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Seroprevalence Of Lyme Disease Among Clinically Suspected Cases In A Tertiary Care Hospital

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Abstract
Background: *Lyme disease is a multisystem zoonotic infection caused by Borrelia burgdorferi and transmitted by Ixodesticks. It remains underdiagnosed and underreported in India despite frequent tick exposure in rural populations.*
Materials and Methods: *A prospective study was conducted over 6 months (March–August 2025) in the Department of Microbiology, Government General Hospital, Guntur. A total of 60 clinically suspected cases were included. Serum samples were tested for Borrelia burgdorferi IgM and IgG antibodies using ELISA.*
Results: *The overall seroprevalence was 21.6% (13/60), with IgM positivity of 13.3% and IgG positivity of 8.3%. Higher seropositivity was observed among individuals with tick exposure and outdoor occupational risk. Common clinical features included erythema migrans, fever, musculoskeletal pain, and neurological symptoms.*
Conclusion: *The study confirms the presence of Lyme disease in this region and highlights the need for increased clinical awareness and larger epidemiological studies.*
Key Words: *Lyme disease, Borrelia burgdorferi, ELISA, Seroprevalence, Tick exposure*

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I. Introduction

Lyme disease is a tick-borne zoonotic infection caused by the spirochete *Borrelia burgdorferi*. It primarily affects the skin, joints, nervous system, and heart. The disease is transmitted by Ixodes ticks, with rodents and birds serving as reservoirs.Clinically, Lyme disease presents with erythema migrans, fever, arthritis, and neurological manifestations such as facial palsy. If left untreated, it may lead to chronic complications including musculoskeletal and cardiac involvement.Although Lyme disease is a well-recognized public health concern worldwide, data from India are limited. Due to frequent exposure to ticks in agricultural and rural settings, there is a need to assess its prevalence in the Indian population.This study aims to estimate the seroprevalence of Lyme disease among clinically suspected cases and correlate it with clinical and demographic factors.

Lyme disease is transmitted by the black-legged tick (*Ixodes scapularis*), which acts as the vector carrying the bacterium *Borrelia burgdorferi*. The infection cycle is maintained in nature by reservoirs such as rodents, birds, and reptiles, which harbor the organism without severe illness. *Borrelia burgdorferi* is a spirochete bacterium responsible for causing Lyme disease. Clinically, patients may present with a characteristic bull's-eye rash known as erythema migrans, along with neurological features like facial palsy and joint involvement such as swollen knee (Lyme arthritis). Diagnosis is mainly based on a modified two-tiered testing approach, where ELISA is used for initial screening and positive results are confirmed by immunoblot (Western blot) for accuracy.

II. Material And Methods

This prospective study was conducted over a period of six months (March–August 2025) in the Department of Microbiology, Government General Hospital, Guntur. A total of 60 patients clinically suspected of Lyme disease, based on symptoms such as fever, erythema migrans, musculoskeletal pain, and neurological manifestations, were included in the study.

Study Design: Prospective observational study

Study Location: Department of Microbiology, Government General Hospital, Guntur

Study Duration: March 2025 to August 2025

Sample Size: 60 patients

Sample Size Calculation: Sample size calculated using the formula $n = Z^2pq/d^2$ with Z = 1.96 (95% confidence), p = 20%, q = 80%, and d = 10%.The calculated sample size was 61.44.It was rounded off to 60 for the study.

Ethical Approval: The study was approved by the Institutional Ethics Committee and Scientific Committee of Government General Hospital, Guntur. Informed consent was obtained from all participants.

Inclusion Criteria:
- Patients of all age groups and both genders
- Clinical features suggestive of Lyme disease (fever, rash, arthritis, neurological or cardiac symptoms)
- History of tick bite or environmental/occupational exposure.
- Patients willing to provide informed consent

Exclusion Criteria:
- Confirmed bacterial or viral infections (e.g., dengue, enteric fever)
- Patients already on antibiotic therapy for Lyme disease prior to sample collection
- Patients with confirmed alternative diagnoses explaining similar clinical features
- Inadequate or hemolyzed serum samples
- Patients who did not provide consent

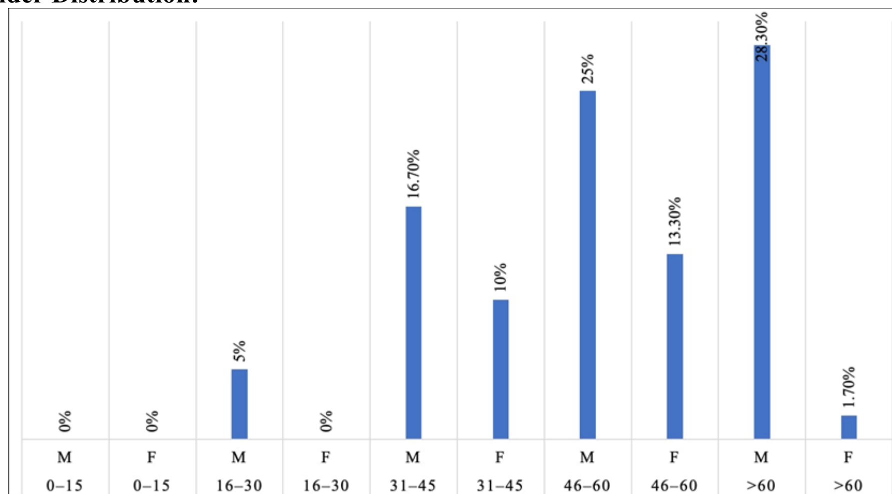
Procedure Methodology:

Venous blood samples were collected aseptically from clinically suspected cases, and serum was separated by centrifugation. The serum samples were then tested for *Borrelia burgdorferi*-specific IgM and IgG antibodies using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The results were interpreted based on standard guidelines provided with the kit[NovaLisa]Detection of Borrelia burgdorferi-specific IgM and IgG antibodies was performed using a commercially available NovaLisa™ ELISA kit (NovaTec Immundiagnostica GmbH, Germany). The assay is based on the indirect ELISA principle, where microtiter wells are coated with Borrelia antigens. Patient serum was added and incubated, allowing specific antibodies to bind. After washing, enzyme-conjugated secondary antibodies were added, followed by substrate, producing a color reaction. The optical density (OD) was measured using an ELISA reader, and results were interpreted according to the manufacturer's instructions.

III. Results

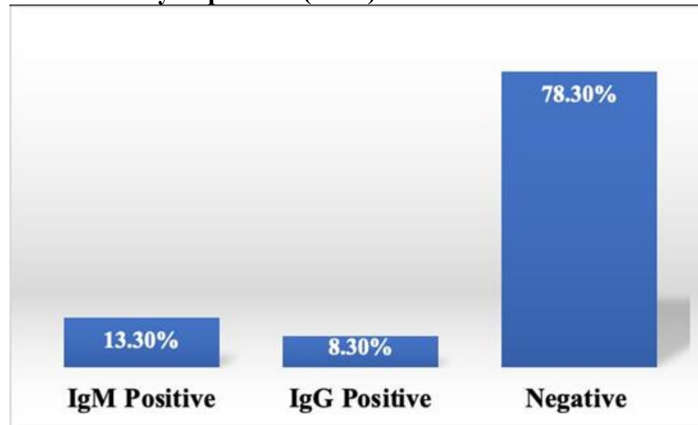
Out of 60 clinically suspected cases, the overall seroprevalence of Lyme disease was 21.6% (13/60), indicating a notable presence of infection in the study population. Among the seropositive cases, IgM antibodies were detected in 13.3% (8/60), suggesting recent or acute infection, while IgG antibodies were found in 8.3% (5/60), indicating past or ongoing exposure. The remaining 78.3% (47/60) cases were seronegative. Most seropositive cases were observed in middle-aged individuals with a male predominance. Clinically, common symptoms included erythema migrans (rash), fever, and joint pain, and many patients reported a history of tick exposure or outdoor activities, supporting the epidemiological pattern of Lyme disease transmission.

Age and Gender Distribution:



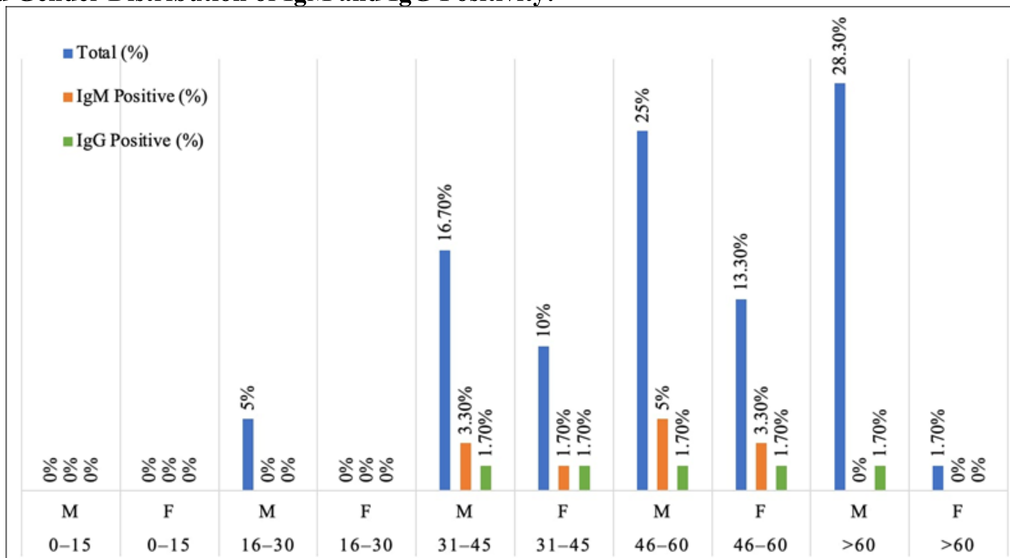
Graph no: 1 The age and gender distribution of the study population (n=60) showed no cases in the 0–15 years group. In the 16–30 years group, 3 cases (5%) were observed, all of which were males. The majority of cases were seen in the 31–45 years (26.7%) and 46–60 years (38.3%) age groups, with a male predominance in both. In the 31–45 years group, 10 males (16.7%) and 6 females (10%) were affected, while in the 46–60 years group, 15 males (25%) and 8 females (13.3%) were reported. Among individuals aged above 60 years, 18 cases (30%) were observed, predominantly in males (28.3%) compared to females (1.7%). Overall, the study population showed a higher prevalence in middle-aged and elderly males

Overall Serological Results of Study Population(n=60)



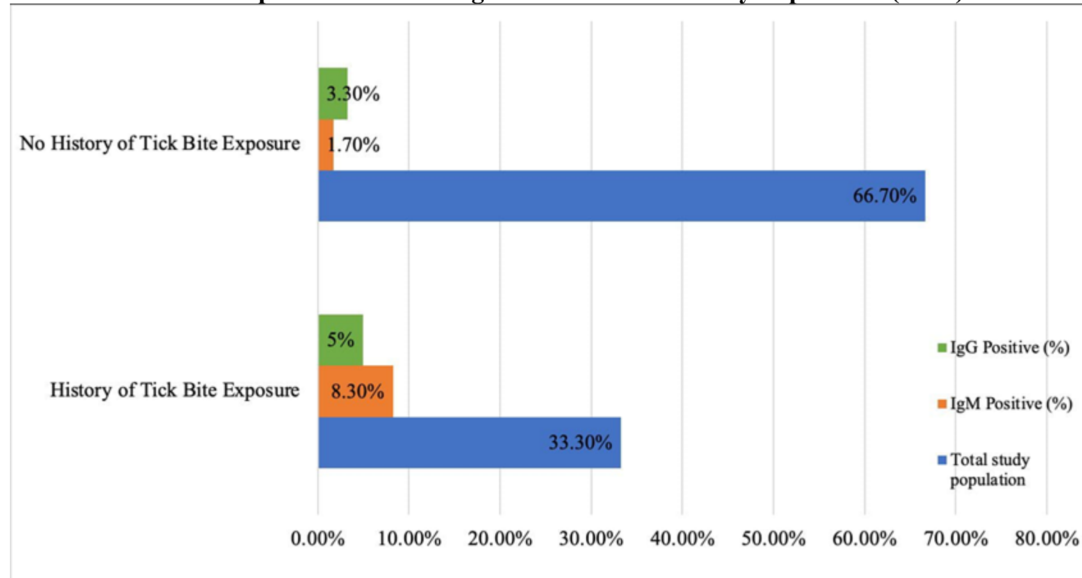
Graph no: 2 The overall seroprevalence of Lyme disease among the study population was 21.6% (13/60). Among the serological findings, IgM positivity was observed in 13.3% (8/60) of cases, indicating recent infection, while IgG positivity was seen in 8.3% (5/60), suggesting past exposure. The majority of patients, 78.3% (47/60), were negative for both IgM and IgG antibodies. These findings indicate that a smaller proportion of clinically suspected cases showed serological evidence of Lyme disease, with most individuals remaining seronegative.

Age and Gender Distribution of IgM and IgG Positivity:



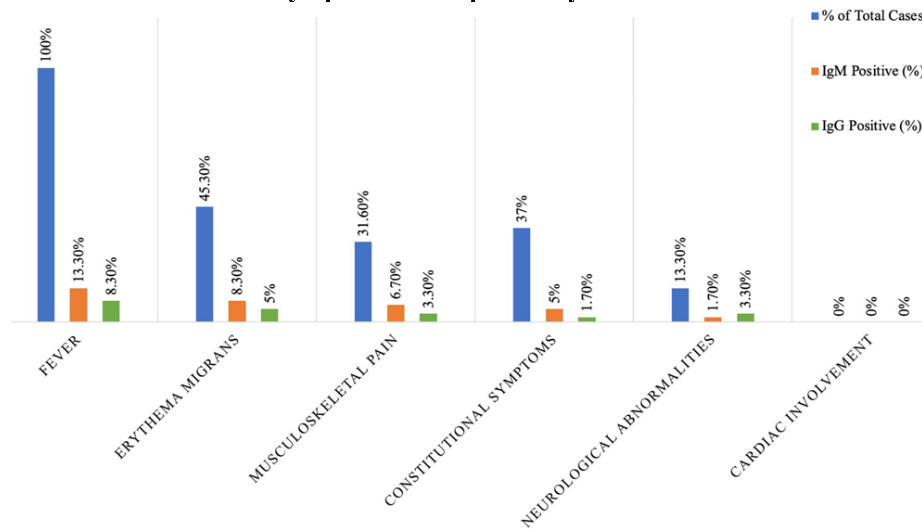
Graph no: 3 The age and gender distribution of seropositive cases showed that IgM positivity was mainly observed in the 31–45 years and 46–60 years age groups. In the 31–45 years group, 2 males (3.3%) and 1 female (1.7%) were IgM positive, while in the 46–60 years group, 3 males (5%) and 2 females (3.3%) were positive. Similarly, IgG positivity was also noted in the same age groups. In the 31–45 years group, 1 male (1.7%) and 1 female (1.7%) were IgG positive, and in the 46–60 years group, 1 male (1.7%) and 1 female (1.7%) were positive. Additionally, in individuals above 60 years, 1 male (1.7%) showed IgG positivity. Overall, seropositivity was more common in middle-aged individuals with a slight male predominance.

Correlation of Tick Bite Exposure with Serological Profile in the Study Population (n=60):



Graph no 4: Out of the total study population, 33.3% had a history of tick bite exposure, while 66.7% had no such history. Among those with tick exposure, IgM positivity was 8.3% and IgG positivity was 5%. In contrast, among those without tick exposure, IgM positivity was 1.7% and IgG positivity was 3.3%. Higher seropositivity was observed in individuals with a history of tick bite exposure.

Serological Correlation of Clinical Symptoms in Suspected Lyme disease:



Graph no: 5 Fever (100%) was observed in all cases, followed by erythema migrans (45.3%), constitutional symptoms (37%), and musculoskeletal pain (31.6%). Neurological abnormalities were present in 13.3% of cases, while no cardiac involvement was noted. IgM and IgG positivity were observed across these clinical features.

IV. Discussion

The present study demonstrated a seroprevalence of 21.6% among clinically suspected cases, indicating that Lyme disease is present in this region though often underdiagnosed. Similar findings have been reported in Indian studies, where serological evidence of Lyme borreliosis has been identified despite low clinical suspicion. Sharma et al. (2019) reported seropositivity in North India, emphasizing that Lyme disease may be more widespread than currently recognized.

In this study, IgM positivity (13.3%) was higher than IgG (8.3%), suggesting that many patients were in the early or recent phase of infection. This observation is consistent with the natural course of Lyme disease, where IgM antibodies appear earlier, followed by IgG in later stages. Marques (2020) highlighted that serological diagnosis using ELISA remains the most practical method in resource-limited settings, although confirmatory

tests like Western blot improve specificity.Clinical correlation in our study showed that erythema migrans, fever, musculoskeletal pain, and neurological symptoms were more commonly associated with seropositive cases. These findings are in agreement with Cardenas-de la Garza et al. (2019), who described the wide clinical spectrum of Lyme disease involving dermatological, neurological, and rheumatological manifestations.

A significant association was observed between seropositivity and risk factors such as tick exposure and outdoor occupational activities. This supports the established epidemiological pattern of Lyme disease transmission through Ixodes ticks, with reservoirs like rodents and birds maintaining the infection cycle. Oei et al. (2021) also emphasized the importance of environmental exposure in the transmission dynamics of Lyme borreliosis.However, the present study has certain limitations. The sample size was relatively small, and confirmatory testing such as Western blot or molecular methods (PCR) was not performed. These limitations may affect the accuracy of diagnosis, as ELISA alone can sometimes give false-positive results. Despite these constraints, the study provides valuable preliminary data on the presence of Lyme disease in this region.

Another important aspect of Lyme disease is the challenge in laboratory diagnosis. Although ELISA is widely used for screening due to its accessibility and cost-effectiveness, it may lack specificity, especially in endemic or cross-reactive conditions. The two-tier testing strategy (ELISA followed by Western blot) is recommended for confirmation, but it is not routinely available in many resource-limited settings. According to Marques (2020) and Oei et al. (2021), advancements in molecular methods such as PCR and improved serological assays may enhance diagnostic accuracy in the future.Furthermore, public health awareness and clinician suspicion play a crucial role in early detection of Lyme disease. In India, low awareness among healthcare professionals and lack of routine screening often lead to missed or delayed diagnosis. Educational initiatives, improved surveillance systems, and inclusion of Lyme disease in differential diagnosis of febrile and neurological illnesses are essential steps. Strengthening vector control measures and promoting preventive strategies, especially among high-risk populations like farmers and forest workers, can help reduce disease transmission.

Overall, the findings highlight the need for increased clinical awareness, improved diagnostic facilities, and larger epidemiological studies to better understand the burden of Lyme disease in India.

V. Conclusion

The overall seroprevalence of Lyme disease was 21.6%, as detected by IgM and IgG ELISA, confirming the presence of *Borrelia burgdorferi* infection in the study population. This finding suggests that Lyme disease, though underrecognized, may be present in this region and should be considered in differential diagnosis of febrile and multisystem illnesses.

Clinical correlation revealed that erythema migrans, fever, musculoskeletal pain, and neurological features were more frequently associated with seropositive cases, supporting the clinical suspicion of Lyme disease. Among these, erythema migrans remains a key early diagnostic indicator, while neurological manifestations indicate possible dissemination of infection.

Seropositivity was observed across different age groups and occupational categories, with relatively higher prevalence among individuals with a history of tick exposure and those engaged in outdoor activities such as farming. This emphasizes the role of environmental and occupational risk factors in disease transmission. However, statistical significance could not be firmly established due to the limited sample size.The use of ELISA as a screening tool proved to be practical and useful for serodiagnosis; however, it may be associated with limitations such as cross-reactivity and inability to distinguish between active and past infection. Confirmatory tests like Western blot, though not performed in this study, could improve diagnostic accuracy.

Overall, these findings highlight the need for increased clinical awareness, early diagnostic testing, and implementation of preventive strategies, especially in high-risk populations. Further large-scale, multicentric studies are required to better understand the epidemiology, risk factors, and true burden of Lyme disease in this region.

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