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UDC 612.361:599.735.53:577.21 **Research article**

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.

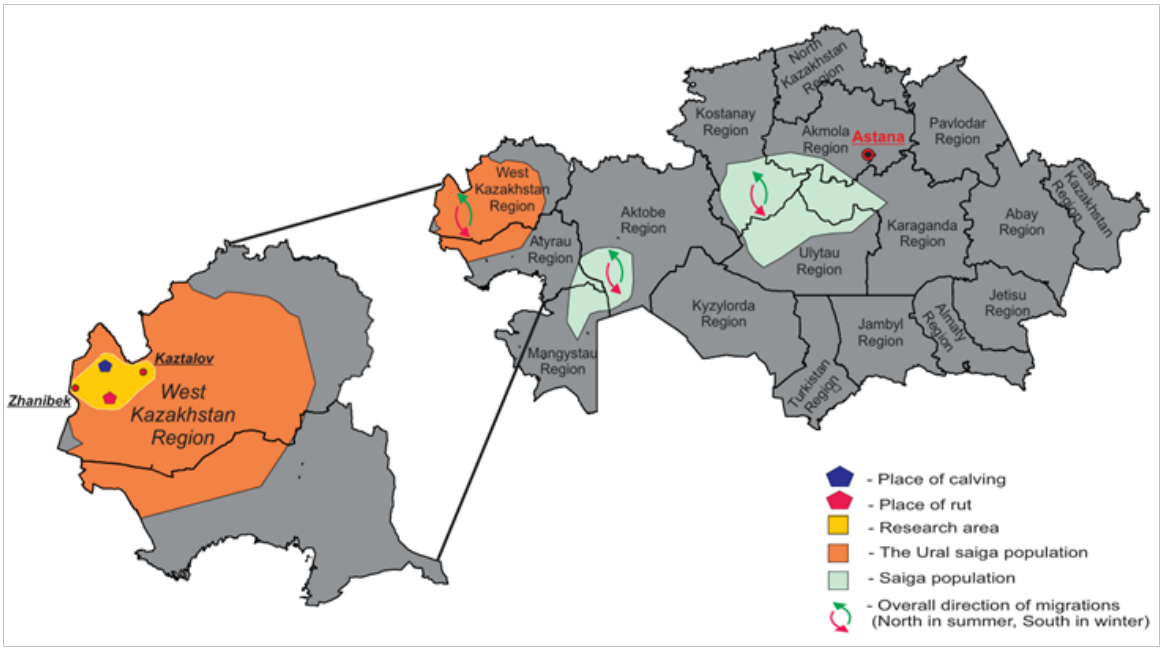

Figure 1. Map of Western Kazakhstan showing the location where saigas from the Ural population

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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