One Health 22 (2026) 101312

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

High throughput sequencing in the diagnosis and surveillance of tick-borne diseases: A narrative review

Yannick-Ibrahim Bisso [a], Olivier Duron [b], Olivier Plantard [c], Gilles Prevost [a], Benoit Jaulhac [a,d], Pierre Boyer [a,d,*]

[a] Université de Strasbourg, UR3073 PHAVI, CHRU de Strasbourg, Instituts de Bactériologie et de Parasitologie, 3, rue Koeberlé, F-67000 Strasbourg, France
[b] MIVEGEC, University of Montpellier, Centre National de la Recherche Scientifique (CNRS), Institut de Recherche pour le Développement (IRD), Montpellier, France
[c] Oniris, INRAE, BIOEPAR, 44300 Nantes, France
[d] CNR des Borrelia, Hôpitaux Universitaires de Strasbourg, F-67000 Strasbourg, France

ARTICLE INFO

Keywords:
High-throughput nucleotide sequencing
Next-generation sequencing
Tick-borne diseases
Ixodidae / microbiology
Disease vectors /microbiology
One health
Microbiota
Zoonoses / epidemiology
Pathogen detection methods
Blood meal analysis

ABSTRACT

Ticks are blood-feeding arthropods that can transmit a wide variety of microorganisms (bacteria, viruses, protozoa, and filarial nematodes) when they feed on various vertebrate hosts. In the recent years, high-throughput sequencing (HTS), also called next-generation sequencing, has become a key tool for detecting and characterizing microorganisms, whether they are pathogens or part of the tick's own microbiota. This narrative review summarizes current applications of HTS for the surveillance and diagnosis of tick-borne diseases within a One Health framework.

From nucleic acids extracted from a tick sample, HTS enables the possibility of simultaneous detection of multiple microorganisms, and provides valuable information on potential reservoir hosts through blood meal analysis. It has revealed a vast diversity of bacterial, viral, protozoan agents and filarial nematodes in various tick species worldwide, including unexpected or novel pathogens. HTS has also improved our understanding of the tick microbiota and how it interacts with pathogens, which could have an impact on vector competence.

In the field of microbiological diagnosis, HTS provides a complementary or alternative approach to traditional diagnostic tests, particularly in cases with non-specific symptoms or when the etiology is unknown. HTS has proven to be effective in detecting rare or novel pathogens, including some transmitted by ticks. It has also enabled the reconstruction of whole genomes of microorganisms from clinical samples or ticks, thereby improving our understanding of the molecular epidemiology of these agents.

By bridging the vector, its pathogens, the reservoir host, and human or animal clinical outcomes, HTS represents a cornerstone technology for future integrated surveillance systems of TBDs within a One Health perspective.

1. Introduction

Ticks are hematophagous arthropods. To pass from one instar to the next, ticks need to take a blood meal on various vertebrate hosts. During this blood meal, ticks can acquire and transmit a wide variety of microorganisms from or to vertebrate hosts: bacteria (*Borrelia, Anaplasma, Neoherlichia, Rickettsia*) [1], viruses (*Bunyavirus, Orthonairovirus, TBE virus* and other *Orthoflaviviridae*) [2], protozoan parasites (such as *Babesia, Theileria,* some *Hepatozoon*, among others) [3,4] and filarial nematodes [5]. When bitten, humans or animals can become infected by these microorganisms and develop pathologies known as Tick Borne Diseases (TBDs). TBDs can be considered from One Health perspective in several ways: their animal dimension, since ticks can parasitize several vertebrate hosts that can act as reservoirs for microorganisms; their environmental dimension, since the ecological conditions can affect tick populations and their hosts; and finally, their human dimension, since humans are accidental hosts of these microorganisms and become sick [6]. Because TBDs are vector-borne diseases (with the population dynamics and the spatial distribution of vectors highly influenced by both the biotic and abiotic environment) and numerous TBDs are zoonotic diseases (Lyme borreliosis, TBE, CCHF…), they are often considered as textbook cases to illustrate the added value of the One Health approach

* Corresponding author at: UR PHAVI Pathogens Host Arthropod Vectors Interfaces – UR 3073-Faculté de Médecine, 1–3 rue Koeberlé, F-67000 Strasbourg, France.
E-mail address: pierreboyer@unistra.fr (P. Boyer).

https://doi.org/10.1016/j.onehlt.2025.101312
Received 9 August 2025; Received in revised form 12 December 2025; Accepted 22 December 2025
Available online 26 December 2025

[7,8]. TBDs occur at the interface between humans, animals and the environments where vectors and natural hosts coexist, and their transmission relies on complex multi-host systems. Therefore, it is necessary to adopt integrated approaches that link all these compartments.

High-throughput sequencing (HTS), also known as next-generation sequencing (NGS), allows for the simultaneous sequencing of millions of nucleic acid fragments. This significantly increases data yield compared to traditional methods like Sanger sequencing. HTS enables the direct analysis of complex DNA/RNA samples containing mixtures of genetic material from multiple organisms [9]. These technologies have revolutionized the way we detect and characterize microorganisms [10]. They represent a powerful tool for integrated surveillance of TBD, insofar as they enable the simultaneous detection and characterization of a wide range of microorganisms transmitted by ticks or forming part of their microbiota, directly from vector ticks, animal reservoirs or clinical samples, while also providing etiological diagnosis of these pathologies. The aim of this narrative review is to provide an overview of the main applications of HTS in vector surveillance, epidemiology and microbiological diagnosis of tick-borne diseases within a One Health framework.

2. Method

This article is a narrative (non-systematic) review based on a targeted search of the literature. Relevant publications were identified in PubMed database using combinations of keywords related to high-throughput sequencing, metagenomics, tick-borne diseases, vector surveillance, tick microbiota and clinical diagnosis, without formal date. Studies were selected for inclusion based on their relevance to the main themes of the review (HTS technologies, applications in tick and reservoir surveillance, microbiota–pathogen interactions and clinical diagnosis), with the aim of providing an overview.

3. HTS technologies: an overview

HTS technologies have profoundly changed microbiology, in settings ranging from basic research to clinical diagnostics. Over the past few decades, several HTS strategies have emerged, each with specific advantages and limitations depending on their intended use.

Among the most widely used approaches is shotgun metagenomic sequencing, which involves sequencing, without targeting, all nucleic acids (DNA and/or RNA) present in a biological sample. This method does not require prior culture nor hypotheses about the microorganism in the sample and therefore makes it possible to detect bacteria, viruses, fungi, and parasites in parallel, including novel or unexpected microorganisms [11]. Its ability to sequence complete or partial genomes provides a superior taxonomic and functional resolution compared to targeted 16S rDNA sequencing [12]. However, its implementation remains challenging: in clinical samples, more than 99 % of reads can be of host origin, leaving microbial DNA in very low relative abundance. As a result, deep sequencing [13], organ-targeted sequencing, host DNA physical removal [14] or microbial DNA enrichment become necessary to achieve sufficient sensitivity, as demonstrated in studies focusing on TBDs surveillance [15]. Furthermore, bioinformatics analysis is computationally intensive and requires specialized infrastructure, while interpretation of results demands multidisciplinary One Health expertise. HTS-based studies on TBD require the combined effort of microbiologists, medical entomologist, infectious disease physicians, veterinarians and bioinformaticians [11]. Such multidisciplinary teams are fully aligned with the principles of the One Health approach. Although often considered as a contaminating background, host nucleic acids recovered through shotgun metagenomic sequencing can also lead to investigating the host's immune response. Increasingly, studies highlight that host gene expression profiles sequencing RNA can help distinguish between different types of infections, such as bacterial versus viral etiologies, by identifying specific inflammatory signatures. While this host-centered approach has not yet been applied in the context of TBDs, it has already shown promising results in other clinical settings. For instance, in osteoarticular infections, host transcriptomic signatures have been used to improve diagnostic stratification [16].

As an alternative, amplicon-based sequencing (e.g., 16S for bacteria, ITS for fungi) uses PCR amplification of conserved gene regions, followed by sequencing of amplicons. This technique is simpler, faster, and less expensive than shotgun metagenomics but it focuses on one or a small number of genes. It does not require as great a sequencing depth as shotgun metagenomics [17]. It generates reliable taxonomic profiles of microbial communities and is less affected by host DNA contamination. However, it provides no further information on virulence or resistance genes and is limited to one microbial group per assay. Additionally, amplification biases can distort relative abundance estimates [18]. Viruses, which lack universal marker genes but though their small genomes sometimes allow for full-length amplification via PCR [19].

To enhance sensitivity in complex samples, targeted capture by hybridization probes represents a valuable alternative to untargeted metagenomic approaches. This technique relies on the selective enrichment of known sequences of interest through hybridization with biotinylated oligonucleotide probes, prior to sequencing. It enables the detection of pathogens present at a very low abundance and the reconstruction of near-complete genomes directly from clinical samples, without the need of culture [20]. It is particularly useful for recovering the genome of a known pathogen, analyzing its phylogeny, and identifying virulence genes. However, designing high-quality probe panels is time-consuming, requiring expert knowledge of target genomic regions. Moreover, this method is intrinsically limited to known targets and, therefore, unsuitable for the discovery of novel or highly divergent emerging pathogens [21].

To minimize interference from host DNA during sequencing, a particularly effective strategy is to perform selective dissection of tick organs prior to nucleic acid extraction. Focusing on the salivary glands is especially relevant for the detection and characterization of tick-borne pathogens, as these tissues are directly involved in pathogen transmission to the vertebrate host. This approach is easier to implement in adult ticks, but salivary glands can also be dissected from nymphs [22], although the procedure is technically more challenging than in adults. In larvae, salivary gland dissection is theoretically possible but highly demanding and, to date, has mainly been used for microscopic or histological studies rather than for HTS applications [23]. Nevertheless, analyzing the salivary glands of immature ticks could provide additional information on vertically transmitted microorganisms and their potential for early transmission during the first blood meal.

Conversely, targeting the ovaries is more appropriate for the investigation of vertically transmitted symbionts and other maternally inherited bacteria.

This organ-specific approach not only increases the relative abundance of microbial DNA in the sample, thereby improving detection sensitivity, but also provides valuable insights into pathogen localization and potential transmission routes. This organ-level dissection enhances the proportion of microbial DNA and provides additional information on pathogen localization and transmission. Moreover, mechanical filtration using a 5 μm filter can help eliminate large host cell nuclei, substantially larger than bacterial cells, further enriching microbial content before sequencing, as demonstrated by Duron et al. [14].

Despite these advances, recovering a complete genome directly from clinical samples or from arthropod vectors remains challenging. Whole-genome sequencing of pure microbial isolates remains the gold standard for molecular epidemiology and strain typing. It enables comprehensive analysis of resistance and virulence genes and high-resolution tracking of outbreaks. However, in some cases, the recovery of a complete genome still relies on the availability of cultivable isolates [24], which poses difficulties for many tick-borne bacteria that grow slowly or not grow at all or require biosafety level 3 or even 4 (for pathogens like Crimean-Congo hemorrhagic fever virus) [24].

Table 1
Principles of HTS strategies and their advantages and limitations.

HTS strategy	Principle	Advantages	Limitations
Shotgun metagenomic	Untargeted sequencing of all DNA/RNA in a sample	• Detects bacteria, viruses, fungi and parasites • Detects unusual or novel microorganisms • Better taxonomic resolution • Access to additional information including host response	• Host DNA/RNA dominates (>99 %) • Microbial reads may be rare • Heavy bioinformatics burden • Higher cost
Amplicon-based sequencing	PCR amplification(s) of conserved regions (e.g., 16S, ITS)	• Faster and cost-effective • Simpler bioinformatics • Higher multiplexing rate in a sequencing reaction • Possibility of multiplexing PCRs to access more information • Lower host contamination	• Often limited to one microbial group • No functional gene info • Amplification biais
Targeted capture (hybrid probes)	Enrichment of specific genomic sequences with biotinylated probes	• High sensitivity for low-abundance pathogens • Near-complete genome recovery without culture	• Probe design is time-consuming • Limited to known targets • Poor for novel/divergent sequences
Whole-genome sequencing of isolates	Sequencing the entire genome of a cultured isolate	• Gold standard • Full resistance/virulence profiling • High-resolution strain typing,	• Requires culturable isolates • Difficult fastidious pathogens

In practice, the choice of HTS platform is driven by the study objectives, the required read length and the error rate [9,11,17]. Short-read Illumina instruments remain the most widely used platforms for metagenomic and amplicon-based studies of tick-borne diseases. They provide highly accurate reads (typically from 150 to 300 base paires) [11,17]. For large surveillance projects, benchtop instruments and mid-output runs allow cost-effective multiplexing of several samples in a single sequencing run [11,17].

Long-read platforms such as PacBio and Oxford Nanopore Technologies offer complementary advantages. PacBio sequencing devices generates long, highly accurate reads that can greatly facilitate complete genome assembly, reconstruction of plasmids, and characterization of repetitive or recombining regions in tick-borne bacteria and viruses [9,11]. ONT devices (e.g. MinION, GridION) provide portable, rapidly deployable sequencing with flexible run times, which is particularly attractive for field investigations, or iterative sequencing of selected sample. Although raw read error rates remain higher than those of Illumina, recent chemistries and base-calling improvements, as well as hybrid strategies combining short- and long-read data, increasingly allow high-quality assemblies and accurate typing.

The optimal run configuration therefore depends on whether the primary goal is broad screening of many samples, detailed characterization of a subset of pathogens, or real-time investigation of emerging tick-borne agents.

Table 1 summarized HTS technology principles, advantages, and limitations.

4. Applications of HTS in vector epidemiology

Epidemiological surveillance of TBDs is mainly based on the detection of microorganisms from ticks collected either from animals or in the field. Traditionally, microorganisms are detected by PCR using standardized protocols, without prejudging the viability of these microorganisms [25]. This model, although imperfect, is widely accepted as reflecting the acarological risk of TBDs [26].

HTS has proven to be as effective as a specific PCR in this context. Most studies have focused on hard ticks: for example, *Ixodes ricinus* in Europe [27] and *I. scapularis* in North America [15]. Several studies also include Asian species (*I. persulcatus*, *Dermacentor nuttalli*, *Hyalomma asiaticum*) [28] or South American species such as *Ornithodoros hasei*, a soft tick collected in Colombia [29]. HTS approaches employed are varied: amplicon sequencing of 16S rDNA for bacterial analysis [29], shotgun metagenomics [30], and RNA-seq for viral analysis [28,31].

4.1. HTS for microorganisms detection in ticks

As regards microorganism detection, there are numerous studies dealing with tick-borne bacteria and viruses, while protozoa have received comparatively less attention, and filarial nematodes are only rarely addressed. For bacteria, pathogenic species of the *Borrelia* spp. genus are widely documented, including *B. garinii*, *B. valaisiana* and *B. miyamotoi* in Europe [27], *B. burgdorferi* sensu stricto in the United States [15]. *B. americana* and *B. carolinensis* were unexpectedly detected in Poland [32], suggesting the need to confirm unexpected results with a second technique. Studies in Asia and South America have also revealed the presence of relapsing fever *Borrelia* [28,29]. Other pathogenic bacteria were also detected, including *Anaplasma phagocytophilum*, *Ehrlichia muris* [15,28], as well as more recently described taxa as members of the *Neoerhlichia* and *Allocryptoplasma* genera [33,34].

RNA viruses represent another reservoir of microorganisms detected by metatranscriptomics, with evidence of well-known zoonotic viruses such as CCHF virus, Alongshan virus or Beiji nairovirus [28], but also of as yet unexplored viral diversity, totaling over 1800 viral genomes identified in China in a wide range of ticks. Many of these newly identified RNA viruses have so far been detected only in ticks, with no confirmed vertebrate hosts and no evidence of pathogenicity. Large virome studies have shown that a substantial fraction of the 'tick virome' corresponds to tick-specific or arthropod-associated viral clades, rather than tick-borne viruses capable of infecting vertebrates [31]. This highlights the need for caution when interpreting viral sequences detected in ticks by HTS.

Some studies report the presence of protozoan parasites such as *Babesia microti* or *Theileria equi* [28] and filarial nematodes of the *Dipetalonema* lineage [5], confirming that ticks can harbor several microorganisms of importance for human and veterinary medicine. Finally, ticks also harbor a variety of fungi on their cuticle, the diversity of which remains poorly characterized, but may include entomopathogenic species [35].

Beyond the simple detection of microorganisms in ticks, these technologies enable direct typing of microorganisms from the collected tick. This was demonstrated by two recent studies focusing on the differentiation of *A. phagocytophilum* variants in *I. scapularis* in the USA. These studies demonstrate that HTS can not only accurately distinguish the pathogenic strain causing human anaplasmosis from non-pathogenic variants but also map their geographic distribution on a large scale. Unlike traditional PCR followed by Sanger sequencing, HTS offers increased sensitivity, improved taxonomic resolution and a multiplexing capability tailored to large-scale surveillance needs [36,37].

It should be noted that in most studies, tick collection is conducted over relatively short periods, typically ranging from a few weeks to a few months during the peak of tick activity (spring-summer). To the best of our knowledge, there are few long-term longitudinal studies spanning multiple years that have applied these techniques, which limits our ability to assess their full potential in terms of precision and impact on the understanding of these pathologies. In addition to the complexity involved in interpreting the results, these techniques also come up against a major obstacle: their cost, which, to date, remains a major barrier to their large-scale implementation, particularly in the context of field epidemiological studies involving the collection and analysis of several hundred ticks.

4.2. Detection of new or unusual microorganisms with HTS

Field studies based on HTS have revealed a much wider range of microbial diversity in ticks than conventional methods would suggest. Numerous studies carried out on several continents have identified new RNA viruses [38–40], little-documented bacteria such as *Rickettsia asiatica* in Europe [41], as well as complex codetection of bacteria, protozoa and pathogenic fungi [42,43]. These results underline the potential of HTS to discover emerging agents and enrich our knowledge of these microorganisms.

However, the ability of HTS to reveal this diversity should not lead to over-interpretation of the pathogenicity of the microorganisms detected [25]. The mere presence of an agent in a tick does not mean that it is pathogenic, nor that the tick is competent to transmit it. Many of the microorganisms detected, such as *Francisella*-like endosymbionts (*Francisella*-LE), are closely related to known pathogens (e.g. *F. tularensis*) but have no proven pathogenicity [14,44]. Indeed, ticks are obligated hematophagous arthropods that rely exclusively on blood as a food source. However, vertebrate blood is deficient in several essential micronutrients, particularly B vitamins. To compensate for this nutritional gap, ticks have established symbiotic relationships with intracellular bacterial endosymbionts, primarily *Coxiella*-like endosymbionts (*Coxiella*-LE) and *Francisella*-LE [45]. Genomic analyses of these endosymbionts have revealed the presence of conserved biosynthetic pathways for three key B vitamins: biotin (vitamin B7), riboflavin (vitamin B2), and folate (vitamin B9) [14,44,46–48]. Experimental studies have confirmed that these vitamins are critical for tick development and survival [14,48–50]. These nutritional endosymbionts, which are essential for the tick's hematophagous lifestyle, are widespread across tick species [51–53]. Notably, they have often been misidentified as pathogenic bacteria, leading to an overestimation of the risk associated with tick-borne infections [44,54,55]. However, the absence of functional virulence genes in their genomes confirms their non-pathogenic status, while the presence of complete B vitamin biosynthesis pathways underscores their fundamental nutritional role [45]. These findings support the idea that mutualistic associations with microbes were a critical evolutionary step in the emergence of hematophagy in ticks. Overall, tick microbial communities are largely composed of such nutritional symbionts [45,56]. Recognizing and characterizing this neglected microbial diversity is now essential to reshape our understanding of ticks, their associated microbes, and the biology of hematophagy [45,56].

Consequently, cross-referencing HTS results with clinical observations, experimental data, and epidemiological context are crucial to assess the true pathogenic significance of the microorganisms detected. A rigorous approach, integrating notions of vector competence and actual pathogenicity, is essential to avoid confusion between presence, transmission and infectious risk. Although HTS offers great prospects for the surveillance of vector-borne agents, its interpretation requires a cautious analytical framework based on converging evidence.

4.3. HTS interaction microbiota/pathogens

HTS gives an exhaustive overview of the tick microbiota, an ecosystem composed of various microorganisms, including endosymbionts, commensals, and pathogens, which interact with each other. In vitro, it has been shown that the composition of tick microbiota when richness is reduced can reduce the presence of a pathogenic microorganism, such as *B. burgdorferi* sensu lato [57]. Conversely, increasing its diversity increases the presence of *B. microti* [58]. It therefore strongly depends on the tick/pathogen couple concerned. Microbiota/pathogen interactions can be competitive or, on the contrary, synergistic. For example, the composition of the microbiota of *D. andersoni* influences its susceptibility to pathogens: an increase in *Rickettsia bellii* reduces infection by *A. marginale*, while *Francisella* endosymbionts favor the acquisition of *F. novicida,* which is used as a surrogate for *F. tularensis* [59]. Moreover, the presence of certain bacteria in the microbiota could interfere with the vector competence of ticks. For example, the presence of the intracellular *Coxiella*-like symbiont in *Amblyomma americanum* suggests a potential role in interfering with the transmission of pathogens such as *Ehrlichia chaffeensis* [60]. As a result, taking into account a more precise microbiota composition could lead to a more refined evaluation of acarological risk and HTS is a suitable tool for this purpose. In the future, it might be possible to identify areas where ticks have a microbiota likely to favor or inhibit the acquisition of a given microorganism. The detection, in ticks, of microorganisms capable of specifically preventing the transmission of certain pathogens (by microbial competition or exclusion, for example) could thus constitute a marker of reduced acarological risk. This prospect remains highly speculative, will first require solid experimental validation using controlled laboratory models, but HTS provide useful data for such investigations [61].

However, it is important to remain attentive to the pre-analytical stages in the study of tick microbiota because the detection of a bacterium in a tick by HTS does not necessarily mean that it is capable of interacting or interfering with a pathogen. For example, Binetruy et al. [62] highlighted a major pitfall associated with the use of HTS from a tick matrix: sample preparation. The comparison of bleach- and ethanol-based sterilization methods showed that ethanol treatment resulted in artificially elevated bacterial diversity due to residual external bacterial DNA. Bleach sterilization proved more effective in eliminating cuticular bacterial DNA without altering internal microbial profiles, making it the preferred method for microbiome studies. This compositional bias can also mask the pathogens of interest or lead to erroneous interpretations of bacterial diversity.

4.4. Study of the reservoirs

The natural reservoirs of TBDs agents can be investigated in two main ways: by collecting samples from wild or domestic hosts, or by analyzing ticks directly to detect the DNA from the host on which they fed.

Various studies have shown that HTS can also be used to study animal reservoirs in TBDs. In small mammals, HTS can be used to identify tick-borne pathogens such as *Anaplasma* spp. or *Rickettsia* spp. in splenic samples [63]. In wild and domestic animals, it reveals a wide diversity of microorganisms, including zoonotic agents shared between hosts [64]. Another remarkable example includes an extensive survey of *Erhlichia* and *Anaplasma* infections in the rainforests of the Amazon biome of French Guiana [65], including 44 species of wild mammals, five species of passerines and 22 species of ticks. HTS based on 16S rDNA bacterial metabarcoding and metagenomics reconstruction revealed a high indigenous biodiversity of infections circulating among humans, wildlife, and ticks inhabiting these ecosystems. Sequencing identifies these infections as highly endemic, with a majority of new strains and putative species specific to French Guiana. They are detected in unusual rainforest wild animals, suggesting they have distinctive sylvatic transmission cycles [65]. Complementary using similar methodological

Table 2
Sample-to-answer overview of what HTS can reveal from tick, animal reservoir and human clinical samples.

	Tick samples	Animal reservoir samples	Human clinical samples
Detection of tick-borne pathogens	**Shotgun:** broad, unbiased detection of known, rare or novel TBD agents. **Targeted:** sensitive detection of predefined pathogens (e.g. 16S rDNA based, capture for selected agents.)	**Shotgun:** detection of a wide range of TBD agents circulating in wildlife or domestic animals. **Targeted:** focused screening of selected pathogen groups.	**Shotgun:** detection of rare or unexpected TBD agents in undiagnosed syndromes. **Targeted:** increased sensitivity for suspected agents at low load (detection of rare or unexpected TBD agents).
Characterization of microbiota and symbionts	**Shotgun:** global description of the tick microbiota virome, including endosymbionts. **Targeted:** bacterial/fungal profiles (16S rDNA, ITS).	Characterization of microbial communities in the reservoir host	N/A
Blood meal / host identification	Recovery of vertebrate DNA/RNA for blood-meal / host identification; sometimes combined with capture.	N/A	N/A
Genome reconstruction and molecular epidemiology	**Shotgun (combine with capture):** near-complete genomes of TBD agents directly from ticks for phylogeny, strain typing and gene content analysis.	**Shotgun (combine with capture):** pathogen genomes from animal reservoirs, comparable to tick and human strains within the same cycle.	**Shotgun/capture:** genomes from clinical samples, enabling detailed molecular epidemiology and linkage to tick/animal strains.
Microbiota–pathogen interactions	Associations between microbiota composition, symbionts and pathogen presence.	N/A	N/A
One Health integration / cross-compartment comparison	HTS data in ticks compared with animal and human data to reconstruct pathogen circulation	HTS data in animals matched with tick and human datasets to identify reservoirs host	HTS data in patients compared with tick and animal data to link clinical cases to the enzootic cycles.

approaches also revealed the presence of 19 novel *Rickettsia* genotypes as well as a novel *Borrelia* species, *B. mahuryensis*, circulating in suburban areas in the same region [66].

In addition to identifying microorganisms in reservoir hosts, HTS can also be applied to determine the origin of the previous blood meal directly in from an unfed tick. A targeted capture approach used to enrich pathogen DNA can be used to recover DNA fragments from the host [67], paving the way for dual characterization from a single tick sample: simultaneous identification of the pathogens transmitted and of the host on which the tick has fed. This provides a significant added value for the detailed study of the epidemiological dynamics of TBDs.

4.5. Comparison of conventional methods and HTS

Generally, comparative studies between conventional methods (PCR/qPCR) and HTS on the same tick DNA extracts show that the two approaches are complementary. HTS, whether shotgun or targeted sequencing, can detect a broader spectrum of microorganisms, including unexpected or new microorganisms, such as emerging viruses [68]. It also allows multiple microorganisms detection in a single manipulation of the same tick, something that PCR can fail to do because of its specific targeting [69,70]. In some cases, discrepancies appear: PCR detects pathogens with a low load that HTS does not capture, while HTS reveals agents not included in the PCR panels. These differences are due to the specific sensitivity of each method, the depth of sequencing, or the presence of amplification bias [68]. In practice, many studies are now adopting an integrated approach in which HTS is used to explore microbial diversity and PCR to validate and refine detections [70–72]. In short, HTS opens the way to a better understanding of the microbial diversity of ticks, while PCR retains its place as a targeted, sensitive and rapid confirmation tool. In some cases, HTS results are validated using complementary molecular techniques, in particular specific PCR or Sanger sequencing, to confirm the identity of the pathogens detected [15,27]. This combined approach increases the reliability of the results, particularly when it comes to distinguishing between closely related species or species of low abundance.

These examples illustrate the holistic dimension of HTS technologies. From a single tick analyzed, it would be possible to simultaneously access information on the microorganisms it harbors (pathogenic or derived from the microbiota), to type them, to identify its potential reservoir via blood meal analysis, and even to determine the species of the vector itself, as shown by the work of Osikowicz et al. [73]. When combined with field collections and studies on animal hosts, these approaches would enable us to better anticipate the emergence of new microorganism-carrying tick populations, and bring an additional degree of precision to the monitoring of population dynamics and associated infectious agents. Table 2 summarizes, in a ‘sample-to-answer’ view, what HTS can reveal from tick, animal reservoir and human clinical samples in the context of tick-borne diseases.

5. HTS for the diagnosis of TBDs

When it comes to HTS in the field of TBDs, one of the pioneering papers by Xu et al. [74] who used non-targeted metagenomic sequencing to identify a novel phlebovirus (Severe Fever with Thrombocytopenia Syndrome Virus or SFTS) of the Bunyaviridae family in patients with acute febrile syndrome in China. This approach enabled them to discover a virus that was unknown at the time. Since then, HTS has continued to advance and is progressively being implemented in microbiology laboratories, particularly for the etiological diagnosis of complex or atypical infections, as well as for cases that remain unresolved with conventional methods, thanks to its ability to perform non-targeted analysis [11].

Since then, HTS has made steady progress and has been democratized in medical microbiology, particularly for the etiological diagnosis of complex, atypical infections or those that remain unresolved using conventional methods. HTS has demonstrated its effectiveness in various medical settings, including pneumocystis and joint infections, as well as bacterial meningitis, with good performances [75]. As reviewed by Rodino and Pritt, HTS, also called clinical metagenomics in this context, offers key advantages for the diagnosis of TBDs. Its ability to detect a wide range of pathogens (bacteria, viruses, parasites) from a

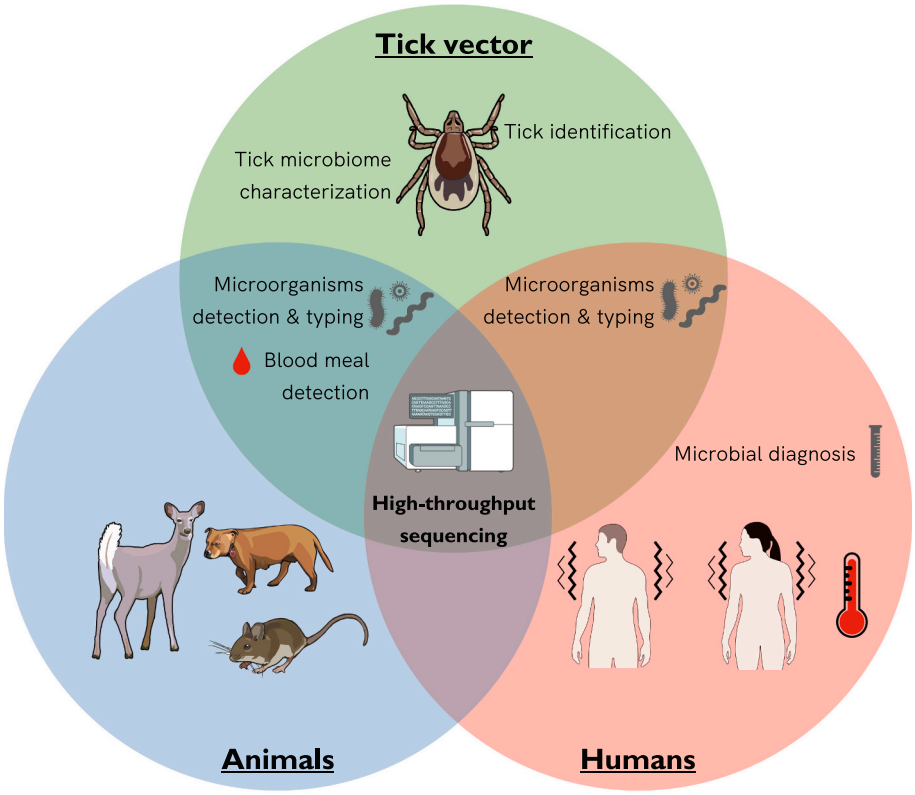

Fig. 1. Multidimensional applications of high-throughput sequencing in ticks, animals, and humans. Illustrations adapted from NIAID Visual & Medical Arts (bioart.niaid.nih.gov).

single clinical sample is particularly valuable in cases of non-specific symptoms or rare etiologies. HTS improves diagnostic yield by reducing the risk of missed or delayed diagnoses. It also enhances patient safety by limiting the risk that important pathogens are not tested for because they are not initially suspected or included in standard diagnostic panels. It also contributes to the discovery of previously unknown or unrecognized tick-borne pathogens. However, its routine implementation still faces major issues, including cost, turnaround time, and the need for clinical validation adapted to organism, sample type, and disease state [76].

5.1. HTS as diagnostic tool

HTS is mainly used for complex clinical situations, especially when diagnoses remain unclear. In some patients, infections with tick-borne microorganisms may be detected incidentally during the diagnostic process. These cases are the subject of case reports and particularly concern immunosuppressed patients. HTS allows, for example, the identification of well-known pathogens, such as *B. afzelii*, *in situ*ations where standard tests, especially serology, remain negative, in patients treated with anti-CD20 antibodies (e.g. rituximab) [77]. It also enables the identification of known microorganisms whose pathogenic potential is currently poorly documented, such as *Spiroplasma ixodetis* [78]. Wang et al. [79] have also shown that HTS from a blood matrix can be used to detect *R. japonica* a pathogen that is rarely identified. Furthermore, this tool is useful for identifying bacterial pathogens in patients with undiagnosed neurological infection, leading to appropriate treatment [80] as in the example of the clinical case reported by Dahdah et al. [77]. Other cases illustrate its contribution: it was used to detect a fragment of the Powassan virus, absent from the current syndromic panel [81]. Similarly, Piantadosi et al. [82] detected several tick-borne pathogens, including Powassan virus, *B. burgdorferi* sl and, more surprisingly, *A. phagocytophilum* in the cerebrospinal fluid (CSF). These agents, sometimes undetectable by conventional PCR in the CSF, have been identified in patients with encephalitis or meningitis. HTS on human samples also led to the description of novel pathogenic bacterial agent like a novel *Anaplasma* species, *A. sparouinense*, infecting human erythrocytes and inducing a novel disease, the Sparouine anaplasmosis but, for which no diagnostic test currently exists. The patient had experienced a prolonged diagnostic odyssey, during which all conventional tests for known pathogens returned negative results. Only 16S rDNA gene barcoding allowed the identification of the infectious agent, now known as *A. sparouinense* [83].

Beyond the elucidation of clinical cases, HTS has also demonstrated its value in larger cohorts of patients. Kingry et al. [84] have developed a targeted metagenomic approach based on 16S rDNA gene amplification followed by HTS, applied to human blood samples to detect a broad spectrum of tick-borne bacteria. This method has made it possible to identify not only expected pathogens, but also poorly characterized agents, such as species related to the *Rickettsia* genus. Rodino et al. [85] improved the approach developed by Kingry et al. [84] by comparing different extraction platforms and two primer sets. This led to an increase in sensitivity and specificity, as well as a reduction in non-specific reads. Branda et al. [86] evaluated the detection of free circulating DNA (also called cell-free DNA) of *B. burgdorferi* sl in plasma by untargeted metagenomic sequencing as a diagnostic tool for early Lyme borreliosis, comparing it with whole blood PCR and serology. This approach identified *B. burgdorferi* sl in 64 % of patients with microbiological confirmation, a sensitivity superior to that of specific blood PCR or acute serology alone, and reached 86 % when combining it with the modified two-tiered testing serological method. Similarly, this method was able to detect low levels of *B. burgdorferi* sl DNA (<3–5 genome equivalent/µL) in 6 of the 9 pediatric patients confirmed by serology [87]. Sanchez-Vicente et al. [88] evaluated the TBDCapSeq approach, a targeted capture method followed by HTS on human blood, which identified tick pathogens in 43 samples compared with 23 by PCR, including

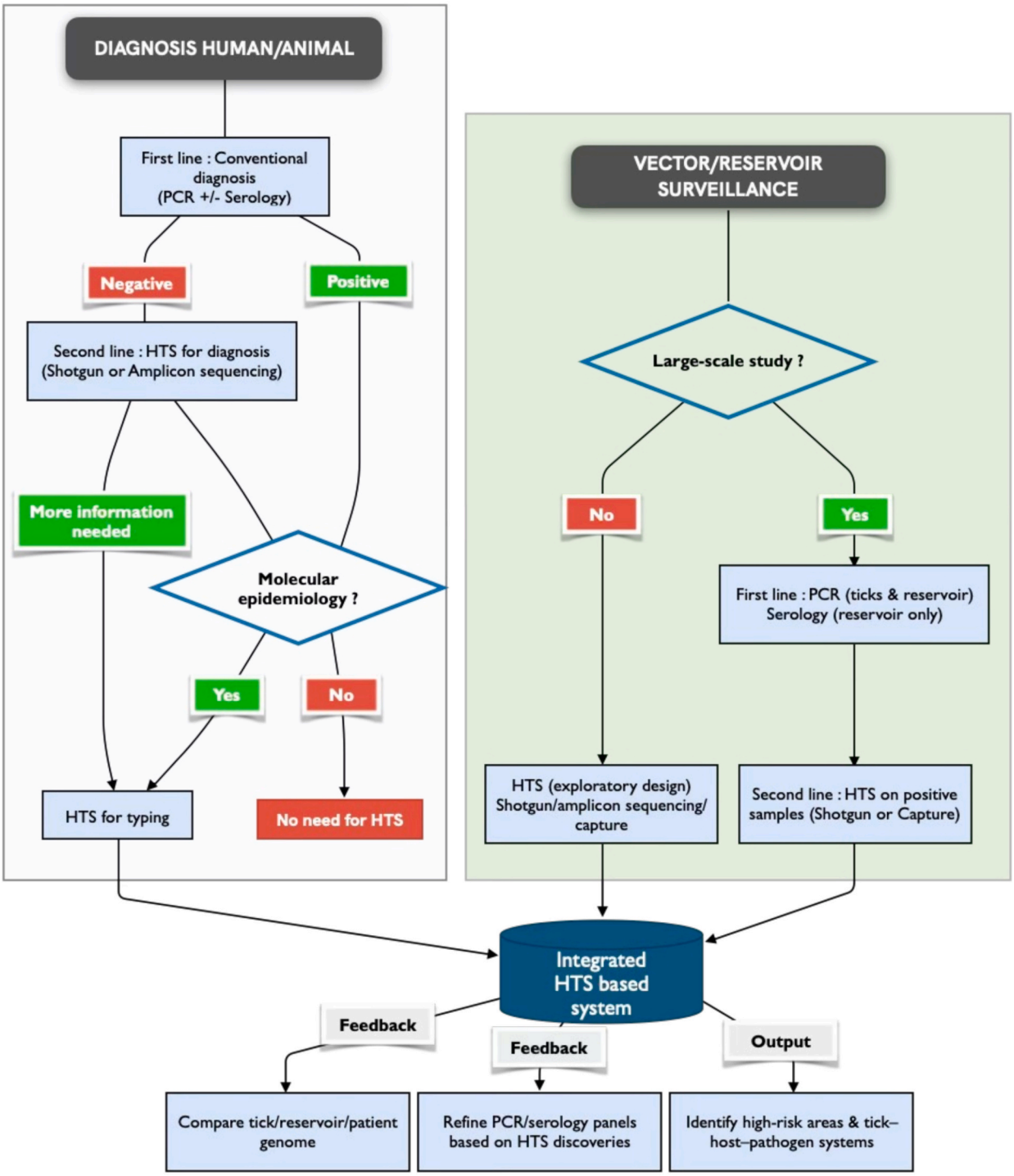

Fig. 2. Decision pathway for the use of high-throughput sequencing versus conventional methods (PCR/serology) in tick-borne disease surveillance and diagnosis within a One Health framework.

B. burgdorferi, *B. microti*, *B. miyamotoi*, *Ehrlichia* spp. and *Rickettsia* spp. In a syndromic approach of fever in tropical settings, the study by Orf et al. [89] conducted in Senegal between 2020 and 2022 used shotgun metagenomics to identify among other *B. crocidurae* in 3.1 % of the clinical blood samples.

5.2. Beyond microbiological diagnosis

In addition to its diagnostic capabilities, HTS can also be used to reconstitute complete genomes from clinical samples, which is a major advantage for the study of pathogens that are difficult or impossible to cultivate. This approach has been particularly used in virology, as shown by several recent studies. Zakotnik et al. [19] have used targeted metagenomics to obtain the complete genome of tick-borne encephalitis virus (TBEV) directly from human cerebrospinal fluid, revealing rare nucleotide substitutions that could be involved in severe clinical forms. D'Addiego et al. [90] have developed a specific probe capture method for sequencing more than 95 % of the CCHF virus genome in clinical samples with a low viral load. This allows them to document the co-circulation of several lineages (I, III, IV, V) in West Africa and to

detect signatures of convergent evolution on the polymerase and glycoprotein genes. In a complementary way, Prayitno et al. [91] demonstrated that nanopore sequencing (MinION) was able to obtain near-complete genomes of the SFTS virus in samples where PCR had produced contradictory results, and to identify a case of inter-clade reassortment, with L, M and S segments belonging to distinct sub-lineages. For bacteria, metagenomic reconstruction was also efficiently used to obtain complete genomes of recently described species, including *A. sparouinense* directly sequenced from a human blood sample [65], *A. amazonensis* from a sloth blood sample, *E. cajennense* (a new close relative of the human pathogen *E. ewingii*) [65] and *B. mahuryensis* from tick bodies [66]. The genome assembly of three tick-borne bacterial species consistently reveals the presence of virulence factors, confirming their medical and veterinary factors [65].

These examples illustrate the ability to go beyond simple diagnosis and gain a deeper understanding of the molecular epidemiology of circulating pathogens. HTS tools allow for an epidemiological connection between pathogens carried by vectors and those that infect humans, through the comparison of circulating tick-borne pathogens to those causing human infection. This has been demonstrated in several studies, especially in virology, even if HTS is not directly used on clinical samples or in conjunction with other detection methods (e.g. serology, classical PCR, culture) [92,93].

6. Conclusion

Fig. 1 summarizes the multidimensional aspect of HTS technologies in the study of ticks and TBDs. They provide an integrated perspective on TBDs, connecting the vector, the microorganisms it harbors, their natural hosts, and the associated clinical cases in humans or animals. HTS makes it possible to detect both known and previously unrecognized pathogens, to describe the tick microbiota in detail, and to identify blood meal sources, and this could be achieved in a single step in the future. This multidimensional capacity strengthens epidemiological surveillance and enhances diagnostic accuracy. One of the major strengths of HTS is its ability to reveal pathogens that are non-cultivable and lack existing diagnostic tests. It can also discriminate between pathogenic and non-pathogenic microorganisms, as in the case of *Coxiella*-like and *Francisella*-like endosymbionts, which lack of virulence genes and instead play essential nutritional roles for the tick. Nevertheless, the technology still faces challenges, including high costs, lack of standardized protocols, and variability in results. Therefore, harmonization of practices is essential to ensure comparability across studies and to fully exploit the potential of HTS as a cornerstone of integrated TBD surveillance systems (Fig. 2).

HTS fits naturally to One Health strategies, as it can generate data from vectors, animal reservoirs and human patients within the same analytical framework. By strengthening the linkage between environmental surveillance and clinical diagnostics, HTS is part of integrated surveillance systems that are essential for a One Health approach to TBDs.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used DeepL Write and Antidote software (Druide informatique) in order to improve the grammar, spelling, and style of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Yannick-Ibrahim Bisso: Writing – original draft. **Olivier Duron:** Writing – original draft. **Olivier Plantard:** Writing – review & editing. **Gilles Prevost:** Writing – review & editing. **Benoit Jaulhac:** Writing – review & editing, Supervision. **Pierre Boyer:** Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data used in this review paper are previously published data which are available online.

References

[1] N. Boulanger, P. Boyer, E. Talagrand-Reboul, Y. Hansmann, Ticks and tick-borne diseases, Med. Mal. Infect. 49 (2019) 87–97, https://doi.org/10.1016/j.medmal.2019.01.007.
[2] J. Shi, Z. Hu, F. Deng, S. Shen, Tick-borne viruses, Virol. Sin. 33 (2018) 21–43, https://doi.org/10.1007/s12250-018-0019-0.
[3] E.M. Galon, A.M. Macalanda, T. Sugi, K. Hayashida, N. Kawai, T. Kidaka, R. H. Ybañez, P.F. Adjou Moumouni, A.E. Ringo, H. Li, S. Ji, J. Yamagishi, A. Ybañez, X. Xuan, Bovine piroplasma populations in the Philippines characterized using targeted amplicon deep sequencing, Microorganisms 11 (2023) 2584, https://doi.org/10.3390/microorganisms11102584.
[4] M. Jalovecka, D. Sojka, M. Ascencio, L. Schnittger, Babesia life cycle – when phylogeny meets biology, Trends Parasitol. 35 (2019) 356–368, https://doi.org/10.1016/j.pt.2019.01.007.
[5] M.A. Bezerra-Santos, L.O. de Macedo, V.-L. Nguyen, R.R. Manoj, Y. Laidoudi, M. S. Latrofa, F. Beugnet, D. Otranto, Cercopithifilaria spp. in ticks of companion animals from Asia: new putative hosts and vectors, Ticks Tick-Borne Dis. 13 (2022) 101957, https://doi.org/10.1016/j.ttbdis.2022.101957.
[6] R. Rosario-Cruz, D.I. Domínguez-García, C. Almazán, F. Rosario Dominguez, A one health epidemiological approach for understanding ticks and tick-borne pathogens, Vector Borne Zoonotic Dis. Larchmt. N 24 (2024) 473–477, https://doi.org/10.1089/vbz.2023.0096.
[7] I. Zortman, M. de Garine-Wichatitsky, E. Arsevska, T. Dub, W. Van Bortel, E. Lefrançois, L. Vial, T. Pollet, A. Binot, A social-ecological systems approach to tick bite and tick-borne disease risk management: exploring collective action in the Occitanie region in southern France, One Health Amst. Neth. 17 (2023) 100630, https://doi.org/10.1016/j.onehlt.2023.100630.
[8] P. Banović, A.A. Díaz-Sánchez, C. Galon, A. Foucault-Simonin, V. Simin, D. Mijatović, L. Papić, A. Wu-Chuang, D. Obregón, S. Moutailler, A. Cabezas-Cruz, A one health approach to study the circulation of tick-borne pathogens: a preliminary study, One Health Amst. Neth. 13 (2021) 100270, https://doi.org/10.1016/j.onehlt.2021.100270.
[9] S. Behjati, P.S. Tarpey, What is next generation sequencing? Arch. Dis. Child. Educ. Pract. Ed. 98 (2013) 236–238, https://doi.org/10.1136/archdischild-2013-304340.
[10] A.M. Nafea, Y. Wang, D. Wang, A.M. Salama, M.A. Aziz, S. Xu, Y. Tong, Application of next-generation sequencing to identify different pathogens, Front. Microbiol. 14 (2023) 1329330, https://doi.org/10.3389/fmicb.2023.1329330.
[11] C.Y. Chiu, S.A. Miller, Clinical metagenomics, Nat. Rev. Genet. 20 (2019) 341–355, https://doi.org/10.1038/s41576-019-0113-7.
[12] C. Lamoureux, L. Surgers, V. Fihman, G. Gricourt, V. Demontant, E. Trawinski, M. N'Debi, C. Gomart, G. Royer, N. Launay, J.-M. Le Glaunec, C. Wemmert, G. La Martire, G. Rossi, R. Lepeule, J.-M. Pawlotsky, C. Rodriguez, P.-L. Woerther, Prospective comparison between shotgun metagenomics and sanger sequencing of the 16S rRNA gene for the etiological diagnosis of infections, Front. Microbiol. 13 (2022) 761873, https://doi.org/10.3389/fmicb.2022.761873.
[13] A.J. McArdle, M. Kaforou, Sensitivity of shotgun metagenomics to host DNA: abundance estimates depend on bioinformatic tools and contamination is the main issue, Access Microbiol. 2 (2020) e000104, https://doi.org/10.1099/acmi.0.000104.
[14] O. Duron, O. Morel, V. Noël, M. Buysse, F. Binetruy, R. Lancelot, E. Loire, C. Ménard, O. Bouchez, F. Vavre, L. Vial, Tick-Bacteria mutualism depends on B vitamin synthesis pathways, Curr. Biol. 28 (2018) 1896–1902.e5, https://doi.org/10.1016/j.cub.2018.04.038.
[15] E.J. Kipp, L.L. Lindsey, B. Khoo, C. Faulk, J.D. Oliver, P.A. Larsen, Metagenomic surveillance for bacterial tick-borne pathogens using nanopore adaptive sampling, Sci. Rep. 13 (2023) 10991, https://doi.org/10.1038/s41598-023-37134-9.
[16] C.R. Fisher, R. Patel, Profiling the immune response to periprosthetic joint infection and non-infectious arthroplasty failure, Antibiot. Basel Switz. 12 (2023) 296, https://doi.org/10.3390/antibiotics12020296.
[17] W. Gu, S. Miller, C.Y. Chiu, Clinical metagenomic next-generation sequencing for pathogen detection, Annu. Rev. Pathol. 14 (2019) 319–338, https://doi.org/10.1146/annurev-pathmechdis-012418-012751.
[18] J. Jovel, J. Patterson, W. Wang, N. Hotte, S. O'Keefe, T. Mitchel, T. Perry, D. Kao, A.L. Mason, K.L. Madsen, G.K.-S. Wong, Characterization of the gut microbiome using 16S or shotgun metagenomics, Front. Microbiol. 7 (2016) 459, https://doi.org/10.3389/fmicb.2016.00459.

[19] S. Zakotnik, N. Knap, P. Bogovič, T.M. Zorec, M. Poljak, F. Strle, T. Avšič-Županc, M. Korva, Complete genome sequencing of tick-borne encephalitis virus directly from clinical samples: comparison of shotgun metagenomic and targeted amplicon-based sequencing, Viruses 14 (2022) 1267, https://doi.org/10.3390/v14061267.
[20] M. Gaudin, C. Desnues, Hybrid capture-based next generation sequencing and its application to human infectious diseases, Front. Microbiol. 9 (2018) 2924, https://doi.org/10.3389/fmicb.2018.02924.
[21] S.N. Naccache, J. Thézé, S.I. Sardi, S. Somasekar, A.L. Greninger, A.C. Bandeira, G. S. Campos, L.B. Tauro, N.R. Faria, O.G. Pybus, C.Y. Chiu, Distinct zika virus lineage in Salvador, Bahia, Brazil, Emerg. Infect. Dis. 22 (2016) 1788–1792, https://doi.org/10.3201/eid2210.160663.
[22] Y. Qiu, R. Nakao, A. Ohnuma, F. Kawamori, C. Sugimoto, Microbial population analysis of the salivary glands of ticks; A possible strategy for the surveillance of bacterial pathogens, PLoS One 9 (2014) e103961, https://doi.org/10.1371/journal.pone.0103961.
[23] S. Higuchi, M. Fujimori, F. Hoshi, S. Kawamura, Y. Yasuda, Development of Babesia gibsoni in the salivary glands of the larval tick, *Rhipicephalus sanguineus*, J. Vet. Med. Sci. 57 (1995) 117–119, https://doi.org/10.1292/jvms.57.117.
[24] X. Didelot, R. Bowden, D.J. Wilson, T.E.A. Peto, D.W. Crook, Transforming clinical microbiology with bacterial genome sequencing, Nat. Rev. Genet. 13 (2012) 601–612, https://doi.org/10.1038/nrg3226.
[25] S. Porcelli, P.L. Deshuillers, S. Moutailler, A.-C. Lagrée, Meta-analysis of tick-borne and other pathogens: co-infection or co-detection? That is the question, Curr. Res. Parasitol. Vector-Borne Dis. 6 (2024) 100219, https://doi.org/10.1016/j.crpvbd.2024.100219.
[26] E. Krupa, A. Dziedziech, R. Paul, S. Bonnet, Update on tick-borne pathogens detection methods within ticks, Curr. Res. Parasitol. Vector-Borne Dis. 6 (2024) 100199, https://doi.org/10.1016/j.crpvbd.2024.100199.
[27] J.S. Lambert, M.J. Cook, J.E. Healy, R. Murtagh, G. Avramovic, S.H. Lee, Metagenomic 16S rRNA gene sequencing survey of Borrelia species in Irish samples of *Ixodes ricinus* ticks, PLoS One 14 (2019) e0209881, https://doi.org/10.1371/journal.pone.0209881.
[28] K. Ergunay, B. Boldbaatar, B.P. Bourke, L. Caicedo-Quiroga, C.L. Tucker, A. G. Letizia, N.G. Cleary, A.G. Lilak, G. Nyamdavaa, S. Tumenjargal, M.E. von Fricken, Y.-M. Linton, Metagenomic nanopore sequencing of tickborne pathogens, Mongolia, Emerg. Infect. Dis. 30 (2024) 105–110, https://doi.org/10.3201/eid3014.240128.
[29] J.D. Carvajal-Agudelo, H.E. Ramírez-Chaves, P.A. Ossa-López, F.A. Rivera-Páez, Bacteria related to tick-borne pathogen assemblages in Ornithodoros cf. hasei (Acari: Argasidae) and blood of the wild mammal hosts in the Orinoquia region, Colombia, Exp. Appl. Acarol. 87 (2022) 253–271, https://doi.org/10.1007/s10493-022-00724-9.
[30] J.L. Occi, D.C. Price, M. Hall, V. Campbell, S. Stronsick, C.F. Sullivan, R. Pesapane, J. Gonzalez, A. Toledo, D.M. Fonseca, Rickettsia and relapsing fever Borrelia in *Alectorobius kelleyi* (Ixodida: Argasidae) from peri domestic bats in the northeastern United States, Ticks Tick-Borne Dis. 14 (2023) 102157, https://doi.org/10.1016/j.ttbdis.2023.102157.
[31] X.-B. Ni, X.-M. Cui, J.-Y. Liu, R.-Z. Ye, Y.-Q. Wu, J.-F. Jiang, Y. Sun, Q. Wang, M.H.-H. Shum, Q.-C. Chang, L. Zhao, X.-H. Han, K. Ma, S.-J. Shen, M.-Z. Zhang, W.-B. Guo, J.-G. Zhu, L. Zhan, L.-J. Li, S.-J. Ding, D.-Y. Zhu, J. Zhang, L.-Y. Xia, X.-Y. Oong, X.-D. Ruan, H.-Z. Shao, T.-C. Que, G.-Y. Liu, C.-H. Du, E.-J. Huang, X. Wang, L.-F. Du, C.-C. Wang, W.-Q. Shi, Y.-S. Pan, Y.-H. Zhou, J.-L. Qu, J. Ma, C.-W. Gong, Q.-Q. Chen, Q. Qin, Tick Genome and Microbiome Consortium (TIGMIC), T.T.-Y. Lam, N. Jia, W.-C. Cao, Metavirome of 31 tick species provides a compendium of 1,801 RNA virus genomes, Nat. Microbiol. 8 (2023) 162–173, https://doi.org/10.1038/s41564-022-01275-w.
[32] J. Dunaj, J. Drewnowska, A. Moniuszko-Malinowska, I. Swiecicka, S. Pancewicz, First metagenomic report of *Borrelia americana* and *Borrelia carolinensis* in Poland - a preliminary study, Ann. Agric. Environ. Med. AAEM 28 (2021) 49–55, https://doi.org/10.26444/aaem/118134.
[33] S. Ouass, N. Boulanger, B. Lelouvier, J.-L.-M. Insonere, C. Lacroux, S. Krief, E. Asalu, N. Rahola, O. Duron, Diversity and phylogeny of the tick-borne bacterial genus Candidatus Allocryptoplasma (Anaplasmataceae), Parasite 30 (2023) 13, https://doi.org/10.1051/parasite/2023014.
[34] T. Azagi, D. Hoornstra, K. Kremer, J.W.R. Hovius, H. Sprong, Evaluation of disease causality of rare *Ixodes ricinus*-borne infections in Europe, Pathog. Basel Switz. 9 (2020), https://doi.org/10.3390/pathogens9020150.
[35] M. Bruley, C. Pasternicki, N. Fattar, J. Amoros, M. Duhayon, K. McCoy, O. Duron, Culturable bacteria and fungi in Ixodes, Dermacentor, Amblyomma and Ornithodoros ticks, Parasite 32 (2025) 20, https://doi.org/10.1051/parasite/2025013.
[36] A. Hojgaard, E. Foster, S.E. Maes, L.M. Osikowicz, C.M. Parise, J. Villalpando, R. J. Eisen, Geographic variation in the distribution of *Anaplasma phagocytophilum* variants in host-seeking *Ixodes scapularis* nymphs and adults in the eastern United States elucidated using next generation sequencing, Ticks Tick-Borne Dis. 15 (2024) 102360, https://doi.org/10.1016/j.ttbdis.2024.102360.
[37] A. Hojgaard, L.M. Osikowicz, M.F. Rizzo, B.N. Ayres, W.L. Nicholson, R.J. Eisen, Using next generation sequencing for molecular detection and differentiation of *Anaplasma phagocytophilum* variants from host seeking *Ixodes scapularis* ticks in the United States, Ticks Tick-Borne Dis. 13 (2022) 102041, https://doi.org/10.1016/j.ttbdis.2022.102041.
[38] Z. Liu, L. Li, W. Xu, Y. Yuan, X. Liang, L. Zhang, Z. Wei, L. Sui, Y. Zhao, Y. Cui, Q. Yin, D. Li, Q. Li, Z. Hou, F. Wei, Q. Liu, Z. Wang, Extensive diversity of RNA viruses in ticks revealed by metagenomics in northeastern China, PLoS Negl. Trop. Dis. 16 (2022) e0011017, https://doi.org/10.1371/journal.pntd.0011017.
[39] C. Martyn, B.M. Hayes, D. Lauko, E. Midthun, G. Castaneda, A. Bosco-Lauth, D. J. Salkeld, A. Kistler, K.S. Pollard, S. Chou, Metatranscriptomic investigation of single *Ixodes pacificus* ticks reveals diverse microbes, viruses, and novel mRNA-like endogenous viral elements, mSystems 9 (2024) e0032124, https://doi.org/10.1128/msystems.00321-24.
[40] S.V. Laredo-Tiscareño, J.A. Garza-Hernandez, C.S. Tangudu, W. Dankaona, C. A. Rodríguez-Alarcón, R. Gonzalez-Peña, J.R. Adame-Gallegos, D.M. Beristain-Ruiz, I.N. Barajas-López, A.M. Hargett, U.G. Munderloh, B.J. Blitvich, Detection of multiple novel viruses in argasid and ixodid ticks in Mexico, Ticks Tick-Borne Dis. 16 (2025) 102455, https://doi.org/10.1016/j.ttbdis.2025.102455.
[41] S. Polsomboon Nelson, K. Ergunay, B.P. Bourke, D.D. Reinbold-Wasson, L. Caicedo-Quiroga, G. Kirkitadze, T. Chunashvili, C.L. Tucker, Y.-M. Linton, Nanopore-based metagenomics reveal a new Rickettsia in Europe, Ticks Tick-Borne Dis. 15 (2024) 102305, https://doi.org/10.1016/j.ttbdis.2023.102305.
[42] A. Brinkmann, O. Hekimoğlu, E. Dinçer, P. Hagedorn, A. Nitsche, K. Ergünay, A cross-sectional screening by next-generation sequencing reveals Rickettsia, Coxiella, Francisella, Borrelia, Babesia, Theileria and Hemolivia species in ticks from Anatolia, Parasit. Vectors 12 (2019) 26, https://doi.org/10.1186/s13071-018-3277-7.
[43] K. Ergunay, M. Mutinda, B. Bourke, S.A. Justi, L. Caicedo-Quiroga, J. Kamau, S. Mutura, I.K. Akunda, E. Cook, F. Gakuya, P. Omondi, S. Murray, D. Zimmerman, Y.-M. Linton, Metagenomic investigation of ticks from Kenyan wildlife reveals diverse microbial pathogens and new country pathogen records, Front. Microbiol. 13 (2022) 932224, https://doi.org/10.3389/fmicb.2022.932224.
[44] M. Buysse, O. Duron, Evidence that microbes identified as tick-borne pathogens are nutritional endosymbionts, Cell 184 (2021) 2259–2260, https://doi.org/10.1016/j.cell.2021.03.053.
[45] O. Duron, Y. Gottlieb, Convergence of nutritional symbioses in obligate blood feeders, Trends Parasitol. 36 (2020) 816–825, https://doi.org/10.1016/j.pt.2020.07.007.
[46] Y. Gottlieb, I. Lalzar, L. Klasson, Distinctive genome reduction rates revealed by genomic analyses of two Coxiella- like endosymbionts in ticks, Genome Biol. Evol. 7 (2015) 1779–1796, https://doi.org/10.1093/gbe/evv108.
[47] M. Buysse, A.M. Floriano, Y. Gottlieb, T. Nardi, F. Comandatore, E. Olivieri, A. Giannetto, A.M. Palomar, B.L. Makepeace, C. Bazzocchi, A. Cafiso, D. Sassera, O. Duron, A dual endosymbiosis supports nutritional adaptation to hematophagy in the invasive tick Hyalomma marginatum, eLife 10 (2021) e72747, https://doi.org/10.7554/eLife.72747.
[48] M.G. Guizzo, L.F. Parizi, R.D. Nunes, R. Schama, R.M. Albano, L. Tirloni, D. P. Oldiges, R.P. Vieira, W.H.C. Oliveira, M.S. de Leite, S.A. Gonzales, M. Farber, O. Martins, I.S. da Vaz, P.L. Oliveira, A Coxiella mutualist symbiont is essential to the development of *Rhipicephalus microplus*, Sci. Rep. 7 (2017) 17554, https://doi.org/10.1038/s41598-017-17309-x.
[49] J. Zhong, A. Jasinskas, A.G. Barbour, Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness, PLoS One 2 (2007) e405, https://doi.org/10.1371/journal.pone.0000405.
[50] M. Ben-Yosef, A. Rot, M. Mahagna, E. Kapri, A. Behar, Y. Gottlieb, Coxiella-like endosymbiont of *Rhipicephalus sanguineus* is required for physiological processes during ontogeny, Front. Microbiol. 11 (2020) 493, https://doi.org/10.3389/fmicb.2020.00493.
[51] M. Buysse, F. Binetruy, R. Leibson, Y. Gottlieb, O. Duron, Ecological contacts and host specificity promote replacement of nutritional endosymbionts in ticks, Microb. Ecol. 83 (2022) 776–788, https://doi.org/10.1007/s00248-021-01773-0.
[52] F. Binetruy, M. Buysse, Q. Lejarre, R. Barosi, M. Villa, N. Rahola, C. Paupy, D. Ayala, O. Duron, Microbial community structure reveals instability of nutritional symbiosis during the evolutionary radiation of Amblyomma ticks, Mol. Ecol. 29 (2020) 1016–1029, https://doi.org/10.1111/mec.15373.
[53] O. Duron, F. Binetruy, V. Noël, J. Cremaschi, K.D. McCoy, C. Arnathau, O. Plantard, J. Goolsby, A.A. Pérez de León, D.J.A. Heylen, A.R. Van Oosten, Y. Gottlieb, G. Baneth, A.A. Guglielmone, A. Estrada-Peña, M.N. Opara, L. Zenner, F. Vavre, C. Chevillon, Evolutionary changes in symbiont community structure in ticks, Mol. Ecol. 26 (2017) 2905–2921, https://doi.org/10.1111/mec.14094.
[54] O. Duron, K. Sidi-Boumedine, E. Rousset, S. Moutailler, E. Jourdain, The importance of ticks in Q fever transmission: what has (and has not) been demonstrated? Trends Parasitol. 31 (2015) 536–552, https://doi.org/10.1016/j.pt.2015.06.014.
[55] O. Duron, The IS1111 insertion sequence used for detection of *Coxiella burnetii* is widespread in Coxiella-like endosymbionts of ticks, FEMS Microbiol. Lett. 362 (2015) fnv132, https://doi.org/10.1093/femsle/fnv132.
[56] O. Duron, Nutritional symbiosis in ticks: singularities of the genus *Ixodes*, Trends Parasitol. 40 (2024) 696–706, https://doi.org/10.1016/j.pt.2024.06.006.
[57] S. Narasimhan, N. Rajeevan, L. Liu, Y.O. Zhao, J. Heisig, J. Pan, R. Eppler-Epstein, K. Deponte, D. Fish, E. Fikrig, Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete, Cell Host Microbe 15 (2014) 58–71, https://doi.org/10.1016/j.chom.2013.12.001.
[58] N. Wei, J. Cao, H. Zhang, Y. Zhou, J. Zhou, The tick microbiota dysbiosis promote tick-borne pathogen transstadial transmission in a babesia microti-infected mouse model, Front. Cell. Infect. Microbiol. 11 (2021) 713466, https://doi.org/10.3389/fcimb.2021.713466.
[59] C.A. Gall, K.E. Reif, G.A. Scoles, K.L. Mason, M. Mousel, S.M. Noh, K.A. Brayton, The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility, ISME J. 10 (2016) 1846–1855, https://doi.org/10.1038/ismej.2015.266.
[60] O. Klyachko, B.D. Stein, N. Grindle, K. Clay, C. Fuqua, Localization and visualization of a Coxiella-type symbiont within the lone star tick, *Amblyomma*

americanum, Appl. Environ. Microbiol. 73 (2007) 6584–6594, https://doi.org/10.1128/AEM.00537-07.
[61] L. Mazuecos, P. Alberdi, A. Hernández-Jarguín, M. Contreras, M. Villar, A. Cabezas-Cruz, L. Simo, A. González-García, S. Díaz-Sánchez, G. Neelakanta, S. I. Bonnet, E. Fikrig, J. de la Fuente, Frankenbacteriosis targeting interactions between pathogen and symbiont to control infection in the tick vector, iScience 26 (2023) 106697, https://doi.org/10.1016/j.isci.2023.106697.
[62] F. Binetruy, M. Dupraz, M. Buysse, O. Duron, Surface sterilization methods impact measures of internal microbial diversity in ticks, Parasit. Vectors 12 (2019) 268, https://doi.org/10.1186/s13071-019-3517-5.
[63] Y. Ge, G. Guo, B. Ge, H. Yin, H. Yin, The spleen microbiota of small wild mammals reveals distinct patterns with tick-borne bacteria, PLoS Negl. Trop. Dis. 12 (2018) e0006499, https://doi.org/10.1371/journal.pntd.0006499.
[64] A.R. Issae, A.S. Katakweba, R.P. Kicheleri, A.A. Chengula, M. van Zwetselaar, C. J. Kasanga, Exploring pathogenic and zoonotic bacteria from wild rodents, dogs, and humans of the Ngorongoro District in Tanzania using metagenomics next-generation sequencing, Zoonotic Dis. 3 (2023) 226–242, https://doi.org/10.3390/zoonoticdis3030019.
[65] M. Buysse, R. Koual, F. Binetruy, B. de Thoisy, X. Baudrimont, S. Garnier, M. Douine, C. Chevillon, F. Delsuc, F. Catzeflis, D. Bouchon, O. Duron, Detection of Anaplasma and Ehrlichia bacteria in humans, wildlife, and ticks in the Amazon rainforest, Nat. Commun. 15 (2024) 3988, https://doi.org/10.1038/s41467-024-48459-y.
[66] F. Binetruy, S. Garnier, N. Boulanger, É. Talagrand-Reboul, E. Loire, B. Faivre, V. Noël, M. Buysse, O. Duron, A novel Borrelia species, intermediate between Lyme disease and relapsing fever groups, in neotropical passerine-associated ticks, Sci. Rep. 10 (2020) 10596, https://doi.org/10.1038/s41598-020-66828-7.
[67] X. Wang, J.J. Brown, M.V. Lilly, L. Plimpton, C.M. Murrell, S.G. Llanos-Soto, J. D. Zehr, Y. Sun, G. Reboul, K. Sams, A. Sabaawy, L. Singh, R.L. Tallmadge, P. K. Mitchell, R.R. Anderson, S.G. Ladd-Wilson, E. Bourgikos, C.B.F. Vogels, X. Olarte-Castillo, M. Diuk-Wasser, L.B. Goodman, TICKHUNTER: a targeted hybridization-capture sequencing approach for the detection and characterization of tick-borne pathogens and blood meals, bioRxiv (2025), https://doi.org/10.1101/2025.06.06.658355, 2025.06.06.658355.
[68] K. Ergunay, E. Dincer, S.A. Justi, B.P. Bourke, S.P. Nelson, H.-M. Liao, M. O. Timurkan, B. Oguz, I. Sahindokuyucu, O.F. Gokcecik, D.D. Reinbold-Wasson, L. Jiang, N.L. Achee, J.P. Grieco, Y.-M. Linton, Impact of nanopore-based metagenome sequencing on tick-borne virus detection, Front. Microbiol. 14 (2023) 1177651, https://doi.org/10.3389/fmicb.2023.1177651.
[69] J. Jiao, Z. Lu, Y. Yu, Y. Ou, M. Fu, Y. Zhao, N. Wu, M. Zhao, Y. Liu, Y. Sun, B. Wen, D. Zhou, Q. Yuan, X. Xiong, Identification of tick-borne pathogens by metagenomic next-generation sequencing in *Dermacentor nuttalli* and *Ixodes persulcatus* in Inner Mongolia, China, Parasit. Vectors 14 (2021) 287, https://doi.org/10.1186/s13071-021-04740-3.
[70] J. Intirach, X. Lv, N. Sutthanont, B. Cai, D. Champakaew, T. Chen, Q. Han, Z. Lv, Molecular and next-generation sequencing analysis of tick-borne pathogens of Rhipicephalus ticks (Acari: Ixodidae) in cattle and dogs, Acta Trop. 252 (2024) 107138, https://doi.org/10.1016/j.actatropica.2024.107138.
[71] G.A. Matulis, J. Sakolvaree, B. Boldbaatar, N. Cleary, R. Takhampunya, B.K. Poole-Smith, A.A. Lilak, D. Altantogtokh, N. Tsogbadrakh, N. Chanarat, N. Youngdech, E. J. Lindroth, J.M. Fiorenzano, A.G. Letizia, M.E. von Fricken, Applying next generation sequencing to detect tick-pathogens in Dermacentor nuttalli, *Ixodes persulcatus*, and *Hyalomma asiaticum* collected from Mongolia, Ticks Tick-Borne Dis. 14 (2023) 102203, https://doi.org/10.1016/j.ttbdis.2023.102203.
[72] S. Su, M.-Y. Cui, L.-L. Xing, R.-J. Gao, L. Mu, M. Hong, Q.-Q. Guo, H. Ren, J.-F. Yu, X.-Y. Si, M. Eerde, Metatranscriptomic analysis reveals the diversity of RNA viruses in ticks in Inner Mongolia, China, PLoS Negl. Trop. Dis. 18 (2024) e0012706, https://doi.org/10.1371/journal.pntd.0012706.
[73] L.M. Osikowicz, S.E. Maes, R.J. Eisen, A. Hojgaard, A next generation sequencing assay combining Ixodes species identification with pathogen detection to support tick surveillance efforts in the United States, Ticks Tick-Borne Dis. 15 (2024) 102343, https://doi.org/10.1016/j.ttbdis.2024.102343.
[74] B. Xu, L. Liu, X. Huang, H. Ma, Y. Zhang, Y. Du, P. Wang, X. Tang, H. Wang, K. Kang, S. Zhang, G. Zhao, W. Wu, Y. Yang, H. Chen, F. Mu, W. Chen, Metagenomic analysis of fever, thrombocytopenia and leukopenia syndrome (FTLS) in Henan Province, China: discovery of a new bunyavirus, PLoS Pathog. 7 (2011) e1002369, https://doi.org/10.1371/journal.ppat.1002369.
[75] H. Cao, Y. Chen, L. Ge, J.S. Kwong, H. Lai, F. Hu, R. Zhang, H. Zhao, L. Hu, R. He, W. Zheng, J. Zhang, An umbrella review of the diagnostic value of next-generation sequencing in infectious diseases, Int. J. Clin. Pharmacol. Ther. 46 (2024) 780–794, https://doi.org/10.1007/s11096-024-01704-2.
[76] K.G. Rodino, B.S. Pritt, Novel applications of metagenomics for detection of tickborne pathogens, Clin. Chem. 68 (2022) 69–74, https://doi.org/10.1093/clinchem/hvab228.
[77] V. Dahdah, K. Chevalier, P. Arias, S. Soliman, A. Raffetin, O. Benveniste, M.-Y. Khitri, Seronegativity and anti-CD20: when a treatment compromises the diagnosis, Rev. Med. Interne 46 (2025) 293–297, https://doi.org/10.1016/j.revmed.2025.03.426.
[78] J. Eimer, L. Fernström, L. Rohlén, A. Grankvist, K. Loo, E. Nyman, A. J. Henningsson, M. Haglund, V. Hultqvist, J. Sjöwall, C. Wennerås, T. Schön, *Spiroplasma ixodetis* infections in immunocompetent and immunosuppressed patients after tick exposure, Sweden, Emerg. Infect. Dis. 28 (2022) 1681–1685, https://doi.org/10.3201/eid2808.212524.
[79] J. Wang, S. Fan, J. He, X. Jiang, R. Lin, Y. Ji, Y. Ge, A case report of fatal *Rickettsia japonica* bloodstream infection in Zhejiang, China, Future Microbiol. 19 (2024) 1529–1534, https://doi.org/10.1080/17460913.2024.2406122.
[80] R. Kanaujia, M. Biswal, A. Angrup, P. Ray, Diagnostic accuracy of the metagenomic next-generation sequencing (mNGS) for detection of bacterial meningoencephalitis: a systematic review and meta-analysis, Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol. 41 (2022) 881–891, https://doi.org/10.1007/s10096-022-04445-0.
[81] M. Farrington, J. Elenz, M. Ginsberg, C.Y. Chiu, S. Miller, S.F. Pangonis, Powassan virus infection detected by metagenomic next-generation sequencing, Ohio, USA, Emerg. Infect. Dis. 29 (2023) 838–841, https://doi.org/10.3201/eid2904.221005.
[82] A. Piantadosi, S.S. Mukerji, S. Ye, M.J. Leone, L.M. Freimark, D. Park, G. Adams, J. Lemieux, S. Kanjilal, I.H. Solomon, A.A. Ahmed, R. Goldstein, V. Ganesh, B. Ostrem, K.C. Cummins, J.M. Thon, C.M. Kinsella, E. Rosenberg, M.P. Frosch, M. B. Goldberg, T.A. Cho, P. Sabeti, Enhanced virus detection and metagenomic sequencing in patients with meningitis and encephalitis, mBio 12 (2021) e0114321, https://doi.org/10.1128/mBio.01143-21.
[83] O. Duron, R. Koual, L. Musset, M. Buysse, Y. Lambert, B. Jaulhac, D. Blanchet, K. D. Alsibai, Y. Lazrek, L. Epelboin, P. Deshuillers, C. Michaud, M. Douine, Novel chronic anaplasmosis in splenectomized patient, Amazon rainforest, Emerg. Infect. Dis. 28 (2022) 1673–1676, https://doi.org/10.3201/eid2808.212425.
[84] L. Kingry, S. Sheldon, S. Oatman, B. Pritt, M. Anacker, J. Bjork, D. Neitzel, A. Strain, J. Berry, L. Sloan, L. Respicio-Kingry, E. Dietrich, K. Bloch, A. Moncayo, G. Srinivasamoorthy, B. Hu, A. Hinckley, P. Mead, K. Kugeler, J. Petersen, Targeted metagenomics for clinical detection and discovery of bacterial tick-borne pathogens, J. Clin. Microbiol. 58 (2020) e00147-20, https://doi.org/10.1128/JCM.00147-20.
[85] K.G. Rodino, M.J. Wolf, S. Sheldon, L.C. Kingry, J.M. Petersen, R. Patel, B.S. Pritt, Detection of tick-borne bacteria from whole blood using 16S ribosomal RNA gene PCR followed by next-generation sequencing, J. Clin. Microbiol. 59 (2021) e03129-20, https://doi.org/10.1128/JCM.03129-20.
[86] J.A. Branda, J.E. Lemieux, L. Blair, A.A. Ahmed, D.K. Hong, S. Bercovici, T. A. Blauwkamp, D. Hollemon, C. Ho, K. Strle, N.S. Damle, T.J. Lepore, N.R. Pollock, Detection of *Borrelia burgdorferi* cell-free DNA in human plasma samples for improved diagnosis of early Lyme borreliosis, Clin. Infect. Dis. 73 (2021) e2355–e2361, https://doi.org/10.1093/cid/ciaa858.
[87] A.S. Handel, A.A. Ahmed, S. Venkatasubrahmanyam, S. Bercovici, Q. Mao, C. Ho, D.D. Hollemon, C. Beneri, Plasma microbial cell-free DNA sequencing for diagnosis of Pediatric Lyme disease, Pediatr. Infect. Dis. J. 44 (2025) 473–475, https://doi.org/10.1097/INF.0000000000004707.
[88] S. Sanchez-Vicente, K. Jain, T. Tagliafierro, A. Gokden, V. Kapoor, C. Guo, E. J. Horn, W.I. Lipkin, R. Tokarz, Capture sequencing enables sensitive detection of tick-borne agents in human blood, Front. Microbiol. 13 (2022) 837621, https://doi.org/10.3389/fmicb.2022.837621.
[89] G.S. Orf, A.D. Ahouidi, M. Mata, C. Diedhiou, A. Mboup, A. Padane, N.M. Manga, A.T. Dela-Del Lawson, F. Averhoff, M.G. Berg, G.A. Cloherty, S. Mboup, Next-generation sequencing survey of acute febrile illness in Senegal (2020−2022), Front. Microbiol. 15 (2024) 1362714, https://doi.org/10.3389/fmicb.2024.1362714.
[90] J. D'Addiego, S. Shah, A.N. Pektaş, B.N.K. Bağci, M. Öz, S. Sebastianelli, N. Elaldı, D.J. Allen, R. Hewson, Development of targeted whole genome sequencing approaches for Crimean-Congo haemorrhagic fever virus (CCHFV), Virus Res. 350 (2024) 199464, https://doi.org/10.1016/j.virusres.2024.199464.
[91] S.P. Prayitno, Y.G. Cho, E.S. Kim, K. Park, S. Lee, A. Natasha, J. Park, J.-W. Song, Y. S. Kim, S.S. Lee, W.-K. Kim, Amplicon-based MinION sequencing complements severe fever with thrombocytopenia syndrome (SFTS) diagnosis via real-time RT-PCR in patients with suspected SFTS, J. Korean Med. Sci. 40 (2025) e69, https://doi.org/10.3346/jkms.2025.40.e69.
[92] H.M. Savage, M.S. Godsey, A. Lambert, N.A. Panella, K.L. Burkhalter, J.R. Harmon, R.R. Lash, D.C. Ashley, W.L. Nicholson, First detection of heartland virus (Bunyaviridae: Phlebovirus) from field collected arthropods, Am. J. Trop. Med. Hyg. 89 (2013) 445–452, https://doi.org/10.4269/ajtmh.13-0209.
[93] A.P. Dupuis, M.A. Prusinski, C. O'Connor, J.G. Maffei, C.A. Koetzner, T. E. Zembsch, S.D. Zink, A.L. White, M.P. Santoriello, C.L. Romano, G. Xu, F. Ribbe, S.R. Campbell, S.M. Rich, P.B. Backenson, L.D. Kramer, A.T. Ciota, Bourbon virus transmission, New York, USA, Emerg. Infect. Dis. 29 (2023) 145–148, https://doi.org/10.3201/eid2901.220283.