobosca equina* were identified as monoxenous species. Fleas collected from captured stray dogs belonged to the family *Pulicidae* and were identified as *Ctenocephalides canis*.

The high prevalence of infestation with ixodid ticks in cattle and small ruminants (39.1% and 34.8%, respectively) indicates a substantial level of tick infestation in these animal species within natural foci. In dogs, the prevalence of infestation was 20.3%. By contrast, horses and chickens showed lower levels of infestation with ixodid ticks, at 9.4% and 2.1%, respectively.

The findings of the present study confirm the data reported by Fedorova [8] regarding the distribution of *Rhipicephalus turanicus*. At the same time, our results demonstrated that *R. turanicus* occurs in all regions of Kyrgyzstan except the Issyk-Kul and Talas Regions. In addition, the two argasid species and representatives of the family *Hippoboscidae* were recorded throughout the republic. In the northern part of the country, *Dermacentor marginatus* and *Hyalomma marginatum* were frequently encountered, whereas in the southern regions *Hyalomma marginatum* was the predominant species. It was also established that *Haemaphysalis sulcata* and *Hyalomma anatolicum* occur only in the Chuy Valley, *Dermacentor ushakovae* in the Issyk-Kul Basin, *Hyalomma asiaticum* in the Jalal-Abad Region, and *Dermacentor niveus* in the Naryn Region.

According to published data, canine hemoparasitic diseases are associated with tick species such as *Rhipicephalus (Boophilus) microplus* [2], as well as *Rhipicephalus sanguineus* and *Rhipicephalus haemaphysaloides* [12]. In our study, however, in addition to *Rhipicephalus sanguineus, Rhipicephalus*

*turanicus* was also frequently recorded and was more often associated with mixed infections involving anaplasmosis and babesiosis.

In Kyrgyzstan, insufficient attention has been paid to blood-feeding flies of the family *Hippoboscidae*, although available data indicate that they may pose a certain epidemiological risk as vectors of transmissible infections. Researchers from France and the United States investigated the potential role of hippoboscid flies in the transmission of *Bartonella* among ruminants [15]. *Bartonella* was detected in 94% of the 83 examined flies, including 48 (71%) adults *Lipoptena cervi,* 17 (100%) adults *Hippobosca equina*, 20 (100%) adults *Melophagus ovinus*, and 10 pupae of *M. ovinus*. These findings suggest that members of the family *Hippoboscidae* may play a role in the transmission of *Bartonella*.

In addition, chewing lice of the family *Trichodectidae* are not uncommonly encountered among animals in Kyrgyzstan. These parasites cause considerable damage to animal husbandry by infesting domestic animals. Specifically, *Bovicola bovis* parasitizes cattle, *Bovicola ovis* sheep, *Bovicola caprae* and *Bovicola limbatus* goats, *Bovicola equi* horses, *Trichodectes canis* dogs, and *Felicola subrostratus* cats. The dog and cat chewing lice serve as intermediate hosts of the parasitic tapeworm *Dipylidium caninum*, the causative agent of dipylidiosis [16].

Blood-sucking flies are also widely distributed among animals in the republic and may pose an epidemiological threat in livestock production. Among them, *Melophagus ovinus* (the sheep ked), a hematophagous ectoparasite belonging to the family *Hippoboscidae* (Diptera: Hippoboscoidea), is particularly common and primarily parasitizes sheep. According to Chinese researchers working in southern Xinjiang, *M. ovinus* not only causes direct harm to its animal hosts but also serves as a vector of infectious agents. Using molecular biological methods, they confirmed the presence of *Anaplasma* spp. in both pupae and adult specimens of *M. ovinus*, as well as the vertical transmission of the pathogen.

## Conclusions

As a result of the present study, 12 tick species belonging to the family *Ixodidae,* two species of the family Argasidae, and two species of blood-feeding insects of the family *Hippoboscidae* were identified in the republic.

The dominant ectoparasite species were as follows: among ixodid ticks, *Rhipicephalus turanicus, Haemaphysalis punctata, Hyalomma marginatum*, and *Dermacentor marginatus*; among argasid ticks, Argas persicus; among blood-sucking insects, *Melophagus ovinus*.

*Rhipicephalus turanicus* was distributed in all regions except the Issyk-Kul and Talas Regions.

In the northern part of the republic, *Dermacentor marginatus* and *Hyalomma marginatum* were frequently recorded, whereas in the southern regions Hyalomma marginatum was the predominant species.

Several tick species showed restricted territorial distribution:

*Haemaphysalis sulcata* and *Hyalomma anatolicum* in the Chuy Valley; *Dermacentor ushakovae* in the Issyk-Kul Basin; *Hyalomma asiaticum* in the Jalal-Abad Region; *Dermacentor niveus* and *D. ushakovae* in the Naryn Region.

A high prevalence of ixodid tick infestation was observed in cattle and small ruminants, amounting to 39.1% and 34.8%, respectively.

## Authors' Contributions

NA and BA: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. EJ and US: conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript.

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**Abstract**
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Materials and Methods.....

Results.....

Conclusion.....( max. 300 words)

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**Discussion**
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## SAMPLE TITLE PAGE

UDC 619.616.98.579.841.935.07

# Using indirect hemagglutination assay for the diagnosis of cattle brucellosis

Mikail M. Mikailov[1], Shahrudin A. Gunashev[1],
Elmira A. Yanikova[1], Ahmed A. Halikov[1], Aitbay. K. Bulashev[2]

[1]Laboratory of Infectious Pathology of Farm Animals, Caspian Zonal Research Veterinary Institute, Republic of Dagestan, Makhachkala, Russia
[2]Department of Microbiology and Biotechnology
Faculty of Veterinary Medicine and Livestock Technology
Saken Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan

**Corresponding author:** Aitbay K. Bulashev: aytbay57@mail.ru
**Co-authors:** (1: MM) mikail.mikailov1981@mail.ru; (2: ShG) sgunashev@mail.ru
(3: EY) nvetmedservis@mail.ru; (4: AH) axmedx93@mail.ru

ORCIDs:
1st Author: https://orcid.org/0000-0002-9620-431X
2nd Author: https://orcid.org/0000-0003-4804-2755
3rd Author: https://orcid.org/ 0000-0002-5561-2499
4th Author: https://orcid.org/ 0000-0002-9765-008X
5th Author: https:// orcid.org 0000-0002-8427-509X

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AB and MM: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. ShG, EY and AH: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscript”.

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