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SHORT COMMUNICATIONS

Urban Breeding Corvids as Disseminators of Ticks and Emerging Tick-Borne Pathogens

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Abstract

Crows (Corvidae) are common city dwellers worldwide and are increasingly important subjects of epidemiology studies. Although their importance as hosts and transmitters of a number of zoonotic parasites and pathogens is well known, there are no studies on their importance as tick hosts. After mosquitoes, ticks are the most important vectors of zoonotic pathogens, especially for those causing emerging zoonotic diseases. Pathogenic bacteria, especially *Borrelia* spp., *Rickettsia* spp., and *Anaplasma* spp., vectored by ticks, are the cause for most vector-borne diseases in Europe. Here we report on ticks and tick-borne pathogens harbored by urban breeding crows. A total of 36 birds (33.33%, $n = 108$) hosted ticks, with 91 individual ticks belonging to 6 species (*Haemaphysalis concinna*, *Haemaphysalis parva*, *Haemaphysalis punctata*, *Hyalomma marginatum*, *Ixodes arboricola*, and *Ixodes ricinus*). *Rickettsia* spp. DNA was found in 6.6% of ticks and 1.9% of bird tissues, whereas *Anaplasma phagocytophilum* was found in 5.9% of ticks and 0.9% of birds. Two rickettsial genospecies were located, *Rickettsia helvetica* and *Rickettsia monacensis*. This is the first study to determine such a diverse tick spectrum feeding on urban corvids, while highlighting their importance as tick hosts and raising concerns about their potential risk to human health.

Keywords: *Anaplasma*, Corvidae, *Rickettsia*, tick, urban

Many crow species (Corvidae) are commonly found in urban areas worldwide, with large populations successfully adapted to live year long even in highly urbanized areas. Most species are omnivorous, extensively using human refuse, and establish large populations within city environments. This is especially the case of rooks (*Corvus frugilegus*) and jackdaws (*Corvus monedula*), both being colonial residents of urban areas in Europe for a long time (Cramp and Perrins 1994). As crows are highly mobile, they may also be important for parasite transfer between rural/natural and urbanized areas. Being social passerines of fairly large size, which feed mainly on the ground, they are well-known hosts for ectoparasites such as ticks (Ixodidae).

Ticks are among the most important vectors of zoonotic pathogens worldwide, especially for those causing emerging zoonotic diseases. Pathogenic bacteria, especially *Borrelia* spp., *Rickettsia* spp., and *Anaplasma* spp., vectored by ticks, are the cause for most vector-borne diseases in Europe (Vorou et al. 2007). Although crows are breeding in most urban centers of the world, up to our knowledge, there is no study reporting on the importance of urban populations of crows as hosts for ticks and the zoonotic pathogens vectored by these ectoparasites. To learn more about these aspects, 108 culled corvids (collected with guns by professional hunters on June 13, 2013, Table 1) collected in Sebeş, Central Romania (45.948229N; 23.562736E), were assessed for the presence of ticks and tick-borne pathogens. The birds were adults and recently fledged young, breeding in the city center, nesting and roosting in trees of an urban park.

The corpses were individually checked for parasites, and tissue samples (heart) were collected from the birds. Ticks were identified using morphological keys (Feider 1965). DNA from ticks and tissue samples was analyzed using PCR to amplify a 360-basepair fragment (for *Rickettsia* spp.) or a 345-basepair fragment (for *Anaplasma phagocytophilum*) of msp2 gene using specific primers (Noaman and Shayan 2009, Mărcuţan et al. 2016). Positive and negative controls were included to assess the specificity of the reaction and the possible presence of contaminants. Positive samples were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced externally (Macrogen Europe, Amsterdam, The Netherlands). Nucleotide sequences were compared with reference sequences available in GenBank, using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

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Table 1. The Number, Species, and Development Stage of Ticks Collected from Corvids, with Pathogens Identified in Ticks

Host	*Tick species*	*Male*	*Female*	*Nymph*	*Larvae*	Rickettsia *spp.*	Anaplasma phagocytophilum
Corvus frugilegus (*n*=57)	*Haemaphysalis concinna*				9	1 L	1 L
	Haemaphysalis parva			1	10		
	Haemaphysalis punctata	1	3	3	18	2 L	
	Ixodes arboricola				1		
	Ixodes ricinus				23	2 L	4 L
Corvus monedula (*n*=51)	*Hae. parva*				1		
	Hae. punctata	1		2	12	1 N	
	Hyalomma marginatum			1			
	I. arboricola				1		
	I. ricinus			2	2		
Total		2	3	9	77	6	5

A total of 36 birds (33.33%) hosted ticks, with 91 individual ticks belonging to 6 species (*Haemaphysalis concinna*, *Haemaphysalis parva*, *Haemaphysalis punctata*, *Hyalomma marginatum*, *Ixodes arboricola*, and *Ixodes ricinus*) being collected (Table 1). Each corvid species hosted five tick species, with no significant differences detected between the individual prevalences.

Six ticks (6.6%) were positive for *Rickettsia* spp. and the sequence analysis showed 100% similarity (one sample) with *Rickettsia helvetica* (Accession No. KJ577821.1) and 99% similarity (five samples) with *Rickettsia monacensis* (Accession No. KU728666.1). Positive ticks were collected from both host species and were belonging to *Hae. concinna* (*n*=1, prevalence 11.1%), *Hae. punctata* (*n*=3, 10%), and *I. ricinus* (*n*=2, 7.4%, Table 1). In addition, a total of five individual ticks belonging to two tick species (*Hae. concinna n*=1, 11.1%; *I. ricinus n*=4, 14.9%) tested positive for *A. phagocytophilum* (collected from one rook). The analysis of tissue samples (heart, *n*=108) resulted in two jackdaws (3.9%) testing positive for *R. monacensis* and one rook being positive for *A. phagocytophilum* DNA (1.7%). All *R. monacensis* sequences amplified were identical to each other.

All recorded pathogens are known agents causing clinical cases, which show an emerging pattern all over Europe. Although there is no record of human clinical cases because of either of these pathogens in Romania, recently in Bucharest several patients were interned with symptoms of SENLAT syndrome caused by other *Rickettsia* spp. because of tick bites (Zaharia et al. 2016).

Crows were listed as important in the maintenance and dispersion of a number of viral, bacterial, and parasitic diseases; however, they are rarely treated as important hosts of ticks and/or tick-borne diseases (Delgado-V and French 2012). The high prevalence and tick diversity found on urban corvids are most likely caused by the host species' extensive foraging forays and colonial nesting habits (Cramp and Perrins 1994). Although even synanthropic birds regularly host ticks (Hornok et al. 2013) and urban parks may harbor large number of ticks and pathogens vectored by these (Corrain et al. 2012), this is the first study to report such a high parasite diversity. All but one (*I. arboricola*) detected species are listed among human parasites, with four (*Hae. concinna*, *Hae. punctata*, *Hy. marginatum*, and *I. ricinus*) known to regularly occur in most development stages on humans (Estrada-Peña and Jongejan 1999).

The diverse range of ticks and the high prevalence of their occurrence in urban breeding corvids highlight the importance of these bird populations as tick hosts and raise concern about their potential risk to human health. Most ticks encountered were of subadult stages (94.5%) and belong to questing species. Such ticks in their later stages of development (after engorgement and molting) may easily pass to people visiting public parks. Moreover, the presence of emerging zoonotic pathogens in these ticks may pose high risks to public health even in highly urbanized areas.

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