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PREVALENCE AND SURVEILLANCE OF LYME BORELIOSIS IN ISTRIA, CROATIA

Abstract: Climate change is the main cause of global warming, which significantly affects human health, among other things, due to the spread of tick-borne pathogens. The paper presents a spatial analysis of collected ticks (from 2020 to 2023), historical data on the prevalence of Lyme boreliosis (LB) in humans (from 2016 to 2023), and the prevalence of the causative agent of LB, bacterias from the genus *Borrelia* sp. extracted from ticks. Pathogenic spirochetes of the *Borrelia burgdorferi* s.l. complex is transmitted by ticks of the genus *Ixodes*, and in the territory of Istria, three vector species for this pathogen stand out: *Ixodes ricinus* (70.72%), *Ixodes hexagonus* (0.13%) and *Ixodes frontalis* (1.40 %). In the observed period, a total of 115 cases of borreliosis were recorded in the examined period. The largest number of human cases of LB are recorded in urban areas. Most ticks are collected in urban areas from dogs, which are sentinel animals for human borreliosis. After proving the presence of *Borrelia* sp. by molecular analyses from ticks, by qPCR method, all obtained data were entered into the QGIS database to monitor the areas of greater infestation of Borrelia-infected ticks. The goal of the research is to collect data on the distribution of tick species in Istria and to investigate their role in the transmission of bacteria from the *Borrelia burgdorferi* s. l. complex. Scientific contribution of this research is the monitoring of ticks and the presence of LB in Istria, the identification of habitats with higher infestations, and creation of the first systematic database on tick-borne diseases in Croatia. The use of GIS technologies significantly contributes to disease surveillance.

Keywords: GIS, ticks, Lyme boreliosis, Istria

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Introduction

Since humans are increasingly invading the various ecological niches of animals, the need to better understand the ecology of parasites of free-ranging wildlife has never been greater. The emergence or resurgence of arthropod-born diseases potentially fueled by increased reporting, has contributed to an increased awareness of the role played by vectors in the transmission of human diseases. As a result, arthropods such as mosquitoes and ticks are drawing more attention from scientists involved in human health issues, therefore, understanding the epidemiology of the many serious diseases that threaten us is essential (Rogers & Randolph, 2000). Monitoring the presence of sources of infectious diseases is essential for the preservation of public health and emphasizes the need for cooperation and communication between different disciplines within the concept known as "One Health" (Zdolec, 2011). Geographic Information Systems (GIS) play a major role in the domain of public health, from infectious disease surveillance to mapping and monitoring the spatial and temporal distribution of infection vectors (Shaw, 2012). In the Republic of Croatia, as in many European countries, there is a lack of spatial data on the distribution and activity of vectors such as ticks that are responsible for the onset of disease (ECDC, 2018). Knowledge of the spatial distribution of ticks in each region is essential for assessing the risk of tick-borne diseases and understanding the ecological dynamics of tick-host interactions (Léger et al., 2013). Lyme borreliosis (LB) is a tick-borne disease caused in Europe by unicellular spirochetes of the *Borrelia burgdorferi* sensu lato complex (*Bbsl*) and consists of 19 species of bacteria from the genus of the same name (Kalenić & Sur, 2019). LB is considered a re-emerging zoonosis and is subject to reporting obligations and is epidemiologically monitored in the Republic of Croatia (NN, 2014; NN, 2021) and at the level of the European Union (HZJZ, 2017a). The symptoms of LB include fatigue, chills, fever, headache, muscle and joint pain, and enlarged lymph nodes, along with a characteristic rash on the skin called *erythema migrans* (Figure 2), an oval or ring-like reddening of the skin that spreads slowly, reaching a diameter usually greater than 5 cm. LB is treated with antibiotics, which are crucial in preventing the development of later stages of the disease, which may include consequences such as arthritis (inflammation of the joints), myocarditis (inflammation of the heart muscle), meningitis and/or neuritis (inflammation of nerves) (HZJZ, 2016).

Analysis of the state of the problems

According to data from the Epidemiology Service of the Croatian Institute of Public Health (HZJZ), 661 cases of Lyme borreliosis (LB) were reported in the Republic of Croatia in 2013, and 470 cases in 2014, with an increasing trend observed over the past ten years. The County of Istria is considered an area with a lower incidence of LB (Ropac, 2019). The most widespread tick species in the County of Istria is the common or forest tick, *Ixodes ricinus* (70.84%) (Cvek et al., 2023), which is known as the primary carrier of LB (Figure 1). Over a period of three years, ticks were collected from various habitats using the flagging/dragging method and manually from different hosts in the area, including humans, pets, and both wild and domestic animals. The ticks were morphologically

identified, and the presence of *Bbsl* bacteria was detected in the tick DNA using the qPCR method. GIS methodology was used to map the locations where ticks were collected and to analyze the presence of *Bbsl*. The results were compared with reported cases of LB. This paper aims to highlight the importance of monitoring zoonotic reservoirs and vectors in Croatia.

Fig. 1. Three developmental stages of *Ixodes ricinus* ticks (nymph, female, and male) collected in Park Forest Šijana, Pula
Source: own work 2020 (Maja Cvek)

Fig. 2. Erythema migrans – a typical symptom of LB
Source*:* HZJZ, 2017b

Materials and methods

Study area. Istria is the largest Adriatic peninsula, located in the westernmost part of Croatia. The County of Istria covers a total area of 2,813 km^2 and has a population of 195,237 spread across 10 cities and 31 municipalities (DZS, 2022). The Mediterranean climate predominates along the Istrian coast, gradually transitioning to a continental climate towards the interior. The main characteristics of the Mediterranean climate include hot, dry summers with an average of 2,400 sunny hours per year. Winters are mild and pleasant, with snow being a rare occurrence. The annual average air temperature along the northern coast is around 14°C, while in the southern areas and islands, it is approximately 16°C (Moj tender, 2020). The specific location of Istria, coupled with these climatic conditions, provides a suitable habitat for ticks.

Collection of Historical Data on Zoonoses (from 2016 to Mid-2023) and Collection of Tick Samples (from 2020 to 2023) in the County of Istria. In accordance with legal regulations (NN, 2023), the Institute of Public Health of the County of Istria (NZZJZIŽ) collects data on the occurrence of zoonoses in humans and submits them to HZJZ. Since 2016, reports on infectious diseases have been submitted through the Agency for Information Systems and Information Support (APIS IT).

A review of annual reports on the occurrence of animal diseases in the Republic of Croatia (NN, 2013) collected data on the occurrence of LB in animals transmitted by ticks.

This paper analyzes data on officially reported cases of LB transmitted by ticks, as reported by doctors and veterinarians in the County of Istria from 2016 to mid-2023.

Tick samples were collected in the County of Istria between 2020 and 2023. Sampling methods included a combined technique of tick tweezing and direct collection from hosts (domestic and wild animals, as well as pets). Ticks were sampled in various habitats, including city parks, recreational areas, picnic spots, uncultivated agricultural areas, Brijuni National Park, and forested regions, using the flagging/dragging method.

Morphological identification of ticks was performed using identification keys for tick species (Estrada-Peña et al., 2004) and a stereo microscope (Olympus SZX9, Olympus Optical Co. Europa GMBH, Hamburg, Germany). All specimens were photographed using a digital camera (Olympus SC50, Münster, Germany). The collected ticks were stored at -80°C for further analysis.

Statistical analysis. All collected ticks were identified to the species level and classified by sex and developmental stage (e.g. larvae, nymphs, and adults). Furthermore, the interactions between individual tick species and their hosts were analyzed (Cvek et al., 2023). In this study, the spatial distribution of tick collections in Istrian County was compared with the spatial occurrence of reported Lyme disease (LB) cases, and the results were visualized using the GIS application QGIS (QGIS v3, Gossau, Switzerland).

Results and discussion

This study provides a comprehensive spatial analysis of tick collections conducted from 2020 to 2023 (Figure 3), alongside historical data on the prevalence of LB between 2016 and 2023, and the detection of *Bbsl* in ticks (Figure 4), all within the Istrian region. A total of 2,349 ticks were collected, representing 12 different species. Among these, three key vector species for Lyme disease were particularly noteworthy: Ixodes ricinus (70.72%), Ixodes hexagonus (0.13%), and Ixodes frontalis (1.40%). Over the course of the study, 115 cases of LB were documented, underscoring the continued relevance of this public health issue in the region.

Most of human LB cases in Istria occurred in urban areas, while fewer cases were reported in rural areas. Most ticks were collected in urban environments, primarily from dogs, which serve as sentinel animals for human borreliosis. After confirming the presence of *Borrelia* spp. in ticks through molecular analysis (qPCR method), all data were entered into the QGIS database. This allowed for the identification of areas with higher tick infestations carrying the pathogen.

The aim of this study was to gather data on the distribution of tick species in Istria and to examine their role in transmitting *Bbsl*. The expected scientific contribution of this research includes continuous monitoring of tick populations and the presence of Lyme disease in the region, identification of habitats with higher infestations, and the creation of the first systematic database on tick-borne diseases in Croatia. This work will significantly enhance efforts to control and prevent zoonotic diseases transmitted by ticks between animals and humans.

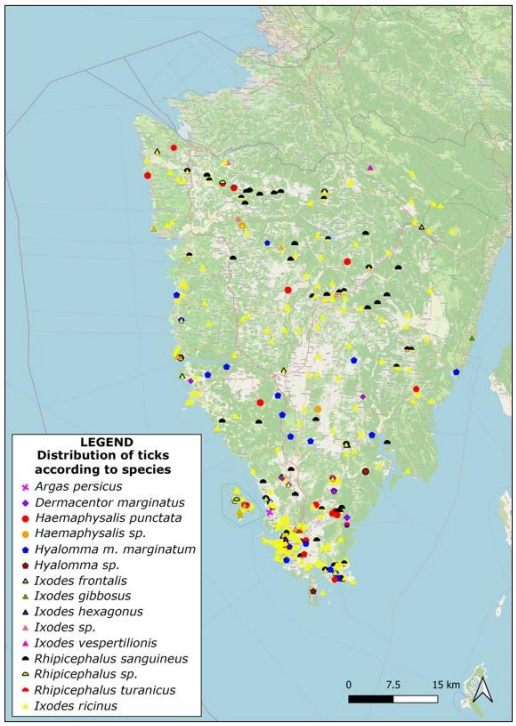

Fig. 3. Spatial distribution of tick species in Istria, collected using flagging/dragging and host methods (2020–2023)
Source: Cvek et al., 2023

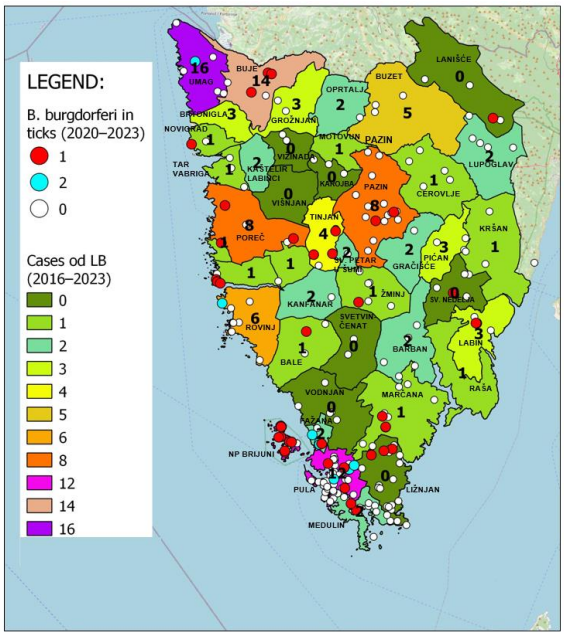

Fig. 4. The prevalence of B. burgdorferi s.l. extracted from ticks is represented by circles (red – confirmed, blue – suspected, white – not determined), alongside the distribution of Lyme disease (LB) cases by local government units for the period 2016–2023. The scale indicates the number of recorded cases, ranging from 0 cases (green) to 16 cases (purple)
Source: own study 2024 (Maja Cvek)

Lyme boreliosis in Istria

Pathogenic spirochetes *Bbsl* which are transmitted by ticks of the genus *Ixodes*, have been found in Istria. The following tick species were identified: *I. ricinus*, *I. hexagonus*, *I. frontalis*, *I. gibbosus*, and *I. vespertilionis*., and the last two of which are not vectors of *Bbsl* (Table 1).

Table 1. Ticks of the genus Ixodes collected in Istrian County from 2020 to 2023

	I. ricinus	*I. hexagonus*	*I. frontalis*	*I. gibbosus*	*I. vespertilionis*
vector *B. burgdorferi*	yes	yes	yes	unknown	no
method of collection: host	550	3	3	1	2
flagging / dragging method	1111		30		
TOTAL NO. *Ixodes sp.*:	**1661**	**3**	**33**	**1**	**2**

Source: own study 2024 (Maja Cvek)

Figure 5 illustrates the percentage of people with LB by age group in Istria County from 2020 to 2023. The data reveals that the disease is most prevalent in older age groups, with 23% of individuals aged 60 and over affected. This is followed by the working-age population, particularly those aged 30-39, at 18%. LB is also notably present in both the older working-age group (50-59 years) and children aged 5–9, each representing 16% of cases. Additionally, the middle-aged and young working-age groups each account for 9%, while 8% of cases are observed in children aged 0–4 years. In adolescents, the disease is nearly absent, with only 1% of cases reported.

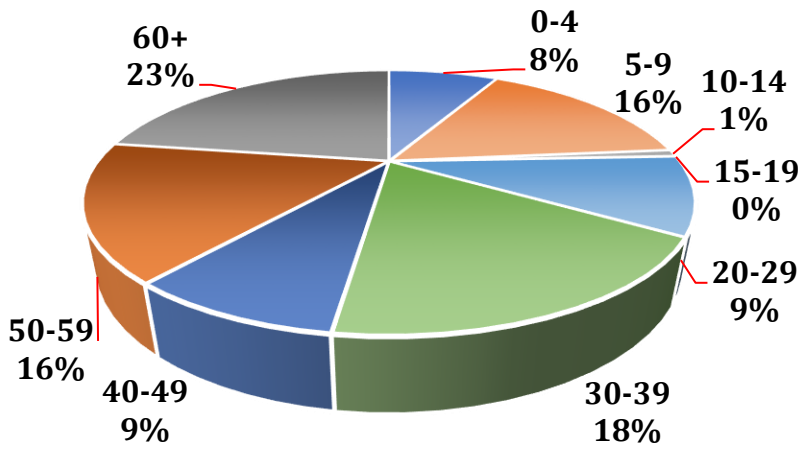

Fig. 5. Percentage of patients with Lyme Borreliosis in Istria County (2016 – mid-2023)
Source: own study 2024 (Maja Cvek)

Figure 6 shows the number of people with Lyme Borreliosis (LB) by year in Istria County during the same period. The highest number of cases (24) was recorded in 2018, the year before the onset of the coronavirus pandemic. This was followed by a slight decrease in cases (20) in 2019, after the introduction of coronavirus measures. A drastic decline in LB cases (4) occurred in 2021, when coronavirus restrictions were fully implemented. However, a resurgence in cases was observed in 2022 and 2023, with 12 cases recorded in each of these years.

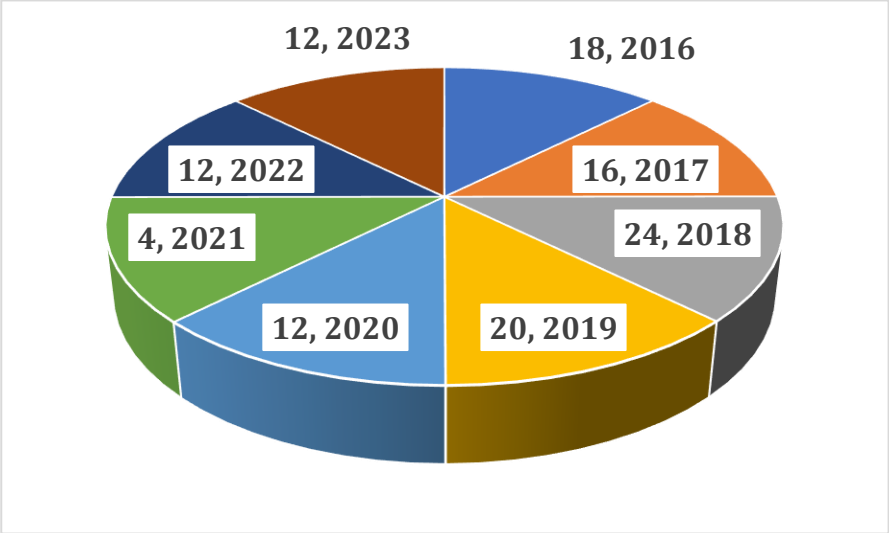

Fig. 6. Number of LB patients by year in Istria County (2020–2023)
Source: own study 2024 (Maja Cvek)

Conclusions

Ticks are significant carriers of pathogens in both human and veterinary medicine, and they have been identified as re-emerging threats to public health. The prevalence of ticks is closely correlated with environmental and climatic factors. Understanding the spatial distribution of ticks in a given region is crucial for assessing the risk of tick-borne diseases (Léger et al., 2013).

Knowledge of the spatio-temporal distribution of competent vectors is essential for identifying the risk of infectious diseases. By mapping the distribution of ticks, we can visualize, integrate, and analyze various data sets, which helps in identifying high-risk areas and informing disease prevention and control measures.

Geographic Information Systems (GIS) provide valuable tools for monitoring tick distribution. GIS allows for the management of spatial data and the prediction of infectious disease vectors' emergence. Ticks, with their strong ties to the ecosystem, are an ideal example of how GIS can be applied in health risk management.

Despite the importance of monitoring tick distribution, many European countries lack spatial data on tick populations and their role as vectors of zoonoses. This gap hinders effective surveillance, risk assessment, and the creation of predictive models for the occurrence of tick-borne diseases.

Improved tick surveillance would enhance public and veterinary risk assessments, ensuring better preparedness for tick-borne diseases.

This paper emphasizes the importance of systematically monitoring zoonotic disease reservoirs and vectors in Croatia. The highest number of human Lyme borreliosis (LB) cases in Istria were recorded in urban areas, with fewer cases observed in rural regions. Interestingly, most ticks were collected from dogs in urban environments, as dogs are sentinel animals for human LB (Duncan et al., 2004). Future research should focus on tracking and linking zoonoses transmitted by arthropods, such as ticks, to both humans and animals. To gain a comprehensive understanding of health risks related to disease vectors like ticks, collaboration across disciplines – such as medicine, biology, veterinary science, and GIS – based on the "One Health" approach, is essential.

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