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Lyme serology in non-endemic countries: Why do we request it and what do we find?

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Abstract

Background The primary objectives of our research were to analyze Lyme serology results from pediatric patients, identify the clinical reasons for ordering these tests, and assess the clinical relevance of the serology results in the context of Lyme disease.

Methods Our study, conducted at a reference pediatric hospital in a non-endemic region for Lyme disease, included all pediatric patients for whom Lyme serology was requested. ELISA and Western blot results were documented. Presenting complaints and findings at the time of admission were recorded. Diagnostic tests were categorized as first-step tests if conducted during the initial visit. Subsequent tests, sent due to the inability to establish a diagnosis based on the results of the first-line tests, were defined as second and third-step tests, respectively.

Results 219 patients, for whom Lyme serology had been requested for varying reasons, were included in our study. The most prevalent complaints and indications observed in the presentations of patients with serology requests were as follows: headache(24.7%), paresis/paresthesia(14.6%), and painful or blurred vision(13.2%). Serology was primarily requested in the presence of neurological(59.4%) and ocular symptoms(13.2%). 68% of the tests were requisitioned during the initial consultation. Nevertheless, it was discerned that no patient received a diagnosis of Lyme disease.

Conclusion According to the guidelines, Lyme serology should only be performed when there is a realistic possibility of exposure to infected ticks, particularly in patients who have had untreated erythema migrans or those with a history of a tick bite presenting with unexplained joint or neurological symptoms. Our data, in line with these guidelines, suggests that unnecessary Lyme serology testing in non-endemic areas, where exposure is highly unlikely, may lead to false-positive results and unnecessary follow-up testing, as illustrated by the high rate of false-positive ELISAs in our cohort.

Keywords Lyme disease, Children, Serology, Nonendemic

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Introduction

Lyme disease is an illness transmitted by specific *Ixodes* spp. ticks caused by different pathogenic genomospecies within the spirochete *Borrelia burgdorferi* sensu lato complex [1].

It is the most commonly encountered tick-borne infection in both Europe and the United States [2]. Humans are not typically part of the natural life cycle of Borrelia spirochetes; therefore, the occurrence of Lyme disease initiates when the uninvited guest, namely humans, enters regions where biological interactions occur among Borrelia-carrying ticks, sheep, deer, and other reservoir animals [3]. Lyme borreliosis has been reported in countries across the Northern Hemisphere. In Europe and Asia, the reported country-wide incidence varies from low to negligible in the United Kingdom, Turkey, and Japan. However, the highest incidence of Lyme disease is observed in Northern and Central Europe, including the Netherlands, Slovenia, Lithuania, and Estonia in Europe, and in China and Mongolia in Asia [4, 5]. In Turkey, ticks of the *I. ricinus* species, which have the potential to transmit Lyme disease, have been identified, and the Ministry of Health has classified Lyme disease as a notifiable disease. However, until 2010, only approximately 60 cases of Lyme disease had been reported in Turkey, and it is known that the total reported cases to date are less than 100. Consequently, Lyme disease does not pose a significant health issue for Turkey [6, 7].

Lyme disease presents with a range of clinical manifestations, with the most frequently affected organ being the skin, followed by the nervous system and joints [5]. Erythema migrans is the predominant clinical manifestation of Lyme disease in both the United States and Europe, occurring in over 80% of patients across these regions [2]. In a community-based prospective study involving 201 children in the United States, the presenting manifestations were as follows: erythema migrans 89%, arthritis 7%, facial palsy 3%, aseptic meningitis 1%, and carditis 0.5% [8]. Due to the diverse clinical presentations of Lyme disease, clinicians often rely on Lyme serology in the differential diagnosis process. Additionally, unsubstantiated claims about Lyme disease circulate in both conventional and social media, leading to increased concern among individuals. Consequently, patients often request healthcare providers perform/order Lyme disease testing. In our study, we aimed to analyze Lyme serology results from pediatric patients to identify patterns in test utilization, assess the clinical conditions that prompted these tests, and determine the accuracy and relevance of the test outcomes.

Materials and methods

Our study is of a retrospective, cross-sectional, and descriptive nature. It was conducted at Ankara Bilkent City Hospital Children's Hospital. Children aged 0–18 who underwent clinical evaluations and had *Borrelia burgdorferi* IgM/IgG serology tests requested, either as outpatients or inpatients, between December 2020 and September 2023, were included in the study. The electronic medical records of the hospital were thoroughly reviewed to identify all patients who had these tests ordered within this period, ensuring a comprehensive evaluation of their cases. Patients for whom clinical information could not be accessed and those who discontinued follow-up at our hospital, leading to an incomplete diagnostic process, were excluded from the study. Diagnostic tests sent for patients presenting with any complaint to any clinic were categorized as first-line tests if the tests were sent at the initial visit. Subsequent tests sent due to the inability to establish a diagnosis based on the results of the first-line tests were defined as second and third-line tests, respectively.

For Lyme serology, the Euroimmun ELISA test (Euroimmun, Lübeck, Germany) was used to detect both IgM and IgG antibodies. The ELISA test provided both IgM and IgG results. Positive or borderline ELISA results were confirmed by a Western Blot (WB) test, performed at the National Public Health Reference Laboratory under the Ministry of Health of Turkey General Directorate of Public Health.

This study was conducted in conformity with the principles of the Declaration of Helsinki and approved by the Republic of Turkey Ministry of Health, the Ethics Committee of Ankara Bilkent City Hospital Ethics Committee, and the Institutional Review Board of the Children's Hospital of Ankara Bilkent City Hospital.

Results

Initially, 353 patients were identified for the study. However, 134 patients were excluded due to inaccessible data and discontinued follow-ups before completing the diagnostic process. Among the 219 patients included in the final analysis, 47% were female, with a mean age of 11.54±4.63 years.

When examining the complaints and symptoms of patients for whom Lyme serology was requested, the following distribution was observed: headache 24.7%, paresis/paresthesia 14.6%, painful or blurred vision 13.2%, facial paralysis 11.4%, joint pain and/or stiffness 10%, sudden vision loss 3.7%, prolonged fever 3.7%, cognitive impairment 3.2%, rash (excluding erythema migrans) 3.2%, lymphadenitis 2.7%, and other 9.6% (including seizures, convulsions, diplopia, acute vision loss, acute psychiatric changes, hematuria, walking difficulties). None of the patients reported a history of tick bites or

erythema migrans. Lyme serology was requested in 59.4% of patients for neurological symptoms, 13.2% for ocular symptoms, 12.3% for infectious symptoms, 10% for rheumatological symptoms, and 5.1% for complaints and findings related to other systems and organs. The distribution of symptoms at diagnostic levels is shown in Table 1.

Serology tests were performed in the first step for 68% of patients, in the second step for 21.5%, and in the third step for 10.5%. Initial screening with ELISA tests for *Borrelia burgdorferi* showed that IgM was positive in five patients and borderline in three, while IgG was positive in nine patients and borderline in three. Simultaneous positivity or borderline results for both IgM and IgG were not observed in any patient. It was also noted that no Western Blot (WB) tests were performed unless an ELISA result was positive or borderline. For all patients with positive or borderline ELISA results, subsequent Western Blot testing was conducted; however, none showed positivity in the Western Blot confirmation tests.

Discussion

In our study conducted in a non-endemic region for Lyme disease, we investigated the specific conditions under which Lyme serology was requested and found that the use of these tests did not result in any confirmed Lyme disease diagnoses. This outcome aligns with existing guidelines, which emphasize that Lyme serology should only be performed when there is a realistic possibility of exposure to infected ticks, particularly in cases where a clear history of tick bites is present or in patients who have had untreated erythema migrans. Our findings indicate that Lyme serology is often ordered early in the diagnostic process without sufficient clinical indication, leading to unnecessary testing and potential false-positive results. Considering that the literature on Lyme disease in Turkey primarily consists of case presentations, case series, and reviews, and international guidelines generally emphasize diagnostic methods, our study contributes significantly to both national and international literature, especially in pediatrics.

Lyme borreliosis is a multisystemic disease transmitted through the bite of specific *Ixodes* spp. ticks, primarily affecting the skin, nervous system, and musculoskeletal system [9]. In Turkey, case series have similarly reported erythema migrans as the most frequent finding [10, 11]. ELISA tests for Lyme disease should be reserved for patients who present with symptoms consistent with Lyme disease and have had potential exposure to ticks in an endemic area [9, 12]. Serologic testing of asymptomatic patients following a tick bite would probably only be useful if part of an acute/convalescent serology pair. If both acute and convalescent samples are positive, this would almost certainly reflect past, irrelevant exposure. Seroconversion could be suggestive of acute infection. Moreover, asymptomatic seropositive patients are at a lower risk of developing disseminated Lyme disease compared to those with erythema migrans [13].

Our study, conducted in a non-endemic region (Turkey), found that none of the patients exhibited erythema migrans or reported a history of tick bites, and no Lyme disease diagnoses were made. Despite this, 7% of patients had positive IgM ELISA results and 4% had positive IgG results, all of which were false positives as confirmed by negative Western blot tests. This underscores the high false-positive rate associated with Lyme ELISAs, particularly IgM, when used alone in non-endemic areas. Existing guidelines clearly state that Lyme serology should only be performed when there is a realistic possibility of exposure to infected ticks. In regions where Lyme disease is uncommon or not typically found and where lone star ticks are prevalent, both physicians and patients might opt for monitoring the condition instead of resorting to Lyme testing or immediate antibiotic treatment for erythema migrans [13]. The findings from our study emphasize that in non-endemic regions like ours, initial serological testing for Lyme disease using ELISA for IgM and IgG antibodies should be avoided unless there is substantial clinical suspicion, including a history of tick bites and specific Lyme disease symptoms. It should also be noted that negative results from serological tests for Lyme disease do not unequivocally rule out the disease, especially in its early stages or in patients with an inadequate immune response. Therefore, the interpretation of serological test results should be made cautiously, considering clinical evaluation and exposure history.

Additionally given that the five IgM positive cases were likely patients with symptoms persisting long enough to warrant this follow up evaluation and testing, it is important to emphasize that isolated IgM ELISA results are not meaningful in patients with symptoms lasting more than 1–2 months. This is because IgM antibodies typically indicate a recent infection, and their presence in prolonged symptomatic cases may lead to misinterpretation

Table 1 Distribution of complaints and findings by systems in Diagnostic steps

	Neurological *n* (%)	Ocular *n* (%)	Rheumatological *n* (%)	Infectious *n* (%)	Psychiatric *n* (%)	Others *n* (%)
1st Step	90 (60.4%)	21 (14.1%)	15 (10.1%)	14 (9.4%)	4 (2.6%)	5 (3.4%)
2nd Step	30 (63.8%)	7 (14.9%)	4 (8.5%)	6 (12.8%)	0 (0%)	0 (0%)
3rd Step	10 (43.6%)	1 (4.3%)	3 (13%)	7 (30.4%)	0 (0%)	2 (8.7%)

and unnecessary concern if not corroborated with additional diagnostic criteria or tests.

Moreover, our data highlights the importance of a two-tier testing strategy. Adding a Western blot test, which has been shown to provide up to 99% specificity [14], could reduce the false positive rate to approximately 1%. In a non-endemic country like Turkey, with a population of approximately 85 million and fewer than 100 confirmed cases of Lyme disease to date, reliance on ELISA alone could misleadingly suggest an incidence rate of up to 11%. In contrast, a two-tier approach with Western blot confirmation would likely indicate a much lower incidence, closer to 1%. However, it is important to emphasize that even this 1% estimate far exceeds the actual incidence in Turkey, where fewer than 100 cases have been confirmed over several decades. Given the extremely low prevalence, even two-tier testing is prone to yielding more false positives than true positive. These findings underscore the necessity of adhering to a two-tier testing strategy to avoid overestimating the prevalence of Lyme disease and prevent unnecessary follow-up testing and treatment.

The overutilization of laboratory tests, testing driven by patient demand, and testing conducted to reassure patients have been documented in studies examining unexplained non-specific symptoms [15]. Studies conducted with general practitioners in the Netherlands have indicated that physicians perceive ordering tests upon patient request as a time-saving measure and often request tests from patients with nonspecific symptoms [16, 17]. The study conducted by Vreugdenhil et al. has elucidated that general practitioners employ serological tests to provide reassurance to patients and exclude Lyme Borreliosis, thus deviating from the national guidelines [18]. Certain studies have indicated that a lack of knowledge or outdated information among healthcare service providers rand patients regarding Lyme disease can impact the utilization of tests with inappropriate indications [19, 20]. Our study highlights that Lyme serology is frequently requested as a first-line diagnostic tool, even in cases without clear indications of Lyme disease. This overuse is likely due to a combination of factors, including a lack of awareness of current guidelines among healthcare providers, a desire to avoid missing a rare diagnosis, and pressure to address patient concerns quickly. It is important to adhere to guidelines to ensure that testing is appropriate and does not lead to unnecessary costs or patient anxiety.

Our study has several limitations that should be acknowledged. Firstly, the retrospective and single-center nature of the study limits the generalizability of the findings to other settings or populations. Additionally, the absence of a detailed survey or qualitative data from clinicians regarding their reasons for ordering Lyme serology tests means that our understanding of the motivations and decision-making processes behind these requests remains incomplete. Another limitation of this study is the lack of detailed travel history and destination data for the patients, which could have provided further context for Lyme disease testing rationale. Due to the retrospective nature of the study, such information was not consistently available in the medical records.

In conclusion, the results of our study highlight a high rate of false-positive Lyme ELISA results, none of which were confirmed by Western blot. This underscores the importance of adhering strictly to a two-tier testing approach. Our data strongly supports the guideline that Lyme serology should only be performed when there is significant clinical suspicion based on exposure history and symptomatology. Conducting Lyme serology tests when not clinically indicated can result in excessive costs and misdiagnoses. To improve clinical outcomes and optimize resource utilization, the development and dissemination of national guidelines and more specific diagnostic algorithms are imperative.

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Author contributions
MY played a pivotal role in conceptualizing the study, designing the research methodology, drafting the initial manuscript, and revising and editing subsequent versions. He has read and approved the final manuscript. MY and FK contributed to the conceptualization and conducted data analysis to ensure the statistical robustness of the findings. They have also read and approved the final manuscript. HSY, YEI, FK and EKK were instrumental in data acquisition, contributed to the drafting of the original manuscript, and participated in the critical review process prior to the approval of the final manuscript. AOP was responsible for the critical review and editing of the manuscript to ensure its accuracy and clarity, and has read and approved the final version. MY and AOP actively participated in the conceptualization of the study, was involved in drafting and editing the original manuscript, and has read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval
This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Ankara Bilkent City Hospital.

Consent to participate
In the present retrospective study, individual informed consent from participants was not required as patient data were anonymized and analyzed without direct identifiers. The need for informed consent was waived by the Ethics Committee of Ankara Bilkent City Hospital (Ethics Committee and Institutional Review Board approval number: E2211095).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet. 2012;379:461–73.
2. Marques AR, Strle F, Wormser GP. Comparison of Lyme disease in the United States and Europe. Emerg Infect Dis. 2021;27:2017.
3. Forrester JD, Vakkalanka JP, Holstege CP, Mead PS. Lyme disease: what the wilderness provider needs to know. Wilderness Environ Med. 2015;26:555–64.
4. Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, Hovius JWR, et al. Lyme borreliosis. Nat Reviews Disease Primers 2016. 2016;2:1.
5. Stanek G, Strle F. Lyme borreliosis–from tick bite to diagnosis and treatment. FEMS Microbiol Rev. 2018;42:233–58.
6. ÖNCEL S. Türkiye'de Lyme Hastalığı. Kocaeli Üniversitesi Sağlık Bilimleri Dergisi. 2018;4:103–6.
7. General directorate of public health Republic of Turkiye Ministry of Health. Infectious Diseases Laboratory Diagnosis Guide. 2014. https://hsgm.saglik.gov.tr/tr/dokumanlar-mikrobiyoloji.html. Accessed 23 Dec 2023.
8. Ichael M, Erber AG, Hapiro UDS, Eorgine G, Urke SB, Arcells AJP, et al. Lyme disease in children in Southeastern Connecticut. N Engl J Med. 1996;335:1270–4.
9. Rauer S, Kastenbauer S, Hofmann H, Fingerle V, Huppertz HI, Hunfeld KP et al. Guidelines for diagnosis and treatment in neurology – lyme neuroborreliosis. GMS German Med Sci. 2020;18.
10. Anlar FY, Durlu Y, Aktan G, Açikgöz E, Bingöl N, Madencioğlu V, et al. Clinical characteristics of Lyme disease in 12 cases. Mikrobiyol Bul. 2003;37:255–9.
11. Akin Belli A, Derviş E, Özbaş Gök S, Midilli K, Gargili A. Istanbul'da eritema migrans ile Başvuran 10 Lyme Olgusunun De1erlendirilmesi. Mikrobiyol Bul. 2015;49:525–31.
12. Kenyon SM, Chan SL. A focused review on Lyme disease diagnostic testing: an update on serology algorithms, current ordering practices, and practical considerations for laboratory implementation of a new testing algorithm. Clin Biochem. 2023;117:4–9.
13. Lantos PM, Rumbaugh J, Bockenstedt LK, Falck-Ytter YT, Aguero-Rosenfeld ME, Auwaerter PG, et al. 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. Clin Infect Dis. 2021;72:E1–48.
14. Steere AC, Dressler F, Whalen JA, Reinhardt BN. Western blotting in the serodiagnosis of Lyme Disease. J Infect Dis. 1993;167:392–400.
15. Whiting P, Toerien M, de Salis I, Sterne JAC, Dieppe P, Egger M, et al. A review identifies and classifies reasons for ordering diagnostic tests. J Clin Epidemiol. 2007;60:981–9.
16. Van Der Weijden T, Van Bokhoven MA, Dinant GJ, Van Hasselt CM, Grol RPTM. Understanding laboratory testing in diagnostic uncertainty: a qualitative study in general practice. Br J Gen Pract. 2002;52:974.
17. Koch H, van Bokhoven MA, ter Riet G, Hessels KM, van der Weijden T, Dinant GJ, et al. What makes general practitioners order blood tests for patients with unexplained complaints? A cross-sectional study. Eur J Gen Pract. 2009;15:22–8.
18. Vreugdenhil TM, Leeflang M, Hovius JW, Sprong H, Bont J, Ang CW, et al. Serological testing for Lyme Borreliosis in general practice: a qualitative study among Dutch general practitioners. Eur J Gen Pract. 2020;26:51.
19. Beck AR, Marx GE, Hinckley AF. Diagnosis, treatment, and Prevention practices for Lyme Disease by clinicians, United States, 2013–2015. Public Health Rep. 2021;136:609–17.
20. Conant JL, Powers J, Sharp G, Mead PS, Nelson CA. Lyme Disease Testing in a high-incidence state: clinician knowledge and patterns. Am J Clin Pathol. 2018;149:234–40.

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