Original Study Article
https://doi.org/10.36233/0372-9311-629



# Experience of applying the metagenomic sequencing method on fragments of the 16S rRNA gene for the detection and identification of natural focal infection pathogens

**Oksana V. Vasilieva[1], Diana V. Ul'shina[1]✉, Anna S. Volynkina[1], Sergey V. Pisarenko[1], Yulia V. Siritsa[1], Olga A. Gnusareva[1], Natalia A. Yatsenko[2], Alexandr N. Kulichenko[1]**

[1]Stavropol Plague Control Research Institute, Stavropol, Russia;
[2]Regional Specialized Clinical Infectious Diseases Hospital, Stavropol, Russia

*Abstract*

**Introduction.** Metagenomic sequencing is one of the most promising methods for both the detection and identification of natural focal infection (NFI) pathogens and for determining the species composition of various bacterial communities.

**The aim** is to detect and identify the NFI pathogens in samples of field and clinical material using metagenomic sequencing of *16S* rRNA gene fragments, and to analyze the taxonomic composition of endosymbiotic microorganisms in the samples.

**Materials and methods.** Samples of field (14 samples) and clinical (2 samples) material with varying loads of DNA from NFI pathogens, determined by PCR (*Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Francisella tularensis*, *Rickettsia* spp., *Coxiella burnetii*), were investigated. Amplification of fragments of the gene encoding *16S rRNA* was performed using primers flanking the variable regions of the gene.

**Results.** In 14 out of 16 studied samples, target NFI pathogens were detected. The species identified included *R. aeschlimannii* (in 57.1% of positive samples), *B. valaisiana* (in 16.6%), *F. tularensis* (in 75%), *C. burnetii* (in 100%), and borreliae — pathogens of relapsing fevers (*B. turcica*, *B. hispanica*) were also found in one sample. The taxonomic structure of the microbiome of *Ixodes ricinus*, *Dermacentor reticulatus*, *Rhipicephalus annulatus, Hyalomma aegyptium, Dermacentor marginatus* ticks collected in the southern regions of the Russian Federation was studied. It was shown that the predominant microorganisms are representatives of the genera *Flavobacterium, Pseudomonas, Serratia, Aeromonas, Pedobacter, Bradyrhizobium, Shingomonas*. DNA markers of microorganisms — endosymbionts of ticks *Candidatus Midichloria mitochondrii*, representatives of the genera *Rickettsiella*, *Coxiella*, non-pathogenic and conditionally pathogenic species of the genus *Francisella* were found in pools of *Ixodes* ticks.

**Conclusion.** The effectiveness of the method of metagenomic sequencing of fragments of the *16S* rRNA gene for the detection and identification of NFI pathogens in samples of clinical and field material was demonstrated. Metagenomic sequencing of *16S rRNA* gene regions can be recommended as an additional laboratory method for detecting and identifying NFI pathogens.

**Keywords:** metagenomic sequencing, *16S rRNA*, natural focal infections, detection, identification, microbiome.

**Ethical approval.** The study was conducted with the informed consent of the patients. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July, 2010). The study protocol was approved by the Local Ethics Committee of the Stavropol State Medical University (protocol No. 112, May 5, 2023).

***Funding source.*** This study was not supported by any external sources of funding.

***Conflict of interest.*** The authors declare no apparent or potential conflicts of interest related to the publication of this article.

***For citation:*** Vasilieva O.V., Ul'shina D.V., Volynkina A.S., Pisarenko S.V., Siritsa Yu.V., Gnusareva O.A., Yatsenko N.A., Kulichenko A.N. Experience of applying the metagenomic sequencing method on fragments of the 16S rRNA gene for the detection and identification of natural focal infection pathogens. *Journal of microbiology, epidemiology and immunobiology.* 2025;102(2):201–212.
DOI: https://doi.org/10.36233/0372-9311-629
EDN: https://www.elibrary.ru/KNLRDI

Оригинальное исследование
https://doi.org/10.36233/0372-9311-629

# Опыт применения метода метагеномного секвенирования по фрагментам гена *16S* рРНК для детекции и идентификации возбудителей природно-очаговых инфекций

**Васильева О.В.[1], Ульшина Д.В.[1]✉, Волынкина А.С.[1], Писаренко С.В.[1], Сирица Ю.В.[1], Гнусарева О.А.[1], Яценко Н.А.[2], Куличенко А.Н.[1]**

[1]Ставропольский противочумный институт Роспотребнадзора, Ставрополь, Россия;
[2]Краевая специализированная клиническая инфекционная больница, Ставрополь, Россия

*Аннотация*

**Введение.** Метагеномное секвенирование — один из наиболее перспективных методов как для детекции и идентификации возбудителей природно-очаговых инфекций (ПОИ), так и для определения видовой структуры различных бактериальных сообществ.

**Цель** работы — выполнить детекцию и идентификацию возбудителей ПОИ в образцах полевого и клинического материала методом метагеномного секвенирования фрагментов гена *16S рРНК*, проанализировать таксономический состав эндосимбиотических микроорганизмов в образцах.

**Материалы и методы.** *И*сследованы образцы полевого (14 проб) и клинического (2 пробы) материала с различной нагрузкой ДНК возбудителей ПОИ, определённой методом полимеразной цепной реакции (*Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum, Francisella tularensis, Rickettsia* spp., *Coxiella burnetii*). Амплификацию фрагментов гена, кодирующего *16S рРНК*, осуществляли с помощью праймеров, фланкирующих вариабельные участки гена.

**Результаты.** В 14 из 16 исследуемых образцов детектированы целевые возбудители ПОИ. До вида идентифицированы *R. aeschlimannii* (в 57,1% положительных образцов)*, B. valaisiana* (в 16,6%)*, F. tularensis* (в 75%), *C. burnetii* (в 100%), также в одном образце выявлены боррелии — возбудители возвратных лихорадок (*B. turcica, B. hispanica*). Исследована таксономическая структура микробиома клещей *Ixodes ricinus, Dermacentor reticulatus, Rhipicephalus annulatus, Hyalomma aegyptium, Dermaceptor marginatus*, собранных в южных регионах России. Выявлено, что преобладающие микроорганизмы — это представители родов *Flavobacterium, Pseudomonas, Serratia, Aeromonas, Pedobacter, Bradyrhizobium, Shingomonas*. В пулах иксодовых клещей обнаружены ДНК-маркеры микроорганизмов — эндосимбионтов клещей *Candidatus Midichloria mitochondrii*, представителей родов *Rickettsiella, Coxiella*, непатогенных и условно-патогенных для человека видов родов *Francisella*.

**Заключение.** Показана эффективность метода метагеномного секвенирования фрагментов гена *16S* рРНК для детекции и идентификации возбудителей ПОИ в пробах клинического и полевого материала. Метагеномное секвенирование по участкам гена *16S рРНК* может быть рекомендовано в качестве дополнительного метода лабораторного исследования образцов с целью детекции и идентификации возбудителей ПОИ.

**Ключевые слова:** *метагеномное секвенирование, 16S рРНК, природно-очаговые инфекции, детекция, идентификация, микробиом*

***Этическое утверждение.*** Исследование проводилось при добровольном информированном согласии пациентов. Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с «Consensus Author Guidelines for Animal Use» (IAVES, 23.07.2010). Протокол исследования одобрен локальным этическим комитетом Ставропольского государственного медицинского университета (заключение № 112 19.05.2023).

***Источник финансирования.*** Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

***Конфликт интересов.*** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

***Для цитирования:*** Васильева О.В., Ульшина Д.В., Волынкина А.С., Писаренко С.В., Сирица Ю.В., Гнусарева О.А., Яценко Н.А., Куличенко А.Н. Опыт применения метода метагеномного секвенирования по фрагментам гена *16S рРНК* для детекции и идентификации возбудителей природно-очаговых инфекций. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2025;102(2):201–212.
DOI: https://doi.org/10.36233/0372-9311-629
EDN: https://www.elibrary.ru/KNLRDI

## Introduction

Natural focal infections (NFI) are widespread in the world and represent an important medical and social problem, the importance of which has been steadily increasing in recent years with the identification of new pathogens, the sources and vectors of which are blood-sucking arthropods, small mammals and birds [1–3]. Continued activity and expansion of the territories of natural foci, as well as high anthropogenic load on the environment lead to a constant increase in the number of people in contact with pathogens and exposed to the risk of infection [4]. It has been proved that simultaneous infection of carriers and vectors with different NFI pathogens is a natural and widespread phenomenon, which, in turn, determines the possibility of combined pathology in humans [5–7].

Currently, molecular genetic methods, primarily polymerase chain reaction (PCR), are widely used for laboratory diagnostics of NFI, which allows detecting the presence of DNA/RNA of NFI pathogens in the material in a short period of time. Most of the developed PCR test systems for detection of pathogens are designed for detection of one or more pathogens [8]. Detection of all potential pathogens requires the use of a set of test systems, which is time-consuming and labor-intensive.

Metagenomic sequencing (MGS) methods are modern approaches that allow simultaneous detection and identification of all microorganisms, both known and new, contained in a sample, and do not require culturing [9]. The use of MGS methods for the identification of infectious agents, including NFI, seems to be especially demanded in cases when traditional laboratory tests do not allow identifying the etiologic agent in atypical course of the disease, as well as in cases of mixed infection with different pathogens [10–12]. Furthermore, MGS of field material samples (ectoparasites, organs of small mammals, birds, etc.) collected during epizootological survey of the territory can be useful for obtaining new comprehensive data on the species composition of pathogenic and endosymbiotic microorganisms associated with different types of carriers and vectors of infections [13].

There are several variants of MGS: targeted sequencing of genome regions encoding evolutionarily conserved genes (*16S rRNA*, etc.) and whole-genome MGS. An approach based on target sequencing of variable regions of the *16S rRNA* gene has been widely used to analyze the taxonomic composition of bacteria in samples and to detect pathogenic bacterial species. The advantages of this method include the possibility of taxonomic classification of a wide range of bacteria, the presence of a stage of preliminary specific enrichment of the target region of the bacterial genome before sequencing, and the relative simplicity of bioinformatics analysis of the results compared to the method of sequencing the complete metagenome [14, 15].

**The aim** of the study was to perform detection and identification of pathogens in samples of field and clinical material by MGS of *16S rRNA* gene fragments, analyze the taxonomic composition of endosymbiotic microorganisms in samples.

## Materials and methods

Sixteen samples of field (collected during the epizootologic survey) and clinical material with different load of PCR-determined DNA of pathogens of bacterial etiology (*Borrelia burgdorferi sensu lato*, *Anaplasma phagocytophilum*, *Francisella tularensis*, *Rickettsia* spp., *Coxiella burnetii*) were studied. The samples contained genetic material of one and several pathogens (**Table 1**).

Work with clinical material was performed with voluntary informed consent of patients. The authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with the "Consensus Author Guidelines for Animal Use" (IAVES, 23.07.2010). Materials from animals used in the study was obtained according to the Plan of epizootological survey of the Stavropol Territory for NFI and particularly dangerous infections for 2024 (agreed by the Head of the Department of Rospotrebnadzor in Stavropol Krai on 21.12.2023, approved by the Chief Physician of the Center of Hygiene and Epidemiology in Stavropol Krai on 21.12.2023). The study protocol was approved by the Local ethical committee of Stavropol State Medical University (conclusion No. 112 dated 19.05.2023).

Ixodid ticks were collected from April through June from animals and vegetation (flagging), species identification of ticks was performed by morphological method [16]. The ticks were used to make pools of 10 specimens each according to Methodological Recommendations 3.1.0322-23[1]. Sample preparation of clinical and field material was performed in accordance with Methodological guidelines 1.3.2569-09[2].

Ticks were treated with 70% ethanol and washed in phosphate-buffered saline. Homogenization parameters for the obtained samples were selected based on the genus of the ticks. The obtained suspension was centrifuged in 300 μL of sterile physiological solution.

Extraction of nucleic acids from human blood serum samples, homogenates of tick pools and flush from the thoracic cavity of the common vole was performed using the RIBO-prep reagent kit (InterLabService).

[1] Methodological recommendations MP 3.1.0322-23 "Collection, accounting and preparation for laboratory examination of blood-sucking arthropods in natural foci of infectious diseases" (approved by the Head of Rospotrebnadzor on 04/13/2023).

[2] Methodological guidelines MU 1.3.2569-09 "Organization of work of laboratories using methods of nucleic acid amplification when working with material containing microorganisms of pathogenicity groups I-IV" (approved by the Head of Rospotrebnadzor on 12/22/2009).

DOI: https://doi.org/10.36233/0372-9311-629


**Table 1.** Data on samples used for MGS analysis

| No. | Sample type | Sample data, location of extraction | PCR-confirmed pathogen | Ct |
|---|---|---|---|---|
| 1 | Tick suspensions | *Ixodes ricinus*, from vegetation, Krasnodar, Sochi | *B. burgdorferi* s.l. | 21.8 |
| 2 | | *I. ricinus*, from vegetation, Krasnodar, Sochi | *B. burgdorferi* s.l. | 22.1 |
| 3 | | *I. ricinus*, from vegetation, Krasnodar, Sochi | *B. burgdorferi* s.l. | 21.1 |
| 4 | | *I. ricinus*, cattle, Republic of South Ossetia | *A. phagocytophilum* | 31.4 |
| 5 | | *I. ricinus*, cattle, Republic of South Ossetia | *A. phagocytophilum* | 23.4 |
| 6 | | *Dermacentor marginatus*, from vegetation, Stavropol | *Rickettsia* spp. | 17.2 |
| 7 | | *Rhipicephalus annulatus*, cattle, Republic of South Ossetia | *Rickettsia* spp. | 23.3 |
| 8 | | *D. marginatus*, from vegetation, Stavropol | *F. tularensis* | 26.6 |
| 9 | Flush from chest cavity | *Microtus arvalis*, Stavropol | *F. tularensis* | 10.1 |
| 10 | Tick suspensions | *Hyalomma aegyptium* from Mediterranean turtle, Republic of Dagestan | *B. burgdorferi* s.l. | 20.2 |
| | | | *Rickettsia* spp. | 16.3 |
| 11 | | *I. ricinus* from vegetation, Krasnodar, Sochi | *B. burgdorferi* s.l. | 25.4 |
| | | | *Rickettsia* spp. | 17.0 |
| 12 | | *I. ricinus* from vegetation, Krasnodar | *B. burgdorferi* s.l. | 25.7 |
| | | | *Rickettsia* spp. | 18.8 |
| 13 | | *Dermacentor reticulatus* from vegetation, Stavropol | *F. tularensis* | 25.5 |
| | | | *Rickettsia* spp. | 17.2 |
| 14 | | *D. reticulatus* from vegetation, Stavropol | *F. tularensis* | 12.6 |
| | | | *Rickettsia* spp. | 21.1 |
| 15 | Blood serum | Human, Stavropol | *C. burnetii* | 21.4 |
| 16 | | Human, Stavropol | *C. burnetii* | 21.3 |

The presence of DNA of NFI pathogens in the samples was determined by PCR using the following reagent kits: AmpliSens *Coxiella burnetii*-FL, AmpliSens *TBEV*, *B. burgdorferi s.l.*, *A. phagocytophilum*, *E. chaffeensis/E. muris-FL* (Central Research Institute of Epidemiology of Rospotrebnadzor), *Francisella tularensis*-RGF gene (Russian Anti-Plague Institute "Microbe"). DNA of rickettsiae in field samples was detected according to the protocol described by O. Mediannikov et al. [17].

Amplification of microbial *16S rRNA* gene fragments contained in the samples for MGS was performed using primers described by I. Abellan-Schneyder et al. [18] (**Table 2**). A separate reaction mixture was prepared for amplification of each variable fragment of the *16S rRNA* gene (V1–V2, V1–V3, V3–V4, V4, V4–V5, V6–V8, V7–V9). The composition of the reaction mixture: primer F (C = 7.2 pmol/μL) — 1.25 μL, primer R (C = 7.2 pmol/μL) — 1.25 μL, BioMaster HS-Taq PCR-Color (2×) PCR mixture (Biolabmix) — 12.5 μL, sample DNA — 10 μL. PCR products were amplified according to the thermocycling program: 95°C — 5 min; 95–20 s, Ta — 30 s, 72°C — 40 s (40 cycles); 72°C — 5 min; 4 — ∞.

The size and purity of the obtained PCR products were assessed by electrophoresis in 1% agarose gel. The procedure for purification of PCR products from excess primers and components of the reaction mixture was performed using the CleanMag DNA kit (Eurogen). Equivalent amounts of amplification products of *16S rRNA* V1–V9 gene fragments were taken for library preparation. The final concentration of target DNA was measured on a Qubit fluorimeter using the Qubit 1X dsDNA High Sensitivity (HS) kit (Invitrogen).

DNA fragment libraries were prepared according to the Ion Xpress Plus gDNA Fragment Library Preparation protocol (Revision K.0) using the Ion Plus Fragment Library Kit (Thermo Fisher Scientific Inc.). Sequencing of libraries prepared from amplicon mixtures was performed on the GeneStudio S5 Plus platform (Thermo Fisher Scientific Inc.).

The Fastp Qs[3], Kallisto[4], STAR [19], Bowtie2[5] programs were used for bioinformatics analysis of MGS data on *16S rRNA* gene regions. The quality of fastq-files was assessed using Fastp Qc and Kallisto programs; reads with quality index Q < 20 were excluded from the analysis. Sequence alignment and filtering were performed using STAR and Bowtie2 software.

[3] URL: https://github.com/OpenGene/fastp
[4] URL: https://github.com/Roslin-Aquaculture/RNA-Seq-kallisto
[5] URL: https://bowtie-bio.sourceforge.net/bowtie2/index.shtml

**Table 2.** Primer sequences for amplification of fragments of the gene encoding *16S rRNA*

| No. | Fragment marking | Fragment length, bp | Annealing temperature, °C | Primer | Sequence 5'–3' |
|---|---|---|---|---|---|
| 1 | V1–V2 | 311 | 57 | 27F | AGAGTTTGATYMTGGCTCAG |
| | | | | 338R | GCTGCCTCCCGTAGGAGT |
| 2 | V1–V3 | 507 | 57 | 27F | AGAGTTTGATYMTGGCTCAG |
| | | | | 534R | ATTACCGCGGCTGCTGG |
| 3 | V3–V4 | 404 | 54 | 341F | CCTACGGGNGGCWGCAG |
| | | | | 785R | GACTACHVGGGTATCTAATCC |
| 4 | V4 | 293 | 54 | 515F | GTGCCAGCMGCCGCGGTAA |
| | | | | 806R | GGACTACHVGGGTWTCTAAT |
| 5 | V4–V5 | 429 | 54 | 515F | GTGCCAGCMGCCGCGGTAA |
| | | | | 944R | GAATTAAACCACATGCTC |
| 6 | V6–V8 | 439 | 57 | 939F | GAATTGACGGGGGCCCGCACAAG |
| | | | | 1378R | CGGTGTGTACAAGGCCCGGGAACG |
| 7 | V7–V9 | 377 | 51 | 1115F | CAACGAGCGCAACCCT |
| | | | | 1492R | TACGGYTACCTTGTTACGACTT |

Assembly of short *de novo* read sequences into longer sequences (contigs) was performed using SPAdes assembler. Taxonomic affiliation of genomic sequences was determined by comparing them with the NCBI database (RefSeq and GenBank using Rapsearch2[6]).

The results of species identification of microorganisms (Borrelia, rickettsiae) obtained by MGS using *16S rRNA* gene sections were confirmed by Sanger sequencing method.

## Results

MGS was performed on the *16S rRNA* gene regions of 16 samples of clinical and field material containing DNA of bacterial pathogens (**Table 3**). Nucleotide sequences obtained by MGS were deposited in the GenBank database (BioProject PRJNA1227530; SAMN46987881-SAMN46987896).

The number of reads satisfying the Q20 parameter for the studied samples was 1127–40,969. The GC value for all samples varied in the range of 49.7–52.4%, which corresponds to the exome regions of *16S rRNA* gene fragments used for MGS analysis. During the processing of the data obtained, it was found that the highest amount of reads after filtration was obtained for sample No. 13 (93,789,000 K). A reduction in the number of reads was observed for samples Nos. 9 and 14 (29,314,000 K and 28,704,000 K). The total number of reads after the filtering step for the 4 samples (Nos. 2, 5, 7 and 10) ranged between 9,172,000–16,651,000 K. The number of filtered reads for the remaining samples ranged 488–7,633,000 K. The least amount of filtered data (Q < 20) after bioinformatics processing was observed for samples Nos. 4, 8 and 11. The highest number of poor quality data (Q < 20) was obtained for samples Nos. 9, 13, and 14. The result of data quality assessment is shown in **Fig. 1**.

### *NFI pathogens identified by MGS using 16S rRNA gene variant regions*

In the study of suspension samples of ixodid ticks (Nos. 1–8, **Table 3**) with PCR-confirmed mono-infection with *Borrelia* genetic complex *B. burgdorferi* s.l., *A. phagocytophilum*, rickettsiae and *F. tularensis,* using the MGS method of *16S rRNA* gene, detected representatives of the *Borrelia* (samples Nos. 1–3), *Francisella* (sample No. 8) (microorganisms identified to genus) genera, as well as *R. aeschlimannii* (samples Nos. 6, 7, microorganism identified to species). The pathogen of human granulocytic anaplasmosis could not be detected by MGS (samples Nos. 4, 5). The results of species identification of *R. aeschlimannii* in samples Nos. 6, 7 were confirmed by Sanger sequencing of a genome fragment.

Mixed-infected samples of ixodid ticks (samples Nos. 10–14, **Table 3**) with a combination of two tick-borne pathogens (*B. burgdorferi* s.l and *Rickettsia* spp.; *F. tularensis* and *Rickettsia* spp.) were studied. All target microorganisms were detected in the samples by the MGS method. *R. aeschlimannii* (samples Nos. 10, 13), *B. valaisiana* (sample No. 12), *F. tularensis* (samples Nos. 13, 14) were identified to species, also microorganisms of the *Rickettsia* (samples Nos. 11, 12, 14) and *Borrelia* (samples Nos. 11, 12, 14) genera whose species could not be identified were detected in the samples. Genetic markers (DNA) of Borrelia, pathogens of relapsing fevers (*B. turcica*, *B. hispanica*) were detect-

[6] URL: https://github.com/zhaoyanswill/RAPSearch2

**Table 3.** Comparison of results obtained by PCR and MGS methods of *16S rRNA* gene fragments

| Sample No. | PCR method | | MGS method of *16S rRNA* gene fragments | |
|---|---|---|---|---|
| | identified pathogens | Ct | Q20, % (number of reads) | pathogens identified (number of reads corresponding to the target pathogen, %) |
| | | | **Mono-infected samples** | |
| 1 | *B. burgdorferi* s.l. | 21.80 | 91.50 (5822) | *Borrelia* spp. (2,90) |
| 2 | *B. burgdorferi* s.l. | 22.10 | 91.90 (20,236) | *Borrelia* spp. (3,10) |
| 3 | *B. burgdorferi* s.l. | 21.10 | 92.20 (10,627) | *Borrelia* spp. (3,20) |
| 4 | *A. phagocytophilum* | 31.40 | 87.50 (12,922) | Unidentified |
| 5 | *A. phagocytophilum* | 23.40 | 91.80 (6618) | Unidentified |
| 6 | *Rickettsia* spp. | 17.20 | 91.60 (8239) | *R. aeschlimannii* (8,90) |
| 7 | *Rickettsia* spp. | 23.30 | 92.10 (22,324) | *R. aeschlimannii* (0,80) |
| 8 | *F. tularensis* | 26.60 | 91.00 (1127) | *Francisella* spp. (2,60) |
| 9 | *F. tularensis* | 10.10 | 91.60 (40,161) | *F. tularensis* (9,90) |
| | | | **Mixed-infected samples** | |
| 10 | *B. burgdorferi* s.l.<br>*Rickettsia* spp. | 20.20<br>16.20 | 90.60 (163,336) | *B. turcica* (27,00)<br>*B. hispanica* (7,60)<br>*R. aeschlimannii* (9,50) |
| 11 | *B. burgdorferi* s.l.<br>*Rickettsia* spp. | 25.40<br>17.00 | 91.90 (11,506) | *Borrelia* spp. (2,40)<br>*Rickettsia* spp. (2,60) |
| 12 | *B. burgdorferi* s.l.<br>*Rickettsia* spp. | 25.70<br>18.80 | 92.40 (5952) | *B. valaisiana* (7,60)<br>*Rickettsia* spp. (2,60) |
| 13 | *F. tularensis*<br>*Rickettsia* spp. | 25.50<br>17.20 | 91.00 (11,506) | *F. tularensis* (9,90)<br>*R. aeschlimannii* (11,30) |
| 14 | *F. tularensis*<br>*Rickettsia* spp. | 12.60<br>21.10 | 91.70 (40,696) | *F. tularensis* (9,90)<br>*Rickettsia* spp. (4,10) |
| | | | **Clinical material** | |
| 15 | *C. burnetii* | 21.40 | 90.60 (8926) | *C. burnetii* (5,30) |
| 16 | *C. burnetii* | 21.30 | 90.00 (7223) | *C. burnetii* (5,00) |

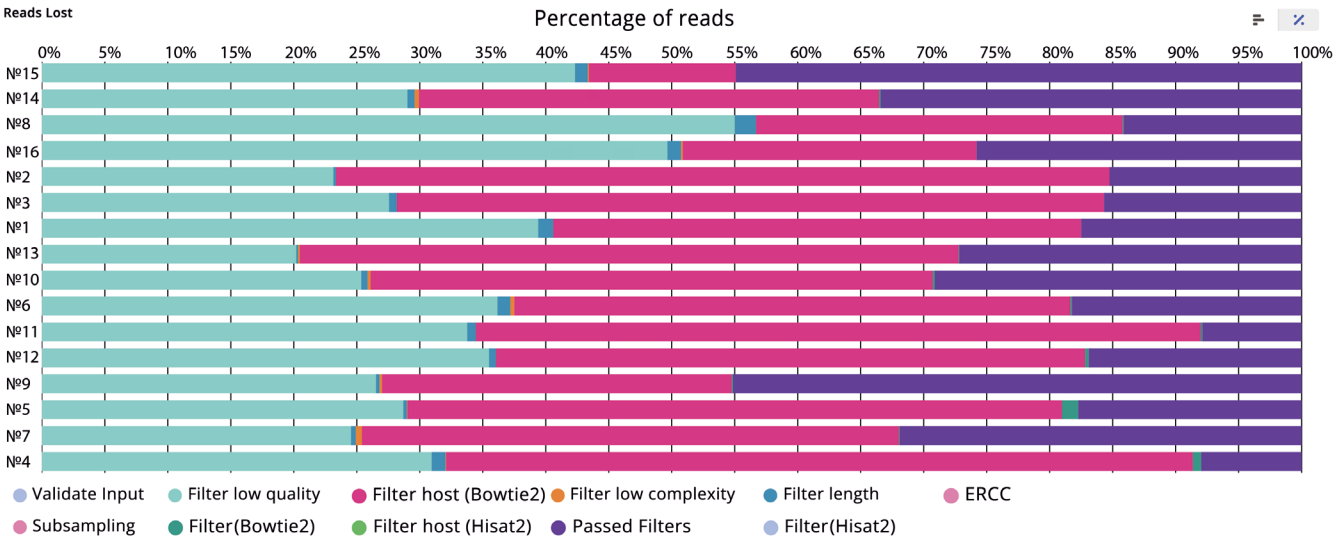


**Fig. 1.** Histogram showing the result of MGS data quality assessment by *16S rRNA* gene regions.
The color of the sectors in the histogram reflects the number of reads for each sample that passed quality filtering (in %, top).
For a color version of the figure, see the journal website.

ed in sample No. 10 by MGS. It was not possible to confirm the results of Borrelia species identification in sample No. 10 by Sanger sequencing, which is due to the mixed-infection of the sample with *H. aegyptium* tick suspension. The results of species identification of the remaining microorganisms detected in the samples were confirmed by sequencing of pathogen genome fragments.

By MGS method in 3 samples containing DNA of the tularemia pathogen (Ct 10.1; 12.6; 25.5) *F. tularensis* was identified to species, in 1 sample (Ct 26.6) the presence of microorganisms of the *Francisella* spp. genus was found, species identification could not be performed.

C. *burnetii* was identified by MGS results using the *16S rRNA* gene region in 2 obviously positive blood plasma samples from patients with Q fever (Ct values 21.3–21.4). The presence of *C. burnetii* DNA was detected in clinical samples 15, 16 from Q fever patients by MGS, the proportion of target reads was 5.0–5.3%. Furthermore, nucleotide sequences of *Methylophilus medardicus* bacteria, as well as representatives of the *Acinetobacter* and *Shingomonas* genera were detected in clinical samples, which may indicate possible contamination of samples at the stages of collection, storage and laboratory examination [20].

We compared the results of the study of field and clinical samples with different DNA load of pathogens of NFI obtained by MGS methods using *16S rRNA* gene regions and PCR. It is shown that as a result of MGS of 6 samples, positive for the presence of borreliae DNA of the *B. burgdorferi* s.l. genetic complex, identification of borreliae to genus (*Borrelia* spp. Ct 21.8; 22.1; 21.1; 25.4) was carried out in 4 samples, while the identification to species (*B. valaisiana* Ct 25.7, *B. turcica*, *B. hispanica* St 20.2) was carried out in 2 samples.

According to MGS results, fragments of *Rickettsia* spp. genome were detected in all obviously positive samples, in 4 samples (Ct values 16.2; 17.2; 17.2 and 23.3) the rickettsia species (*R. aeschlimannii*) was identified, in 3 samples (Ct values 17.0; 18.8 and 21.1) species identification of rickettsia could not be performed. The presented results of identification of *Borrelia* and rickettsiae in the studied material (Table 3) are confirmed by the literature data on the difficulty of species identification by MGS of representatives of the *Rickettsia* and *Borrelia* genera [8, 15]. Accurate species identification of *Rickettsia* and *Borrelia* using MGS is difficult due to high homology of nucleotide sequences of *16S rRNA* gene for these bacterial pathogens [12, 13]. In the case of detection of microorganisms of the *Rickettsia* and *Borrelia* genera by MGS of the *16S rRNA* gene, further identification to species by Sanger sequencing may be necessary.

The only pathogen that could not be confirmed by MGS was *A. phagocytophilum*.

### *Taxonomic composition of the microbiome of ixodid ticks*

The study of the taxonomic structure of the microbiome of ticks was carried out in accordance with their species affiliation, place and territory of collection (**Fig. 2**).

Main taxonomic groups of the tick microbiome:

- for representatives of *I. ricinus* (samples Nos. 1–3): *Flavobacterium* spp. (57–81%), *Pseudomonas* spp. (7–27%), *Serratia* spp. (2–4%), *Pedobacter* spp. (2–4%);
- for representatives of *I. ricinus* (samples Nos. 4, 5): *Candidatus Midichloria mitochondrii* (31–87%), *Clostridium* spp. (6–61%), *Sphingomonas* spp. (3%), *Staphylococcus* spp. (1–10%), *Bradyrhizobium* spp. (1%);
- for representatives of *I. ricinus* (samples Nos. 11, 12): *Pseudomonas* spp. (7–49%), *Serratia* spp. (4–12%), *Rickettsiella endosymbiont of Pandinus imperator* (3–19%), *Rhodobacterales* spp. (3%);
- for representatives of *D. reticulatus* (samples Nos. 13, 14): *Flavobacterium* sp. Nj (25–53%), *Cardinium endosymbiont of Bemisia tabaci* (19%), *Clostridium* spp. (15%), *Francisella-like endosymbiont of Dermacentor reticulatus* (9–21%), *Francisella persica* (2%), uncultured *Francisella* spp. (1–6%), *Bradyrhizobium* spp. (1–3%), *Dyadobacter* spp. (1–3%);
- for representatives of *R. annulatus* (samples No. 7): *Rickettsiella endosymbiont of Pandinus imperator* (5%), uncultured *Coxiella* spp. (10%), *Wolbachia pipientis* (9%), *Candidatus Coxiella mudrowiae* (6%), *Coxiella endosymbiont of Rhipicephalus microplus* (3%), *Coxiella endosymbiont of Rhipicephalus geigyi* (1%), *Coxiella endosymbiont of Rhipicephalus turanicus* (3%), *Shingomonas* spp. (5%), *Staphilococcus* spp. (5%), *Bradyrhizobium* spp. (3%), *Flavobacterium* spp. (2%), *Leptotrichia wadei* (2%);
- for representatives of *H. aegyptium* (samples No. 10): *Rickettsia endosymbiont of Bemisia tabaci* (3%), *Flavitalea flava* (1%), uncultured *Borrelia* spp. (9%), *Blastopirellula marina* (4%), *Dyadobacter alkalitolerans* (2%), *Bradyrhizobium* (1%);
- for representatives of *D. marginatus* (samples Nos. 6, 8): *Pseudomonas* spp. (9–33%), uncultured *Arsenophonus* spp. (11%), uncultured *Alteromonas* spp. (2%), *Alphaproteobacteria bacterium* (2%), *Coxiella endosymbiont of Dermacentor marginatus* (2%).

## Discussion

In this study, we applied the MGS method using *16S rRNA* gene regions for detection and identification

JOURNAL OF MICROBIOLOGY, EPIDEMIOLOGY AND IMMUNOBIOLOGY. 2025; 102(2)
DOI: https://doi.org/10.36233/0372-9311-629
ORIGINAL RESEARCHES

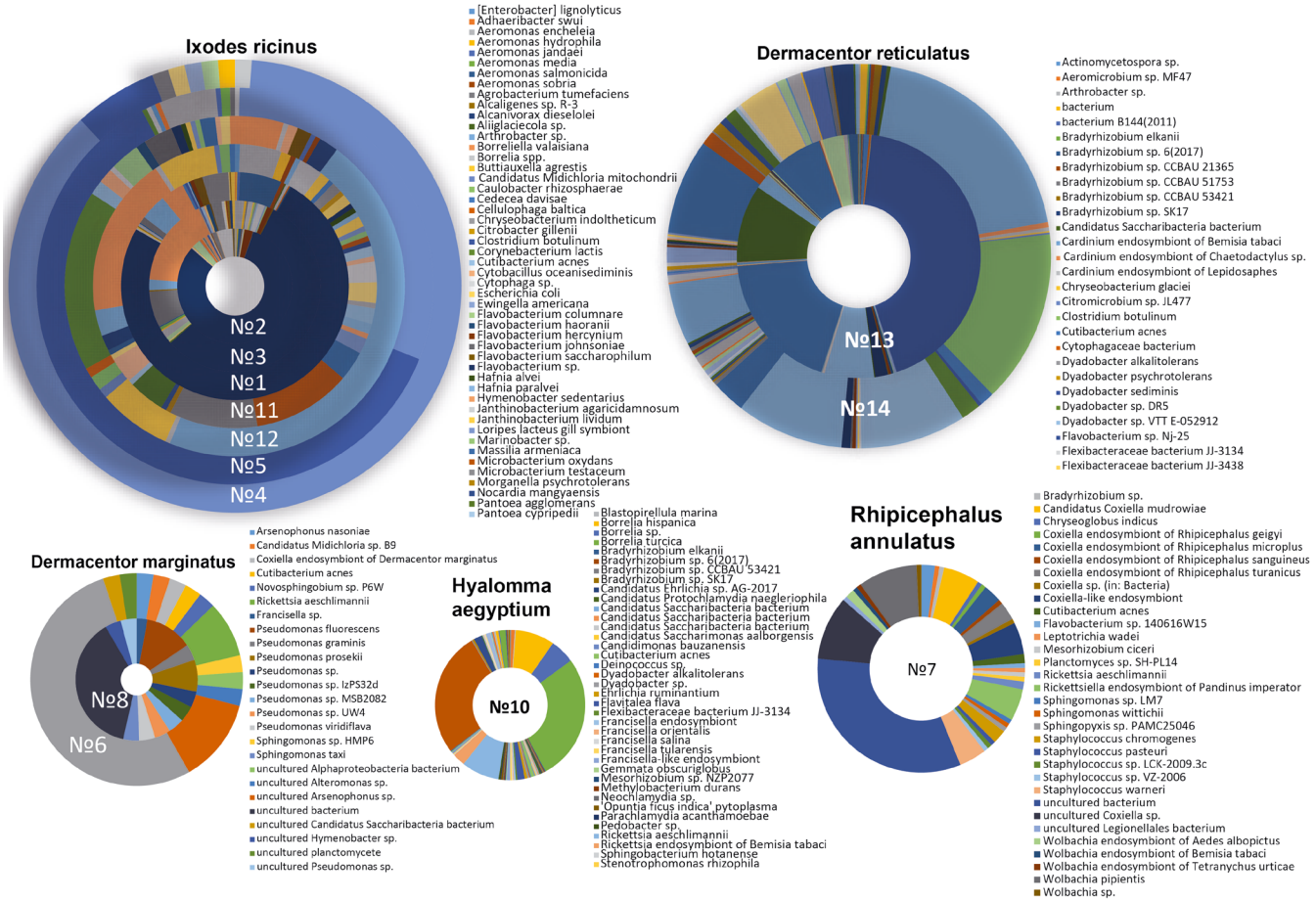


**Fig. 2.** Taxonomic composition of microbiomes of ixodid ticks (sample numbers are indicated by numbers).
Due to the availability of a large amount of data, only the most represented taxa are marked with color markers.
For a color version of the figure, see the journal's website

of known pathogens of bacterial etiology in samples of clinical and field material, and investigated the possibility of its use in simultaneous detection of different types of pathogens. Mixed infection with two pathogens of NFI (borreliosis, tick-borne rickettsiosis, tularemia) in several pools of ixodid ticks was determined. The negative result in the detection of *A. phagocytophilum* may be due to low concentration of the bacterial pathogen in the tested material, as well as insufficient quality and quantity of data obtained after bioinformatics processing.

The results of using the method of targeting MGS by *16S rRNA* gene regions to detect pathogens of NFI in samples of clinical and field material are presented in a number of publications. Thus, L. Kingry et al., using the MGS method at the *16S rRNA* gene region in clinical samples from febrile patients, detected tick-borne pathogens: *B. burgdorferi* s.l., *B. mayonii*, *B. miyamotoi*, *B. hermsii*, *A. phagocytophilum*, *Ehrlichia chaffeensis*, *E. muris* subsp. *eauclarinsis*, *E. ewingii*, and *F. tularensis* [8]. R. Takhampunya et al. detected microorganisms of the *Anaplasma*, *Bartonella*, *Coxiella*, *Leptospira*, *Orientia* genera in the blood of patients with fever of unclear genesis [15]. Furthermore, other authors have obtained the results of the study using the *16S rRNA* MGS method of samples of ixodid ticks for the entire spectrum of tick-borne pathogens [20–22].

One of the demanded areas of application of the MGS method for the *16S rRNA* gene region is the study of clinical samples from patients with fevers of unclear genesis in cases when traditional methods of research (PCR, enzyme immunoassay, serologic methods, etc.) failed to identify the pathogen. Detection of microorganisms belonging to genera including pathogens of NFI in the material from febrile patients will allow further in-depth molecular genetic analysis to confirm the presence of DNA of the detected pathogens in the sample.

In the literature, there are numerous reports of human cases of combined forms of NFI caused by associations of microorganisms, the clinical course of which is significantly more severe compared to mono-infections, and laboratory confirmation of the diagnosis is difficult [23, 24]. In the etiologic interpretation of such cases, the metagenomic approach acquires special relevance and clearly demonstrates its advantage.

As a result of bioinformatics processing of MGS data using variable fragments of the *16S rRNA gene*, the taxonomic composition of the microbiome associated

with *I. ricinus*, *D. reicinus*, *R. annulatus*, *H. aegyptium*, *D. marginatus* ticks collected in the southern regions of Russia (Fig. 2) was determined. The microbiome of all ticks was dominated by the following microorganisms: *Flavobacterium* spp., *Pseudomonas* spp., *Serratia* spp., *Aeromonas* spp., *Pedobacter* spp., *Bradyrhizobium* spp. and *Shingomonas* spp. Probably, some of these bacteria entered the organism of mites in the process of their vital activity or inhabit their chitinous exoskeleton and digestive system, while not being symbionts of arthropods [25].

Furthermore, DNA markers of microorganisms — endosymbionts of ticks, including *Candidatus Midichloria mitochondrii* (samples Nos. 4, 5), representatives of genera *Rickettsiella*, *Coxiella*, *Candidatus Coxiella mudrowiae* (sample No. 7), non-pathogenic and conditionally pathogenic for human species *Francisella* spp. (*F. frigiditurris*, *F. philomiragia*, *F. persica*) (sample No. 13).

It is interesting to note that the composition of the bacterial community of the ixodid tick pool of sample No. 10 based on the data of MGS sites of the *16S rRNA* gene differed significantly from the other samples, which may be related to the peculiarities of the tick feeder and the species of the vector of tick-borne infections — the Mediterranean turtle. Bacteria of the *Bradyrhizobium* genus — symbiotic microorganisms of plants, *Blastopirellula marina* and *Dyadobacter alkalitolerans*, being natural inhabitants of saline water bodies and sandy soils, were detected in small amounts. The obtained results, presented in Table 3 and Fig. 2, are consistent with the literature data on bacterial pathogens carried by *H. aegyptium* ticks and found in the blood of reptiles (pythons, lizards and turtles) [26, 27]. Information has been published on the detection of markers of pathogens (borreliosis, tick-borne rickettsiosis) during the study of biological material from reptiles and ticks removed from reptiles: *R. aeschlimannii* [28], *B. turcica* [29, 30], *B. hermsii* [31], *B. crocidurae* [32] and *B. hispanica* [33]. The above data on the high occurrence of *Borrelia* — pathogens of relapsing fevers in animals confirm the wide distribution of these bacterial pathogens in a number of regions and have almost ubiquitous character.

The use of MGS in the study of ixodid ticks can obviously be effective in obtaining comprehensive information on the species spectrum of NFI pathogens, as well as endosymbionts associated with different species of ixodid ticks inhabiting different regions. As a consequence, new perspectives in the study of the species spectrum of pathogens, as well as the selection of microorganisms to assess the specificity of existing and developing PCR test systems for the study of field samples [34]. The information obtained in this work about the species of endosymbiotic microorganisms detected in ixodid tick pools is consistent with previously published data [34].

It is necessary to take into account the limitations of the method when determining the species affiliation of closely related microorganisms, including for a number of Borrelia and Rickettsia species based on MGS data [35, 36]. It has been shown that the results of taxonomic classification may differ depending on the variation regions used [37–39]. In this case, the use of a mixture of primers targeting different hypervariable regions of the *16S rRNA* gene [40–42] contributes to increasing the discriminatory power of the method, which was applied in the present study.

## Conclusion

We analyzed the taxonomic composition of microorganisms, as well as the detection and identification of pathogens in samples by MGS method using *16S rRNA* gene sections, and experimentally confirmed the effectiveness of this method for the detection of pathogens in clinical and field samples. Microorganisms belonging to *Rickettsia* spp., *Borrelia* spp., *Francisella* spp. were detected, including human pathogenic species, as well as species identification of pathogens with different DNA load in the studied material, in particular, *R. aeschlimannii* (Ct at PCR up to 23.3), *C. burnetii* (Ct < 21.4), *F. tularensis* (Ct < 26.6), *Borrelia* spp. *burgdorferi* s.l. (*B. valaisiana*, Ct < 25.7), borreliae of the pathogens of relapsing fevers (*B. turcica*, *B. hispanica* Ct < 20.2). The taxonomic structure of the microbiome of *I. ricinus*, *D. reticulatus*, *R. annulatus*, *H. aegyptium*, *D. marginatus* ticks collected in the southern regions of Russia was studied. It is shown that microorganisms from genera *Flavobacterium*, *Pseudomonas*, *Serratia*, *Aeromonas*, *Pedobacter*, *Bradyrhizobium* and *Shingomonas* predominate. DNA markers of microorganisms — endosymbionts of ticks *Candidatus Midichloria mitochondrii*, representatives of genera *Rickettsiella*, *Coxiella*, non-pathogenic and conditionally pathogenic for human species of the genus *Francisella* — were found in the pools of ixodid ticks.

Continued work in this area will allow a more accurate assessment of the resolution of the method for the detection and identification of pathogens. The study of patterns of existence of pathogens in the structure of the tick microbiome is a promising area for further research.

The main advantage of the MGS method using the *16S rRNA* gene region in the study of field and clinical samples is the possibility to perform simultaneous detection and identification of all bacteria in the sample, including known pathogens, without the necessity for several diagnostic tests. Targeted MGS can be used for etiologic interpretation in case of atypical course and abbreviated clinical picture of the disease, in case of mixed-infection with several pathogens of bacterial etiology, when there is a difficulty with the diagnosis using traditional laboratory methods of investigation. MGS can also be used to obtain information on the tax-

onomic composition of the bacterial microbiome in the organism of different species of carriers and vectors of NFI pathogens.

СПИСОК ИСТОЧНИКОВ | REFERENCES

1. Рудаков Н.В., Ястребов В.К. Эволюция учения о природной очаговости болезней человека. *Эпидемиология и инфекционные болезни. Актуальные вопросы*. 2014;(4):4–8. Rudakov N.V., Yastrebov V.K. Evolution of teaching of the natural nidality of human diseases. *Epidemiology and Infectious Diseases. Current Items.* 2014;(4):4–8. EDN: https://elibrary.ru/swnymz
2. Ястребов В.К. Клещевой энцефалит в Сибири: эпидемиология, сочетанность природных очагов. *Бюллетень Сибирского отделения Российской академии медицинских наук*. 2007;27(4):89–93. Yastrebov V.K. Tick-borne encephalitis in Siberia: epidemiology, combined natural foci. *Bulletin of Siberian Medicine.* 2007;27(4):89–93. EDN: https://elibrary.ru/pjdxjp
3. Смелянский В.П., Жуков К.В., Бородай Н.В. и др. Современное состояние проблемы природно-очаговых инфекций на территории Волгоградской области. *Здоровье населения и среда обитания – ЗНиСО*. 2021;29(11):83–93. Smelyansky V.P., Zhukov K.V., Borodai N.V., et al. The problem of natural focal infectious diseases in the Volgograd region: a state-of-the-art review. *Public Health and Life Environment — PH&LE.* 2021;29(11):83–93. DOI: https://doi.org/10.35627/2219-5238/2021-29-11-83-93 EDN: https://elibrary.ru/qrtwmi
4. Истомин А.В. Региональный мониторинг природно-очаговых инфекций. *Псковский регионологический журнал*. 2006;(1):122–35. Istomin A.V. Regional monitoring over natural-focal infections. *Pskov Regionology Journal.* 2006;(1):122–35. EDN: https://elibrary.ru/nuhqfd
5. Демидова Т.Н., Шарапова Н.Е., Горшенко В.В. и др. Эпидемиологическое проявление сочетанных природных очагов туляремии, лептоспирозов и геморрагической лихорадки с почечным синдромом: микстинфекции. *Эпидемиология и вакцинопрофилактика*. 2022;21(2):38–45. Demidova T.N., Sharapova N.E., Gorshenko V.V., et al. Epidemiological manifestation of combined natural foci of tularemia, leptospirosis and hemorrhagic fever with renal syndrome: mixed infections. *Epidemiology and Vaccinal Prevention.* 2022;21(2):38–45. DOI: https://doi.org/10.31631/2073-3046-2022-21-2-38-45 EDN: https://elibrary.ru/fsnrdv
6. Коренберг Э.И. Изучение и профилактика микст-инфекций, передающихся иксодовыми клещами. *Вестник Российской академии медицинских наук*. 2001;(11):41–5. Korenberg E.I. The study and prevention of mixed infections transmitted by ticks. *Annals of the Russian Academy of Medical Sciences.* 2001;(11):41–5.
7. Шкарин В.В., Благонравова А.С., Чумаков М.Э. Эпидемиологические особенности сочетанных природно-очаговых инфекций. *Эпидемиология и вакцинопрофилактика.* 2017;16(5):43–52. Shkarin V.V., Blagonravova A.S., Chumakov E.M. Epidemiological features of combined natural-focal infections. *Epidemiology and Vaccinal Prevention.* 2017;16(5):43–52. EDN: https://elibrary.ru/zqolrv
8. Kingry L., Sheldon S., Oatman S., et al. Targeted metagenomics for clinical detection and discovery of bacterial tick-borne pathogens. *J. Clin. Microbiol*. 2020;58(11):e00147–20. DOI: https://doi.org/10.1128/JCM.00147-20
9. Handelsman J., Rondon M.R., Brady S.F., et al. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem. Biol*. 1998;5(10):R245–9. DOI: https://doi.org/10.1016/s1074-5521(98)90108-9
10. Thoendel M. Targeted metagenomics offers insights into potential tick-borne pathogens. *J. Clin. Microbiol*. 2020;58(11):e01893–20. DOI: https://doi.org/10.1128/JCM.01893-20
11. Ghosh A., Mehta A., Khan A.M. Metagenomic analysis and its applications. In: *Encyclopedia of Bioinformatics and Computational Biology. Volume 3.* Elsevier;2019:184–93. DOI: https://doi.org/10.1016/B978-0-12-809633-8.20178-7
12. Rodino K.G., Wolf M.J., Sheldon S., et al. Detection of tick-borne bacteria from whole blood using 16S ribosomal RNA gene PCR followed by next-generation sequencing. *J. Clin. Microbiol*. 2021;59(5):e03129-20. DOI: https://doi.org/10.1128/jcm.03129-20
13. Carpi G., Cagnacci F., Wittekindt N.E., et al. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. *PloS One.* 2011;6(10):e25604. DOI: https://doi.org/10.1371/journal.pone.0025604
14. Kumar P.S., Brooker M.R., Dowd S.E., Camerlengo T. Target region selection is a critical determinant of community fingerprints generated by 16S pyrosequencing. *PloS One*. 2011;6(6):e20956. DOI: https://doi.org/10.1371/journal.pone.0020956
15. Takhampunya R., Korkusol A., Pongpichit C., et al. Metagenomic approach to characterizing disease epidemiology in a disease-endemic environment in Northern Thailand. *Front. Microbiol*. 2019;10:319. DOI: https://doi.org/10.3389/fmicb.2019.00319
16. Филлипова Н.А. Иксодовые клещи подсем. *Amblyomminae*. В кн.: *Фауна России и сопредельных стран. Паукообразные.* СПб.;1997. Filippova N.A. Ixodidae ticks of Amblyomminae subfamily. In: *Fauna of Russia and Bordering Countries. Arachnids.* St. Petersburg;1997.
17. Mediannikov O., Diatta G., Fenollar F., et al. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl. Trop. Dis*. 2010;4(9):e821. DOI: https://doi.org/10.1371/journal.pntd.0000821
18. Abellan-Schneyder I., Matchado M.S., Reitmeier S., et al. Primer, pipelines, parameters: issues in *16S rRNA* gene sequencing. *mSphere* 2021;6(1):e01202–20. DOI: https://doi.org/10.1128/mSphere.01202-20
19. Dobin A., Davis C.A., Schlesinger F., et al. STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15–21. DOI: https://doi.org/10.1093/bioinformatics/bts635
20. Salter S.J., Cox M.J., Turek E.M., et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol*. 2014;12:87. DOI: https://doi.org/10.1186/s12915-014-0087-z
21. Keller A., Graefen A., Ball M., et al. New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing. *Nat. Commun*. 2012;3:698. DOI: https://doi.org/10.1038/ncomms1701
22. Mayne P.J. Emerging incidence of Lyme borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia. *Int. J. Gen. Med*. 2011;4:845–52. DOI: https://doi.org/10.2147/IJGM.S27336
23. Krause P.J., Telford S.R., Spielman A., et al. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *JAMA*. 1996;275(21):1657–60.
24. Belongia E.A. Epidemiology and impact of coinfections acquired from Ixodes ticks. *Vector Borne Zoonotic Dis*. 2002;2(4):265–73. DOI: https://doi.org/10.1089/153036602321653851
25. Курильщиков А.М., Ливанова Н.Н., Фоменко Н.В., Тикунова Н.В. Метагеномный анализ бактериального сообщества, ассоциированного с клещами *Ixodes persulcatus*. *Вестник Новосибирского государственного университета. Серия: Биология, клиническая медицина.* 2013;11(4):13–21. Kurilchshikov A.M., Livanova N.N., Fomenko N.V., Tikunova N.V. Metagenomic analysis of bacterial community associated with *Ixodes persulcatus* ticks. *Vestnik*

*NSU. Series: Biology and Clinical Medicine.* 2013;11(4):13–21. EDN: https://elibrary.ru/rtukux
26. Norte A. C., Harris D. J., Silveira D., et al. Diversity of microorganisms in *Hyalomma aegyptium* collected from spur-thighed tortoise (*Testudo graeca*) in North Africa and Anatolia. *Transbound. Emerg. Dis*. 2022;69(4):1951–62. DOI: https://doi.org/10.1111/tbed.14188
27. Akveran G.A., Karasartova D., Keskin A., et al. Bacterial and protozoan agents found in *Hyalomma aegyptium* (L., 1758) (*Ixodida*: Ixodidae) collected from *Testudo graeca* L., 1758 (Reptilia: Testudines) in Corum Province of Turkey. *Ticks Tick Borne Dis*. 2020;11(5):101458. DOI: https://doi.org/10.1016/j.ttbdis.2020.101458
28. Benyahia H., Diarra A.Z., Gherissi D.E., et al. Molecular and MALDI-TOF MS characterisation of *Hyalomma aegyptium* ticks collected from turtles and their associated microorganisms in Algeria. *Ticks Tick Borne Dis*. 2022;13(1)101858. DOI: https://doi.org/10.1016/j.ttbdis.2021.101858
29. Kalmár Z., Cozma V., Sprong H., et al. Transstadial transmission of *Borrelia turcica* in *Hyalomma aegyptium* ticks. *PLoS One*. 2015;10(2):e0115520. DOI: https://doi.org/10.1371/journal.pone.0115520
30. Hepner S., Fingerle V., Duscher G.G., et al. Population structure of *Borrelia turcica* from Greece and Turkey. *Infect. Genet. Evol*. 2020; 77:104050. DOI: https://doi.org/10.1016/j.meegid.2019.104050
31. Barbour A.G., Gupta R.S. The family *Borreliaceae* (*Spirochaetales*), a diverse group in two genera of tick-borne spirochetes of mammals, birds, and reptiles. *J. Med. Entomol*. 2021;58(4):1513–24. DOI: https://doi.org/10.1093/jme/tjab055
32. Abdelbaset A.E., Nonaka N., Nakao R. Tick-borne diseases in Egypt: a one health perspective. *One Health*. 2022;15:100443. DOI: https://doi.org/10.1016/j.onehlt.2022.100443
33. Moraga-Fernández A., Muñoz-Hernández C., Sánchez-Sánchez M., et al. Exploring the diversity of tick-borne pathogens: the case of bacteria (*Anaplasma*, *Rickettsia*, *Coxiella* and *Borrelia*) protozoa (Babesia and Theileria) and viruses (*Orthonairovirus*, tick-borne encephalitis virus and louping ill virus) in the European continent. *Vet. Microbiol*. 2023;286:109892. DOI: https://doi.org/10.1016/j.vetmic.2023.109892
34. Carpi G., Cagnacci F., Wittekindt N.E., et al. Metagenomic profile of the bacterial communities associated with *Ixodes ricinus ticks. PloS One.* 2011;6(10):e25604. DOI: https://doi.org/10.1371/journal.pone.0025604
35. Plummer E., Twin J., Bulach D.M., et al. A comparison of three bioinformatics pipelines for the analysis of preterm gut microbiota using *16S rRNA* gene sequencing data. *J. Proteomics Bioinform*. 2015;8(12):283–91. DOI: https://doi.org/10.4172/jpb.1000381
36. Pinna N.K., Dutta A., Monzoorul Haque M., Mande S.S. Can targeting non-contiguous V-regions with paired-end sequencing improve 16S rRNA-based taxonomic resolution of microbiomes?: an *in silico* evaluation. *Front. Genet*. 2019;10:653. DOI: https://doi.org/10.3389/fgene.2019.00653
37. Bukin Y.S., Galachyants Y.P., Morozov I.V., et al. The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Sci. Data.* 2019;6:190007. DOI: https://doi.org/10.1038/sdata.2019.7
38. Martinez-Porchas M., Villalpando-Canchola E., Ortiz Suarez L.E., Vargas-Albores F. How conserved are the conserved 16S-rRNA regions? *PeerJ*. 2017;5:e3036. DOI: https://doi.org/10.7717/peerj.3036
39. Barb J.J., Oler A.J., Kim H.S., et al. Development of an analysis pipeline characterizing multiple hypervariable regions of 16S rRNA using mock samples. *PLoS One*. 2016;11(2):e0148047. DOI: https://doi.org/10.1371/journal.pone.0148047
40. Hiergeist A., Reischl U., Gessner A. Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high inter-center variability. *Int. J. Med. Microbiol.* 2016;306(5):334–42. DOI: https://doi.org/10.1016/j.ijmm.2016.03.005
41. Takahashi S., Tomita J., Nishioka K., et al. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS One.* 2014;9(8):e105592. DOI: https://doi.org/10.1371/journal.pone.0105592
42. Yang B., Wang Y., Qian P.Y. Sensitivity and correlation of hypervariable regions in *16S rRNA* genes in phylogenetic analysis. *BMC Bioinformatics.* 2016;17:135. DOI: https://doi.org/10.1186/s12859-016-0992-y

DOI: https://doi.org/10.36233/0372-9311-629


***Information about the authors***

*Oksana V. Vasilieva* — Cand. Sci. (Med.), Head, Laboratory for diagnostics of bacterial infections, Stavropol Plague Control Research Institute, Stavropol, Russia, vasileva_ov@snipchi.ru, https://orcid.org/0000-0002-8882-6477

*Diana V. Ul'shina*✉ — Cand. Sci. (Biol.), senior researcher, Laboratory for diagnostics of bacterial infections, Stavropol Plague Control Research Institute, Stavropol, Russia, vladidiana@yandex.ru, https://orcid.org/0000-0001-7754-2201

*Anna S. Volynkina* — Cand. Sci. (Biol.), Head, Laboratory for diagnostics of viral infections, Stavropol Plague Control Research Institute, Stavropol, Russia, volyn444@mail.ru, https://orcid.org/0000-0001-5554-5882

*Sergey V. Pisarenko* — Cand. Sci. (Chem.), leading researcher, Laboratory of biochemistry, Stavropol Plague Control Research Institute, Stavropol, Russia, pisarenko_sv@mail.ru, https://orcid.org/0000-0001-6458-6790

*Yulia V. Siritsa* — researcher, Laboratory for diagnostics of bacterial infections, Stavropol Plague Control Research Institute, Stavropol, Russia, merendera@mail.ru, https://orcid.org/0000-0001-9442-6966

*Olga A. Gnusareva* — researcher, Laboratory for diagnostics of bacterial infections, Stavropol Plague Control Research Institute, Stavropol, Russia, gnusarevao@mail.ru, https://orcid.org/0000-0002-9044-1808

*Natalia A. Yatsenko* — Chief Physician, Regional Specialized Clinical Infectious Diseases Hospital, Stavropol, Russia, natali.yanet@yandex.ru; https://orcid.org/0000-0003-4353-4777

*Alexandr N. Kulichenko* — D. Sci. (Med.), Professor, Academician of RAS, Director, Stavropol Research Anti-Plague Institute, Stavropol, Russia, stavnipchi@mail.ru, https://orcid.org/0000-0002-9362-3949

***Authors' contribution:*** *Vasilyeva O.V.* — research concept, writing the text; *Ulshina D.V.* — data analysis, writing the text; *Volinkina A.S.* — science editing the article; *Pisarenko S.V.* — genomic research; *Siritsa Yu.V., Gnusareva O.A., Yatsenko N.A.* — laboratory research; *Kulichenko A.N.* — research concept, article science editing. All authors confirm that their authorship meets the criteria of the International Committee of Medical Journal Editors, have made a significant contribution to the search and analytical work and preparation of the article, read and approved the final version before publication.

The article was submitted 20.01.2025;
accepted for publication 29.03.2025;
published 28.04.2025

***Информация об авторах***

*Васильева Оксана Васильевна* — канд. мед. наук, зав. лаб. диагностики бактериальных инфекций Ставропольского противочумного института, Ставрополь, Россия, vasileva_ov@snipchi.ru, https://orcid.org/0000-0002-8882-6477

*Ульшина Диана Васильевна*✉ — канд. биол. наук, с. н. с. лаб. диагностики бактериальных инфекций Ставропольского противочумного института, Ставрополь, Россия, vladidiana@yandex.ru, https://orcid.org/0000-0001-7754-2201

*Волынкина Анна Сергеевна* — канд. биол. наук, зав. лаб. диагностики вирусных инфекций Ставропольского противочумного института, Ставрополь, Россия, volyn444@mail.ru, https://orcid.org/0000-0001-5554-5882

*Писаренко Сергей Владимирович* — канд. хим. наук, в. н. с. лаб. биохимии Ставропольского противочумного института, Ставрополь, Россия, pisarenko_sv@mail.ru, https://orcid.org/0000-0001-6458-6790

*Сирица Юлия Владимировна* — н. с. лаб. диагностики бактериальных инфекций Ставропольского противочумного института, Ставрополь, Россия, merendera@mail.ru, https://orcid.org/0000-0001-9442-6966

*Гнусарева Ольга Александровна* — н. с. лаб. диагностики бактериальных инфекций Ставропольского противочумного института, Ставрополь, Россия, gnusarevao@mail.ru, https://orcid.org/0000-0002-9044-1808

*Яценко Наталья Александровна* — главный врач Краевой специализированной клинической инфекционной больницы, Ставрополь, Россия, natali.yanet@yandex.ru, https://orcid.org/0000-0003-4353-4777

*Куличенко Александр Николаевич* — д-р мед. наук, проф., академик РАН, директор Ставропольского противочумного института, Ставрополь, Россия, stavnipchi@mail.ru, https://orcid.org/0000-0002-9362-3949

***Участие авторов:*** *Васильева О.В.* — концепция исследования, написание текста; *Ульшина Д.В.* — анализ данных, написание текста; *Волынкина А.С.* — научное редактирование статьи; *Писаренко С.В.* — геномные исследования; *Сирица Ю.В.*, *Гнусарева О.А.*, *Яценко Н.А.* — лабораторные исследования; *Куличенко А.Н.* — концепция исследования, научное редактирование статьи. Все авторы подтверждают соответствие своего авторства критериям Международного комитета редакторов медицинских журналов, внесли существенный вклад в проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию до публикации.

Статья поступила в редакцию 20.01.2025;
принята к публикации 29.03.2025;
опубликована 28.04.2025