

Article

Molecular Surveillance of *Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* in Ticks from Urbanized Areas of Lithuania

Justina Snegiriovaitė , Indrė Lipatova , Miglė Razgūnaitė , Algimantas Paulauskas * and Jana Radzijeuskaja

Faculty of Natural Sciences, Vytautas Magnus University, K. Donelaičio Str. 58, LT-44248 Kaunas, Lithuania; justina.snegiriovaite@vdu.lt (J.S.); indre.lipatova@vdu.lt (I.L.); migle.razgunaite@vdu.lt (M.R.); jana.radzijeuskaja@vdu.lt (J.R.)

* Correspondence: algimantas.paulauskas@vdu.lt

Abstract

Neoehrlichia mikurensis and *Anaplasma phagocytophilum*, both members of the Anaplasmataceae family, are pathogens that can cause diseases in animals and humans. Ixodid ticks are the primary vectors for both species. While urban green spaces offer various ecological and social benefits, there is increasing evidence suggesting potential public health risks, particularly increased exposure to vector-borne diseases. The aim of the present study was to assess the prevalence and co-occurrence of *A. phagocytophilum* and *N. mikurensis* in ticks from urban environments in Lithuania. A total of 3599 *Ixodes ricinus* and 29 *Dermacentor reticulatus* were collected from 31 urban and 21 peri-urban areas. Ticks were examined for pathogens using duplex real-time PCR. *Anaplasma phagocytophilum* was detected in 4.47% of tested ticks, while *N. mikurensis* in 6.17%. Co-infection was found in 0.42% of *I. ricinus* specimens. Phylogenetic analysis of the *groEl* gene revealed low genetic variability of *N. mikurensis* and the circulation of two ecotypes (I and II) of *A. phagocytophilum*. Additionally, *Ehrlichia muris* was identified in *I. ricinus* ticks. This study is the first report of *N. mikurensis* detection in ticks from Lithuania. Our findings highlight the potential risk posed by tick-borne pathogens in urban and peri-urban areas of the country.

Keywords: *Ixodes ricinus*; *Dermacentor reticulatus*; tick-borne pathogens; zoonosis



Academic Editor: Alina Rodríguez Mallon

Received: 2 June 2025

Revised: 25 June 2025

Accepted: 27 June 2025

Published: 28 June 2025

Citation: Snegiriovaitė, J.; Lipatova, I.; Razgūnaitė, M.; Paulauskas, A.; Radzijeuskaja, J. Molecular Surveillance of *Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* in Ticks from Urbanized Areas of Lithuania. *Pathogens* **2025**, *14*, 642. <https://doi.org/10.3390/pathogens14070642>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Ticks are competent vectors of a wide range of pathogens, including bacteria, viruses, and protozoa, which can cause various diseases in humans and animals [1]. Global warming is influencing the distribution and abundance of ticks, potentially increasing the risk of tick-borne diseases [2]. The two widely distributed tick species in Lithuania, *Ixodes ricinus* and *Dermacentor reticulatus*, are among the most abundant and epidemiologically significant tick species in Europe [3,4]. The prevalence and distribution of these ticks, as well as the pathogens they transmit, are influenced by ecological factors such as biotope type and climate conditions [5].

The family Anaplasmataceae is a group of small, Gram-negative, pleomorphic, obligate intracellular bacteria within the order Rickettsiales [6]. This family includes five genera: *Anaplasma*, *Ehrlichia*, *Neoehrlichia*, *Neorickettsia*, and *Wolbachia*. Members of Anaplasmataceae family infect various host cells, including erythrocytes, monocytes, macrophages, neutrophils, and platelets [7–9]. Ixodidae ticks serve as vectors for numerous Anaplasmat-

aceae bacteria, which can cause disease in humans and a wide range of domestic and wild animals [10].

Neoehrlichia mikurensis is a newly identified tick-borne bacterium belonging to the family Anaplasmataceae [11]. This pathogen was first time reported in wild rats in Japan [12]. To date, its presence has been recorded in at least 20 European countries [13]. Ticks of the genus *Ixodes* are recognized as the primary vector of *N. mikurensis* [11,14]. Although the pathogen has also been detected in *Dermacentor* spp. ticks, the role of this tick genus in its transmission remains unclear [15]. Rodents are considered the main reservoir host, although *N. mikurensis* has also been identified in other wild and domestic animals [13].

Anaplasma phagocytophilum is another member of the Anaplasmataceae family, which causes human granulocytic anaplasmosis (HGA), tick-borne fever (TBF), and granulocytic infections in animals [16]. While wild ruminants and small mammals are thought to play a central role in maintaining *A. phagocytophilum* in nature, various other animals, such as bears, foxes, wild boars, horses, and reptiles, may also serve as occasional hosts or reservoir species [17]. *Anaplasma phagocytophilum* has a broad geographic distribution in Europe and exhibits significant genetic diversity, with different strains showing both host and regional specificity [10,18].

In Lithuania, *A. phagocytophilum* has previously been detected in questing ticks, ticks collected from migratory birds, cervids, and European bison, as well as in samples from domestic dogs, raccoon dogs, roe deer, and European bison [19–24]. However, there is a lack of data on the presence of *A. phagocytophilum* in ticks from urban environments. Furthermore, no studies have been conducted to investigate the presence of *N. mikurensis*. The aim of this study was to assess the prevalence and co-occurrence of *A. phagocytophilum* and *N. mikurensis* in ticks from urban environments in Lithuania.

2. Materials and Methods

2.1. Tick Collection and DNA Extraction

A total of 52 green spaces (31 urban and 21 peri-urban) across 22 cities and ten counties in Lithuania were selected for tick sampling (Table S1). Priority was given to heavily frequented recreational areas with suitable ecological conditions for ticks. Questing ticks were collected between April and June during the years 2021–2024 using standard flagging and dragging techniques. All ticks were identified to the species level, developmental stage, and sex using taxonomic keys [25] and were preserved in 70% ethanol.

Genomic DNA was extracted from individual tick specimens by lysis in 2.5% ammonium hydroxide solution [26]. The extracted DNA was then stored at $-20\text{ }^{\circ}\text{C}$ for subsequent molecular analyses.

2.2. PCR Assay and Sequencing

A duplex real-time PCR assay was used for the simultaneous detection of *A. phagocytophilum* and *N. mikurensis* in tick samples, targeting 98 bp and 129 bp fragments of the *msp2* and *groEL* genes, respectively [27,28]. Reactions were performed on a Rotor-Gene Q (Qiagen, Venlo, The Netherlands) using 2x Sensi MixTM II Probe No-ROX (Bioline, London, UK). Nucleotide sequences of the primers and TaqMan probes used for the detection of *A. phagocytophilum* and *N. mikurensis* are listed in Table 1. Each 15 μL reaction mixture contained 2 μL of template DNA, 1x Sensi MixTM II Probe No-ROX, 1 pM of each primer, and 0.5 pM of each probe. The thermal cycling conditions were as follows: initial denaturation at $95\text{ }^{\circ}\text{C}$ for 2 min, followed by 50 cycles of denaturation at $95\text{ }^{\circ}\text{C}$ for 20 s, annealing at $60\text{ }^{\circ}\text{C}$ for 1 min, and extension at $72\text{ }^{\circ}\text{C}$ for 20 s. Both negative and positive controls were included in each run. Positive controls consisted of *I. ricinus* tick DNA samples previously

confirmed as positive for *A. phagocytophilum* and *N. mikurensis* by sequencing. Results were considered positive when repeats yielded cycle threshold (Ct) values < 38.

Table 1. Nucleotide sequences of primers and probes used in this study.

Primers and Probes	Sequences (5'-3')	Target Gene	Amplicon Size	References
Anaplasma_F Anaplasma_R Anaplasma_Zr	GGACAACATGCTTGTAGCTATGGAAGG CCTTGGTCTTGAAGCGCTCGTA VIC-TCTCAAGCTCAACCCTGGCACCACCA-BHQ1	<i>msp2</i>	98 bp	[26]
Neo2f Neo2r Neo2m	GCAAATGGAGATAAAAACATAGGTAGTAAA CATACCGTCAGTTTTTCAACTTCTAA Cy5-TTACAGTTGAGGAAAGTAAGGGA-BHQ2	<i>groEL</i>	129 bp	[27]
HS1 HS6a HS43 HSVR	AITGGGCTGGTAITGAAAT CCICCIIGGIACIAIACCTTC ATWGCWAARGAAGCATAGTC CTCAACAGCAGCTCTAGTAGC	<i>groEL</i>	1450 bp 1300 bp	[28,29]

Positive *A. phagocytophilum* and *N. mikurensis* samples identified by real-time PCR were further confirmed using a nested PCR (nPCR) assay. Two primer sets, HS1a/HS6a and HS43/HSVR, were used in nPCR to amplify an approximately 1300 bp fragment of the *groEL* gene in species of the Anaplasmataceae family [29,30]. Each 25 µL reaction mixture for both PCR rounds contained 1x Dreamtaq Green Master Mix (ThermoFisher Scientific, Vilnius, Lithuania), 0.4 µM of each primer, nuclease-free water, and 2 µL of DNA template for the first round, or 1 µL of the first-round PCR product for the second round. The amplification program starts with three cycles of DNA denaturation at 94 °C for 1 min, annealing at 48 °C for 2 min, and extension at 70 °C for 1 min 30 s. This was followed by 37 cycles of denaturation at 88 °C for 1 min, annealing at 52 °C for 2 min in the first-round PCR and at 55 °C for 2 min in the second round. The final extension step was then carried out at 68 °C for 5 min, and the reaction was held at 10 °C.

PCR amplicons from *A. phagocytophilum* and *N. mikurensis* positive samples were excised from 1.5% agarose gels and purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Vilnius, Lithuania) according to the manufacturer's instructions. The purified products were sent for sequencing to Macrogen (Amsterdam, The Netherlands). The obtained sequences were edited, aligned with one another, and compared with the sequence from GenBank using the software Mega 11 version 11.0.3 [31] and the BLASTn algorithm. The phylogenetic tree for the *groEL* gene was constructed using the Maximum Likelihood method with the Tamura 3-parameter model and bootstrap analysis with 1000 replicates.

The sequences obtained in this study were submitted to the GenBank database under the accession numbers PV711389–PV711402 for *A. phagocytophilum* and PV711403–PV711412 for *N. mikurensis*.

2.3. Statistical Analysis

A statistical analysis was performed using Statistica for Windows (version 7.0, StatSoft, Tulsa, OK, USA). A chi-square test and 95% confidence intervals (95% CI) were used to compare differences in the prevalence of detected pathogen species across tick species, development stages, sex, and the area types. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Prevalence of *A. phagocytophilum* and *N. mikurensis*

A total of 3628 ticks were collected from 43 out of 52 urban and peri-urban locations. The ticks were morphologically identified as *I. ricinus* ($n = 3599$) and *D. reticulatus* ($n = 29$) (Table 2). *Anaplasma phagocytophilum* was detected in 4.47% of tested tick samples (162/3628) (95% CI: 3.79–5.14), while *N. mikurensis* was found in 6.17% (224/3628) (95% CI: 5.39–6.96). Among the identified tick species, only one *D. reticulatus* female (3.45%) was found to be infected with *A. phagocytophilum*, while no specimens were positive for *N. mikurensis*. In contrast, infections with both pathogens were predominantly detected in *I. ricinus*, which showed significantly higher prevalence rates (4.47% for *A. phagocytophilum* and 6.22% for *N. mikurensis*). No substantial differences in infection rates were observed between urban and peri-urban areas for either pathogen species: *A. phagocytophilum* was detected in 4.63% (95% CI: 3.95–5.32) of ticks from urban areas and 4.31% (95% CI: 3.64–4.97) from peri-urban areas; *N. mikurensis* was found in 5.87% (95% CI: 5.11–6.64) and 6.46% (95% CI: 5.66–7.26), respectively. Similarly, infection rates by habitat type showed little variation, with *A. phagocytophilum* prevalence at 4.53% (95% CI: 3.85–5.20) in forested sites and 4.35% (95% CI: 3.69–5.02) in non-forested areas, while *N. mikurensis* was found in 5.85% (95% CI: 5.09–6.61) and 6.76% (95% CI: 5.95–7.58), respectively. However, infection prevalence varied more substantially across individual sampling locations, ranging from 0.71% to 23.53% for *A. phagocytophilum* and from 0.87% to 16.67% for *N. mikurensis* (Table S1).

Table 2. Number of collected ticks and prevalence of *Anaplasma phagocytophilum* and *Neorhlichia mikurensis*. N—number of collected ticks; n—number of infected ticks.

		N	Prevalence, n (%) (95% CI)		Pathogen Co-Occurrence
			<i>Anaplasma phagocytophilum</i>	<i>Neoehrlichia mikurensis</i>	
Tick species	<i>Ixodes ricinus</i>	3599	161 (4.47) (3.80–5.15)	224 (6.22) (5.44–7.01)	15
	<i>Dermacentor reticulatus</i>	29	1 (3.45) (2.85–4.40)	0	0
Tick sex	Female	985	53 (5.38) (4.65–6.11)	66 (6.70) (5.89–7.51)	1
	Male	1040	65 (6.25) (5.46–7.04)	69 (6.63) (5.82–7.44)	8
Tick stage	Adults	2025	118 (5.83) (5.06–6.59)	135 (6.66) (5.85–7.48)	9
	Nymphs	1603	44 (2.74) (2.21–3.27)	89 (5.55) (4.81–6.29)	6
Area type	Urban	1770	82 (4.63) (3.95–5.32)	104 (5.87) (5.11–6.64)	5
	Peri-urban	1858	80 (4.31) (3.64–4.97)	120 (6.46) (5.66–7.26)	10
Habitat type	Forested	2342	106 (4.53) (3.85–5.20)	137 (5.85) (5.09–6.61)	13
	Non-forested	1286	56 (4.35) (3.69–5.02)	87 (6.76) (5.95–7.58)	2

A total of 15 cases of co-infection (0.42%; 95% CI: 0.21–0.63) with *A. phagocytophilum* and *N. mikurensis* were detected in *I. ricinus* ticks. Co-occurrence of both pathogens was significantly more frequent in males than females ($\chi^2 = 4.83$, $p = 0.03$), and in adult ticks compared to nymphs ($\chi^2 = 0.21$, $p = 0.65$). Co-infections were more commonly observed in peri-urban sites (10 cases) than in urban ones (5 cases) ($\chi^2 = 1.38$, $p = 0.24$). Similarly, the vast majority of co-infection cases (13 out of 15) were detected in forested habitats ($\chi^2 = 3.76$, $p = 0.05$) (Table 2).

3.2. Phylogenetic Analysis

Analysis of the obtained partial *groEL* gene sequences revealed that *I. ricinus* ticks were infected with three pathogen species: *A. phagocytophilum*, *N. mikurensis*, and *Ehrlichia muris* (Figure 1). The *A. phagocytophilum* sequences showed 99.9–100% identity to reference

sequences deposited in GenBank. Most of the sequences from this study clustered within ecotype I, while a few were assigned to ecotype II. Four distinct sequence variants of the *A. phagocytophilum groEL* gene were identified, with 19 variable nucleotide positions. The Lithuanian *N. mikurensis* sequences were 99.8–100% identical to *N. mikurensis* sequences from other European countries. In this study, six sequence variants of the *N. mikurensis groEL* gene with six variable nucleotide positions were identified, differing by one to six nucleotides among the identified variants. Moreover, ten of the sequences matched *E. muris* reference sequences in GenBank with 99.9–100% identity, supporting their classification as *E. muris*. Sequence comparison revealed two *E. muris* variants, differing by a single nucleotide substitution (G→C).

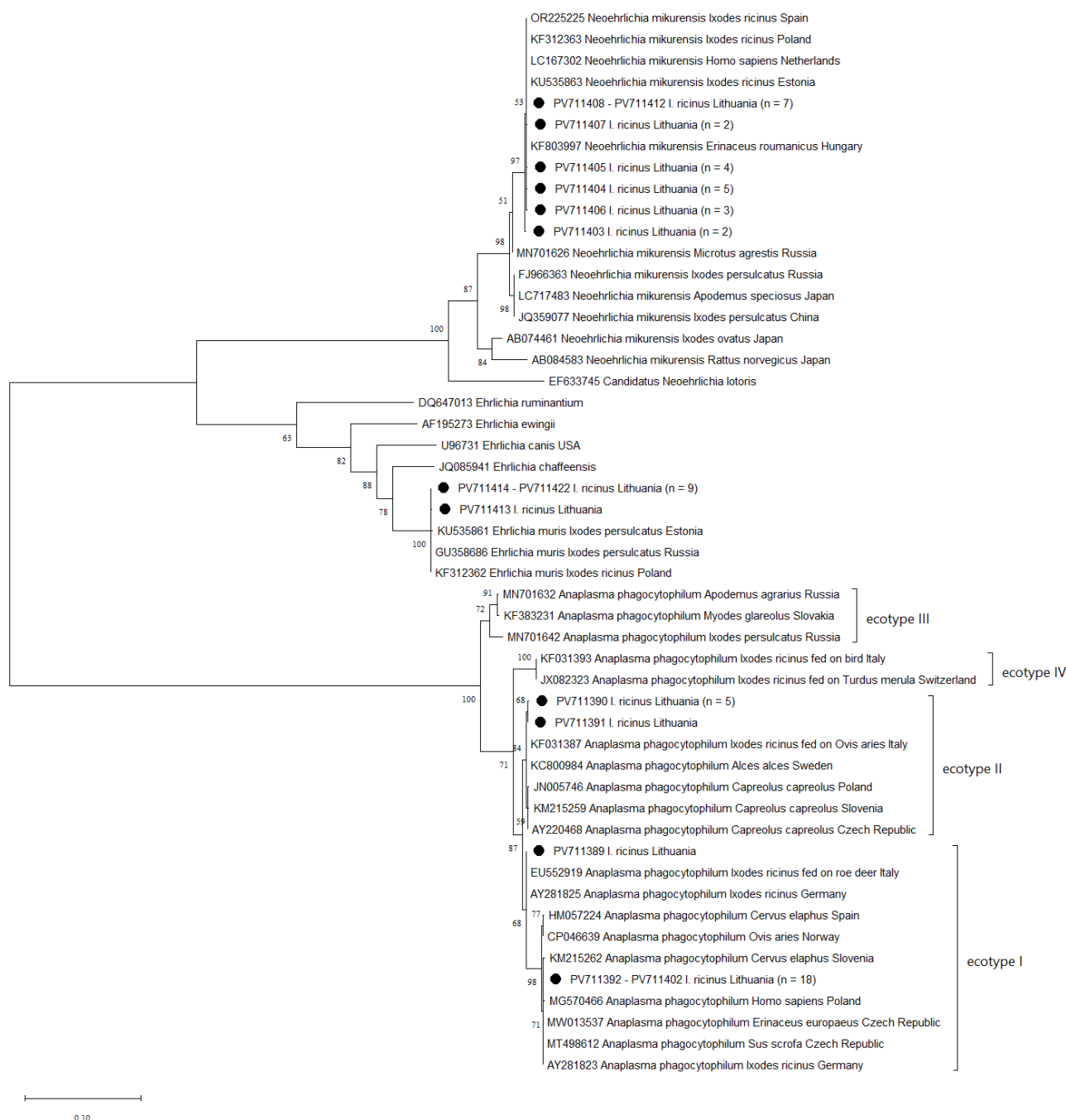


Figure 1. Maximum-likelihood phylogenetic tree based on partial *groEL* gene sequences. Samples isolated from ticks in this study are marked with ●. The number of samples represented by the sequence is given in parentheses ($n = x$).

4. Discussion

To the best of the authors' knowledge, this is the first report on the detection of *N. mikurensis* in ticks from Lithuania. *N. mikurensis* was found in *I. ricinus* ticks collected from both urban and peri-urban areas. This study confirmed the presence of *A. phagocytophilum* in *I. ricinus* and *D. reticulatus* ticks from urbanized environments, extending previous findings that had primarily identified this pathogen in ticks from natural habitats in Lithuania [19,21].

In Lithuania, Lyme borreliosis and tick-borne encephalitis are the only tick-borne diseases routinely diagnosed in humans. Other potential tick-borne diseases, such as anaplasmosis, babesiosis, ehrlichiosis, and rickettsiosis, which can cause flu-like symptoms, are not investigated in clinical practice. In comparison, HGA has been clinically confirmed and verified through laboratory testing in Austria, Italy, Poland, and several other European countries [32]. Our study demonstrates the presence of *A. phagocytophilum* and *N. mikurensis* in frequently visited public green spaces in Lithuania. Therefore, expanding the list of tick-borne diseases included in medical surveillance and diagnostics is essential for improved disease recognition and management.

In this study, the overall prevalence of *A. phagocytophilum* was 4.47%. By comparison, studies from Poland, Latvia, and Estonia reported significantly lower prevalence rates (1.7%, 1.1%, and 0.5%, respectively) [33–35]. *Anaplasma phagocytophilum* has been identified in multiple animal species, including both domestic and wild mammals. A recent global meta-analysis reported a 15.18% prevalence of *A. phagocytophilum* in animal reservoirs, with an infection rate approximately twice as high in wildlife compared to domestic animals, highlighting the importance of wild hosts in the maintenance and transmission of this pathogen [36]. In Europe, *A. phagocytophilum* is divided into four main ecotypes based on *groEL* gene sequences [37]. Sequences obtained in this study from ticks collected in urban and peri-urban areas belonged to ecotype I and ecotype II, with ecotype I being predominant. Ecotype I has been detected in a wide range of hosts and is associated with HGA and TBF. In contrast, ecotype II has primarily been found in roe deer and moose and is currently considered non-zoonotic [37,38]. The predominance of ecotype I in tick samples from urban and peri-urban areas highlights a potential risk of infection, particularly among people and pets spending time in urban green spaces.

The detection of *N. mikurensis* at an overall prevalence rate of 6.22% highlights its established circulation in urban tick populations in Lithuania, suggesting a potential risk of human infection. The prevalence varied considerably across different sampling locations within the country, ranging from 0.87% to 16.67%, indicating spatial heterogeneity in pathogen distribution. The prevalence of *N. mikurensis* also varies among Lithuania's neighboring countries, with lower rates reported in Estonia (2.4%) and higher rates in Poland (19.3%) [9,39]. Rodents are important reservoir hosts for *N. mikurensis*, playing a key role in its maintenance and circulation in both urban and natural habitats [40]. In this study, *N. mikurensis* was detected only in *I. ricinus* ticks. A similar observation was reported in studies from Germany and Poland, where *D. reticulatus* tested negative for this pathogen [5,41]. Furthermore, previous studies have reported low genetic diversity in *N. mikurensis* *groEL* gene sequences across various European countries [28,42,43]. Similarly, our phylogenetic analysis of the *groEL* gene sequences revealed limited genetic variation among the strains detected in this study. The low genetic variability of *N. mikurensis* likely results from its adaptation to a narrow ecological niche, involving a limited range of reservoir hosts and vectors [43]. No clear evidence currently links genetic variation with differences in *N. mikurensis* pathogenicity. This pathogen often causes mild or asymptomatic infections, especially in immunocompetent individuals [44]. Further research is necessary to clarify its pathogenic potential.

In this study, *E. muris* was incidentally detected in several *I. ricinus* tick samples. The detection is consistent with previous reports, which showed that *groEL* gene primers also amplify non-target *Ehrlichia* species [29,45]. *Ehrlichia muris* primarily affects various rodent species but can also infect humans [46–48]. It was also reported from dogs [49]. Hard tick species, such as *Ixodes persulcatus* and *I. ricinus* in Eurasia, and *Ixodes scapularis* in the USA, are suggested as a potential vectors for this pathogen [48,50]. Although the prevalence of *E. muris* was not systematically assessed in our study, its incidental detection may indicate the presence of co-infection with the target pathogen. Further targeted investigations would be necessary to confirm such co-infections and evaluate their epidemiological significance.

In this study, co-infection with *A. phagocytophilum* and *N. mikurensis* was also detected. Ticks harboring more than one pathogen represent a potential source of co-infection for both humans and animals [51]. Co-occurrence of *N. mikurensis* and *A. phagocytophilum* has previously been reported in *I. ricinus* ticks from Central Europe [11]. In Poland, a significantly higher occurrence of Anaplasmatidae species co-infections was observed in *I. ricinus* ticks from urban areas compared to natural habitats [52]. These pathogens are known to share similar mammalian reservoir hosts, such as rodents, and it has been suggested that they may facilitate each other's transmission by ticks, as shown for *A. phagocytophilum* and *Borrelia* spp. [52,53]. However, further studies are needed to confirm this interaction.

5. Conclusions

The present findings confirm the presence of tick-borne pathogens in urban and peri-urban areas of Lithuania. Although no human cases of anaplasmosis, neorickettsiosis, or ehrlichiosis have been officially registered in Lithuania so far, these infections are likely underdiagnosed or misclassified in clinical practice as other flu-like illnesses. Therefore, expanding diagnostic awareness and integrating these pathogens into routine surveillance is warranted.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pathogens14070642/s1>, Table S1: Tick collection sites, number of collected ticks and prevalence of *A. phagocytophilum* and *N. mikurensis*.

Author Contributions: Conceptualization, A.P. and J.R.; methodology, J.S., I.L., M.R., A.P. and J.R.; investigation, J.S., I.L. and M.R.; resources, J.S., I.L., M.R., A.P. and J.R.; writing—original draft preparation, J.S. and I.L.; writing—review and editing, J.S., I.L., M.R., A.P. and J.R.; project administration, J.R.; funding acquisition, A.P. and J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the Research Council of Lithuania, grant number S-MIP-19/23.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. de la Fuente, J.; Estrada-Pena, A.; Venzal, J.M.; Kocan, K.M.; Sonenshine, D.E. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Front. Biosci.* **2008**, *13*, 6938–6946. [CrossRef] [PubMed]
2. Nuttall, P.A. Climate change impacts on ticks and tick-borne infections. *Biologia* **2022**, *77*, 1503–1512. [CrossRef]
3. Černý, J.; Lynn, G.; Hrnková, J.; Golovchenko, M.; Rudenko, N.; Grubhoffer, L. Management options for *Ixodes ricinus*-associated pathogens: A review of prevention strategies. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1830. [CrossRef]

4. Kuyucu, A.C.; Hekimoglu, O. Predicting the distribution of *Ixodes ricinus* in Europe: Integrating microclimatic factors into ecological niche models. *Parasitology* **2024**, *151*, 1012–1023. [\[CrossRef\]](#)
5. Zając, Z.; Obregon, D.; Foucault-Simonin, A.; Wu-Chuang, A.; Moutailler, S.; Galon, C.; Kulisz, J.; Woźniak, A.; Bartosik, K.; Cabezas-Cruz, A. Disparate dynamics of pathogen prevalence in *Ixodes ricinus* and *Dermacentor reticulatus* ticks occurring sympatrically in diverse habitats. *Sci. Rep.* **2023**, *13*, 10645. [\[CrossRef\]](#)
6. Teixeira, S.C.; Teixeira, T.L.; Tavares, P.C.B.; Alves, R.N.; da Silva, A.A.; Borges, B.C.; Martins, F.A.; Dos Santos, M.A.; de Castilhos, P.; E Silva Brígido, R.T.; et al. Subversion strategies of lysosomal killing by intracellular pathogens. *Microbiol. Res.* **2023**, *277*, 127503. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Rosenberg, E. The Family Anaplasmataceae. In *The Prokaryotes*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 79–80.
8. Nicholson, W.N.; Pritt, B.S. Family Anaplasmataceae (Anaplasmosis, Ehrlichiosis, Neorickettsiosis, and Neoehrlichiosis). In *Principles and Practice of Pediatric Infectious Diseases*; Long, S.S., Ed.; Elsevier: Amsterdam, The Netherlands, 2023; pp. 937–942.e2.
9. Szczotko, M.; Kubiak, K.; Michalski, M.M.; Moerbeck, L.; Antunes, S.; Domingos, A.; Dmitryjuk, M. *Candidatus Neoehrlichia mikurensis*—A new emerging tick-borne pathogen in north-eastern Poland? *Pathogens* **2023**, *12*, 307. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Huang, X.B.; Tang, T.; Chen, J.J.; Zhang, Y.Y.; Lv, C.L.; Xu, Q.; Wang, G.L.; Zhu, Y.; Wei, Y.H.; Hay, S.I.; et al. The global distribution and risk prediction of Anaplasmataceae species: A systematic review and geospatial modelling analysis. *EBioMedicine* **2025**, *115*, 105722. [\[CrossRef\]](#)
11. Derdákóvá, M.; Václav, R.; Pangráčová-Blaňárová, L.; Selyemová, D.; Koči, J.; Walder, G.; Špitalská, E. *Candidatus Neoehrlichia mikurensis* and its co-circulation with *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks across ecologically different habitats of Central Europe. *Parasit. Vectors* **2014**, *7*, 160. [\[CrossRef\]](#)
12. Kawahara, M.; Rikihisa, Y.; Isogai, E.; Takahashi, M.; Misumi, H.; Suto, C.; Shibata, S.; Zhang, C.; Tsuji, M. Ultrastructure and phylogenetic analysis of ‘*Candidatus Neoehrlichia mikurensis*’ in the family Anaplasmataceae, isolated from wild rats and found in *Ixodes ovatus* ticks. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 1837–1843. [\[CrossRef\]](#)
13. Portillo, A.; Santibáñez, P.; Palomar, A.M.; Santibáñez, S.; Oteo, J.A. ‘*Candidatus Neoehrlichia mikurensis*’ in Europe. *New Microbes New Infect.* **2018**, *22*, 30–36. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Wennerås, C. Infections with the tick-borne bacterium *Candidatus Neoehrlichia mikurensis*. *Clin. Microbiol. Infect.* **2015**, *21*, 621–630. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Liu, H.; Xiao, W.; Du, X.; Xue, J.; Wang, H.; Wang, Q.; Wang, Y.; Jia, H.; Song, H.; Qiu, S. Molecular detection of tick-borne bacterial pathogens in ticks and rodents from the China-Vietnam border. *Vet. Sci.* **2025**, *12*, 256. [\[CrossRef\]](#)
16. Stuenkel, S.; Granquist, E.G.; Silaghi, C. *Anaplasma phagocytophilum*—A widespread multi-host pathogen with highly adaptive strategies. *Front. Cell. Infect. Microbiol.* **2013**, *3*, 31. [\[CrossRef\]](#)
17. Szekeres, S.; Claudia Coipan, E.; Rigó, K.; Majoros, G.; Jahfari, S.; Sprong, H.; Földvári, G. *Candidatus Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* in natural rodent and tick communities in Southern Hungary. *Ticks Tick-Borne Dis.* **2015**, *6*, 111–116. [\[CrossRef\]](#)
18. Rar, V.; Tkachev, S.; Tikunova, N. Genetic diversity of *Anaplasma* bacteria: Twenty years later. *Infect. Genet. Evol.* **2021**, *91*, 104833. [\[CrossRef\]](#)
19. Radzijeuskaja, J.; Paulauskas, A.; Rosef, O. Prevalence of *Anaplasma phagocytophilum* and *Babesia divergens* in *Ixodes ricinus* ticks from Lithuania and Norway. *Int. J. Med. Microbiol.* **2008**, *298*, 218–221. [\[CrossRef\]](#)
20. Paulauskas, A.; Radzijeuskaja, J.; Rosef, O. *Anaplasma* in ticks feeding on migrating birds and questing ticks in Lithuania and Norway. *Clin. Microbiol. Infect.* **2009**, *15*, 34–36. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Paulauskas, A.; Radzijeuskaja, J.; Rosef, O. Molecular detection and characterization of *Anaplasma phagocytophilum* strains. *Comp. Immunol. Microbiol. Infect. Dis.* **2012**, *35*, 187–195. [\[CrossRef\]](#)
22. Radzijeuskaja, J.; Tamoliūnaitė, D.; Sabūnas, V.; Aleksandravičienė, A.; Paulauskas, A. Prevalence and co-infection of mosquito- and tick-borne pathogens in domestic dogs suspected for canine babesiosis in Lithuania. *Biologija* **2020**, *66*, 94–102. [\[CrossRef\]](#)
23. Radzijeuskaja, J.; Snegiriovaitė, J.; Kibiša, A.; Ražanskė, I.; Paulauskas, A. Molecular characterization of *Anaplasma phagocytophilum* infection in the cervids and feeding ticks from Lithuania. *Biologija* **2020**, *66*, 136–144. [\[CrossRef\]](#)
24. Lipatova, I.; Černevičienė, D.; Gričiuvienė, L.; Ražanskė, I.; Aleksandravičienė, A.; Kibiša, A.; Radzijeuskaja, J.; Olech, W.; Anusz, K.; Didkowska, A.; et al. *Anaplasma phagocytophilum* in European bison (*Bison bonasus*) and their ticks from Lithuania and Poland. *Ticks Tick-Borne Dis.* **2023**, *14*, 102246. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Hillyard, P.D. *Ticks of North-West Europe, Synopses of the British Fauna No. 52 (New Series)*; The Linnean Society of London: London, UK, 1996.
26. Rijpkema, S.; Golubić, D.; Molkenboer, M.; Verbeek-De Kruif, N.; Schellekens, J. Identification of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. *Exp. Appl. Acarol.* **1996**, *20*, 23–30. [\[CrossRef\]](#)

27. Razanske, I.; Rosef, O.; Radzijeuskaja, J.; Bratchikov, M.; Gričuvienė, L.; Paulauskas, A. Prevalence and co-infection with tick-borne *Anaplasma phagocytophilum* and *Babesia* spp. in red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) in Southern Norway. *Int. J. Parasitol. Parasites Wildl.* **2019**, *8*, 127–134. [\[CrossRef\]](#)
28. Jenkins, A.; Raasok, C.; Pedersen, B.N.; Jensen, K.; Andreassen, Å.; Soleng, A.; Edgar, K.S.; Lindstedt, H.H.; Kjelland, V.; Stuenkel, S.; et al. Detection of *Candidatus* Neoehrlichia mikurensis in Norway up to the northern limit of *Ixodes ricinus* distribution using a novel real time PCR test targeting the groEL gene. *BMC Microbiol.* **2019**, *19*, 199. [\[CrossRef\]](#)
29. Sumner, J.W.; Nicholson, W.L.; Massung, R.F. PCR amplification and comparison of nucleotide sequences from the groESL heat shock operon of *Ehrlichia* species. *J. Clin. Microbiol.* **1997**, *35*, 2087–2092. [\[CrossRef\]](#)
30. Liz, J.S.; Sumner, J.W.; Pfister, K.; Brossard, M. PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). *J. Clin. Microbiol.* **2002**, *40*, 892–897. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Matei, I.A.; Estrada-Peña, A.; Cutler, S.J.; Vayssier-Taussat, M.; Varela-Castro, L.; Potkonjak, A.; Zeller, H.; Mihalca, A.D. A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe. *Parasit. Vectors* **2019**, *12*, 599. [\[CrossRef\]](#)
33. Stańczak, J.; Cieniuch, S.; Lass, A.; Biernat, B.; Racewicz, M. Detection and quantification of *Anaplasma phagocytophilum* and *Babesia* spp. in *Ixodes ricinus* ticks from urban and rural environment, northern Poland, by real-time polymerase chain reaction. *Exp. Appl. Acarol.* **2015**, *66*, 63–81. [\[CrossRef\]](#)
34. Capligina, V.; Seleznova, M.; Akopjana, S.; Freimane, L.; Lazovska, M.; Krumins, R.; Kivrane, A.; Namina, A.; Aleinikova, D.; Kimsis, J.; et al. Large-scale countrywide screening for tick-borne pathogens in field-collected ticks in Latvia during 2017–2019. *Parasit. Vectors* **2020**, *13*, 351. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Vikentjeva, M.; Geller, J.; Bragina, O. Ticks and tick-borne pathogens in popular recreational areas in Tallinn, Estonia: The underestimated risk of tick-borne diseases. *Microorganisms* **2024**, *12*, 1918. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Karshima, S.N.; Ahmed, M.I.; Mohammed, K.M.; Pam, V.A.; Momoh-Abdullateef, H.; Gwimi, B.P. Worldwide meta-analysis on *Anaplasma phagocytophilum* infections in animal reservoirs: Prevalence, distribution and reservoir diversity. *Vet. Parasitol. Reg. Stud. Rep.* **2023**, *38*, 100830. [\[CrossRef\]](#)
37. Jahfari, S.; Coipan, E.C.; Fonville, M.; van Leeuwen, A.D.; Hengeveld, P.; Heylen, D.; Heyman, P.; van Maanen, C.; Butler, C.M.; Földvári, G.; et al. Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. *Parasit. Vectors* **2014**, *7*, 365. [\[CrossRef\]](#)
38. Gandy, S.; Hansford, K.; McGinley, L.; Cull, B.; Smith, R.; Semper, A.; Brooks, T.; Fonville, M.; Sprong, H.; Phipps, P.; et al. Prevalence of *Anaplasma phagocytophilum* in questing *Ixodes ricinus* nymphs across twenty recreational areas in England and Wales. *Ticks Tick-Borne Dis.* **2022**, *13*, 101965. [\[CrossRef\]](#)
39. Ivanova, A.; Geller, J.; Katargina, O.; Värvi, K.; Lundkvist, Å.; Golovljova, I. Detection of *Candidatus* Neoehrlichia mikurensis and *Ehrlichia muris* in Estonian ticks. *Ticks Tick-Borne Dis.* **2017**, *8*, 13–17. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Svitáľková, Z.H.; Haruštiaková, D.; Mahríková, L.; Mojšová, M.; Berthová, L.; Slovák, M.; Kocianová, E.; Vayssier-Taussat, M.; Kazimírová, M. *Candidatus* Neoehrlichia mikurensis in ticks and rodents from urban and natural habitats of South-Western Slovakia. *Parasit. Vectors* **2016**, *9*, 2. [\[CrossRef\]](#)
41. Richter, D.; Kohn, C.; Matuschka, F.R. Absence of *Borrelia* spp., *Candidatus* Neoehrlichia mikurensis, and *Anaplasma phagocytophilum* in questing adult *Dermacentor reticulatus* ticks. *Parasitol. Res.* **2013**, *112*, 107–111. [\[CrossRef\]](#)
42. Ondruš, J.; Balážová, A.; Baláž, V.; Zechmeisterová, K.; Novobilský, A.; Šíroky, P. *Candidatus* Neoehrlichia mikurensis is widespread in questing *Ixodes ricinus* ticks in the Czech Republic. *Ticks Tick-Borne Dis.* **2020**, *11*, 101371. [\[CrossRef\]](#)
43. Zając, Z.; Kulisz, J.; Kunc-Kozioł, R.; Woźniak, A.; Foucault-Simonin, A.; Banović, P.; Corduneanu, A.; Moutailler, S.; Cabezas-Cruz, A. *Neoehrlichia mikurensis*: An emerging pathogen in Southeastern Poland—Prevalence in *Ixodes ricinus* ticks and phylogenetic characterization. *Ann. Agric. Environ. Med.* **2025**, *32*, 146–150. [\[CrossRef\]](#)
44. Azagi, T.; Hoornstra, D.; Kremer, K.; Hovius, J.W.R.; Sprong, H. Evaluation of disease causality of rare *Ixodes ricinus*-borne infections in Europe. *Pathogens* **2020**, *9*, 150. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Félix, M.L.; Muñoz-Leal, S.; Carvalho, L.A.; Queirolo, D.; Remesar Alonso, S.; Nava, S.; Armúa-Fernández, M.T.; Venzal, J.M. Molecular characterization of novel *Ehrlichia* genotypes in *Ixodes auritulus* from Uruguay. *Curr. Res. Parasitol. Vector Borne Dis.* **2021**, *1*, 100022. [\[CrossRef\]](#)
46. Smetanová, K.; Boldis, V.; Kocianová, E.; Spitalská, E. Detection of *Ehrlichia muris* in a yellow-necked mouse (*Apodemus flavicollis*) in Central Slovakia. *Acta Virol.* **2007**, *51*, 69–71. [\[PubMed\]](#)
47. Johnson, D.K.; Schiffman, E.K.; Davis, J.P.; Neitzel, D.F.; Sloan, L.M.; Nicholson, W.L.; Fritsche, T.R.; Steward, C.R.; Ray, J.A.; Miller, T.K.; et al. Human infection with *Ehrlichia muris*-like Pathogen, United States, 2007–2013(1). *Emerg. Infect. Dis.* **2015**, *21*, 1794–1799. [\[CrossRef\]](#) [\[PubMed\]](#)

48. Xu, G.; Foster, E.; Ribbe, F.; Hojgaard, A.; Eisen, R.J.; Paull, S.; Rich, S.M. Detection of *Ehrlichia muris* eauclairensis in blacklegged ticks (*Ixodes scapularis*) and white-footed mice (*Peromyscus leucopus*) in Massachusetts. *Vector Borne Zoonotic Dis.* **2023**, *23*, 311–315. [\[CrossRef\]](#)
49. Hegarty, B.C.; Maggi, R.G.; Koskinen, P.; Beall, M.J.; Eberts, M.; Chandrashekar, R.; Breitschwerdt, E.B. *Ehrlichia muris* infection in a dog from Minnesota. *J. Vet. Intern. Med.* **2012**, *26*, 1217–1220. [\[CrossRef\]](#)
50. Croci, C.; Erriquez, L.; Bisaglia, B.; Bellinzona, G.; Olivieri, E.; Sassera, D.; Castelli, M. Genome sequence of *Ehrlichia muris* from *Ixodes ricinus* collected in Italy on a migratory bird provides epidemiological and evolutionary insights. *Ticks Tick-Borne Dis.* **2024**, *15*, 102409. [\[CrossRef\]](#)
51. Porcelli, S.; Deshuillers, P.L.; Moutailler, S.; Lagrée, A.C. Meta-analysis of tick-borne and other pathogens: Co-infection or co-detection? That is the question. *Curr. Res. Parasitol. Vector Borne Dis.* **2024**, *6*, 100219. [\[CrossRef\]](#)
52. Kowalec, M.; Szewczyk, T.; Welc-Faleciak, R.; Siński, E.; Karbowski, G.; Bajer, A. Rickettsiales occurrence and co-occurrence in *Ixodes ricinus* ticks in natural and urban areas. *Microb. Ecol.* **2019**, *77*, 890–904. [\[CrossRef\]](#)
53. Thomas, V.; Anguita, J.; Barthold, S.W.; Fikrig, E. Coinfection with *Borrelia burgdorferi* and the agent of human granulocytic ehrlichiosis alters murine immune responses, pathogen burden, and severity of Lyme arthritis. *Infect. Immun.* **2001**, *69*, 3359–3371. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.