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ECOLOGY

The Role of Birds in the Maintenance of Tick-Borne Infections in the Tomsk Anthropurgic Foci

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Abstract—The role of birds in the focus of tick-borne infections was studied from 2006 to 2011. The frequency index of ticks carried by ground dwelling birds is about 49.7%. The index of their abundance is 3.8. The larvae of ticks have been found on birds in 43.8% of cases. Nymphs and adult ticks have been found in 39.9 and 16.3%, respectively. It was revealed that *Ixodex pavlovskyi* was transferred and dominated in the urban microfoci because of its ornithophily. The markers of infectious agents have been recorded in 42 of 60 bird species under study.

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INTRODUCTION

The role played by birds in the circulation of tick-borne encephalitis within the Tomsk anthropurgic focus has been studied since the 1950s (Fedorov, 1958; Moskvitin, 1969; et al.). In recent decades, the number of tick-borne pathogens has increased due to advances in methodological approaches. New infectious agents have been found in areas where they were previously absent. In particular, the West Nile virus (WNV) was found in wild birds (Ternovoi et al., 2004; Kononova et al., 2006) and humans in Novosibirsk region. This finding allowed us to suggest that there is a foci here supported by birds and mosquitoes. In 2006, WNV was also recorded in Tomsk region (Moskvitina et al., 2008). It was evidence that a new foci of WNV infection with participation ixodid ticks and vertebrates are emerged in West Siberia. Previously, such structure of the foci with ticks as a component was proved experimentally (Azarova and Mishaeva, 2002). Subsequently, the existence of this foci were confirmed by cases of sickness, including those related to tick bites reported from the outskirts of Tomsk (Il'inskikh et al., 2009).

The aim of this work is to estimate the role of birds in the foci of tick-borne infections with various degrees of anthropogenic transformation.

MATERIALS AND METHODS

The material was collected during 2006–2011 in the biotopes of Tomsk: University grove, Southern cemetery, dendropark in the botanical garden of Tomsk State University, as well as the northern (TNKhK) and southern (Kolarovo) outskirts. The biotopes were described by us in a previous study (Moskvitina et al., 2008). Additional surveys were carried out at a considerable distance from Tomsk (Khaldeevo, 56°37′56″ N, 85°34′17″ E, and the Maloyuksinskii Nature Reserve, 57°11′54″ N, 85°30′14″ E). The material was collected every year from April to August. The birds were caught by mist nets. Some of them were hunted with special permission. The examination of birds and the sampling of ectoparasites were performed by the standard methods (Dubinina, 1971). The degree of tick infestation of birds was expressed in the indices of frequency and abundance (Zhmaeva et al., 1964). The living ticks were identified to species using a light microscope. Before having been analyzed for pathogen infection, ticks from each bird were either kept alive in separate tubes in a refrigerator at a temperature of 4°C or frozen. In order to find the markers of infectious agents, brain, liver, and spleen samples were taken from the caught birds. The samples were frozen in liquid nitrogen. The analysis was carried out at the State Research Center of Virology and Biotechnology "Vector."

In all, in 2006–2011, 736 bird specimens were examined. They had 804 ticks (larvae, nymphs, and imago) belonging to the genus *Ixodes* Latr., 1795. To analyze the infection with pathogens, the organs of 443 birds from 60 species and 9 orders (passerines, anseriformes, charadriiformes, columbiformes, galliformes,

Table 1. Infection of birds with *Ixodes persulcatus* and *I. pavlovskyi*

Species*	Number of caught birds/those carrying ticks/abundance index	*I. persulcatus*				*I. pavlovskyi*			
		total number of ticks	Abundance index			total number of ticks	Abundance index		
			larvae	nymphs	imagoes		larvae	nymphs	imagoes
Anthus trivialis (L.)	4/4/13.25	34	4.5	4	0	19	3.75	1	0
Turdus pilaris L.	91/57/5.73	146	0.68	0.85	0.08	364	1.55	1.4	1.05
T. iliacus L.	9/8/3.33	5	0	0.56	0	25	0.89	1.11	0.78
Corvus cornix L.	5/2/2.2	8	0	0	1.6	3	0	0	0.6
Luscinia calliope (Pall.)	9/6/1.55	9	0.33	0.67	0	4	0.33	0.11	0
L. luscinia L.	2/1/1.5	1	0	0.5	0	2	1	0	0
Passer montanus (L.)	3/3/1.33	1	0	0.33	0	3	0.67	0.33	0
Phoenicurus phoenicurus (L.)	49/13/1.3	10	0.14	0.06	0	55	0.69	0.43	0
Fringilla coelebs L.	46/16/1.06	14	0.2	0.11	0	30	0.37	0.28	0
F. montifringilla L.	11/3/0.73	5	0.18	0.27	0	1	0	0.09	0
Pica pica (L.)	2/1/0.5	0	0	0	0	1	0	0	0.5
Sitta europaea L.	6/3/0.5	1	0	0.17	0	3	0.17	0.17	0.17
Carpodacus erythrinus (Pall.)	7/2/0.28	1	0	0.14	0	1	0.14	0	0
Acrocephalus dumetorum Blyth	64/12/0.28	5	0.06	0.02	0	9	0.06	0.06	0.02
Chloris chloris (L.)	6/1/0.16	0	0	0	0	1	0.17	0	0
Parus major L.	121/10/0.1	2	0.02	0	0	8	0.02	0.05	0
Sylvia curruca L.	21/1/0.06	1	0	0.05	0	0	0	0	0
Ficedula hypoleuca (Pall.)	111/4/0.05	1	0	0.01	0	2	0.02	0	0

* List of species is given according to the decrease in the total abundance of ticks being fed.

piciformes, apodiformes, cuculiformes, and falconiformes) were studied, as well as 378 larvae, nymphs, and adult ticks collected from them. All the samples were analyzed for tick-borne encephalitis virus (TBEV) and WNV using the methods of reverse transcriptase-polymerase chain reaction (RT-PCR) and immune-enzyme analysis (IEA). What is more, in the years 2009–2011, the samplings were studied for bacterial (*Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Bartonella* spp.) and protozoan (*Babesia* spp.) infections. Similar methods for such kind of analysis were described by us earlier (Chausov et al., 2010).

RESULTS

Ticks from the genus *Ixodes* were found on 18 bird species (Table 1), even though the list of their hosts in Western Siberia includes 97 species (Fedorov, 1973; *Taezhnyi kleshch* ..., 1985). Generally, any bird may be attacked by adult, nymphal, and larval ticks if it stays on the ground for a short period of time and makes contact with herbaceous vegetation.

The first meeting ticks of all development stages were recorded on birds at the beginning of May, shortly after the adult ticks were found during flagging. At this moment, the majority of migratory birds inhabiting forests continue their migrations and, thus, transmit ticks. The maximum tick infestation of birds was observed in June–July. It coincided with or lagged behind the activity peak of adult ticks, which were collected by flagging. The last meeting ticks were found on birds in the middle of August. In July–August, the ticks were recorded on thrushes (fieldfare *Turdus pilaris* L. and redwing *T. iliacus* L.) during their post-nesting migrations (Moskvitin, 1974), which is evidence that they may be distributed along the territory in this period as well.

Let us consider possible transmission of the ticks by birds in an isolated park (University grove), where ixodid ticks were collected by flagging from 1996 until 2011. At the beginning of this period, the abundance of ticks fluctuated from 0.1 to 1.2 specimens/km over six years. Then, they were found neither by flagging nor on rodents inhabiting this area in the course of four years. In 2006, their imago appeared again. Since 2008, their larvae and nymphs were found on small-sized mammals (Moskvitina et al., 2013), while birds carried ticks of all developmental stages. Now, the total abundance of imaginal ticks has increased from 0.2 to 4.5 specimens/km.

Among different stages of ticks carried by birds, larvae (43.8%) and nymphs (39.9%) were found most frequently; imago were registered in 16.3% of birds. In 46.2%, we found various combinations of ticks at different development stages on one and the same bird. The highest number of ticks is carried by *T. pilaris* L. (Fig. 1); up to 70 specimens were found on one and the same bird (among them, there were 18 adults). Small-sized birds are hosts of mainly nymphs and larvae. Adults ticks were not commonly found on them. Birds having larger sizes (hooded crow *Corvus cornix* L. and magpie *Pica pica* (L.)) carried only imaginal ticks. Obviously, the highest role in the transmission of ticks is played by birds inhabiting the ground layer, which nest and/or often feed on the earth surface (thrushes, galliformes, corvids, pipits, finches). The frequency index (I_F) of ticks in this group (n = 185) was about 49.70 ± 3.68%, and the abundance index (I_A) equaled 3.80 ± 0.66. Tick infestation was significantly lower in birds inhabiting the ground and bushy layer (n = 281, I_F = 16.70 ± 2.23%, I_A = 0.30 ± 0.06). Those living within the tree and bushy layer carried even fewer ticks (n =229, I_F = 3.50 ± 1.21%, I_A = 0.05 ± 0.05).

Fig. 1. Fieldfare *Turdus pilaris* L. is the main feeder of ixodid ticks (photo by S.I. Gashkov).

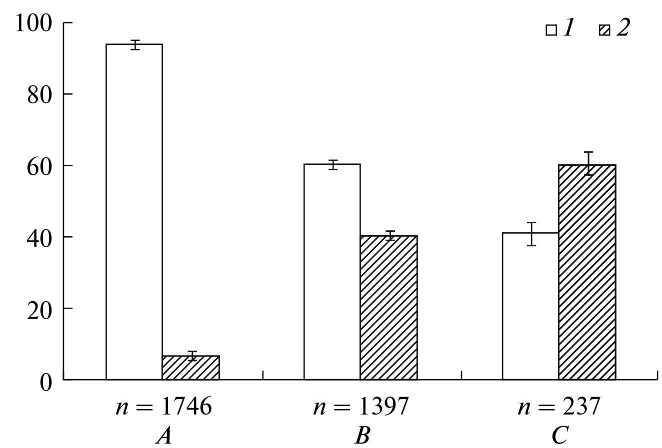

Fig. 2. Ratio of *Ixodes persulcatus* (*1*) and *I. pavlovskyi* (*2*) taken from the flag (*A*), small-sized mammals (*B*), and birds (*C*) in suburban areas.

Five species of ixodid ticks were found in the foci under study. Among them, there were dominant species dangerous to humans—*I. persulcatus* P. Sch., 1930 and *I. pavlovskyi* Pom., 1946 (Romanenko and Kondrat'eva, 2011). Both species parasitized birds. *I. persulcatus* dominated in the areas located at a distance from the city, while *I. pavlovskyi* was common in urban areas. The same ratio was recorded during flagging. In the outskirts inhabited by two species of ticks, *I. pavlovskyi* was found on birds more often than on mammals or during flagging (Fig. 2).

I. pavlovskyi dominated on birds, compared to *I. persulcatus*, at all development stages. These differences are significant for imago and larvae ($P < 0.05$). Probably, the adults of *I. pavlovskyi* were more frequently found due to the behavioral features of this tick, which does not climb very high and prefers to attack its prey out of the low grass or even from the litter (Bolotin et al., 1977; Romanenko, 2007; Romanenko and Kondrat'eva, 2011). The ornithophily of *I. pavlovskyi* was also registered earlier (Ushakova et al., 1969). In 50% of cases, both species of ticks in their common habitats used the same bird species as hosts (n = 86).

In all, 42 (70%) of 60 bird species under study had markers of 1 to 8 infectious agents: TBEV, WNV, *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Bartonella* spp. and *Babesia* spp. Basically, the group of infectious agents includes forest and semi-aquatic or aquatic bird species (anseriformes, sandpipers, the sand martin *Riparia riparia* L., the synanthropic rock dove *Columba livia* Gm., and the tree sparrow *Passer*

Table 2. Infections (%) of birds and ticks parasitizing them

Focus component	TBEV	WNV	Borreliae	Rickettsiae	Babasiae	Bartonellae	Ehrlichiae	Anaplasmae
Birds	42.4 ± 2.8 (n = 316)	32.9 ± 2.6 (n = 316)	12.2 ± 2.3 (n = 197)	18.8 ± 2.8 (n = 197)	12.7 ± 3.3 (n = 102)	15.7 ± 3.6 (n = 102)	6.9 ± 2.5 (n = 102)	13.7 ± 3.4 (n = 102)
Ixodes persulcatus	9.8 ± 3.9 (n = 59)	1.7 ± 1.7 (n = 59)	17 ± 5.9 (n = 41)	4.9 ± 3.4 (n = 41)	8 ± 5.4 (n = 25)	4 ± 3.9 (n = 25)	4 ± 3.9 (n = 25)	8 ± 5.4 (n = 25)
I. pavlovskyi	4.7 ± 1.2 (n = 319)	2.8 ± 0.9 (n = 319)	9 ± 1.8 (n = 245)	1.2 ± 0.7 (n = 245)	2.1 ± 1.2 (n = 144)	3.5 ± 1.5 (n = 144)	2.1 ± 1.2 (n = 144)	6.9 ± 2.1 (n = 144)

TBEV is tick-borne encephalitis; WNV is West Nile virus; n is number of specimens under study.

montanus (L)). The latter were caught in the parks of Tomsk, where it is also very likely to be attacked by ticks.

In each urban and suburban area, the entire spectrum of pathogens (eight) was registered in birds. Six infectious agents were found at a distance from the city, but we do not exclude that this is related to the lower number of birds that were caught and analyzed. The share of birds infected with pathogens was higher in the city than in its outskirts. For example, the total TBEV and WNV infection rate in the city (52.8 ± 5.3 and 42.7 ± 2.2%, respectively), which was determined by the methods of IEA and PCR, was significantly different ($P < 0.05$) from that observed in the outskirts (36.7 ± 3.5 and 28.7 ± 1.9) and, if we consider the WNV infection rate, from that in remote areas (30.8 ± 2.8). A similar trend was observed in *Borrelia* spp., *Rickettsia* spp., *Babesia* spp. and *Anaplasma* spp., which allows to suggest that they pose a higher potential threat to the urban foci.

Mixed infections were registered in more than 50% of all birds (67.6% of cases). The majority of birds carrying mixed infections (87.2%) had 2–3 pathogens. The maximum number of the pathogen markers carried by one specimen was found in *T. pilaris* L. (six) and the common redstart *Phoenicurus phoenicurus* (L.) (seven).

The ticks collected from birds were less infected with viral diseases than the birds themselves (Table 2). A similar situation was observed in bacteria and protozoa. The only exception was that *I. persulcatus* was highly infected with *Borrelia* spp. The infection rate of *I. persulcatus* and *I. pavlovskyi* sampled from birds is almost the same based on all infectious agents. Both tick species were more infected with TBEV than with WNV, which is also reflected in the infection rate of birds (Table 2).

DISCUSSION

Having studied the modern state of natural infections in the Tomsk anthropurgic focus, we managed to reveal some of its specific features that are most likely to pose a high epidemic threat. In particular, there is a considerable number of infectious agents characterized by high genetic diversity in the territory of Tomsk (Chausov et al., 2010; Mikryukova et al., 2013), as well as a high share of mixed infections and species diversity of ticks.

It was revealed that birds are tick prey and transmit infectious agents. Thus, their role in maintaining and changing the natural foci seems to be very important and manifested in a similar way within different areas. Firstly, in spring, birds migrate back from various geographical locations, which are different in their epidemic status. It is known that birds inhabiting Tomsk undergo winter migrations to Europe, Equatorial Africa, the Near East, the Caspian, Hindustan, and Japan (Moskvitin et al., 2010). Secondly, in spring, when small-sized mammals (Moskvitina, 1999) are not numerous, birds are the main prey of ticks. At the same time, ticks may spread as birds keep on migrating, until the beginning of nesting. Thus, infections may transmit from one area to another. Thirdly, since birds are very active during their nesting and post-nesting periods, they, along with mammals, carry a lot of ticks at all stages. It should be emphasized that birds, which are mainly active in the daylight, widen the daily spectrum of tick feeding, in which small-sized mammals occupy another period of time: as a rule, they are active in twilight, at night, and in early morning (Kravchenko, 1999). Finally, during the post-nesting period and migrations, as well as in spring, birds transmit ticks carrying pathogens considerable distances from their actual nesting sites. Generally, being highly mobile, birds favor connection of isolated areas, as well as development and transformation of components in the tick-borne infection foci (Chunikhin, 1969; Alekseev et al., 2001).

With regard to the obtained data, special attention should be paid to the role played by *I. pavlovskyi*, recently detected in the acarofauna of Tomsk and its outskitrs, in the focus and its relationship with birds. This species is characterized by a disjunctive habitat: it is found in Primorskii krai, Altai, and Gornaya Shoria. It is dominant in some of these areas and even outnumbers *I. persulcatus* (Filippova and Ushakova, 1967; Sapegina, 1969; Ushakova et al., 1969). *I. pavlovskyi* was first recorded in Tomsk and its outskirts in the early 19th century (Romanenko and Chekalkina, 2004). In the Novosibirsk Research Center, where a well-studied natural TBE focus of high epidemic threat is found in the park zone (*Infektsii* ..., 2011), this species has recently made up 60.5% of the ixodid population (Chicherina et al., 2011).

A large number of researchers consider only the epidemiological role played by *I. persulcatus* in the Tomsk focus (Nikitin et al., 2008; Chikanova et al., 2011). The finding of *I. pavlovskyi*, which had been reported by Romanenko and Chekalkina (2004), was not noticed. Now, researchers from Novosibirsk have made an attempt to estimate the role of this species (Livanova et al., 2011; etc.). In the work of Korenberg et al. (2010), it was shown that *I. pavlovskyi* in the environs of Tomsk can maintain the natural foci of borreliosis by itself when *I. persulcatus* is either low in number or absent. According to our data, *I. persulcatus* and *I. pavlovskyi* (imago taken from vegetation) are equally infected with TBEV (7.9 ± 0.6 and 9.3 ± 0.8%, respectively) and WNV (9 ± 0.7 and 9.6 ± 0.9). TBEV infection in Tomsk is two times higher than in Novosibirsk region. Both tick species are also equally infected with *Borrelia* spp. At the same time, four infectious agents (*Anaplasma* spp., *Babesia* spp., *Bartonella* spp. and *Ehrlichia* spp.) were not found in *I. pavlovskyi* collected by flagging. Rickettsiae were more common in *I. persulcatus*. It is likely to be related to a longer coexistence of components in the natural foci, the key element of which was *I. persulcatus*. Nevertheless, *I. pavlovskyi* taken from birds had all eight pathogens. The rate of these infections was the same as in *I. persulcatus*. Therefore, the role played by *I. pavlovskyi* is very significant, especially in the foci located within anthropogenically affected territories.

It is widely accepted that WNV is transmitted by mosquitoes and hosted by aquatic birds and corvids, which take part in the formation of natural foci of this disease (L'vov, 2000). Ticks, as a rule, play a secondary role in this process. However, it has been found in recent years that ticks taken from corvids in the WNV endemic areas were more significantly infected than mosquitoes (L'vov et al., 2009). It follows from the data obtained by us that ixodid ticks are involved in WNV circulation in the taiga zone of Western Siberia (Moskvitina et al., 2008) and are fed by the majority of birds that contact them. The evidence that birds are infected with WNV by transmission through ticks is the finding of the WNV pathogen in nonmigratory birds (great tit *Parus major* L., *P. pica*, etc.) and young specimens of migratory birds during their nesting period before they fly out. Along with this, the infections can be annually transferred into Tomsk region from the southern areas, where birds stay in winter, and migration pathways, which is proved by WNV in migratory semi-aquatic species—peewit *Vanellus vanellus* (L.), shore flies.

The ornithophily of *I. pavlovskyi*, which was mentioned above, is of special interest, because it may be the reason for changes in the components of foci, such as composition of carriers and causative agents, which can be brought by birds from other territories. For example, this is related to the described dynamics of ticks in one of the isolated areas (University grove), where the abundance of ticks significantly increased and their ratio shifted towards the dominance of *I. pavlovskyi*. Therefore, transmission of ticks at different development stages by birds is the most likely reason for the formation of microfoci in the city, which are maintained by both birds and small-sized mammals (Moskvitina et al., 2013).

Although *I. pavlovskyi* does not commonly attack humans due to its behavioral features (Romanenko and Kondrat'eva, 2011), it becomes the main carrier of infectious agents in the areas along recreational sites, where its concentrations are large. Thus, the role of *I. pavlovskyi* in the maintenance of the populations of infectious agents is comparable to that played by *I. persulcatus* and can even become more important as this species is confined to urban and suburban areas.

Mutual parasitism on birds of different tick species or ticks at different stages, which was registered by us in almost half of all cases, enables a transptial transmission of pathogens (Alekseev, 1993), the importance of which must increase as the species diversity of pathogens and carriers gets higher.

Another feature of urban foci is that they are characterized by a higher share of birds infected by the majority of infectious agents when compared to those in suburban and remote areas. In addition, birds carrying 6–7 pathogens were found only in the city. This shows that urban foci, the circulation of tick-borne infections in which is determined by birds, are potentially dangerous.

Consequently, birds can transmit at considerable distances both infectious agents and their carriers. They do not only connect urban, suburban, and natural remote localities, thus favoring changes in them, but are also reservoirs of tick-borne infections, which increases the infection risks for human population living in these territory of foci.

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