Infectious diseases now 54 (2024) 104959

Contents lists available at ScienceDirect

Infectious Diseases Now

journal homepage: www.sciencedirect.com/journal/infectious-diseases-now

Original article

Tick-borne diseases at the crossroads of the Middle East and central Europe

Pavle Banović [a,b,c,*,1], Dejan Jakimovski [d,e,f,1], Ivana Bogdan [c,g], Verica Simin [c,g], Dragana Mijatović [c,h], Mile Bosilkovski [d,e], Sofija Mateska [e,f], Adrian A. Díaz-Sánchez [i], Angelique Foucault-Simonin [j], Zbigniew Zając [k], Jaonna Kulisz [k], Sara Moutailler [j], Alejandro Cabezas-Cruz [j,*]

[a] Clinic for Lyme Borreliosis and Other Tick-Borne Diseases, Department of Prevention of Rabies and Other Infectious Diseases, Pasteur Institute Novi Sad, Novi Sad 21000, Serbia
[b] Department of Microbiology with Parasitology and Immunology, Faculty of Medicine, University of Novi Sad, Novi Sad 21000, Serbia
[c] Diagnostics and Laboratory Research Task Force, Balkan Association for Vector-Borne Diseases, 21000, Novi Sad, Serbia
[d] Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, 1000 Skopje, North Macedonia
[e] University Clinic for Infectious Diseases and Febrile Conditions, 1000 Skopje, North Macedonia
[f] Clinical medicine Task Force, Balkan Association for Vector-Borne Diseases, 21000, Novi Sad, Serbia
[g] Department of Microbiology, Pasteur Institute Novi Sad, 21000 Novi Sad, Serbia
[h] Department for Research & Monitoring of Rabies & Other Zoonoses, Pasteur Institute Novi Sad, 21000 Novi Sad, Serbia
[i] Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, S7N 5E2, Canada
[j] ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France
[k] Department of Biology and Parasitology, Medical University of Lublin, Radziwiłłowska 11, 20-080 Lublin, Poland

ARTICLE INFO

Keywords:
Ticks
Tick-borne diseases (TBDs)
Tick-borne pathogens (TBPs)
Epidemiology
Balkans

ABSTRACT

Objectives: The Balkan Peninsula, acting as a crossroad between central Europe and the Middle East, presents diverse ecosystems supporting various tick species capable of transmitting TBDs. This study focuses on Serbia and North Macedonia, both endemic for TBDs, aiming to investigate human-biting ticks' prevalence, TBD prevalence, and major TBPs in blood samples.

Patients and Methods: This prospective observational study was conducted in 2022 at two medical centers, involving 45 patients from Novi Sad, Serbia, and 17 patients from Skopje, North Macedonia. All participants had either a tick still attached or had had one removed within the preceding 48 h. The study consisted in clinical evaluations of patients and testing of patient samples and ticks for tick-borne pathogens using a High-Throughput pathogen detection system based on microfluidic real-time PCR. In addition, the study assessed the genetic diversity of the identified pathogens.

Results: Ixodes ricinus was the most prevalent tick species, with varying infestation rates across various body parts. Tick species and feeding times differed between Novi Sad and Skopje. TBPs were prevalent, with *Rickettsia* spp. dominant in Skopje and a mix including *Rickettsia aeschlimannii*, *Rickettsia monacensis*, *Anaplasma phagocytophilum*, and *Borrelia afzelii* in Novi Sad. Subclinical bacteremia occurred in 8.06% of cases, mostly involving *Anaplasma* spp. Clinical manifestations, primarily local hypersensitivity reactions, were observed in six patients. Phylogenetic analysis confirmed *R. aeschlimannii* and *R. monacensis* identity, highlighting genetic differences in *gltA* gene sequences.

Conclusions: This study sheds light on the prevalence and diversity of TBPs in tick-infested individuals from Serbia and North Macedonia, contributing valuable insights into the epidemiology of TBDs in the Balkan region.

1. Introduction

The Balkan Peninsula transitions between temperate and subtropical climatic zones and geographic landscapes [1]. In addition to *I. ricinus*, the diverse ecosystems of the Balkans support various tick species capable of infesting humans and transmitting tick-borne pathogens

* Corresponding authors.
E-mail address: alejandro.cabezas@vet-alfort.fr (A. Cabezas-Cruz).
[1] Equal contribution.

https://doi.org/10.1016/j.idnow.2024.104959
Received 30 January 2024; Received in revised form 30 May 2024; Accepted 24 July 2024
Available online 28 July 2024

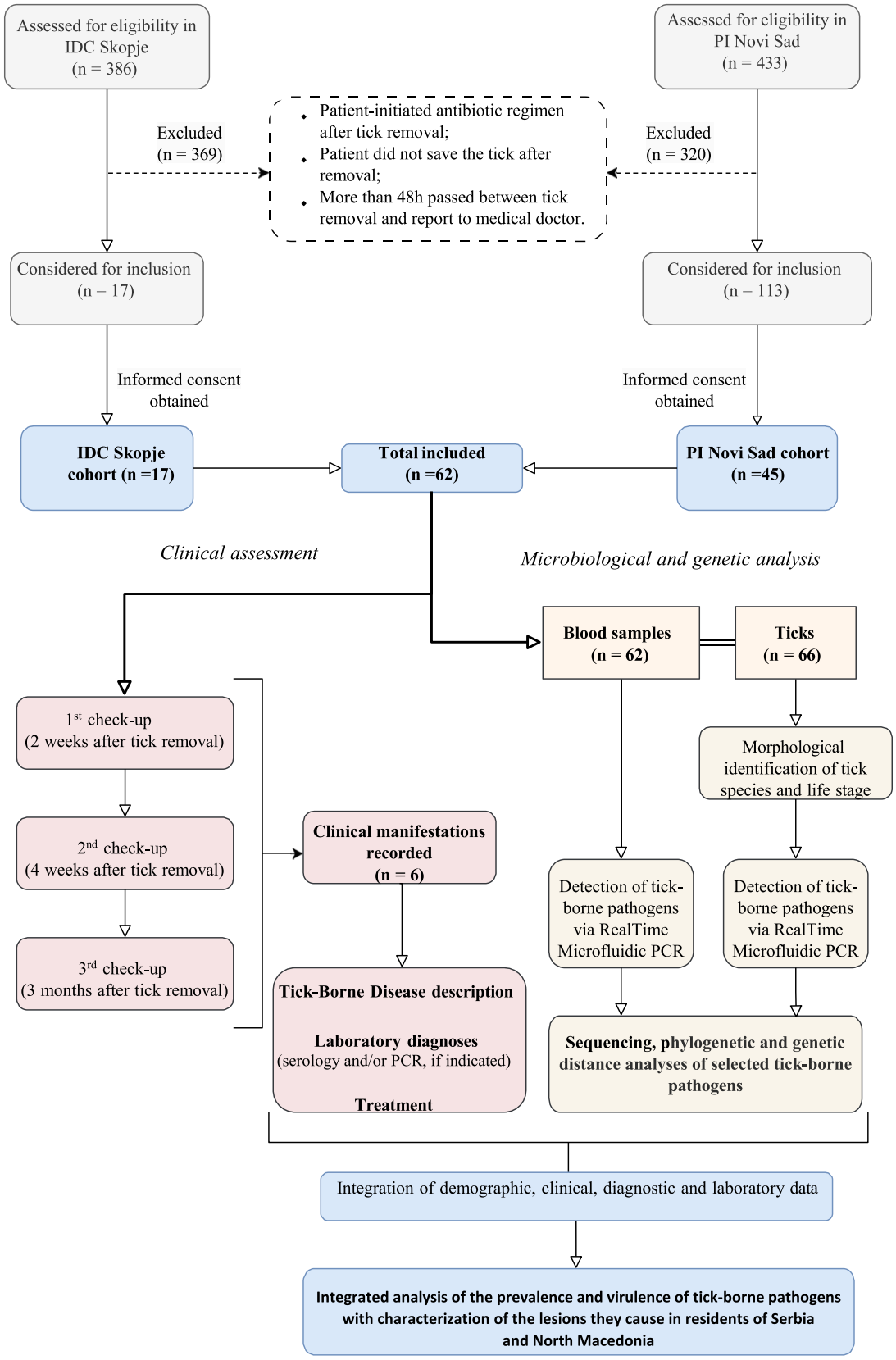

Fig. 1. Study flowchart. IDC Skopje – Infectious Disease Clinic in Skopje; PI Novi Sad – Clinic for Lyme borreliosis and other Tick-Borne Diseases of the Pasteur Institute Novi Sad.

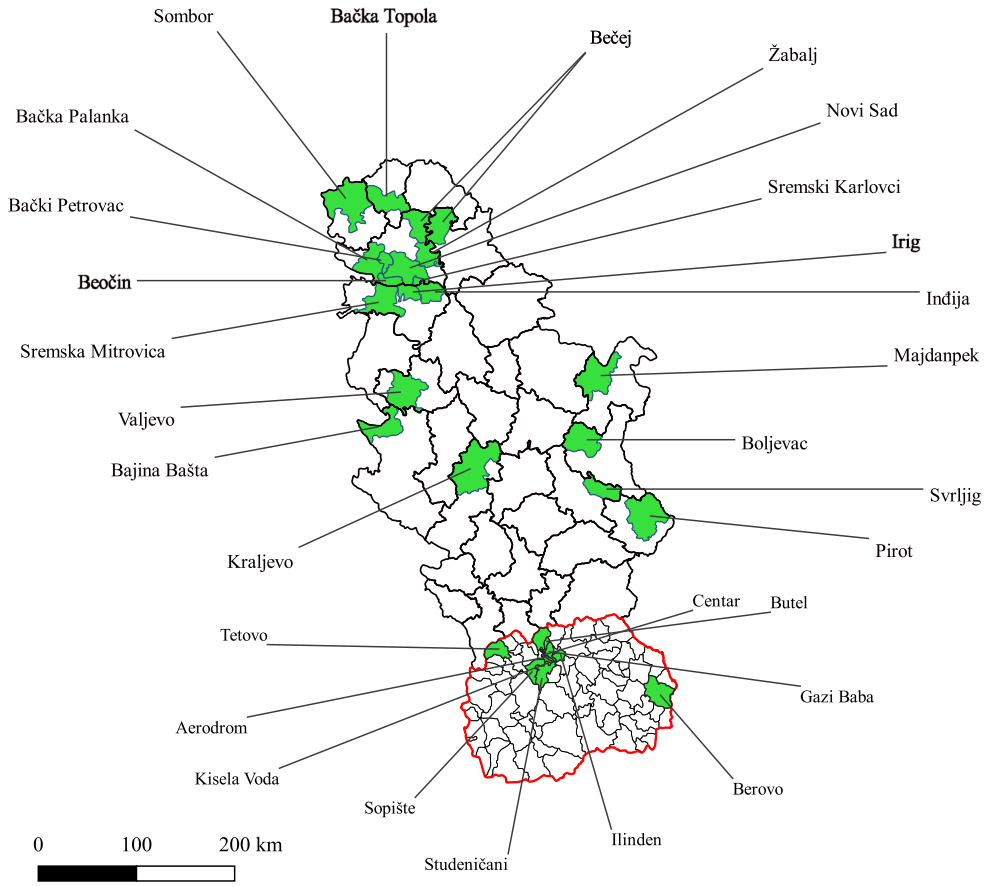

Fig. 2. Geographical locations where the tick infestations occurred in patients reported to Pasteur Institute Novi Sad and Infectious Disease Clinic in Skopje. The location distribution is presented by municipalities, across Serbia and North Macedonia. Two cases of infestation that occurred outside Serbia and North Macedonia are not presented on the map. The Serbian and Macedonian shape file for mapping at municipality levels is available at the GADM database of Global Administrative Areas (v4.1, July 2022, https://gadm.org/). The map was generated by using QGIS v3.12 (QGIS Development Team, 2020).

(TBPs) (e.g., *Hyalomma* spp., *Rhipicephalus* spp., *Dermacentor* spp., and *Haemaphysalis* spp.) [2–4]. Consequently, the Balkan countries have regions endemic for various TBDs, including Crimean-Congo Hemorrhagic fever (CCHF) [2,5,6], Mediterranean Spotted Fever (MSF) [7,8], and Lyme borreliosis [9,10].

Serbia has a moderate continental climate, and previously reported tick-borne infections caused by members of the *Borrelia burgdorferi sensu lato* (s.l.) complex and emerging Spotted Fever Group *Rickettsia* (SFGR) afflicting tick-infested Serbian residents [10,11]. Despite this, clinical reports of emerging TBDs are still scarce in Serbia, suggesting potential exposure to neglected TBPs, such as *Anaplasma phagocytophilum*, *Borrelia miyamotoi* and Tick-Borne Encephalitis virus (TBEV) [12–14].

North Macedonia, bordering Serbia to the north, exhibits continental and Mediterranean climates transitioning from the northern regions to the southern regions bordering Greece. Currently, data on tick species infesting humans in North Macedonia are limited, and burden of TBP in ticks removed from Macedonians has not been investigated [9]. The re-emergence of CCHF in 2023 [6] and a case of MSF-like illness in 2022 [15] underscore the need for a comprehensive examination of TBD prevalence and TBP exposure in the Macedonian population.

Therefore, our study aims to examine the prevalence of human-biting ticks, as well as TBD prevalence of in tick-infested human cohorts from Serbia and North Macedonia, and to identify major TBPs in the blood samples of the infected patients.

2. Materials and methods

2.1. Ethical statement

This study received approval from the ethics committee of Medicine Faculty Skopje, University of Ss. Cyril and Methodius in Skopje (Ethical approval No. 03–1835/2) and the ethics committee of Medicine Faculty Novi Sad, University of Novi Sad (Ethical approval No. 01–39/24/1). The research was conducted in compliance with the principles outlined in the Declaration of Helsinki and adhered to the Patient Rights Law of the Republic of North Macedonia and Republic of Serbia, respectively.

2.2. Patient recruitment and follow-up

Patients eligible for the study presented at the Clinic for Lyme borreliosis and other TBDs of the Pasteur Institute Novi Sad (PI Novi Sad) or the Infectious Disease Clinic in Skopje (IDC Skopje) with a tick still attached or removed within the previous 48 h. Patients who self-initiated antibiotic treatment or had pre-existing conditions were excluded.

After obtaining informed consent, patients were scheduled for follow-up visits at two weeks, four weeks, and three months. Epidemiological data including affected body compartments were recorded, and patients reported any symptoms between visits. Six months later, patients were contacted by telephone for late-stage TBD symptom assessment.

Clinical examinations focused on skin and tick bite sites, with observations for fever, headache, muscle pain, and itching. Cutaneous

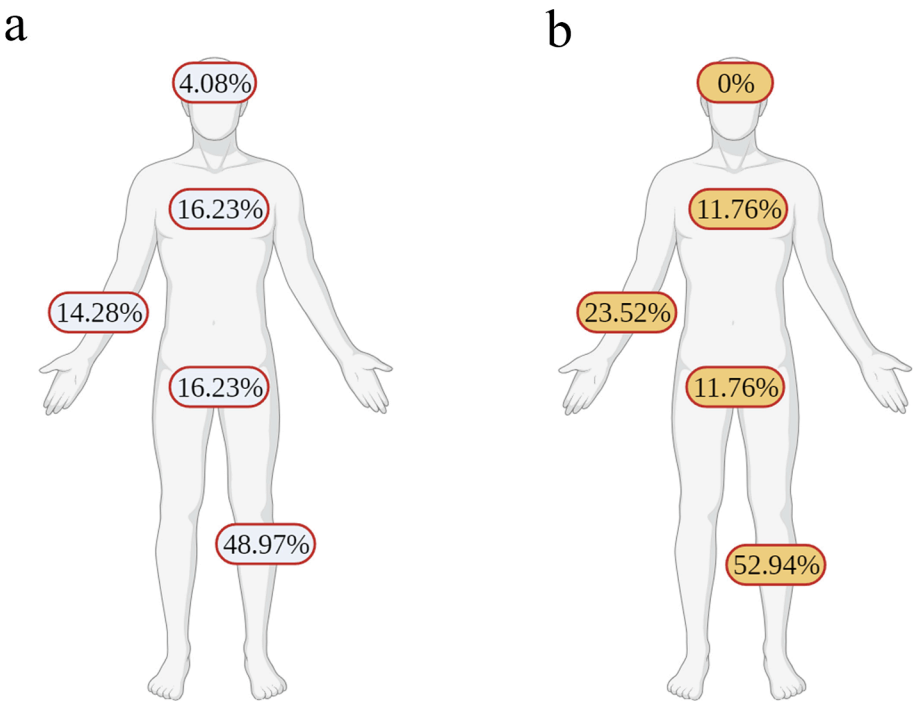

Fig. 3. Comparison of tick infestation frequency in different body compartments. a) Pasteur Institute Novi Sad cohort. Ticks were the most frequently removed from legs, inguinal/genital region and thorax/abdomen compartment; b) Infectious Disease Clinic in Skopje cohort. Tick infestation was the most commonly registered in legs and arms. Created with BioRender.com.

hypersensitivity reactions were likewise monitored. If symptoms appeared, further diagnostic procedures were carried out.

2.3. Tick collection and identification

Ticks from the Novi Sad cohort were mainly removed by patients or local Community Health Center ambulances, while in Skopje, they were primarily removed at the local Emergency Department. Removal in healthcare facilities involved tweezers, with patients often using them or their fingers; sometimes ticks were picked up after detachment. After removal, patients were instructed to place ticks in plastic containers and bring them to their respective centers.

Attached ticks ($n = 66$) were taxonomically identified following standard keys by Estrada Pena et al. [16], considering sex and life stage. Molecular identification of *I. ricinus* and *Dermacentor reticulatus* used microfluidic real-time PCR with species-specific primers/probes by Michelet et al. [17]. Attachment time and likely encounter location were assessed through patient information and when unsure, scutal and coxal indices were used to calculate feeding time [18]. Collected ticks were placed in 70 % ethanol and conserved at − 80 °C until DNA extraction.

2.4. Blood sample collection and sera extraction

After informed consent and within 48 h after tick removal was acquired from each patient or patient's caretakers (in the case of underage individuals), 2 ml of blood were collected in BD Vacutainer® spray-coated Na-citrate tubes (BD, Oakville, CA, United States) using BD PrecisionGlide™ needles and BD Vacutainer® Safety-Lok™ system (BD, Oakville, CA, United States) for adults and young patients, respectively. Blood samples were stored at − 80 °C until DNA extraction. Blood and tick samples were labeled to allow for pairwise analysis of pathogen detection in ticks and humans. For serological analyses (see below), one blood sample (3 ml) was collected in patients who developed lesions suggestive of rickettsial disease or Lyme borreliosis at least three weeks after tick removal, using BD Vacutainer® SST™ Tubes (BD, Franklin Lakes, NJ, United States). Blood samples were allowed to clot at room temperature and, after centrifugation at 2000 × g for 10 min, serum was extracted and stored at − 80 °C until further use. If a patient developed any kind of TBD-suggestive signs and symptoms (See 2.5. Clinical and serological diagnosis of TBDs), a second serum sample was acquired four weeks after tick removal for the purposes of paired serum examination.

2.5. Clinical and serological diagnosis of TBDs

Diagnosis of TBDs followed the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines [19]. Patients meeting the criteria for early-stage Lyme borreliosis, including erythema migrans, were diagnosed if symptoms appeared ≥ 72 h post-tick removal. Other signs such as eschar or redness were considered for localized rickettsial infection [11]. Non-specific lesions developing ≥ 72 h post-tick removal were evaluated against the ESCMID criteria [19]. Patients without TBD signs in 6-month follow-up were discharged. Molecular and serological analyses confirmed clinical diagnoses retrospectively (see below).

2.6. Detection of anti-Borrelia and anti-Rickettsia antibodies

Serum samples were heat-inactivated at 56 °C for seroreactivity detection. Commercial ELISA kits were employed for anti-*Borrelia* spp. IgG reactivity (recomWell Borrelia IgG, Mikrogen Diagnostik GmbH, Neuried, Germany, Cat. No. 4204). The kits included OspC and VlsE antigens from *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, and *B. afzelii*. Assays followed the kit instructions and were validated using the provided controls. Results were interpreted qualitatively (positive/negative). Values < 24 units/mL were negative, >24 units/mL positive. Units/mL were calculated from optical density (O.D.) at 450 nm.

To quantify anti-*Rickettsia* IgM and IgG, another commercial ELISA kit was used (Vircell S.l., Grenada, Spain, Cat. No. G/M1005), with plates coated with cells containing *Rickettsia conorii*. Results were interpreted qualitatively, and an antibody index (AI) was calculated by dividing sample O.D. by mean O.D. from the kit's cut-off serum, and then multiplying by 10. An AI<11 was considered negative, >11

Table 1
Prevalence of vector-borne pathogens detected by microfluidic real-time PCR in ticks removed from patients.

Vector-borne pathogen(s)	Total	%
IDC Skopje cohort	17	
Total infected ticks (≥1 pathogen)	**16**	**94.12**
Rickettsia spp.	15	88.24
Rickettsia felis	1	5.88
Single infections	**16**	**94.12**
Rickettsia spp.	15	88.24
Rickettsia felis	1	5.88
TBPs not-detected	1	5.88
PI Novi Sad cohort	49	
Total infected ticks (≥1 pathogen)	**43**	**87.76**
Anaplasma spp.	3	6.12
Anaplasma phagocytophilum	5	10.20
Neoehrlichia mikurensis	1	2.04
Borrelia afzelii	3	6.12
Borrelia lusitaniae	1	2.04
Francisella-like Endosymbionte	6	12.24
Rickettsia spp.	13	26.53
Rickettsia aeschlimannii	4	8.16
Rickettsia helvetica	5	10.20
Rickettsia felis	2	4.08
Single infections	**23**	**46.94**
Anaplasma spp.	1	2.04
A. phagocytophilum	2	4.08
Borrelia afzelii	1	2.04
Francisella-like Endosymbionte	1	2.04
Rickettsia spp.	7	14.29
Rickettsia helvetica	5	10.20
Rickettsia aeschlimannii	4	8.16
Neoehrlichia mikurensis	1	2.04
Rickettsia felis	1	2.04
Mixed infections	**10**	**20.41**
Mixed infections with two pathogens	**10**	**20.41**
Rickettsia spp. + *Anaplasma* spp.	1	2.04
Rickettsia spp. + *A. phagocytophilum*	1	2.04
Rickettsia aeschlimannii + *Francisella-like Endosymbiont*	3	6.12
Rickettsia felis + *Francisella-like Endosymbiont*	1	2.04
Rickettsia felis + *Borrelia afzelii*	1	2.04
Borrelia lusitaniae + *Anaplasma* spp.	1	2.04
Borrelia afzelii + *A. phagocytophilum*	1	2.04
Borrelia afzelii + *Rickettsia* spp.	1	2.04
TBPs not detected	6	12.24

positive. O.D. was measured using an ELX800 ELISA reader (BioTek, Wisconsin, VT, USA). Due to the known cross-reactivity of anti-SFGR antibodies, detection of specific IgM and IgG was done only at the genus level [19].

2.7. Molecular detection of pathogens

2.7.1. DNA extraction and DNA pre-amplification for microfluidic real-time PCR

DNA extraction and pre-amplification followed established protocols [10,11,20]. Blood and tick samples were processed using the Nucleospin Tissue kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instructions, yielding DNA eluted into 50 μL elution buffer and stored at − 80 °C. Pre-amplification of total DNA utilized a pooled primer mix targeting all pathogens and PreAmp Master Mix (Standard Biotools, CA, USA) as per manufacturer guidelines. Pre-amplified reactions were diluted 1:10 in Milli-Q ultrapure water and stored at − 20 °C until further use.

2.7.2. Microfluidic real-time PCR

The microfluidic real-time PCR method used in this study has been previously detailed [10,11,20]. It targets major TBPs such as *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Coxiella* spp., *Babesia* spp., and other parasites (Supplementary Table S1). For comprehensive information on the development and validation of this high-throughput tool, including sensitivity, specificity, and control measures, please consult references [17] and [21].

2.7.3. Validation of microfluidic real-time PCR system results by PCR

To validate microfluidic real-time PCR system outcomes, *Rickettsia*-positive samples underwent additional scrutiny via conventional and nested PCR assays. Unique primers, distinct from those in the BioMark™ real-time PCR system, were used [22–25]. Targeted fragments of *Rickettsia* outer membrane protein B (*ompB*) and citrate synthase (*gltA*) were amplified using previously outlined primers and PCR conditions [11]. Sequencing was performed by Eurofins MWG Operon (Ebersberg, Germany), with assembly conducted using BioEdit software (Ibis Biosciences, Carlsbad). The accession numbers for nucleotide sequence data from this study are OQ678867, OR288098, OR288097, OR288100, OR288099, and OR288101 in the GenBank, EMBL, and DDBJ databases.

2.8. Phylogenetic and genetic distance analyses

Basic Local Alignment Search Tool (BLAST) [26] was used to analyze *Rickettsia gltA* and *ompB* sequences for species and genetic diversity. GenBank was then searched for sequences within SFRG species, including those from Serbia and/or North Macedonia, Europe, and other continents. Sequences were aligned using MAFFT for accuracy, excluding redundant ones. The Tamura 3-parameter (T92) model, determined as the best-fit model, was applied for phylogenetic tree reconstruction in MEGA X [27]. Maximum likelihood (ML) phylograms were constructed with the 'complete deletion' option, and branch reliability was assessed using the bootstrapping method (1000 replicates) in MEGA X.

2.9. Genetic distance analysis

Genetic distances between *Rickettsia aeschlimannii* or *Rickettsia monacensis* (targeting *gltA* or *ompB* gene) sequences from this study and those from the same species in phylogenetic trees were calculated as p-distance in MEGA X. These values assessed genetic diversity of *Rickettsia* strains identified here and elsewhere in Serbia, North Macedonia, and globally. The first comparison involved sequences from this study and others in the trees (excluding those from Serbia and/or North Macedonia). The second compared sequences from Serbia and/or North Macedonia (excluding those from this study). The third compared all sequences in the tree, excluding those from Serbia and/or North Macedonia obtained in this or previous studies.

2.10. Classification of SFGR infection cases

Following retrospective assessment of diagnostic and xenodiagnostic findings combined with clinical manifestations observed in patients, SFGR infection cases were classified as in [11]. Briefly, SFGR infection was considered confirmed if (i) SFGR DNA was detected in eschar crust or in capillary blood sample collected from the lesion or (ii) four-fold rise of anti-SFGR IgG was detected in the paired serum sample. Probable SFGR infection was considered when even though SFGR DNA was not detected in eschar crust or capillary blood sample, the patient had compatible clinical manifestations with detection of anti-SFGR IgG in single serum sample and positive xenodiagnostic finding (i.e., SFGR DNA was detected in tick removed from skin). Finally, a case was classified as suspected SFGR infection if the patient had developed compatible clinical manifestations with positive xenodiagnostic finding, but without positive serology or SFGR DNA detection in eschar crust or the capillary blood sample collected at lesion site.

2.11. Bias identification and management

The most significant bias identified during this clinical observational study was the proximity of our centers to patients' homes. Patients living in urban and suburban areas of Novi Sad and Skopje are more likely to

Table 2
Demographic, clinical, xenodiagnostic and laboratory findings in persons with different manifestations of tick-borne diseases.

	Patient number					
	59/22	**161/22**	**282/22**	**306/22**	**337/22**	**356/22**
General patient and tick-related data						
Age/gender	61y/F	67y/M	61y/F	66y/F	45y/F	72y/M
Date of tick removal	04.05.2022.	20.05.2022	13.06.2022	14.06.2022	22.06.2022	28.06.2022
Suspected location of infestation	Popovica village, Serbia	Sremska Kamenica, Serbia	Hopovo monastery, Serbia	Sremski Karlovci, Serbia	Andrevlje, Serbia	Bešenovo village, Serbia
Date of illness	09.05.2022	03.06.2022.	07.07.2022.	27.06.2022.	30.06.2022	13.07.2022.
Incubation time	5 days	14 days	21 days	13 days	8 days	15 days
Tick species	*I. ricinus*	*I. ricinus*	*I. ricinus*	*I. ricinus*	*I. ricinus*	*I. ricinus*
Tick life stage	Larva	Adult female	Adult female	Adult female	Nymph	Nymph
Tick feeding time	N/A	Less than 12 h	Less than 12 h	5 days	2 days	2 days
Signs and symptoms						
Fever	No	No	No	No	No	No
Headache	No	No	No	No	No	No
Myalgia	No	No	No	No	No	No
Eschar	No	No	No	No	No	No
Enlargedlymph nodes	No	No	No	No	No	No
Non-expanding local redness	Yes	Yes	Yes	Yes	No	Yes
Expanding local redness	No	No	No	No	Yes	No
Itching sensation at lesion site	Yes	Yes	Yes	Yes	Yes	No
Serological findings in serum collected on the day of tick removal						
anti-*Borrelia* IgG	(−)	(−)	(−)	(−)	(−)	(−)
anti-*Rickettsia* IgM	(−)	(−)	(−)	(−)	(−)	(−)
anti-*Rickettsia* IgG	(−)	(−)	(−)	(−)	(−)	(−)
Serological findings in serum collected 4 weeks after tick removal						
anti-*Borrelia* IgG	(−)	(−)	(−)	(−)	(+)	(−)
anti-*Rickettsia* IgM	(−)	(−)	(−)	(+)4-fold increase	(−)	(−)
anti-*Rickettsia* IgG	(−)	(−)	(−)	(−)	(−)	(−)
Clinical diagnosis						
	LPR	LPR	LPR	Local SFGR infection	Lyme borreliosis	LPR
Detection of TBPs						
Patient blood	(−)	(−)	(−)	*Anaplasma* spp.	(−)	(−)
Tick	(−)	*A. phagocytophilum*	*Rickettsia* spp.	*Rickettsia aeschlimannii*	*B. afzelii,*	*A. phagocytophilum*

N/A- not accessible, (+) – positive, (−) – negative.

report to IDC Skopje/PI Novi Sad for medical attention compared to those who need to travel longer distances for control examinations. We managed this bias by instructing general practitioners to strongly encourage patients to report to IDC Skopje/PI Novi Sad if they acquired a tick within or outside their home city. This approach allowed us to analyze ticks from various locations across Serbia and North Macedonia, not only from the municipalities of their residence.

2.12. Statistical analysis

For comparison of parametric variables between two cohorts, the two-tailed Student *t*-test was used. Differences in *p*-distance values between the analyzed DNA sequences were assessed in GraphPad software v.9 (GraphPad Software Inc., La Jolla, CA, USA). The Shapiro-Wilk normality test rejected 'normal distribution' of the *p*-distance values obtained. Therefore, the nonparametric Mann Whitney *U* test was used. Statistical significance was considered when $p < 0.05$.

3. Results

3.1. Participant enrollment

In 2022, a total of 433 patients at PI Novi Sad and 386 patients at IDC Skopje attended medical consultations and reported tick bites and tick-associated infections. Following the initial consultation phase, a thorough selection process was undertaken to identify participants who met the study's criteria. After excluding patients that did not meet these selection criteria, we successfully formed two distinct cohorts for further analysis: the IDC Skopje cohort, consisting of 17 participants, and the PI Novi Sad cohort, with 45 participants. Fig. 1 illustrates this selection process, including the clinical and molecular analyses conducted on the samples collected from the 62 patients and the 66 ticks that were attached to them.

3.2. Demographics of the enrolled patients

Demographic analysis showed that patients with tick infestations from IDC Skopje were significantly older (59 years; 95 % CI: 51.8—66.9) than those reporting to PI Novi Sad (42 years; 95 % CI: 37.8—46.7; $t = 3.86, p < 0.001$). Males constituted the majority in both

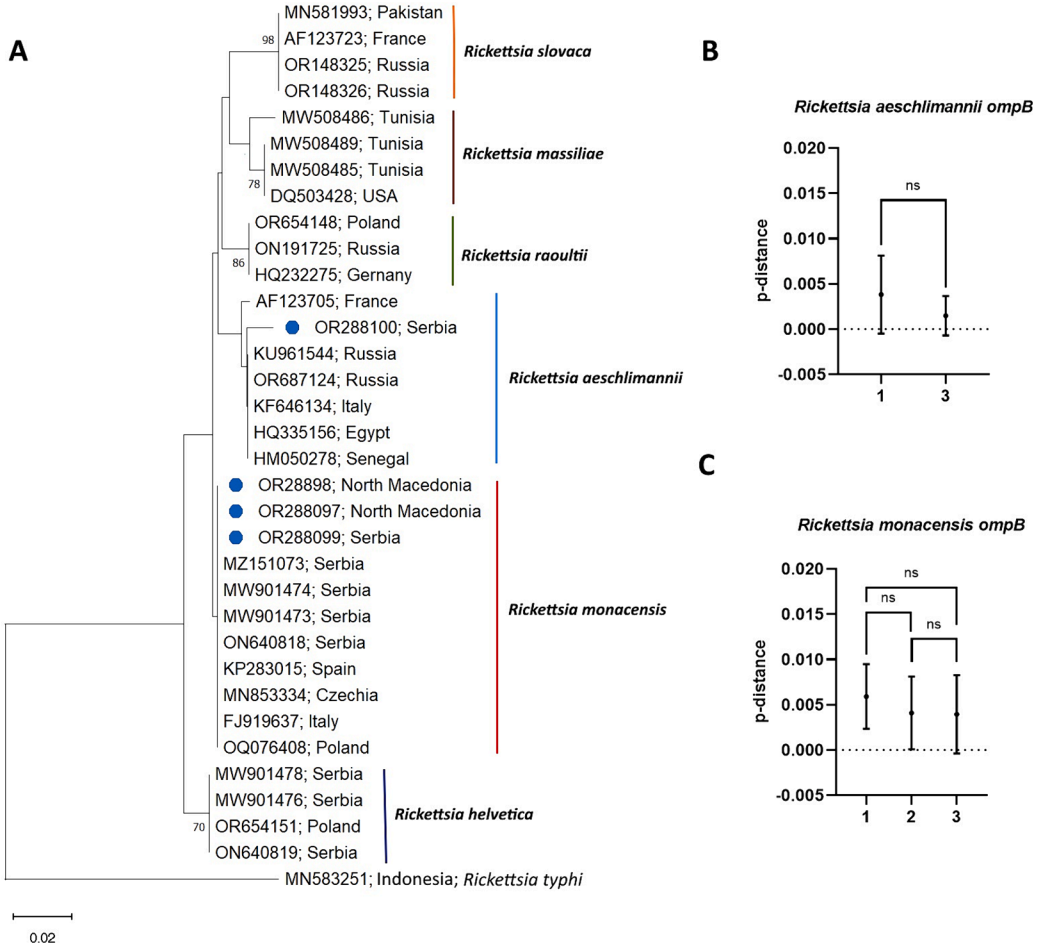

Fig. 4. Genetic diversity of spotted fever group rickettsiae (SFGR) strains targeting *ompB* gene. Phylogram **(A)** shows position of the sequences obtained in the current study (marked blue) and other sequences available in GenBank (accession numbers and country of origin are displayed). The phylogenetic tree was inferred using the maximum likelihood method with the Tamura 3-parameter evolutionary model. The tree is drawn to scale, with branch lengths measured in terms of number of substitutions per site. Numbers on internal branches are the bootstrap values (only values ≥ 60 % are shown). The genetic distances between *R. aeschlimannii* **(B)** and *R. monacensis* **(C)** nucleotide sequences included in phylogenetic tree were calculated as p-distances. Three sets of pairwise p-distance values were collected and compared. P-distance values resulting from comparisons between the sequences identified in this study and others included in each tree (excluding sequences previously identified in Serbia and/or Norh Macedonia) are labelled as '1′. P-distance values resulting from comparisons between the sequences previously identified in Serbia and/or Norh Macedonia and others included in the trees (excluding sequences identified in this study) are labelled as '2′, if available. P-distance values resulting from comparisons between all sequences of *R. monacensis* and *R. aeschlimannii* in the tree (excluding sequences obtained in Serbia and/or Norh Macedonia, in this or previous studies) are labelled as '3′. Significance of comparison is marked (ns – non statistically significant). Differences between p-distances in '1′, '2′ and '3′ were assessed using the Mann Whitney *U* test ($p < 0.05$ was considered significant). Diagrams (**B, C**) show mean values of p-distance including standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cohorts: with Skopje (13/17; 76.47 %); Novi Sad (33/45; 73.33 %). Epidemiological survey supported by determination of tick feeding time revealed that tick infestation occurred in total of 33 municipalities, including 11 and 20 municipalities within North Macedonia and Serbia, respectively (Fig. 2). In the Serbian cohort, tick infestation most frequently occurred in municipalities proximal to Fruška Gora Mountain (i.e., municipalities of Novi Sad, Bačka Palanka, Irig, Beočin and Sremska Mitrovica), while Macedonians were most frequently infested within the Skopje region (valley in the north surrounded by many mountains and proximal to the Serbian border).

3.3. Characteristics of the ticks removed from patients and assessment of tick attachment time

Most patients in both groups had one tick; some had two. *Ixodes ricinus* was predominant in Serbia (95.91 %) and North Macedonia (70.58 %). Nymphs slightly outnumbered adult females in Serbia (53.19 % vs. 44.68 %), while females dominated in North Macedonia (83.33 %). Additional tick species found in Serbia included *Haemaphysalis* spp. and *Dermacentor* spp., while Skopje patients also displayed *Rhipicephalus sanguineus* and *Haemaphysalis inermis*.

Ticks in both cohorts mainly targeted the lower extremities (Fig. 3). In Serbia, the inguinal/genital region showed susceptibility similar to thorax/abdomen (Fig. 3a), while in Macedonia, infestation descended from arms to thorax/abdomen (Fig. 3b). Average tick feeding time was 2.85 days (95 % CI: 2.11—–3.59) in Macedonian and 1.94 days (95 % CI: 1.45—–2.43) in Serbian patients.

3.4. Presence of TBPs in ticks removed from patients

3.4.1. IDC Skopje cohort

In the IDC Skopje cohort, 94.11 % (16/17) of ticks removed tested positive for at least one of the pathogens examined (Table 1). All PCR-positive samples harbored a single infection, with no detected evidence of mixed infections. Positive PCR results for the presence of TBPs were observed in *I. ricinus* (100 %, 12/12, 10 females and two nymphs), *R. sanguineus* (100 %, 3/3, two males, one female), and *H. inermis* (50 %, 1/2, one female). DNA of *Rickettsia* spp. was prevalent in almost all ticks

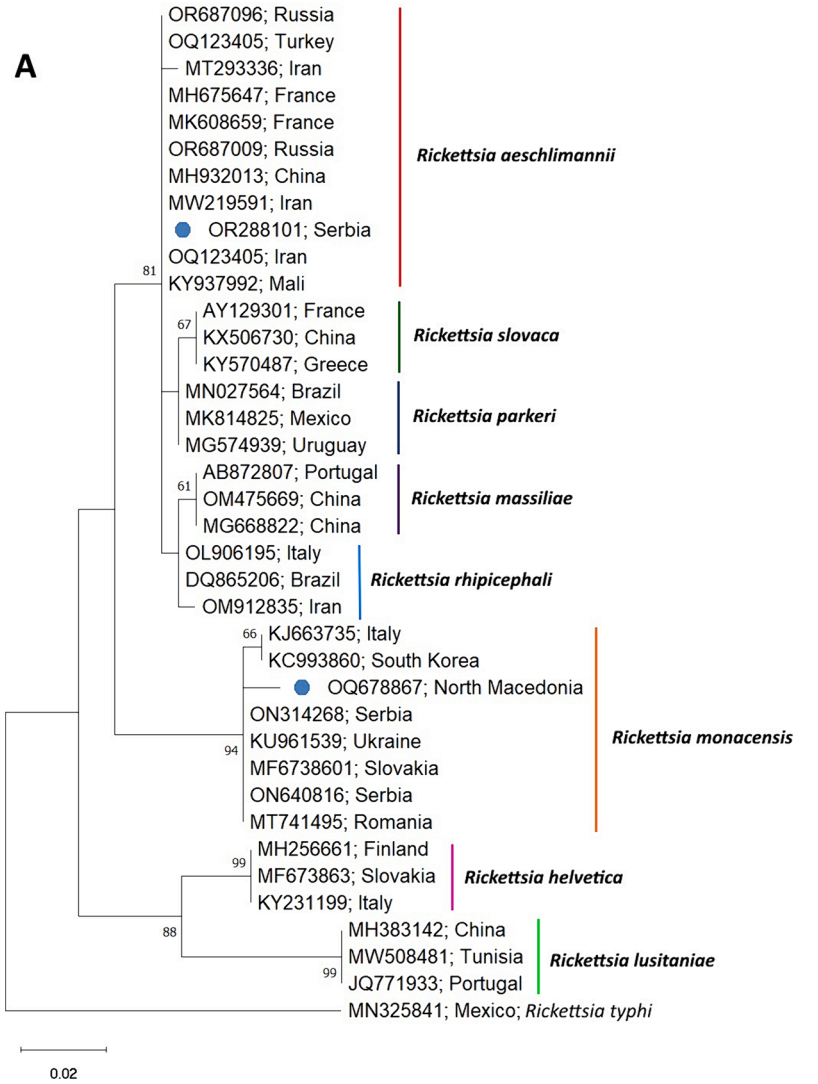

Fig. 5. Genetic diversity of spotted fever group rickettsiae (SFGR) strains targeting *gltA* gene. Phylogram **(A)** shows position of the sequences obtained in the current study (marked blue) and other sequences available in GenBank (accession numbers and country of origin are displayed). The phylogenetic tree was inferred using the maximum likelihood method with the Tamura 3-parameter evolutionary model. The tree is drawn to scale, with branch lengths measured in terms of number of substitutions per site. Numbers on internal branches are the bootstrap values (only values $\geq$ 60 % are shown). The genetic distances between *R. aeschlimannii* **(B)** and *R. monacensis* **(C)** nucleotide sequences included in the phylogenetic tree were calculated as p-distances. Three sets of pairwise p-distance values were collected and compared. P-distance values resulting from comparisons between sequences identified in this study and others included in each tree (excluding sequences previously identified in Serbia and/or Norh Macedonia) are labelled as '1′. P-distance values resulting from comparisons between all sequences in the tree (excluding sequences obtained in Serbia and/or North Macedonia, in this or previous studies) are labelled as '3′. The significance of comparison is marked (asterisk – statistically significant). Differences between p-distances in '1′ and '3′ were assessed using the Mann Whitney *U* test ($p < 0.05$ was considered significant). Diagrams (**B, C**) show mean values of p-distance including standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

collected from Macedonian patients (15/17, 88.24 %), while *Rickettsia felis* was found in one tick sample (5.88 %). Further DNA sequencing analysis revealed *Rickettsia monacensis* infection in *I. ricinus* (two females) (Accession numbers OQ678867, OR288097) and *R. sanguineus* (one male) (Accession number OR288098) tick species.

3.4.2. PI Novi Sad cohort

In the PI Novi Sad cohort, 87.76 % (43/49) of the ticks removed from Serbian residents tested positive for at least one of the pathogens examined (Table 1). Out of 43 PCR-positive samples, 46.94 % (23/49) showed a single infection, while 20.41 % (10/49) were co-infected with two pathogens. Positive PCR results for the presence of TBPs were found in *I. ricinus* (74.46 %, 35/47, 17 females and 18 nymphs) and *Dermacentor* spp. (100 %, 1/1, one female). Overall, 10 different pathogens with variable prevalence were identified using species-specific primers. The most prevalent identified TBP was SFGR (44.89 %; 22/49), followed by *Anaplasma phagocytophilum* (10.20 %; 5/49), and *Borrelia afzelii* (6.12 %; 3/49). Further DNA sequencing analysis confirmed the presence of *Rickettsia aeschlimannii* (Accession numbers OR288100, OR288101) and *Rickettsia monacensis* (Accession number OR288099) in *I. ricinus* ticks (1 nymph, 2 females).

3.5. Presence of subclinical bacteriaemia after tick removal

Subclinical bacteriaemia following tick removal was identified in a total of five patients (8.06 %; 5/62). All patients were infested with *I. ricinus* (adult female). Among TBPs, *Anaplasma* spp. was the most prevalent (60 %; 3/5), with *A. phagocytophilum* (20 %; 1/5) and Apicomplexa (20 %; 1/5) each detected in one patient. During follow-up, only one patient showed disease signs, which did not align with

anaplasmosis. The disease manifested 13 days post-tick removal, and was diagnosed as localized SFGR infection (Table 2).

3.6. Clinical manifestations in tick-infested patients

In this study of 62 patients having undergone three clinical check-ups, six from the Novi Sad cohort developed manifestive disease after a minimum 72-hour incubation period (Table 2). None of the patients developed signs and/or symptoms of TBD between 2nd and 3rd check-ups (time interval between four weeks and four months after tick removal). Common clinical findings included persistent circular redness and itching sensation at lesion sites. No eschars, lymph node enlargement, or fever were recorded. Local Hypersensitivity Reaction (LPR) after a tick bite was the most common diagnosis (66.66 %; 4/6), with one case each of erythema migrans and local rickettsial infection. Patients with LPR were treated with loratadine (10 mg once daily) for seven days, resolving itching sensation during treatment. Microfluidic real-time PCR of ticks from these patients showed *Rickettsia* spp. or *A. phagocytophilum* in three cases (75 %; 3/4) (Table 2). Blood samples were PCR-negative for TBPs, suggesting a non-infectious etiology. Lyme borreliosis and rickettsial infection cases were treated with doxycycline (2 × 100 mg daily) for 10 days, resulting in complete recovery. PCR analysis of ticks from Lyme borreliosis and rickettsial infection cases revealed *B. afzelii* positivity. Serologic testing confirmed recent exposure to these TBPs with anti-Borrelia IgG and anti-*Rickettsia* IgM seroconversion, respectively.

3.7. Phylogenetic analysis and genetic distance of SFGR strains

The phylogenetic analysis confirmed *R. aeschlimannii* and *R. monacensis* identity (Figs. 4, 5). The constructed tree, based on *gltA* gene sequences, showed *R. aeschlimannii* forming a regional monophyletic cluster with low genetic diversity compared to Asia and Africa. Similarly, *ompB* gene analysis revealed comparable diversity (Figs. 4, 5). *Rickettsia monacensis ompB* sequences clustered with Europe and Asia, while *gltA* showed broader diversity (Figs. 4, 5). Genetic distances of *gltA* sequences from our study differed significantly from others, but *ompB* showed no such differences (Figs. 4, 5).

4. Discussion

This study represents the first comprehensive observational investigation in two neighboring countries, combining clinical data from individuals exposed to tick bites with morphological tick analysis and screening for TBPs in ticks and matched human blood samples.

The prevalence of ticks recovered in the Serbian cohort aligns with findings from 2019 to 2021, reporting *I. ricinus* as the most frequently encountered human-infesting tick species [12,20]. While *Hyalomma* spp. have been reported to infest animals [28] and humans [6,15], none of the Macedonian patients in our study were infested with this tick species. The identification of *H. inermis* infestation in Macedonian patients is noteworthy, given that in Serbia, human infestation with *H. inermis* has been reported only in the southeastern region [29]. Further research is needed to elucidate the potential public health impact of *H. inermis* in the Balkan Peninsula.

Interestingly, Serbian patients were exposed to a broader spectrum of TBPs compared to Macedonians. Ticks removed from humans in Serbia exhibited higher prevalence of *Rickettsia* spp. infection than in many other European countries where SFGRs circulate [30–33], and this trend seems to have been consistent over the years [10,12,20]. On the other hand, the remarkable prevalence of *Rickettsia* spp. in the ticks removed from Macedonians is not common in other European countries. These findings are corroborated by a recent report on MSF-like illness caused by *Rickettsia sibirica mongolitimonae* in a patient from Skopje [15]. In contrast, tick-borne rickettsial diseases in Serbia have been mostly mild or atypical [11], possibly due to lesser SFGR virulence in the Serbian environment. Skopje may not be the only area in North Macedonia where TBP exposure occurs, as evidenced by detection of autochthonous CCHFV strains having caused outbreaks in other municipalities (i.e., Municipality of Karbinci) [6,34]. The reason for the discrepancy between Serbia and North Macedonia is not entirely clear, but it may be that the SFGR members found in ticks and patients from Serbia are less virulent than those in the Mediterranean and sub- Mediterranean climate of North Macedonia [9]. These environmental factors could contribute to the virulence and prevalence of *Borrelia* spp. and other TBPs in the ticks removed from Serbian and Macedonian cohorts.

While the *Borrelia burgdorferi* sensu lato (s.l.) complex was not found in ticks from the North Macedonia cohort, it was detected in ticks from the Serbian cohort. Subclinical bacteremia after tick removal was caused mainly by *Anaplasma* spp., a finding consistent with previous Serbian studies [12]. Analysis of blood from asymptomatic patients subsequent to tick removal offers valuable insights into tick competency in transmission of TBPs to non-reservoir hosts in real-world scenarios. Importantly, detection of TBPs in patient blood does not necessarily mean that the individual will develop a related disease, as most patients experience post-tick bite transient bacteremia without subsequent evidence of TBD [12]. These observations support the prevailing view that humans are generally resistant to infections from most TBPs, probably due to innate immune mechanisms such as phagocytosis and resistance acquired through anti-alpha-gal antibodies [12,35]. In this study, involving 62 participants, only six exhibited diseases manifestly linked to tick bites. Specifically, patient 306/22 developed a disease consistent with SFGR infection, corroborated by pathogen presence in the corresponding tick and a five-day infestation period. Other ticks may have caused *Anaplasma* spp. bacteremia before detection. A previous study found *Borrelia* spp. seroprevalence in Novi Sad seven times higher than in Skopje [9]. Of note, *Borrelia* spp. infection has not been reported in North Macedonia.

This study confirms the presence of *Borrelia* spp., *Neoehrlichia* spp., and *Anaplasma* spp., along with the circulation of SFGR members like *R. aeschlimannii* and *R. monacensis* in Serbia [10,20]. Phylogenetic analysis shows similarities between our sequences and those from other European regions, especially in the *gltA* gene, indicating higher genetic diversity, a pattern consistent with the findings of a previous study from Serbia [11].

Six cases of TBDs were identified, with confirmed infectious ethology in two instances: one Lyme borreliosis and one local SFGR infection. Four cases were classified as LPR. Hypersensitivity reactions, considered a defense against TBPs [35,36], have been noted in Serbia [11,20]. '*Acquired tick resistance*' refers to reactions where the immune response is activated after tick antigen recognition [36], while '*allergic klendusity*' is a disease-escaping ability produced by the development of hypersensitivity to an allergen [35], both of which involve IgE and basophils [34,35]. As a resuly, TBPs can be neutralized at the tick attachment site, preventing the production of TBP-specific IgM and IgG antibodies. Although some findings suggest that "*Acquired tick resistance*" and/or '*allergic klendusity*' take place against tick vectors and TBPs, further research on the mechanism underlying these phenomena is needed.

5. Conclusions

The study reveals regional variations in tick-borne disease epidemiology. *Ixodes ricinus* is the primary tick species, with considerable differences between the two cohorts. The Macedonian cohort showed higher *Rickettsia* spp. prevalence, challenging previous European infection rates for these pathogens. Interestingly, North Macedonian ticks lack the *Borrelia burgdorferi sensu lato* (s.l.) complex members present in Serbian ticks. Clinical manifestations, including subclinical bacteremia, occurred in a small percentage of patients, with Local Hypersensitivity Reaction as the most common diagnosis. This study underscores a need for further investigations conducive to comprehension of TBD complexity in the Balkans.

Ethical statement

This study received approval from the ethics committee of Medicine Faculty Skopje, University of Ss. Cyril and Methodius in Skopje (Ethical approval No. 03–1835/2) and the ethics committee of Medicine Faculty Novi Sad, University of Novi Sad (Ethical approval No. 01–39/24/1). The research was conducted in compliance with the principles outlined in the Declaration of Helsinki and adhered to the Patient Rights Laws of the Republic of North Macedonia and the Republic of Serbia, respectively.

CRediT authorship contribution statement

Pavle Banović: Conceptualization, Supervision, Methodology, Investigation, Formal analysis, Visualization, Resources, Writing – original draft, Writing – review & editing. **Dejan Jakimovski:** Conceptualization, Supervision, Methodology, Investigation, Formal analysis, Visualization, Resources, Writing – original draft, Writing – review & editing. **Ivana Bogdan:** Investigation, Writing – review & editing. **Verica Simin:** Investigation, Validation, Writing – review & editing. **Dragana Mijatović:** Validation, Resources, Writing – review & editing. **Mile Bosilkovski:** Investigation, Writing – review & editing. **Sofija Mateska:** Investigation, Writing – review & editing. **Adrian A. Díaz-Sánchez:** Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Angelique Foucault-Simonin:** Investigation, Formal analysis, Data curation, Validation, Writing – review & editing. **Zbigniew Zając:** Formal analysis, Data curation, Visualization, Writing – review & editing. **Jaonna Kulisz:** Formal analysis, Data curation, Visualization, Writing – review & editing. **Sara Moutailler:** Supervision, Methodology, Resources, Writing – review & editing. **Alejandro Cabezas-Cruz:** Conceptualization, Supervision, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by The Balkan Association for Vector-Borne Diseases (BAVBD) www.bavbd.org.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idnow.2024.104959.

References

[1] Peneva E, Matov M, Tsekov M. Mediterranean influence on the climatic regime over the Balkan Peninsula from 1901–2021. Climate 2023;11:68. https://doi.org/10.3390/cli11030068.
[2] Sherifi K, Rexhepi A, Berxholi K, Mehmedi B, Gecaj RM, Hoxha Z, et al. Crimean-congo hemorrhagic fever virus and borrelia burgdorferi sensu lato in ticks from Kosovo and Albania. Front Vet Sci 2018;5:38. https://doi.org/10.3389/fvets.2018.00038.
[3] Omeragic J. Ixodid ticks in bosnia and herzegovina. Exp Appl Acarol 2011;53:301–9. https://doi.org/10.1007/s10493-010-9402-8.
[4] Krčmar S, Klobučar A, Vucelja M, Boljfetić M, Kučinić M, Madić J, et al. DNA barcoding of hard ticks (Ixodidae), notes on distribution of vector species and new faunal record for Croatia. Ticks Tick Borne Dis 2022;13:101920. https://doi.org/10.1016/j.ttbdis.2022.101920.
[5] Christova I, Gladnishka T, Taseva E, Kalvatchev N, Tsergouli K, Papa A. Seroprevalence of crimean-congo hemorrhagic fever virus. Bulgaria Emerg Infect Dis 2013;19:177–9. https://doi.org/10.3201/eid1901.120299.
[6] Jakimovski D, Grozdanovski K, Rangelov G, Pavleva V, Banović P, Cabezas-Cruz A, et al. Cases of Crimean-Congo haemorrhagic fever in North Macedonia, July to August 2023. Euro Surveill 2023;28:2300409. https://doi.org/10.2807/1560-7917.ES.2023.28.34.2300409.
[7] Baltadzhiev I, Kevorkyan A, Popivanova N. Mediterranean spotted fever in child and adult patients: investigation from an endemic region in Bulgaria. Cent Eur J Public Health 2020;28:187–92. https://doi.org/10.21101/cejph.a5608.
[8] Brouqui P, Parola P, Fournier PE, Raoult D. Spotted fever rickettsioses in southern and eastern Europe. FEMS Immunol Med Microbiol 2007;49:2–12. https://doi.org/10.1111/j.1574-695X.2006.00138.x.
[9] Jakimovski D, Mateska S, Dimitrova E, Bosilkovski M, Mijatović D, Simin V, et al. Tick-borne encephalitis virus and borrelia burgdorferi seroprevalence in balkan tick-infested individuals: A two-centre study. Pathogens 2023;12:922. https://doi.org/10.3390/pathogens12070922.
[10] Banović P, Díaz-Sánchez AA, Galon C, Simin V, Mijatović D, Obregón D, et al. Humans infested with Ixodes ricinus are exposed to a diverse array of tick-borne pathogens in Serbia. Ticks Tick-borne Dis 2020;12:101609. https://doi.org/10.1016/j.ttbdis.2020.101609.
[11] Banović P, Díaz-Sánchez AA, Foucault-Simonin A, Mateos-Hernandez L, Wu-Chuang A, Galon C, et al. Emerging tick-borne spotted fever group rickettsioses in the Balkans. Infect Genet Evol 2023;107:105400. https://doi.org/10.1016/j.meegid.2022.105400.
[12] Banović P, Piloto-Sardiñas E, Mijatović D, Foucault-Simonin A, Simin V, Bogdan I, et al. Differential detection of tick-borne pathogens in human platelets and whole blood using microfluidic PCR. Acta Trop 2022;238:106756. https://doi.org/10.1016/j.actatropica.2022.106756.
[13] Banović P, Díaz-Sánchez AA, Đurić S, Sević S, Turkulov V, Lendak D, et al. Unexpected TBEV Seropositivity in serbian patients who recovered from viral meningitis and encephalitis. Pathogens 2022;11:371. https://doi.org/10.3390/pathogens11030371.
[14] Banović P, Mijatović D, Bogdan I, Simin V, Meletis E, Kostoulas P, et al. Evidence of tick-borne encephalitis virus neutralizing antibodies in Serbian individuals exposed to tick bites. Front Microbiol 2023;14:1314538. https://doi.org/10.3389/fmicb.2023.1314538.
[15] Jakimovski D, Mateska S, Simin V, Bogdan I, Mijatović D, Estrada-Peña A, et al. Mediterranean spotted fever-like illness caused by Rickettsia sibirica mongolitimonae, North Macedonia, June 2022. Euro Surveill 2022;27:2200735. https://doi.org/10.2807/1560-7917.ES.2022.27.42.2200735.
[16] Estrada-Peña A, Mihalca AD, Petney TN. Ticks of Europe and North Africa: A Guide to Species Identification. Springer International Publishing; 2018.
[17] Michelet L, Delannoy S, Devillers E, Umhang G, Aspan A, Juremalm M, et al. High-throughput screening of tick-borne pathogens in Europe. Front Cell Infect Microbiol 2014;4. DOI: 10.3389/fcimb.2014.00103.
[18] Gray J, Stanek G, Kundi M, Kocianova E. Dimensions of engorging Ixodes ricinus as a measure of feeding duration. Int J Med Microbiol 2005;295:567–72. https://doi.org/10.1016/j.ijmm.2005.05.008.
[19] Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjoërsdorff A, Blanco JR, et al. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect 2004;10:1108–32. https://doi.org/10.1111/j.1469-0691.2004.01019.x.
[20] Banović P, Díaz-Sánchez AA, Simin V, Foucault-Simonin A, Galon C, Wu-Chuang A, et al. Clinical aspects and detection of emerging rickettsial pathogens: a "one health" approach study in Serbia, 2020. Front Microbiol 2022;12:797399.
[21] Grech-Angelini S, Stachurski F, Vayssier-Taussat M, Devillers E, Casabianca F, Lancelot R, et al. Tick-borne pathogens in ticks (Acari: Ixodidae) collected from various domestic and wild hosts in Corsica (France), a Mediterranean island environment. Transbound Emerg Dis 2020;67:745–57. https://doi.org/10.1111/tbed.13393.
[22] Choi Y-J, Lee S-H, Park K-H, Koh Y-S, Lee K-H, Baik H-S, et al. Evaluation of PCR-based assay for diagnosis of spotted fever group rickettsiosis in human serum samples. Clin Diagn Lab Immunol 2005;12:759–63. https://doi.org/10.1128/CDLI.12.6.759-763.2005.
[23] Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 1991;173:1576–89. https://doi.org/10.1128/jb.173.5.1576-1589.1991.
[24] Rar VA, Fomenko NV, Dobrotvorsky AK, Livanova NN, Rudakova SA, Fedorov EG, et al. Tickborne pathogen detection, Western Siberia. Russia Emerg Infect Dis 2005;11:1708–15. https://doi.org/10.3201/eid1111.041195.
[25] Masatani T, Hayashi K, Andoh M, Tateno M, Endo Y, Asada M, et al. Detection and molecular characterization of Babesia, Theileria, and Hepatozoon species in hard ticks collected from Kagoshima, the southern region in Japan. Ticks Tick Borne Dis 2017;8:581–7. https://doi.org/10.1016/j.ttbdis.2017.03.007.
[26] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.
[27] Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018;35:1547–9. https://doi.org/10.1093/molbev/msy096.
[28] Mertens M, Vatansever Z, Mrenoshki S, Krstevski K, Stefanovska J, Djadjovski I, et al. Circulation of crimean-congo hemorrhagic fever virus in the former Yugoslav Republic of Macedonia revealed by screening of cattle sera using a novel enzyme-linked immunosorbent assay. PLoS Negl Trop Dis 2015;9:e0003519.
[29] Pavlović I, Lazarević D. Biodiversity and seasonal distribution of ticks in southeastern Serbia. Timočki Medicinski Glasnik 2023;48:45–8. https://doi.org/10.5937/tmg2302045P.

[30] Lernout T, De Regge N, Tersago K, Fonville M, Suin V, Sprong H. Prevalence of pathogens in ticks collected from humans through citizen science in Belgium. Parasit Vectors 2019;12:550. https://doi.org/10.1186/s13071-019-3806-z.
[31] Audino T, Pautasso A, Bellavia V, Carta V, Ferrari A, Verna F, et al. Ticks infesting humans and associated pathogens: a cross-sectional study in a 3-year period (2017–2019) in northwest Italy. Parasit Vectors 2021;14:136. https://doi.org/10.1186/s13071-021-04603-x.
[32] Koczwarska J, Pawełczyk A, Dunaj-Małyszko J, Polaczyk J, Welc-Falęciak R. Rickettsia species in Dermacentor reticulatus ticks feeding on human skin and clinical manifestations of tick-borne infections after tick bite. Sci Rep 2023;13:9930. https://doi.org/10.1038/s41598-023-37059-3.
[33] Jahfari S, Hofhuis A, Fonville M, van der Giessen J, van Pelt W, Sprong H. Molecular detection of tick-borne pathogens in humans with tick bites and erythema migrans, in the Netherlands. PLoS Negl Trop Dis 2016;10:e0005042.
[34] Jakimovski D, Banović P, Spasovska K, Rangelov G, Cvetanovska M, Cana F, et al. Detection of autochthonous virus strain responsible for the recent outbreak of Crimean-Congo haemorrhagic fever in North Macedonia, July to August 2023 2024. DOI: 10.21203/rs.3.rs-4360716/v1.
[35] Cabezas-Cruz A, Hodžić A, Mateos-Hernández L, Contreras M, de la Fuente J. Tick-human interactions: from allergic klendusity to the α-Gal syndrome. Biochem J 2021;478:1783–94. https://doi.org/10.1042/BCJ20200915.
[36] Ng YQ, Gupte TP, Krause PJ. Tick hypersensitivity and human tick-borne diseases. Parasite Immunol 2021;43:e12819.