



# Successful diagnosis and treatment of *Borrelia miyamotoi* in a patient with joint and muscle pains, ME/CFS and cognitive dysfunction following tick bites: a case report

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## Results

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## Abstract

**Introduction:** Diagnosing *Borrelia miyamotoi* disease (BMD) presents challenges due to its overlap with Lyme disease (LD) symptoms and the lack of reliable laboratory diagnostics. This case study demonstrates the successful use of phage-based PCR (phb-PCR) in identifying *B. miyamotoi* in a patient with multiple tick bites. A 46-year-old female presented with joint and muscle pain, chronic fatigue, and cognitive impairment after being bitten by ticks in Europe. Standard diagnostic tests, including Enzyme-linked immunosorbent assay (ELISA), immunoblot for LD, and antibody tests for *Bartonella*, *Anaplasma*, and autoimmune conditions, all returned negative results. However, phb-PCR identified the presence of *B. miyamotoi*. The patient was treated with intravenous ceftriaxone, oral azithromycin, and intravenous vitamin and mineral therapy, resulting in significant improvement in symptoms, including reduced pain, improved cognitive function, and decreased fatigue. This case emphasises the importance of direct diagnostic methods like phb-PCR for accurately identifying BMD, especially when conventional serological tests fail. Clinicians should consider testing for *B. miyamotoi* in cases of complex tick-borne diseases for timely and effective management.

## Introduction

Diagnosing tick-borne diseases (TBDs) like Lyme disease (LD) and tick-borne relapsing fever (TBRF) is challenging because they share overlapping symptoms, such as fever, chills, headaches, muscle and joint pain, fatigue, arthritis and neurological manifestations (Guérin et al., 2023). Although both LD and TBRF diagnoses are difficult, the presence of the erythema migrans (EM) rash in approximately 60%–80% of LD patients provides a slight advantage in identifying LD (Steere et al., 2016; Grażlewska and Holec-Gąsior, 2023). In contrast, the absence of this characteristic rash in TBRF complicates its diagnosis (Jakab et al., 2022).

The geographic distribution of LD-causing *Borrelia* species varies greatly. For instance, *Borrelia burgdorferi* sensu stricto (s.s.) is the most common LD-causing species in the United States, whereas *B. garinii* and *B. afzelii* are predominant in Europe and Asia. Additionally, other *Borrelia* species such as *B. mayonii*, *B. spielmanii*, *B. bissettii* and *B. valaisiana* are also linked to LD, although they are encountered less frequently (Marques et al., 2021). This geographic specificity begs the question of the need for regionally tailored diagnostic and treatment strategies.

The increasing complexity of relapsing fever (RF)-causing *Borrelia* species has significantly complicated their diagnosis and management. *Borrelia* species commonly causing TBRF include *B. coriaceae*, *B. lonestari*, *B. duttonii*, *B. crocidurae*, *B. hispanica*, *B. parkeri*, *B. turicatae* and *B. hermsii*, all of which are transmitted by soft-bodied ticks (Ras et al., 1996). In contrast, *B. recurrentis*, which also causes RF, is transmitted by the human body louse (Cutler et al., 1997). Additionally, *B. miyamotoi*, discovered in Japan in 1995, is an exception among TBRF *Borrelia* species because it is transmitted by hard-bodied ticks, the same vectors responsible for LD (Fukunaga et al., 1995). Consequently, a single bite from a hard-bodied tick could potentially transmit both LD and TBRF. This co-transmission of LD and TBRF has overwhelmed diagnostic resources, highlighting the need for improved detection methods and more adaptable testing protocols that are practical for clinical use.

Diagnosing *B. miyamotoi* disease (BMD), a type of TBRF, is challenging because it shares symptoms with LD but lacks the characteristic EM rash (Platonov et al., 2011; Krause and Barbour, 2015). Due to these diagnostic challenges, laboratory detection of *B. miyamotoi* is crucial. However, current serological detection methods are unsuitable due to cross-reactivity (Koetsveld et al., 2020; Grażlewska and Holec-Gąsior, 2023). The CDC (Centers for Disease Control and Prevention) recommends PCR testing to detect *B. miyamotoi*, but bacteria-based PCR methods often have low sensitivity (Karan et al., 2018; Boyer et al., 2020).

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**Table 1.** Summary of diagnostic test results for the patient

Test	Method	Result	Interpretation
<i>B. burgdorferi</i> sensu lato - ELISA	Enzygnost® <i>Borrelia</i> Lyme link VlsE/IgG and IgM ELISA	IgG: <4 U/mL, IgM: Negative	No evidence of current or past LD infection
<i>B. burgdorferi</i> sensu lato - Immunoblot	<i>Borrelia</i> Europe plus Tpn17 LINE by Sekisui Virotech	No reactive bands detected	No evidence of current or past LD infection
<i>B. henselae</i> - IFA	Vircell IFA Kit	IgG: <1:64	Negative, no detectable immune response
<i>B. quintana</i> - IFA	Vircell IFA Kit	IgG: <1:64	Negative, no detectable immune response
<i>A. phagocytophilum</i> - IFA	Focus Diagnostics IFA Kits	IgM: 1:20 (Inconclusive), IgG: <1:64	Inconclusive IgM result, negative IgG result
Antinuclear antibodies (ANA)	Indirect Immunofluorescence (IIF) on HEp-2 cells	Positive at 1/320, homogeneous irregular/speckled	Suggestive of an autoimmune aetiology
Anti-Ro/SS-A antibodies	ELISA	Negative	No evidence of specific autoimmune disease
Anti-La/SS-B antibodies	ELISA	Negative	No evidence of specific autoimmune disease
Anti-Sm antibodies	ELISA	Negative	No evidence of specific autoimmune disease
Anti-RNP antibodies	ELISA	Negative	No evidence of specific autoimmune disease
Anti-Scl-70 antibodies	ELISA	Negative	No evidence of specific autoimmune disease
Anti-dsDNA antibodies	ELISA IgG + IgM	<30 U/mL (Reference: <50 U/mL)	No significant presence of anti-dsDNA antibodies
phb-PCR	PCR targeting <i>B. miyamotoi</i> phage genes	Positive	Confirmed presence of <i>B. miyamotoi</i> , validated by sequencing

To enhance PCR sensitivity, phage-based PCR (phb-PCR) has emerged as a promising solution for the sensitive detection of bacterial species. Unlike traditional PCR, which targets bacterial DNA directly, phb-PCR detects multiple-copy phage sequences associated with the bacteria, providing higher sensitivity (Luo *et al.*, 2010; Shan *et al.*, 2021). This approach leverages the intrinsic specificity of phages for their bacterial hosts, ensuring that phage detection strongly indicates the presence of the target bacteria (Mandilara *et al.*, 2006; Shivaram *et al.*, 2023). Researchers have successfully used phb-PCR to detect tuberculosis, *Borrelia burgdorferi* s.l. and *Borrelia miyamotoi* (Swift *et al.*, 2020; Shan *et al.*, 2021; Shan *et al.*, 2023).

In this case report, we showcased the positive impact of phb-PCR in diagnosing BMD and developing an effective treatment plan. After encountering multiple negative serological tests and struggling to identify the causative agent, we turned to phb-PCR. Recognising the increasing cases of *B. miyamotoi*, we specifically tested for this pathogen (Wormser *et al.*, 2019; Xu *et al.*, 2021; Cleveland *et al.*, 2023). Our approach exemplifies the potential of phb-PCR in identifying *B. miyamotoi* infections and guiding appropriate treatment strategies. Testing for *B. miyamotoi* when serological tests yield negative results in complicated tick-borne diseases could be a wise decision when coupled with clinical judgement. Clinicians, scientists and patient charity workers should collaborate to raise awareness of tick-borne diseases and BMD, ensuring patients receive timely and accurate diagnostics and treatment.

## Methods

We explored the complex medical history of a 46-year-old female patient who experienced three distinct tick exposures. The first exposure occurred in July 2006, the second in April 2016, and the third in June 2016. Following these exposures, the patient presented with symptoms including joint and muscle pains, fever, chill, myalgic encephalomyelitis/chronic fatigue syndrome

(ME/CFS) and cognitive dysfunction ('brain fog'), but did not exhibit the hallmark EM rash of LD.

The various diagnostic tests conducted for the patient are summarised in Table 1. The patient underwent diagnostic evaluation at the National Reference Centre for Lyme Diseases in Strasbourg, France. Initial screening used Enzygnost® *Borrelia* Lyme IgM and Enzygnost® *Borrelia* Lyme link VlsE/IgG Enzyme-linked immunosorbent assay (ELISA) by Siemens to detect the patient's immune response to *B. burgdorferi* s.l. species (Hjetland *et al.*, 2014). Immunoblot testing employed the *Borrelia* Europe plus Tpn17 LINE immunoblot by Sekisui Virotech to analyse the detailed immune response to specific *Borrelia* antigens (De Bont *et al.*, 2022).

Subsequent diagnostic efforts included multiple tests to identify the causative agent. Vircell's *Bartonella* indirect immunofluorescence assay (IFA) kits were used to screen for IgG antibodies against *B. henselae* and *B. quintana* (Aydin *et al.*, 2014), while Focus Diagnostics IFA kits were employed to detect IgG and IgM antibodies against *Anaplasma phagocytophilum* (Chochlak *et al.*, 2009).

To explore potential autoimmune disorders, an antinuclear antibodies (ANA) test was conducted using the indirect immunofluorescence technique on HEp-2 cells (Sack *et al.*, 2009). Additionally, specific antibodies against extractable nuclear antigens (ENAs) and other nuclear and cytoplasmic components were tested using ELISA for specific autoantibodies included anti-Ro/SS-A, anti-La/SS-B, anti-Sm, anti-RNP, anti-Scl-70 and anti-dsDNA antibodies. Finally, anti-double stranded DNA (anti-dsDNA) were assessed using ELISA for IgG and IgM (Subasic *et al.*, 2009).

A proprietary phb-PCR test designed to detect *B. miyamotoi* was employed (Shan *et al.*, 2018). This test, offered by RED Laboratories, was performed in compliance with Good Laboratory Practice and conducted according to the MIQE guidelines (Bustin *et al.*, 2009). The phb-PCR test has been evaluated using analytical, clinical and tick samples and its accuracy and reliability have been validated and peer-reviewed, with results published in scientific

**Table 2.** Autoantibodies, their target antigens, associated diseases in autoimmune conditions

Autoantibody	Target antigen	Associated diseases
Anti-Ro/SS-A	Ro/SS-A antigens are ribonucleoproteins found in the nucleus and cytoplasm.	Sjögren's syndrome, Systemic lupus erythematosus (SLE)
Anti-La/SS-B	La/SS-B antigens are ribonucleoproteins that interact with nascent RNA transcripts.	Sjögren's syndrome, SLE
Anti-Sm (Anti-Smith)	Sm antigens are nuclear ribonucleoproteins that are major components of the eukaryotic spliceosome.	SLE
Anti-RNP (Anti-Ribonucleoprotein)	U1-RNP antigens are components of small nuclear ribonucleoproteins (snRNPs) involved in pre-mRNA splicing.	Mixed connective tissue disease (MCTD), SLE, systemic sclerosis
Anti-Scl-70 (Anti-Topoisomerase I)	Scl-70 antigen is the topoisomerase I enzyme involved in DNA replication and repair.	Systemic sclerosis, particularly the diffuse cutaneous form of the disease
Anti-dsDNA	Double stranded DNA	SLE

literature (Shan et al., 2021, 2023). The PCR product was sequenced using the Sanger method.

To evaluate the patient's progress post-treatment, a series of detailed follow-up assessments were conducted:

- Quality of life measurements:** Quality of life was assessed using the Short Form Health Survey (SF-36) (Ware et al., 1992). The SF-36 evaluates multiple dimensions of health, including physical functioning, bodily pain, general health perceptions, vitality, social functioning, emotional role functioning and mental health.
- Symptom severity tracking:** Symptom severity was tracked using the Fatigue Severity Scale (Shahid et al., 2012) and the Visual Analogue Scale (Hawker et al., 2011) for pain assessment. These scales provide objective data on symptom progression and treatment response, which is essential for effectively managing and adjusting therapeutic strategies.
- Cognitive function assessment:** Cognitive function in patients was evaluated using the Montreal Cognitive Assessment (Nasreddine et al., 2005). This assessment is crucial for detecting cognitive impairments commonly seen in LD and TBDs and tracking improvements with treatment.
- Laboratory testing:** We conducted phb-PCR on the patient's sample after completing the treatment to confirm the absence of *B. miyamotoi*.

## Results

### 1. Diagnostic results

For clarity and ease of access, detailed diagnostic results and interpretations are summarised in Table 1. Each individual test and its results are explained in the text below.

The patient's diagnostic evaluation for LD yielded negative results. The IgG and IgM ELISA showed IgG levels below the diagnostic threshold (<4 U/mL) and a negative IgM result. Additionally, the immunoblot detected no reactive bands. These findings collectively suggest that the patient has not been exposed to *B. burgdorferi sensu lato* and does not have an active or previous infection.

Subsequent diagnostic efforts using IFA tests revealed no evidence of exposure to *B. henselae* or *B. quintana*, as indicated by negative IgG results (<1:64) for both pathogens. For *A. phagocytophilum*, the IFA tests showed an inconclusive IgM

result at 1:20 and a negative IgG result (<1:64). These findings jointly suggest that there is no detectable immune response indicative of infection by *B. henselae*, *B. quintana* or *A. phagocytophilum* in the patient.

The ANA test for autoimmune markers showed a positive result at a dilution of 1:320 with a homogeneous irregular/speckled pattern. This positive ANA suggests a possible autoimmune aetiology. However, specific autoantibodies, including anti-Ro/SS-A, anti-La/SS-B, anti-Sm, anti-RNP and anti-Scl-70 (detailed in Table 2), were all negative, indicating no evidence of specific autoimmune diseases commonly associated with these antibodies. Additionally, anti-dsDNA antibodies tested negative, with levels measured at <30 U/mL, which is below the reference value of <50 U/mL.

In summary, while the positive ANA result suggests a potential autoimmune condition, the absence of specific autoantibodies does not support the initial ANA finding, thereby excluding certain autoimmune diseases.

Given the negative results from the serological tests, we decided to switch from antibody-based methods to a direct test for detecting *B. miyamotoi*. The phb-PCR assay detected *B. miyamotoi*, and sequencing of the PCR product confirmed its identity. All controls (positive, negative, internal and extraction) in the PCR functioned correctly.

### 2. Therapeutic interventions

Following the detection of *B. miyamotoi* from the patient's blood, a targeted treatment regimen was established, focusing on antibiotic therapy against *B. miyamotoi*, complemented by comprehensive nutritional support (Eikeland et al., 2024). The treatment regimen involved:

#### Antibiotic therapy

- Intravenous Ceftriaxone: Administered in 12-day cycles.
- Oral Azithromycin: Administered concurrently with the intravenous ceftriaxone cycles.
- Rest Periods: Each antibiotic cycle was followed by a three-week rest period.

#### Nutritional support

During the antibiotic treatment phase, the patient received intravenous nutrient infusions for 18 days. This nutritional

**Table 3.** Improvement in symptoms before and after treatment

Parameter	Pre-treatment	Post-treatment	Interpretation
General health (SF-36)	40%	60%	Significant improvement in overall well-being and physical health.
Energy (SF-36)	25%	70%	Substantial increase in energy levels indicates enhanced physical and mental stamina.
Social (SF-36)	60%	75%	Improved social functioning reflects better social interactions.
Health Change (SF-36)	25%	100%	Improvement in perceived health change shows a positive perception of health progress.
Fatigue (FSS Score)	40	26	Fatigue severity decreased from high to low, demonstrating effective alleviation of fatigue.
Pain (VAS Score)	75 mm	45 mm	Pain severity reduction from severe to moderate demonstrates the efficacy of the treatment.
Cognitive Function (MoCA)	22	26	Cognitive function improved from low to acceptable range.
Phb-PCR	Positive	Negative	The absence of <i>B. miyamotoi</i> proves the treatment's effectiveness.

support was critical in maintaining the patient's overall health and aiding recovery (Yadav *et al.*, 2023). The infusions included:

- Vitamin C
- B complex vitamins
- Essential minerals
- Calcium gluconate
- Neurobion (Vitamin B1)
- Spasmag (Magnesium Sulphate)
- Cyanocobalamin

### Symptom improvement

Table 3 illustrates the significant clinical improvements observed with the treatment regimen. The SF-36 scores showed an overall enhancement in quality of life. The patient experienced a marked reduction in fatigue and pain severity scores, along with improved cognitive function and enhanced general well-being. Fatigue and pain scores dropped from high and severe to low and moderate, while cognitive function improved from low to acceptable, indicating substantial recovery. Additionally, the negative phb-PCR result post-treatment confirms the clearance of *B. miyamotoi* infection. This result provides strong microbiological evidence of the treatment's effectiveness.

Following the completion of the treatment regimen, the patient underwent regular follow-up assessments to monitor for potential recurrence of the infection and to evaluate overall health and symptomatology. The therapeutic effects have been sustained for eight months, during which the patient has not experienced any relapse of symptoms. After this period, the patient experienced some flare-ups, which were successfully managed with non-antibiotic therapies, including non-corticosteroid anti-inflammatory medications and painkillers.

### Discussion

Given the complexities and challenges associated with diagnosing TBDs, and in light of the guidance provided by the CDC, IDSA (Infectious Diseases Society of America) and ECDC (European Centre for Disease Prevention and Control), our decision to use phb-PCR for diagnosing *B. miyamotoi* was justified by several factors. Initially, the patient underwent standard serological testing for LD and other common co-infections such as *Bartonella* and *A. phagocytophilum*, which all returned negative results. These

tests are typically prioritised due to the higher prevalence of LD and its co-infections, as well as the more established diagnostic protocols available for these diseases. Additionally, comprehensive autoimmune screenings were conducted to rule out conditions that could mimic TBD symptoms.

Despite these negative serological results, the clinical presentation of the patient continued to suggest a tick-borne aetiology, warranting further investigation. The CDC and IDSA both acknowledge that *B. miyamotoi* is an emerging pathogen and recommend considering it in differential diagnoses, particularly when standard tests do not yield conclusive results, but clinical suspicion remains high (Lantos *et al.*, 2020; Kubiak *et al.*, 2021; Burde *et al.*, 2023; Coder *et al.*, 2024). Phb-PCR offers enhanced sensitivity and specificity by targeting the phage genes associated with *B. miyamotoi*, which are part of the pathogen's epigenetic makeup and present in multiple copies within the bacterial cells.

Therefore, the use of phb-PCR was a logical next step to accurately identify *B. miyamotoi*, allowing for the timely initiation of an effective treatment regimen. This approach underscores the importance of utilising advanced diagnostic techniques when standard methods fail to provide definitive answers, ultimately enhancing patient care and outcomes.

After detecting *B. miyamotoi*, we initiated a treatment regimen involving intravenous ceftriaxone and oral azithromycin, supplemented with nutrient infusions. This approach leverages the distinct mechanisms of action of two antibiotics, thereby reducing the risk of antibiotic resistance development. The inclusion of nutritional supplementation was not merely an adjunct but a pivotal component that mitigates the side effects commonly associated with prolonged antibiotic use, such as gastrointestinal disturbances and nutrient depletion. This strategy supports the body's natural defences and facilitates a more effective recovery.

We recognise the complexities in TBDs. This case report advocates for clinicians to recognise the rising incidence of *B. miyamotoi* in ticks and patients when making differential diagnoses of TBDs. Utilising sensitive direct diagnostic tests, such as phage-based PCR, enhances diagnostic accuracy. This prevents delayed diagnoses and misdiagnoses, reduces patient suffering and addresses antibiotic resistance issues. This case exemplifies the synergy between laboratory diagnostics and clinical judgment, ultimately improving patient care. By integrating advanced diagnostics with clinical expertise, we can refine the diagnostic algorithm for TBRF and significantly enhance patient outcomes.



## Conclusions

This case report highlights the critical importance of direct diagnostic methods for accurately identifying BMD. Diagnosing BMD is challenging due to its symptom overlap with LD and unreliable serological tests. In this case, multiple negative serological tests prompted the use of phb-PCR, which successfully detected *B. miyamotoi* in the patient's blood. This detection facilitated a tailored treatment plan that included intravenous ceftriaxone, oral azithromycin and nutritional support, resulting in significant symptom improvement.

The success of phb-PCR in diagnosing BMD underscores its value in managing complex TBDs. Clinicians should consider *B. miyamotoi* testing when serological tests for LD and other common TBDs yield negative results, yet the clinical presentation suggests a tick-borne aetiology. Incorporating BMD testing into diagnostic protocols will ensure timely and accurate treatment.

**Data availability statement.** The findings of this case report are detailed within the article. Due to privacy and ethical considerations, individual patient data is not publicly accessible. For inquiries about the study's methodologies and anonymized data, please contact the corresponding author, Jinyu Shan, at the provided email address. Data requests will be considered in line with ethical standards and patient confidentiality.

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**Author contributions.** Louis Teulières and Jinyu Shan co-conceived the idea for this paper. Louis Teulières handled the clinical aspects of the work and provided the outline for the diagnostic and treatment approaches. Jinyu Shan, with the assistance of Ying Jia, was responsible for the meticulous drafting and critical writing of the manuscript with constant conversation with Louis Teulières. Martha Clokie proofread the manuscript.

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**Competing interests.** The authors declare no conflict of interest.

**Ethics statement.** The research conducted and presented in this paper was carried out with strict adherence to ethical principles, respecting the dignity, privacy and rights of the patient involved. Prior to conducting this study, informed consent was obtained from the patient, ensuring she was fully aware of the research's nature, objectives, potential benefits and risks. The study was designed and executed with a commitment to maintaining confidentiality and safeguarding the patient's personal and medical information, in accordance with the Declaration of Helsinki and relevant local regulations.

The authors have taken meticulous care to ensure that the research methodologies employed were ethically sound, scientifically justified and conducted under the appropriate oversight.

Furthermore, this paper does not contain any data that could lead to the identification of the patient, and all personal information has been anonymised to protect her privacy. The collaboration between the authors from PhelixRD Charity and the University of Leicester was founded on mutual respect, integrity and a shared goal of advancing medical knowledge for the benefit of patients suffering from tick-borne diseases.

## Connections references

**Lawrence N and Brailey A (2023).** What is the current and expected evolution of prevalence, geographical spread and impact of ticks and tick-borne diseases, and what strategies are needed to improve management, testing, diagnosis and treatment of these diseases amongst patients and animal populations? Research Directions: One Health, 1-4. <https://doi.org/10.1017/one.2023.11>.

## References

- Aydin N, Bülbül R, Telli M and Gültekin B (2014)** Seroprevalence of *Bartonella henselae* and *Bartonella quintana* in blood donors in Aydin province, Turkey. *Mikrobiyoloji Bulteni* 48(3), 477–483. <https://doi.org/10.5578/mb.7598>.
- Boyer PH, Koetsveld J, Zilliox L, Sprong H, Talagrand-Reboul É, Hansmann Y, de Martino S J, Boulanger N, Hovius JW, Jaulhac BE (2020)** Assessment of *Borrelia miyamotoi* in febrile patients and ticks in Alsace, an endemic area for Lyme borreliosis in France. *Parasites & Vectors* 13(1), 199. <https://doi.org/10.1186/s13071-020-04071-9>.
- Burde J, Bloch EM, Kelly JR and Krause PJ (2023)** Human *Borrelia miyamotoi* infection in North America. *Pathogens* 12(4), 553. <https://doi.org/10.3390/pathogens12040553>.
- Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009)** The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* 55(4), 611–622. <https://doi.org/10.1373/clinchem.2008.112797>.
- Chochlakis D, Psaroulaki A, Kokkini S, Kostanatis S, Arkalati E, Karagrannaki E, Tsiatis K, Tselentis Y, Gikas A (2009)** First evidence of *Anaplasma* infection in Crete, Greece. Report of six human cases. *Clinical Microbiology and Infection* 15, 8–9. <https://doi.org/10.1111/j.1469-0691.2008.02695.x>.
- Cleveland DW, Anderson CC and Brissette CA (2023)** *Borrelia miyamotoi*: a comprehensive review. *Pathogens* 12(2), 267. <https://doi.org/10.3390/pathogens12020267>.
- Coder BL, Price KJ, Tewari D, Witmier BJ, Chapman HA, Chroscinski MS, Long L, Livengood JL and Boyer CN (2024)** Statewide surveillance of *Ixodes scapularis* (Acari: Ixodidae) for the presence of the human pathogen *Borrelia miyamotoi* (Spirochaetales: Spirochaetaceae), a relapsing fever spirochete in Pennsylvania, USA, 2019–2020. *Journal of Medical Entomology* 61(4), 1086–1090.
- Cutler SJ, Moss J, Fukunaga M, Wright DJM, Fekade D, Warrell D (1997)** *Borrelia recurrentis* characterization and comparison with relapsing-fever, Lyme-associated, and other *Borrelia* spp. *International Journal of Systematic Bacteriology* 47(4), 958–968. <https://doi.org/10.1099/00207713-47-4-958>.
- De Bont E, Lagrou K and Depypere M (2022)** Comparison of the Euroimmun *Borrelia* 'antibody index' with Virotech immunoblot-based detection of intrathecal *Borrelia* antibody production for the diagnosis of Lyme neuroborreliosis. *European Journal of Clinical Microbiology & Infectious Diseases* 41(1), 155–161. <https://doi.org/10.1007/s10096-021-04343-x>.
- Eikeland R, Henningsson AJ, Lebech AM, Kerlefsen Y, Mavin S, Vrijlandt A, Hovius JW, Lernout T, Lim C, Dobler G, Fingerle V, Gynthersen RM, Lindgren P-E and Reiso H (2024)** Tick-borne diseases in the North Sea region - A comprehensive overview and recommendations for diagnostics and treatment. *Ticks and Tick-borne Diseases* 15(2), 102306.
- Fukunaga M, et al. (1995)** Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *International Journal of Systematic Bacteriology* 45(4), 804–810. <https://doi.org/10.1099/00207713-45-4-804>.
- Grażlewska W and Holec-Gąsior L (2023)** Antibody cross-reactivity in serodiagnosis of Lyme disease. *Antibodies* 12(4), 63.
- Guérin M, Shawky M, Zedan A, Octave Séphane A, Avelle Béangère A, Maffucci I and Padiolleau-Lefèvre S (2023)** Lyme borreliosis diagnosis: state of the art of improvements and innovations. *BMC Microbiology* 23(1), 204.
- Hawker GA, Mian S, Kendzerska T and French M (2011)** Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Research* 63(S11), S240–S252.
- Hjetland R, Nilsen R M, Grude N, Ulvestad E (2014)** Seroprevalence of antibodies to *Borrelia burgdorferi* sensu lato in healthy adults from western Norway: risk factors and methodological aspects. *APMIS* 122(11), 1114–1124. <https://doi.org/10.1111/apm.124.122.issue-11>.

- Jakab Á., Kahlig P, Kuenzli E and Neumayr A (2022) Tick borne relapsing fever - a systematic review and analysis of the literature. *PLOS Neglected Tropical Diseases* **16**(2), e0010212.
- Karan L, Makenov M, Kolyasnikova N, Stukolova O, Toporkova M, Olenkova O (2018) Dynamics of spirochetemia and early PCR detection of *Borrelia miyamotoi*. *Emerging Infectious Diseases* **24**(5), 860–867. <https://doi.org/10.3201/eid2405.170829>.
- Koetsveld J, Platonov A E, Kuleshov K, Wagemakers A, Hoornstra D, Ang W, Szekeres S, van Duijvendijk G L A, Fikrig E, Embers M E, Sprong H, Hovius J W (2020) *Borrelia miyamotoi* infection leads to cross-reactive antibodies to the C6 peptide in mice and men. *Clinical Microbiology and Infection* **26**(4), 513.e1–513.e6. <https://doi.org/10.1016/j.cmi.2019.07.026>.
- Krause PJ and Barbour AG (2015) *Borrelia miyamotoi*: the newest infection brought to us by deer ticks. *Annals of Internal Medicine* **163**(2), 141–142. <https://doi.org/10.7326/M15-1219>.
- Kubiak K, Szczotko M and Dmitryjuk M (2021) *Borrelia miyamotoi*—An emerging human tick-borne pathogen in Europe. *Microorganisms* **9**(1), 154.
- Lantos PM, Rumbaugh J, Bockenstedt LK, Falck-Ytter YT, Agüero-Rosenfeld ME, Auwaerter P G, Baldwin K, Bannuru RR, Belani KK, Bowie WR, Branda JA, Clifford DB, FJ DiMario, Halperin JJ, Krause PJ, Lavergne V, Liang MH, Meissner HC, Nigrovic LE, Nocton J, Osani MC, Pruitt AA, Rips J, Rosenfeld LE, Savoy ML, Sood SK, Steere AC, Strle F, Sundel R, Tsao J, Vaysbrot EE, Wormser GP, Zemel LS (2020) Clinical practice guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 guidelines for the prevention, diagnosis and treatment of Lyme disease. *Clinical Infectious Diseases* **72**(1), e1–e48. <https://doi.org/10.1093/cid/ciaa1215>.
- Luo RF, Scahill MD and Banaei N (2010) Comparison of single-copy and multicopy real-time PCR targets for detection of *Mycobacterium tuberculosis* in paraffin-embedded tissue. *Journal of Clinical Microbiology* **48**(7), 2569–2570. <https://doi.org/10.1128/JCM.02449-09>.
- Mandilara GD, Smeti EM, Mavridou AT, Lambiri MP, Vatopoulos AC, Rigas FP (2006) Correlation between bacterial indicators and bacteriophages in sewage and sludge. *FEMS Microbiology Letters* **263**(1), 119–126. <https://doi.org/10.1111/fml.2006.263.issue-1>.
- Marques AR, Strle F and Wormser GP (2021) Comparison of Lyme Disease in the United States and Europe. *Emerging Infectious Diseases* **27**(8), 8–2024. <https://doi.org/10.3201/eid2708.204763>.
- Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL and Chertkow H (2005) The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society* **53**(4), 695–699.
- Platonov A E, Karan L S, Kolyasnikova N M, Makhneva N A, Toporkova M G, Maleev V V, Fish D, Krause P J (2011) Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerging Infectious Diseases* **17**(10), 1816–1823. <https://doi.org/10.3201/eid1710.101474>.
- Ras NM, *et al.* (1996) Phylogenesis of relapsing fever *Borrelia* spp. *International Journal of Systematic Bacteriology* **46**, 859–865.
- Sack U, Conrad K, Csernok E, Frank I, Hiepe F, Krieger T, Kromminga A, Von Landenberg P, Messer G, Witte T and Mierau R (2009) Autoantibody detection using indirect immunofluorescence on HEp-2 cells. *Annals of the New York Academy of Sciences* **1173**(1), 166–173. <https://doi.org/10.1111/nyas.2009.1173.issue-1>.
- Shahid A, *et al.* (2012) Fatigue Severity Scale (FSS). In Shahid A, *et al.* (eds.), *STOP, THAT, One Hundred Other Sleep Scales*, New York, NY: Springer, pp. 167–168.
- Shan J, Jia Y, Teulières L, Patel F, Clokie M R J (2021) Targeting multicopy prophage genes for the increased detection of *Borrelia burgdorferi* Sensu Lato (s.l.), the Causative Agents of Lyme Disease, in Blood. *Frontiers in Microbiology* **12**, 651217. <https://doi.org/10.3389/fmicb.2021.651217>.
- Shan J, Jia Y, Hickenbotham P, Teulières L, Clokie M R J (2023) Combining citizen science and molecular diagnostic methods to investigate the prevalence of *Borrelia burgdorferi* s.l. and *Borrelia miyamotoi* in tick pools across Great Britain. *Frontiers in Microbiology* **14**, 1126498. <https://doi.org/10.3389/fmicb.2023.1126498>.
- Shan J, Clokie MR and Teulières L (2018) Phage-based detection of borreliosis and means therefor, US11739388B2, PCT/GB2017/053323, WO 2018/083491.
- Shivaram K B, Bhatt P, Verma M S, Clase K, Simsek H (2023) Bacteriophage-based biosensors for detection of pathogenic microbes in wastewater. *Science of The Total Environment* **901**, 165859. <https://doi.org/10.1016/j.scitotenv.2023.165859>.
- Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JWR and Mead PS (2016) Lyme borreliosis. *Nature Reviews. Disease Primers* **2**(1), 16090.
- Subasic D, Karamelic J, Gavrankapetanovic F, Hodzic H, Kasumovic C, Delic-Sarac M and Prljaca-Zececic L (2009) ELISA subtyping of anti-ENA autoantibodies in clinical management of autoimmune diseases in Bosnia and Herzegovina. *Medicinski arhiv* **63**(6), 312–316.
- Swift BMC, Meade N, Barron ES, Bennett M, Perehenic T, Hughes V, Stevenson K, Rees CED (2020) The development and use of Actiphage<sup>®</sup> to detect viable mycobacteria from bovine tuberculosis and John's disease-infected animals. *Microbial Biotechnology* **13**(3), 738–746. <https://doi.org/10.1111/mbt2.v13.3>.
- Ware JEJ and Sherbourne CD (1992) The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Medical Care* **30**(6), 473–483.
- Wormser GP, Shapiro ED and Fish D (2019) *Borrelia miyamotoi*: an emerging tick-borne pathogen. *American Journal of Medicine* **132**(2), 136–137. <https://doi.org/10.1016/j.amjmed.2018.08.012>.
- Xu G, Luo C-Y, Ribbe F, Pearson P, Ledizet M, Rich SM (2021) *Borrelia miyamotoi* in human-biting ticks, United States, 2013–2019. *Emerging Infectious Diseases* **27**(12), 3193–3195. <https://doi.org/10.3201/eid2712.204646>.
- Yadav N, Shah A, George R, Baral T and Miraj SS (2023) Chapter 63 - Role of nutrients in combating infection. In Bagchi D, Das A, Downs BW (eds.), *Viral, Parasitic, Bacterial, and Fungal Infections*, Academic Press, pp. 815–826.