

Prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* and *Dermacentor reticulatus* and Coinfection with *Borrelia burgdorferi* and Tick-Borne Encephalitis Virus in Western Ukraine

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Abstract

Introduction: Tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi*, the causative agent of Lyme disease (LD), are widespread in Western Ukraine. However, relatively little is known about *Anaplasma phagocytophilum* in this region. This study examined patterns of infection with *A. phagocytophilum* in two tick vectors compared with the better studied TBEV and *B. burgdorferi*.

Materials: Ticks were collected in three different ecosystems of the Western Ukraine during 2009–2014. Samples were examined for pathogen detection using real-time polymerase chain reaction (PCR), and logistic regression models were developed to assess the significance of different factors.

Results: Among the three selected ecological systems of the Western region of Ukraine, 5130 ticks belonging to *Ixodes ricinus* and *Dermacentor reticulatus* were collected between 2009 and 2014. They were grouped into 366 pools and were tested by PCR for *A. phagocytophilum*. A subsample (1620 ticks, 162 pools) of the ticks was concurrently tested by PCR for *A. phagocytophilum*, *B. burgdorferi*, and TBEV. Overall, there was no trend in the proportion of positive ticks across years ($p > 0.05$). However, the prevalence of *A. phagocytophilum* was higher (27.4%) in *I. ricinus* than in *D. reticulatus* (15.9%) (OR = 2.69; 95% CI, 1.52–4.94 (Lower, Upper 95% CI)). Infection was more common in forested habitats (OR = 1.89; 95% CI, 1.07–3.36) and during the later summer–early autumn (3.78; 95% CI, 1.79–8.06). *B. burgdorferi* was found in 29.3% and 31.9% of *I. ricinus* and *D. reticulatus*, respectively; and TBEV was found in 6.3% and 14.5% of *I. ricinus* and *D. reticulatus*. Coinfection of *A. phagocytophilum* and *B. burgdorferi* occurred more often than chance and was more frequent than any other combination of pathogens ($p = 0.031$).

Conclusions: Our study is the first to explore the potential relationship between the ecosystems, vectors, and the presence of Human Granulocytic Anaplasmosis (HGA) and other tick-borne infections in Western Ukraine. *Anaplasma* demonstrated a greater prevalence in *I. ricinus* in the forested area in Western Ukraine. Altogether, HGA, LD, and tick-borne encephalitis (TBE) pathogens are actively circulating in these ecosystems and have the potential to coinfect vectors that might increase the risk of transmitting multiple pathogens to humans during host feeding by individual ticks.

Keywords: human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, tick-borne encephalitis virus, tick-borne infections, ticks, Ukraine

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Introduction

TICK-BORNE DISEASES AFFECT humans and other mammals when infectious ticks feed on them, most commonly ticks of the *Ixodidae* family. Clinical presentation and disease severity in humans mostly depend on the infecting pathogen (Hamel et al. 2013, Reye et al. 2013, Vynograd 2014). Although multiple tick species may act as vectors and transmit the same pathogen to vertebrate hosts, coinfections in ticks with multiple pathogens also can occur so that one tick bite can transmit one or more pathogens to the infested host (Pańczuk et al. 2016, Asman et al. 2017). The strong seasonality of tick-borne diseases depends on the tick activity, which, in turn, depends on temperature and hygrometry (i.e., climate and environment). Altogether, climate and ecosystems favorable to tick reproduction, natural host abundance for various tick life stages, human occupations and recreational activities, as well as an active circulation of various pathogens in the region, drive the zoonotic risk for both humans and animals (Alexander et al. 2012, Dantas-Torres 2015).

Previous studies in Ukraine (Movila et al. 2009) have provided insight into tick-borne encephalitis (TBE), a disease caused by a Flavivirus, and Lyme disease (LD), caused by the spirochete, *Borrelia burgdorferi* (Biletska et al. 2008). From 2004 to 2014, the highest rate of registered cases of LD was 4.1 per 100,000 people in Ukraine (Biletska et al. 2014). From 2002 through 2015, inclusive, 100 cases of TBE also were locally reported (and four imported cases were identified) indicating the presence of virus in areas with different landscape and climatic characteristics (Biletska et al. 2011, 2012, Lozynskyi et al. 2013, Pavlikovska et al. 2014).

Human Granulocytic Anaplasmosis (HGA) is not a subject of mandatory reporting in Ukraine so there are no official data about the disease incidence. The presence of HGA in Ukraine was first described in 2007 in a pilot study conducted in nine oblasts of Ukraine (Ben and Biletska 2008). ELISA was used to detect IgM and IgG to *Anaplasma phagocytophilum* in 98 suspected cases of tick-borne infections (fever after tick bite). The first 13 cases of HGA were identified in Volyn (3), Dnipropetrovsk (1), Zaporizhzhya (4), Lviv (1), Poltava (1), Rivne (1), and Cherkassy (2) Oblasts in 2007 (Ben and Biletska 2008, 2009). In addition, 328 healthy persons were tested with ELISA in Western Ukraine (Volyn, Zakarpattia, and Lviv Oblasts) in 2007–2009. The level of positive IgG to *A. phagocytophilum* was between 4.8–10.3% in population of Volyn Oblast; 3.3–6.4% in Lviv Oblast; and 1.4% in Zakarpattia Oblast (Biletska and Ben 2009). ELISA was also used to test 82 patients with tick bites for presence of IgG to *A. phagocytophilum* in Eastern Ukraine (Kharkiv Oblast) in 2007–2009; 7.7% cases were positive (Malyj et al. 2010). Despite serological evidence of HGA pathogen circulation in Western Ukraine, there appears to be limited knowledge of HGA prevalence in ticks.

Lviv and Volyn oblasts of Western region of Ukraine cover three landscape zones: forest (= Ukrainian Polissia); forest steppe; and Ukrainian Carpathian Mountains (Ben and Biletska 2015). This area is characterized by a mild wet climate, a wide range of plants and animals, the presence of vector ticks, and reservoir hosts (Biletska et al. 2012). In addition, HGA has been reported in countries neighboring this region of Ukraine (Kiewra et al. 2014).

The goals of this study were: (1) to identify the likely vectors of *A. phagocytophilum* in Western Ukraine and (2) to compare the prevalence and risk factors of *Anaplasma* infection with other, better studied tick pathogens (*B. burgdorferi* and TBEV) in the region.

Materials and Methods

Study period

Sample collection and testing were performed from April to September (except July when tick activity naturally decreases) every year during 2009–2014.

Study sites

Ticks were collected in Lviv and Volyn oblasts of Western Ukraine in the forest (Ukrainian Polissia) (16 districts), forest-steppe (12 districts), and Ukrainian Carpathian zone (5 districts) range characterized by wetlands with pastures and meadows (Fig. 1).

Collection of ticks

Sample collection was performed in locations identified as sampling sites for national routine vector surveillance. Ixodid ticks were collected by flagging (Chong et al. 2013) and identified to species using external characteristics (Balashov Yu 1998). Live ticks were pooled to represent groupings of the same species, sex, the date, and site of collection. Of the total 5130 ticks, 3170 ticks collected by our institute were pooled as adults in groups of 10 individuals. The remaining 1960 ticks were collected as a part of routine monitoring for tick-borne infections with the staff of the Volyn Oblast Laboratory Center of the Ministry of Health of Ukraine and were pooled as 40 individuals in a pool (49 pools). Pools were stored at -70°C until the end of each season of collection. All pools were tested for DNA of *A. phagocytophilum*; samples collected from areas where tick-borne infections had been previously reported were also tested for presence of *B. burgdorferi* and TBEV to assess risk of human coinfection after a tick bite.

Polymerase chain reaction

Pools of ticks were tested for the causative agents of tick-borne infections using real-time polymerase chain reaction (qPCR). DNA was extracted with established methods (Grzeszczuk 2006) using UltraClean™ Tissue® & Cell DNA Isolation Kit® (MO BIO Laboratories, Inc.) according to the manufacturer's instructions. The quality of extracted DNA was tested with a spectrophotometer to confirm extraction of quality DNA (NanoDrop ND-2000; Thermo Scientific®). PCR for *Anaplasma sp.* DNA detection used the commercial diagnostic test system Ampli-HGA® (Omnix, St. Petersburg, Russian Federation). Multiprime-AmpliSens® (InterLabService, Russian Federation) was used for simultaneous detection of DNA for *Anaplasma* and *Borrelia* and cDNA fragments of TBEV, *B. burgdorferi*, and *A. phagocytophilum* (Karan 2012). During amplification of the study samples, we used amplification controls: negative control (C–)–10 mL of DNA buffer and positive control (C+)–10 mL of cDNA of TBEV, *B. burgdorferi s.l.*, and *A. phagocytophilum*. The total volume of reaction composition was 25 mL.

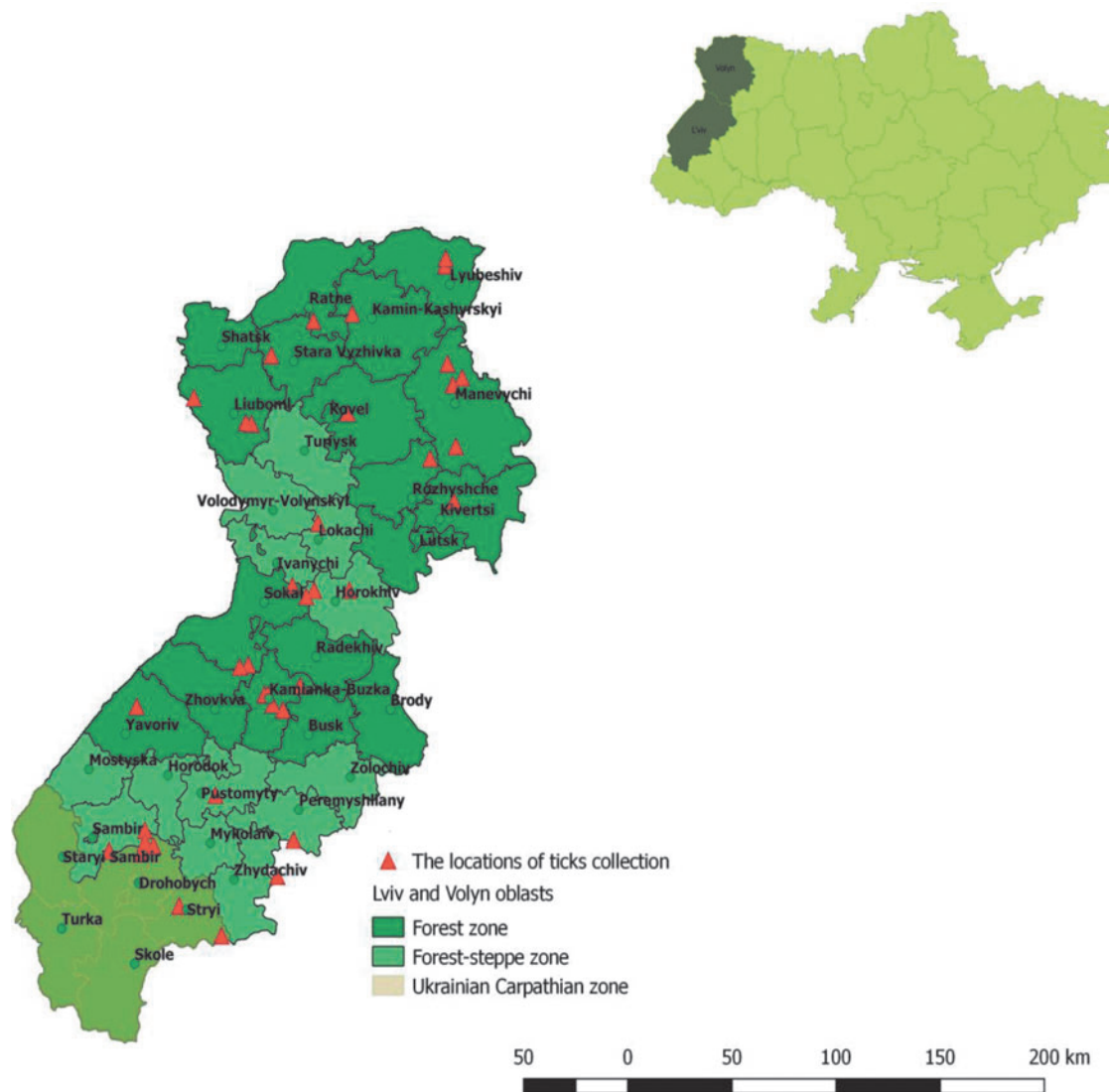


FIG. 1. Locations of tick collections in Lviv and Volyn oblasts, Ukraine, 2009–2014.

Amplification and detection of *A. phagocytophilum*, *B. burgdorferi*, and TBEV used a thermocycler (Rotor-Gene™ 6000) and software Thermal Cycler System (Mysterud et al. 2013). A system for real-time detection of fluorescent signal used the following cycling conditions: initial denaturation at 95°C for 15 min; then 5 cycles of 95°C 10 s, 60°C 35 s, 72°C 15 s; and 40 cycles of 95°C 10 s, 56°C 35 s, 72°C 15 s. Detection of fluorescent signal was performed at FAM, JOE, ROX fluorophore channels for TBEV and *A. phagocytophilum* and FAM, JOE channels for *B. burgdorferi* s.l.

Statistical analyses

Logistic regression models were developed to assess the significance of tick species, seasonal habitat effects, and coinfection conditions for detecting of any of the three pathogens. Estimation of odds ratios and confidence intervals was performed using R software. To evaluate whether coinfections were more common than expected under chance, alone, the prevalence of pathogen combinations in individual tick pools

was tested by chi-squared test with Yates' continuity correction. Adjusted *p* values were generated using Holm's (Holm 1979) adjustment to account for multiple comparisons. *P* value <0.05 was regarded as statistically significant.

Results

A total of 5130 ticks (366 pools) were analyzed for *A. phagocytophilum*. They belonged to only two species: *Ixodes ricinus*—3210 ticks (190 pools) and *Dermacentor reticulatus*—1920 ticks (176 pools) (Tables 1 and 2). *A. phagocytophilum* was found in both tick vectors with 21.9% of all pools testing positive by PCR (Table 1). After controlling for year, season, and eco-zone, *A. phagocytophilum* was more frequent in *I. ricinus* than in *D. reticulatus* (OR = 2.69; 95% CI, 1.52–4.94 Table 3).

Infection with *A. phagocytophilum* was more commonly found in ticks collected from forest habitats (27.9%) and were less likely in forest steppe (19.2%) or the Carpathian zone (8.3%); multiple logistic regression showed significant

TABLE 1. THE RESULTS OF SCREENING FOR DNA OF *ANAPLASMA PHAGOCYTOPHILUM* WITH PCR IN TICKS COLLECTED IN DIFFERENT ECOSYSTEMS OF LVIV AND VOLYN OBLASTS, 2010–2014

Landscape-geographical zones	The results of screening for <i>A. phagocytophilum</i> with PCR					
	Total		By species			
	Number of ticks tested/number of pools tested	Number of positive pools (% positive)	<i>I. ricinus</i>		<i>Dermacentor reticulatus</i>	
Ticks tested/pools tested			Number of positive pools (% positive pools)	Ticks tested/pools tested	Number of positive pools (% positive pools)	
Forest	3010/172	48 (27.9%)	2070/94	35 (37.2%)	940/78	13 (16.7%)
Forest steppe	1640/146	28 (19.2%)	990/81	15 (18.5%)	650/65	13 (20%)
Carpathians	480/48	4 (8.3%)	150/15	2 (13.3%)	330/33	2 (6.1%)
Total	5130/366	80 (21.9%)	3210/190	52 (27.4%)	1920/176	28 (15.9%)

association with forest habitats (OR = 1.89; 95% CI, 1.07–3.36). Collecting in urban areas was strongly associated with higher chances of detecting the pathogen in ticks (OR = 8.26; 95% CI, 2.93–24.82).

A. phagocytophilum had a bimodal seasonal pattern of infection in ticks (Fig. 2). PCR positive ticks were found between April and September (no surveys were performed in July of any year of the study period). The prevalence of infected ticks had a spring-summer peak reaching nearly 21% in May before decreasing in late summer. Prevalence peaked again in autumn (September) at more than 30%. There was a significant increase in the proportion of positive pools with *A.*

phagocytophilum (OR = 3.78; 95% CI, 1.79–8.06) identified in the autumn compared to the early summer (Table 3). There was no evidence of an annual trend in detecting *A. phagocytophilum* during the study period (Table 3; variable Year, OR = 1.01; $p > 0.05$).

Among 366 tick pools tested for *A. phagocytophilum*, 190 pools (99 of *I. ricinus* and 91 of *D. reticulatus*) were additionally tested for presence of *B. burgdorferi*; 162 of them (79 of *I. ricinus* and 83 of *D. reticulatus*) were also tested for presence of *B. burgdorferi* and TBEV (Table 2).

The prevalence of *B. burgdorferi* in all pools was 30.5%; with 31.9% in *D. reticulatus* and 29.3% in *I. ricinus*. In

TABLE 2. NUMBER OF TICKS POSITIVE FOR *ANAPLASMA PHAGOCYTOPHILUM*, *BORRELIA BURGDORFERI*, TICK-BORNE ENCEPHALITIS VIRUS AND COINFECTIONS BY DIFFERENT RISK FACTORS

Variable	Value	<i>Anaplasma phagocytophilum</i> (n = 366)		<i>Borrelia burgdorferi</i> (n = 190)		Tick-borne encephalitis virus (n = 162)	
		POS	NEG	POS	NEG	POS	NEG
Tick Species	<i>Dermacentor reticulatus</i>	28	148	29	62	12	71
	<i>Ixodes ricinus</i>	52	138	29	70	5	74
Eco-zone	Forest	48	124	27	63	12	67
	Forest steppe	28	118	29	51	4	68
	Carpathians	4	44	2	18	1	10
Area	Rural	66	280	53	122	17	130
	Urban	14	6	5	10	0	15
Collection Month	Apr	3	22	4	15	1	18
	May	27	102	9	18	0	27
	Jun	28	112	25	76	15	66
	Aug	1	11	6	2	-	-
	Sep	21	39	14	21	1	34
Collection Year	2009	1	7	3	3	0	6
	2010	14	71	3	46	7	42
	2011	2	22	2	1	0	3
	2012	45	137	41	54	10	85
	2013	6	16	8	2	0	2
	2014	12	33	1	26	0	7
A results ^a	POS	—	—	25	42	—	—
	NEG	—	—	33	90	—	—
A or B results ^b	POS	—	—	—	—	6	81
	NEG	—	—	—	—	11	64
Subtotals		80	286	58	132	17	145

POS, positive test result; NEG, negative test result.

^a*Anaplasma phagocytophilum* (A) presence.

^b*Anaplasma phagocytophilum* (A) or *Borrelia burgdorferi* (B) presence.

TABLE 3. RESULTS FROM THE MULTIPLE LOGISTIC REGRESSIONS FOR THREE PATHOGENS

Parameter Estimates	Value	<i>Anaplasma phagocytophilum</i>			<i>Borrelia burgdorferi</i>			<i>Tick-borne encephalitis virus</i>		
		OR	LL	UL	OR	LL	UL	OR	LL	UL
Intercept		0.07*	0.02	0.2	0.3*	0.1	0.82	0.07*	0.01	0.37
Tick Species	<i>Derma-centor reticulatus</i>	Ref	—	—	Ref	—	—	Ref	—	—
	<i>Ixodes ricinus</i>	2.69*	1.52	4.94	0.82	0.38	1.74	1.01	0.24	4.22
Eco-zone	Forest	1.89*	1.07	3.36	0.55	0.26	1.15	4.29*	1.2	18.98
	Forest steppe	Ref	—	—	Ref	—	—	Ref	—	—
	Carpathians	Ref	—	—	Ref	—	—	Ref	—	—
Area	Rural	Ref	—	—	Ref	—	—	Ref	—	—
	Urban ^a	8.26*	2.93	24.82	1.84	0.4	8.26	—	—	—
Month	Apr-May	0.92	0.48	1.77	0.81	0.29	2.17	0.12*	0.01	0.81
	Jun	Ref	—	—	Ref	—	—	Ref	—	—
	Aug-Sep	3.78*	1.79	8.06	1.96	0.83	4.57	0.10*	0.01	0.66
A result ^b	NEG	—	—	—	Ref	—	—	—	—	—
	POS	—	—	—	1.66	0.8	3.45	—	—	—
A or B results ^c	NEG	—	—	—	—	—	—	Ref	—	—
	POS	—	—	—	—	—	—	0.46	0.12	1.62
Year (continuous)		1.01	0.8	1.26	1.12	0.84	1.51	1.22	0.7	2.13

Year variable has been recoded from 2009 to 2014 into 1–6.

*Indicates a significant effect on 95% Confidence Level.

^aArea type is not included in the regression for TBEV to avoid problems with numerical estimation.

^b*Anaplasma phagocytophilum* (A) presence.

^cA, *phagocytophilum* (A) or *Borrelia burgdorferi* (B) presence.

OR, odds ratio; 95% CI, 95% confidence interval.

LL, lower limit of the confidence interval.

UL, upper limit of the confidence interval.

contrast to *A. phagocytophilum*, *B. burgdorferi* positive pools were not associated with tick species, eco-zone, or area.

The prevalence of TBEV in total was found to be 10.5%, with 6.3% in *I. ricinus* and 14.5% in *D. reticulatus*. TBEV infection patterns differed from *A. phagocytophilum* and *B. burgdorferi* (Table 3). As with *A. phagocytophilum*, there were significant effects of eco-zones with forest habitats significantly more likely to yield positive pools than forest-steppe zones (OR=4.29; 95% CI, 1.20–18.98). However, both *I. ricinus* and *D. reticulatus* were comparable in yielding positive pools (OR=1.01; 95% CI, 0.24–4.22). TBEV also showed a seasonal variation in prevalence. However, in contrast to *A. phagocytophilum* and *B. burgdorferi*, the proportion of positive pools was highest in the summer (June) and lowest in the spring (OR=0.12; 95% CI, 0.01–0.81) or the autumn (OR=0.10; 95% CI, 0.01–0.66). As with the previous pathogens, the prevalence of infection did not differ significantly across years of the study.

When analyzing frequencies of the three pathogens and their combination, the most frequently observed pathogen or pathogen combination was *A. phagocytophilum* (A), followed by *B. burgdorferi* (B) and the combination of these two pathogens (A+B) (Fig. 3). Thirty-five samples (21.6%) had only *A. phagocytophilum* (A), 26 samples (16%) were only positive for *B. burgdorferi* (B), and 20 samples (12.3%) had both *A. phagocytophilum* and *B. burgdorferi* (A+B). TBEV was found in 11 samples (6.8%). The smallest observed prevalence belonged to *A. phagocytophilum* and *B. burgdorferi* pathogen combinations with TBEV (A+E, B+E, or A+B+E) with about 1% each.

After adjusting for multiple comparisons, we concluded that tick samples that were positive exclusively for TBEV were significantly less common than for *A. phagocytophilum* or *B. burgdorferi* (p value 0.001 and 0.04, respectively). In addition, the pathogen combination of *A. phagocytophilum* and *B. burgdorferi* occurred significantly more often than any

other pathogen combination (p value 0.03). Despite the difference in infection prevalence, the tick pools with multiple pathogens occurred randomly. None of the combinations had odds ratios that differed significantly from 1.0 (Table 3 and Fig. 3).

Discussion

Our study is the first to explore the potential relationship between the ecosystems, vectors, and the presence of HGA and other tick-borne infections in Western Ukraine. The prevalence of *Anaplasma* in *I. ricinus* and *D. reticulatus* is an important indicator of epidemicity of natural foci provided by immediate evidence of the ticks' roles in development and persistence of natural foci of HGA, rather than relying on serological evidence from vertebrate species.

The presence of the HGA pathogen in ticks (21.9% of all pools) showed that the agent is common in Western Ukraine. Our results are similar to the data obtained in the active natural foci of HGA in many European countries, including those adjoining Ukraine: Poland, Belarus, and Russia, but with rates higher than in Hungary (Grzeszczuk and Stańczak 2006, Rigó et al. 2011, Nordberg 2012, Víchová et al. 2014). The absence of any annual trends in infection prevalence during the 6 years study (Tables 2 and 3) suggests that the infection is endemic and is not dramatically increasing.

Based on our data (Tables 1–3), there are at least two vector species maintaining *A. phagocytophilum*. Although there are eco-zone and seasonal differences in infection prevalence, *Anaplasma* was consistently more common in *I. ricinus* (OR=2.69; 95% CI, 1.52–4.94) compared with *D. reticulatus*. As reported in other Eurasian countries (Belarus, Lithuania, Serbia, and Russian Asia) (Nordberg, 2012, Stuen et al. 2013), among the 10 species of Ixodid ticks identified in the Western region of Ukraine, these two species are the most abundant in the region. Consequently, during our

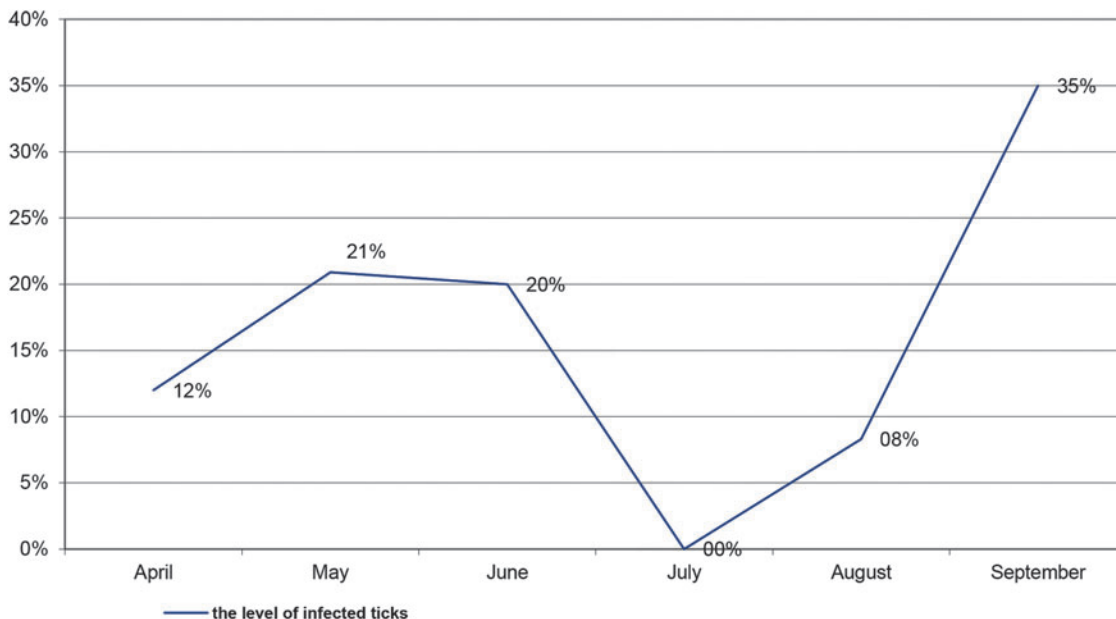


FIG. 2. Seasonal distribution of infected with *A. phagocytophilum* ticks by months, % of positive pools in PCR, in 2009–2014 in Lviv and Volyn Oblast.

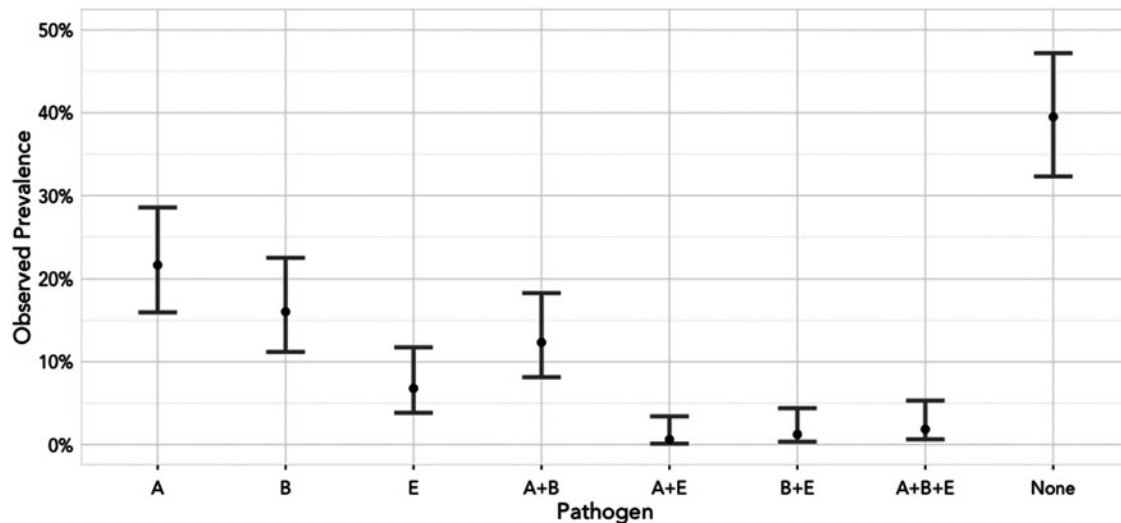


FIG. 3. Observed pathogen and pathogen combination prevalence with 95% Confidence Interval for samples that were tested for all three pathogens. A, *A. phagocytophilum*; B, *B. burgdorferi*; E—TBEV, None—none of the three pathogens was detected in the sample.

5-year study period, *I. ricinus* was the main vector of *Anaplasma*, while *D. reticulatus* served as a secondary vector (Stuen et al. 2013). *I. ricinus* is mainly found in forest areas and caught during April–October. *D. reticulatus* is more often found in forest-steppe and Carpathian areas; its activity starts in late March and may last until November. The involvement of two vector species in the transmission of *Anaplasma* increased the duration of the risk season (up to 8–9 months per year), as well as the geographic range of the risk area, thus, increasing the risk of human infection.

To determine the epidemiological significance of coinfection in Ixodid ticks, we simultaneously tested the presence of additional pathogens: LD and TBE. While 12.3% of pools were positive for both *A. phagocytophilum* and *B. burgdorferi*, the co-occurrence of *A. phagocytophilum* and/or *B. burgdorferi* with TBEV was much less common, at about 1% each. The data are consistent with observations reported in other countries (Nordberg 2012, Asman and Nowak 2013). Observations of coinfection in *I. ricinus* with the pathogens of two (HGA-LD) agents in Europe have been described since 2000 in Italy (Aureli et al. 2012) and were equal to 0.9% in Germany (Tappe et al. 2014), 1.6% in the Netherlands (Wielinga et al. 2006), 5% in Slovakia (Derdakova et al. 2003), from 0.93% (Wójcik-Fatla et al. 2009) to 8.3% (Stańczak et al. 2004) in Poland, and 9.3% in Bulgaria (Nader et al. 2018). Infection of ticks with three pathogens occurs much less frequently—0.3% of sampled *I. persulcatus* in Russia (Swanson et al. 2006).

The presence of two or three pathogens in the same vector allows simultaneous infection and development of mixed infection in individual humans. In future studies, patterns of coinfection should examine individual ticks rather than pools for the presence of the three causative agents (HGA, LD, and TBE). This would better clarify if individuals were carrying multiple human pathogens compared to identifying sites where multiple pathogens are circulating in close proximity of space and time (pools were grouped by site and date).

In general, it would be beneficial for future studies to standardize tick collection technique among participating

institutions, namely, the approach to selection of study sites and sample number and, if using pools, the number of ticks in a pool. While we tested only ticks that were collected by flagging, examination of ticks collected from humans would provide an important insight about the level of risk for human infection. In addition, it is also important to compare infection rate in ticks and rodents in future studies to assess the role of mammal host species in maintaining endemicity.

Understanding the spatial and temporal ecology of the tick-borne pathogens, including ticks and natural hosts (wild vertebrate), and the risk and vulnerability of human population (density, proximity, behavior, etc.) with respect to environmental factors (e.g., season, chorology, altitude, and so on) appears essential for the control and prevention of tick-borne diseases. In addition, efficient survey and control of HGA in Ukraine will encompass eco-epidemiology of other tick-borne pathogens, including parasitic or viral diseases, such as Babesiosis and TBE.

Conclusions

Active foci of HGA were identified in the Western region of Ukraine. Of the two main tick species circulating in Western Ukraine, the predominantly observed vector of *A. phagocytophilum* in Lviv and Volyn oblasts is wood tick *I. ricinus* with the average prevalence of 27.4%. The secondary vector is the marsh tick *D. reticulatus*, which had average infection rates of 15.9%. Based on tests of these samples, HGA was the second most common infection after LD. The recognition of HGA in Western Ukraine is the key rationale for the public health system to develop protocols for differential diagnosis and targeted preventive measures against tick-borne infections. Such high prevalence of *A. phagocytophilum* strengthens the choice of doxycycline as agent of choice for LB treatment since active also against HGA.

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Author Disclosure Statement

No competing financial interests exist.

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