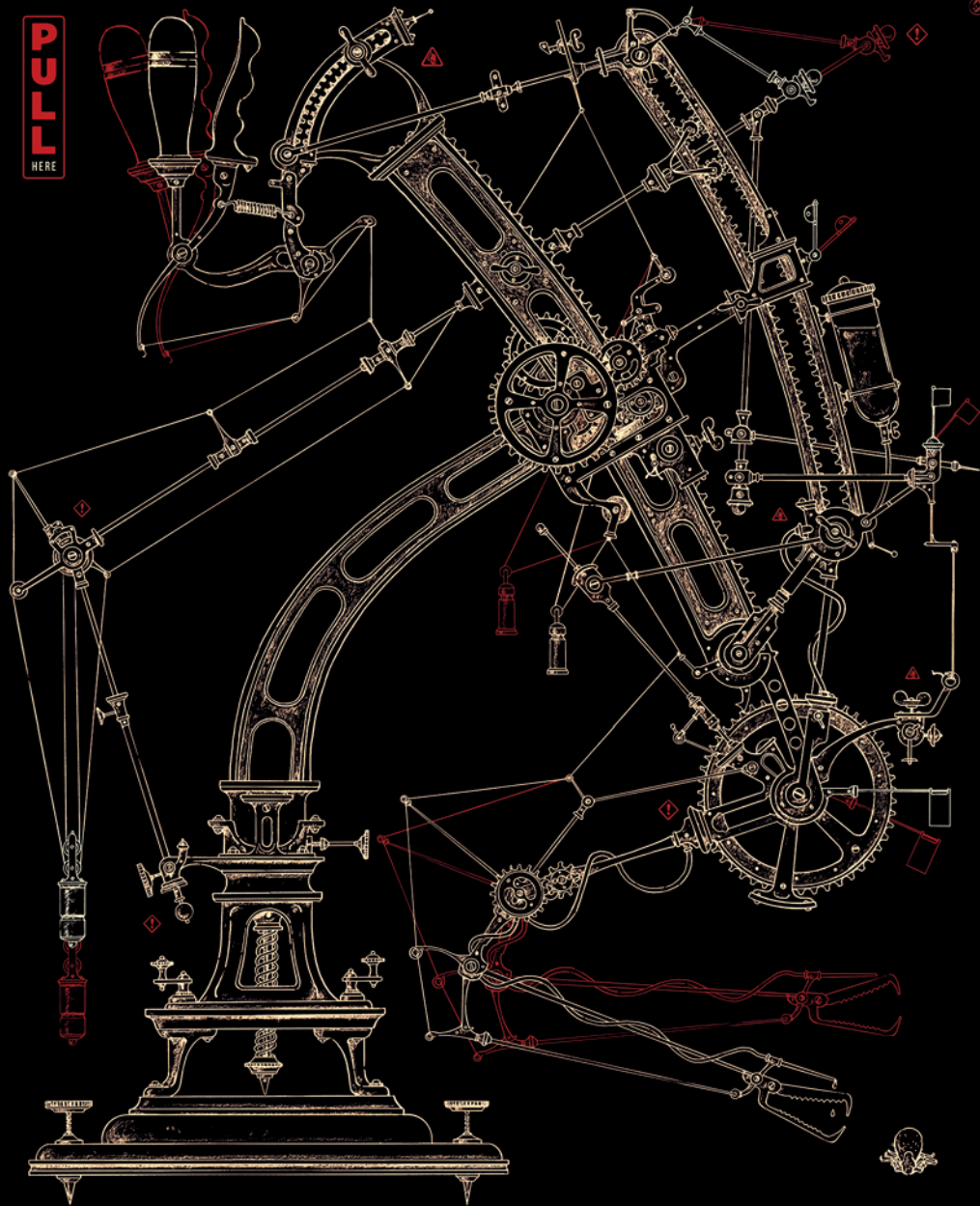


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Molecular screening of *Borrelia miyamotoi* and Tick-Borne Encephalitis Virus in ixodid ticks in urban green areas in Serbia

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Introduction: Zoonotic tick-borne diseases (TBDs) represent an increasing threat in urban areas, including city parks and green recreational areas. *Borrelia miyamotoi* is pathogenic to humans but there is still little information about its circulation in nature and potential local impact on human health, while tick-borne encephalitis virus (TBEV) is endemic in many European countries and sporadic autochthonous cases in humans have been reported in Serbia.

The objective of our study was to determine the prevalence of two emerging zoonotic tick-borne pathogens (TBPs) – *B. miyamotoi* and TBEV in urban areas in the city of Belgrade (24 localities), four localities in Eastern Serbia, one in the province of the Vojvodina.

Methods: At 29 localities, a total of 480 ticks were collected from March to June 2021, and identified to the species level by using morphological keys. DNA and RNA were extracted from individual tick samples, while for molecular detection, probe-based qPCR and nested PCR were applied.

Results: Among 480 ticks, 445 were identified as *Ixodes ricinus* (213 males, 176 females, 56 nymphs), 30 as *Dermacentor reticulatus* (18 females, 12 males), three *Dermacentor marginatus* females, one *Haemaphysalis concinna* female, and one *Haemaphysalis punctata* male. Using probe-based qPCR, the prevalence of *B. miyamotoi* in ticks was 1.04% (DNA was detected in five *I. ricinus*, two females and two males collected from three Belgrade localities and one male from Eastern Serbia), while sequencing was successful in samples of two *I. ricinus* females collected from two Belgrade localities and one male from Eastern Serbia. TBEV RNA was not detected in any sample.

Conclusions: This is the first report on the presence of the emerging zoonotic pathogen *B. miyamotoi* in *I. ricinus* ticks in urban green areas in Serbia, indicating the risk of *B. miyamotoi* disease. Risk areas within cities should be identified and knowledge regarding TBPs and TBDs among the general population in urban areas should be increased.