# TICK-BORNE PATHOGENS IN URBAN GREEN SPACES IN CHISINAU'S CITY PARKS

*Alexandr MOROZOV[1], ORCID: 0000-0002-6792-7957*
*Ion TODERAS[1], ORCID: 0000-0001-6159-5325*
*Vadim NIRCA[2], ORCID:*
*Anna VICTOROVA[2], ORCID:*
*[1] Center of Research of Biological Invasions, Institute of Zoology, Chisinau, Moldova*
*[2] Molecular Biology Department, Imunotehnomed Ltd., Chisinau, Moldova*

**CZU: 595.421(478-21)**

e-mail: AcarologMD@gmail.com
iontoderas@yahoo.com

*City parks of Chisinau, Moldova, were examined for the occurrence of host-seeking ticks and collected specimens were examined for presence of Borrelia spp. and Anaplasma phagocitophilum pathogens. In the spring season 2021, the collected individuals were Dermacentor reticulatus (n = 42), Demacentor marginatus (n = 21) and Ixodes ricinus (n = 146), Haemaphysalis punctata (n=2) . Totally, 5.2% of tested ticks were PCR-positive with A. phagocytophilum and 13.2% gave positive PCR results for B. burgdoorferi s.l.. We did not detect any mixed infections. These results indicate that city parks in Chisinau are habitat for ticks of high medical and veterinary importance. Due to the risk of acquiring tick-borne pathogens, the urban environment such as parks and home gardens, should be monitored for the presence of ticks and their infection with human pathogens. Screening of these areas is an important preventive element of environmental epidemiology.*

***Keywords:*** *Anaplasma phagocytophilum, Borrelia spp.,* Chisinau, city parks, ticks.

## INTRODUCTION:

Ticks are hematophagous arachnids known to be primary vectors for various pathogens that cause diseases in humans and animals. These vector-borne diseases, including Lyme borreliosis and anaplasmosis, are of increasing public health concern globally [1]. Urban green spaces, with their rich biodiversity and frequent human interaction, can often serve as reservoirs for tick populations, thus increasing the potential for disease transmission [2]. Chisinau, the capital of Moldova, known as the "Green Capital", boasts of vast verdant spaces, parks, and recreational areas. These green spaces, while providing relaxation and a touch of nature amidst urban settings, may harbor ticks and consequently the pathogens they carry [3]. Given the recreational significance of these parks to the city's inhabitants and visitors, there is a pressing need to understand the tick distribution and associated pathogens within them. This study aims to provide an in-depth analysis of tick prevalence across different parks in Chisinau and investigate

the presence of specific tick-borne pathogens. The findings will not only inform local health authorities and park management but also emphasize the importance of awareness campaigns for the general public.

## MATERIALS AND METHODS

**Study Area:**

Chisinau, the capital city of Moldova, spans across the central region of the country. It is often referred to as the "Green Capital" owing to its numerous parks and verdant spaces. The climate of Chisinau is continental with hot summers and cold winters. This city is an important cultural, economic, and political center of Moldova and is home to numerous historical monuments and government buildings. Chisinau, as of the last census prior to 2021, had a population approaching 700,000 inhabitants [4].

For the purpose of tick collection and research, samples were taken from three significant city parks of Chisinau frequented by residents and tourists alike: "La Izvor" Park, Park Rascani, and "Valea Morilor" Park.

**Short Characteristics of the Collection Sites:**

"La Izvor" Park: Covering an area of approximately 30 hectares, "La Izvor" Park is located in the western part of the city. Established in the late 20th century, the park surrounds a reservoir and features vast open spaces, playgrounds, and recreational zones. It is primarily populated by a variety of deciduous trees, including oaks and lindens, and is home to several species of birds and small mammals [5].

"Parcul Râșcani" (or "Rascani Park"): Spanning approximately 45 hectares, "Parcul Râșcani" is nestled in the northern sector of Chisinau. With origins dating back to the mid-20th century, this park is a blend of natural landscapes. It boasts a serene pond that attracts both local bird species and those migrating, providing a tranquil spot for visitors to relax. The park is dotted with pathways, making it a favored destination for joggers and walkers. A mix of evergreen conifers and broadleaf trees, such as maples and beeches, provide shade and add to the park's aesthetic appeal [6]. Children's play areas, coupled with open spaces for picnics and gatherings, make it a favorite among families. Additionally, the park often serves as a venue for local community events, bringing together residents in celebration and unity.

"Valea Morilor" Park: With an area of roughly 102 hectares, "Valea Morilor" Park is one of the largest green spaces in the city. Established in the 1950s, the park features a lake suitable for boating during summer and ice-skating in winter. The surrounding terrain is diverse, featuring cliffs, stairways, and a variety of tree species including pine, oak, and chestnut. The park is a hub of activity, offering various recreational activities and hosting several cultural events throughout the year [7].

**Tick collections**

The harvest period was in the spring months, April-May 2021. The ticks were col-

lected from vegetation using the flagging method, sweeping vegetation with flannel flags to a height of 1 m. The collected arachnids were identified according to the key of hard tick species identification under a stereomicroscope using the identification keys of Nosek (1972), Fillipova (1979, 1998) and Apanaskevich (2006, 2008) [8,9,10].

**DNA analysis**

DNA was isolated by the Prep-NA commercial kit (DNA Tehnology, Moskow, Russia) followed the manufacturer protocol [11], separately from each individual tick. Pools (5 isolates each) were made for screening, after which all positive pools (PCR+ = presence of pathogen) were retested for individuals.

The detection of *Borrelia spp.* was performed with Borrelia-gen commercial kit [12] (DNA Tehnology, Moskow, Russia).

The detection of *A. phagocytophilum* was carried out using real-time PCR that focused on a 77 bp segment of the msp2 gene, following established methods [13]. To verify positive outcomes for *A. phagocytophilum*, we employed a nested PCR that aimed at a 497 bp section of the 16S rRNA gene [14]. We then observed the nested PCR products using 2% agarose gel electrophoresis, and for staining, we utilized Gel Red by Biotium (Fremont, CA, USA).

## RESULTS AND DISCUSSION

The objective of this study was to assess the prevalence and distribution of ticks in various parks in Chisinau, Moldova, and to determine the presence of specific pathogens within these tick populations. The results from the collection data, spanning two months (Table 1), provide an in-depth overview of the diversity and distribution of tick species across La Izvor, Parcul Râșcani, and Valea Morilor.

*Ixodes ricinus*, commonly known as castor bean tick, was the most prominent species across all the parks. When analyzed month by month, there seemed to be a clear uptick in the number of these ticks collected in May, as it always should be, based on the standard life cycle of ticks in our region. This suggests a possible seasonal increase in their activity, aligning with the spring season. Of interest, Valea Morilor's higher tick counts could be attributed to its larger size, richer fauna providing more hosts for ticks, and greater distance from urban influences, offering a more natural tick habitat.

*Table 1*

**Monthly distribution of tick species across Chisinau parks in spring 2021.**

| Tick species/ Park | April | | | | May | | | |
|---|---|---|---|---|---|---|---|---|
| | La Izvor | Parcul Rascani | Valea Morilor | | La Izvor | Parcul Rascani | Valea Morilor | Total |
| *D.reticulatus* (imago) | 2 | 1 | 8 | | 5 | 3 | 20 | **39** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *D.reticulatus* (nymphs) | 0 | 0 | 0 | | 0 | 0 | 3 | **3** |
| *D. marginatus* (imago) | 1 | 0 | 4 | | 2 | 2 | 11 | **20** |
| *D. marginatus* (nymphs) | 0 | 0 | 0 | | 0 | 0 | 1 | **1** |
| *Haemaphisalis punctata* (imago) | 1 | 0 | 0 | | 0 | 0 | 1 | **2** |
| *I. ricinus* (imago) | 6 | 3 | 22 | | 13 | 6 | 48 | **98** |
| *I. ricinus* (nymphs) | 1 | 1 | 5 | | 8 | 3 | 30 | **48** |
| **Total** | **12** | **5** | **47** | | **27** | **14** | **106** | **211** |

The second table focuses on the screening results, highlighting the prevalence of two significant tick-borne pathogens, *B. burgdorferi* sensu lato and *A. phagocytophilum*, within the *I. ricinus* and *D. reticulatus* populations (Table 2). The data suggests that there's a significant presence of these pathogens, especially the *B. burgdorferi* sensu lato, which is the causative agent for Lyme disease. This is particularly evident within the adult *I. ricinus* populations, with Valea Morilor once again standing out as a hotspot.

*Table 2*

**Prevalence of pathogens in ticks from Chisinau parks**

| **Tick species, Park** | ***Borrelia burgdorferi* sensu lato** | ***Anaplasma phagocytophilum*** | **Total** |
|---|---|---|---|
| *I. ricinus* (imago), "La Izvor", | 3 | 1 | 4 |
| *I. ricinus* (imago), "Parcul Rascani" | 2 | 1 | 3 |
| *I. ricinus* (imago), "Valea Morilor" | 11 | 5 | 16 |
| *I. ricinus* (nymphs), "La Izvor" | 1 | 0 | 1 |
| *I. ricinus* (nymphs), "Parcul Rascani" | 5 | 2 | 7 |
| *I. ricinus* (nymphs), "Valea Morilor" | 5 | 2 | 7 |
| *D. reticulatus* (imago), "Valea Morilor" | 1 | 0 | 1 |
| **Total** | **28** | **11** | **39** |

Such findings emphasize the importance of public health awareness. People frequenting these parks, particularly Valea Morilor, should be informed about the risks associated with tick bites, the potential symptoms of Lyme disease, and other tick-borne illnesses. Given that Lyme disease can present with diverse clinical manifestations, ranging from skin rashes to neurological complications if left untreated, this becomes even more crucial.

On the other hand, *A. phagocytophilum*, the agent of human granulocytic anaplasmosis, while less prevalent, is still of notable concern. The consequences of this pathogen can be severe, especially for individuals with compromised immune systems, the elderly, or those with co-infections.

Interestingly, while *D. reticulatu*s ticks were found in substantial numbers, only one adult from Valea Morilor tested positive for *B. burgdorferi* sensu lato, suggesting that the *I. ricinus* populations may be the primary carriers of this pathogen in the studied areas.

Considering the data from both tables, the urban environment's potential as a habitat for ticks of medical and veterinary significance cannot be understated. The high prevalence of these ticks in city parks like those in Chisinau implies that urban green spaces can be significant reservoirs for tick-borne diseases.

In 2006 Dr. Movila's study reported that 9% of tested *I. ricinus* ticks were infected with *A. phagocytophilum*, a figure that aligns closely with our findings of 7.5% among *I. rinicus* ticks [15]. However, there is a notable difference in the prevalence of *B. burgdorferi s.l.* While the 2006 study found a 25.2% positive PCR rate for *B. burgdorferi s.l.*, our recent findings indicate a lower prevalence of 19.2% among *I. rinicus* ticks. Although in raw comparison to the study conducted by Dr. Movila, our current research indicates a decline in the prevalence of pathogens in ticks from Chisinau parks there's no statistically significant difference, given a 95% confidence level. The critical z-value for a 95% confidence level is 1.96. If the computed z-value is greater than 1.96 or less than -1.96, the difference in proportions is statistically significant at the 95% confidence level.

For *B. burgdorferi s.l.*: The calculated z-value is approximately 1.72. This is less than 1.96, so the difference for *B. burgdorferi s.l.* is not statistically significant at the 95% confidence level.

For *A. phagocytophilum*: The calculated z-value is approximately 0.482. This is also less than 1.96, meaning the difference in proportions for *A. phagocytophilum* is not statistically significant at the 95% confidence level either.

Lastly, this study underlines the importance of environmental epidemiology in urban settings. Regular monitoring and screening of these areas, combined with public awareness campaigns, could play a pivotal role in disease prevention. In future studies, it would be beneficial to assess other potential tick-borne pathogens and possibly expand the study area to include other urban and suburban green spaces.

IN CONCLUSION, the presence of tick-borne pathogens in city parks in Chisinau, particularly in the *I. ricinus* population, accentuates the need for continuous monitoring and awareness campaigns. It's not just rural areas that present risks associated with ticks; urban green spaces can be just as perilous if not adequately addressed.

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