Detection of tick-borne pathogens in wild birds and their ticks in Western Siberia and high level of their mismatch

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Abstract: The Tomsk region located in the south of Western Siberia is one of the most high-risk areas for tick-borne diseases due to elevated incidence of tick-borne encephalitis and Lyme disease in humans. Wild birds may be considered as one of the reservoirs for tick-borne pathogens and hosts for infected ticks. A high mobility of wild birds leads to unpredictable possibilities for the dissemination of tick-borne pathogens into new geographical regions. The primary goal of this study was to evaluate the prevalence of tick-borne pathogens in wild birds and ticks that feed on them as well as to determine the role of different species of birds in maintaining the tick-borne infectious foci. We analysed the samples of 443 wild birds (60 species) and 378 ticks belonging to the genus *Ixodes* Latraille, 1795 collected from the wild birds, for detecting occurrence of eight tick-borne pathogens, the namely tick-borne encephalitis virus (TBEV), West Nile virus (WNV), and species of *Borrelia*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Bartonella* and *Babesia* Starcovicv, 1893, using RT-PCR/or PCR and enzyme immunoassay. One or more tick-borne infection markers were detected in 43 species of birds. All markers were detected in samples collected from fieldfare *Turdus pilaris* Linnaeus, Blyth’s reed warbler *Acrocephalus dumetorum* Blyth, common redstart *Phoenicurus phoenicurus* Lat., using RT-PCR/or PCR and enzyme immunoassay. One or more tick-borne infection markers were detected in the majority of cases (75.5%), there were mismatches of pathogens in birds and ticks collected from them. Wild birds and their ticks may play an extremely important role in the dissemination of tick-borne pathogens into different geographical regions.

Keywords: birds, *Ixodes*, WNV, TBEV, *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., *Bartonella* spp., *Ehrlichia* spp., *Babesia* spp.

The prevalence of vector-borne diseases in the world has remained a very relevant concern. Numerous studies have demonstrated an important role of wild birds in spreading parasites and pathogens. Wild birds also serve as reservoirs for viruses, bacteria and protists that are pathogenic for humans (Hubálek 2004, Tsiodras et al. 2008, Hasle 2013). The Tomsk region, where we conducted this study, is one of the most high-risk areas for tick-borne diseases due to elevated incidence of tick-borne encephalitis and Lyme disease in humans (State Report Tomsk 2017). The major vector for these diseases in the western part of Russia is *Ixodes persulcatus* Schulze, 1930. Since the beginning of the 21st century, another tick with similar ecology, *Ixodes pavlovskyi* Pomerantsev, 1946, has been found in the same habitats (Romanenko and Chekalkina 2004) and currently predominates over *I. persulcatus* in urban areas. Although both ticks can bite humans, *I. pavlovskyi* is also known as an ornithophilic tick (Ushakova et al. 1969, Moskvitina et al. 2014). Its disjunct habitat range includes the mountain territories in the Russian Far East, Altai, and Gornaya Shoriya, and its appearance in the Tomsk region is probably caused by wild birds. The prevalence of tick-borne encephalitis virus (TBEV) and *Borrelia* spp. in questing ticks in the Tomsk region has been estimated to be up to 9.3% and 28.5%, respectively (Moskvitina et al. 2014, Pankina et al. 2015).

There are 333 species of birds in the Tomsk region and more than 250 of them have annual migration (Ryabitsev et al. 2001). The geographical location of this region in the Eurasian continent provides a wide range of directions for the migration of wild birds to the wintering grounds. According to the data of ringing (Moskvitin and Dubovik 1969, 1977, Moskvitin and Strelkov 1977, Moskvitin 1992, Ryabitsev 2001), Siberian birds spend the winter in Africa, Europe, West Asia, India, and Southeast Asia (Fig. 1). This creates preconditions for the dispersion of pathogens from...
different areas with various epidemiological settings. Numerous pathogens have been found in ticks and vertebrate hosts in the Tomsk region (Chausov et al. 2010). In addition to TBEV and *Borrelia* spp., some other pathogens such as species of *Rickettsia*, *Anaplasma*, *Babesia*, *Ehrlichia*, and *Bartonella* were also found. Surprisingly, West Nile virus (WNV) was found in ticks from the genus *Ixodes* Latraille, 1795, which attack both humans and vertebrates. We assumed that the circulation of WNV in the tick vertebral system is theoretically possible (Moskvitina et al. 2008, 2014), which has been previously demonstrated experimentally (Azarova and Mishaeva 2002). Although several studies have focused on the detection of various pathogens in birds and ticks collected from them (Hildebrandt et al. 2010, Špitalská et al. 2011, Dubska et al. 2012, Kang et al. 2013, Movila et al. 2013, Toma et al. 2014, Diakou et al. 2016), there is no clear information on the relationship between pathogens, vectors, and hosts. Therefore, this study was conducted to identify the pathogens that were detected in different species of birds and ticks parasitising on them and to evaluate their interdependence.

**MATERIALS AND METHODS**

**Bird capture and tick collection**

Fieldwork was conducted from April to August during 2006–2011. The study material was collected in Tomsk (56.4826N, 84.9950E) and their nearest suburbs within 15 km zone. Altogether, 736 wild birds were examined for the presence of feeding ticks, of which 293 birds were captured using mist nets and subsequently released and 443 were shot during the hunt and subsequently analysed for the presence of pathogens. A total of 804 larvae, nymphs and adults from the genus *Ixodes* were collected from the birds. Feeding ticks were analysed for the presence of pathogens. We have previously reported the prevalence and intensity of tick infection in birds (Moskvitina et al. 2014). Ticks from each bird were collected separately in Eppendorf tubes and after their species identification using a taxonomic guide (Filippova 1977), they were stored alive at 4°C or frozen at –20°C until the detection of pathogens. The identification of bird species was performed according to a field guide (Ryabitsev 2001). All procedures involving wild birds were conducted according to permits 70 No. 024401 and 70 No. 024399 from the local government and regulations for working with wild animals.

**Detection of selected pathogens**

A total of 443 specimens of 60 species of birds (spleen, liver, and brain) and 378 ticks (all stages) were examined for the presence of eight pathogens (TBEV, WNV, spp. of *Borrelia*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Bartonella*, and *Babesia*).

**Enzyme immunoassay**

TBEV antigens were detected via enzyme immunoassay analysis as described previously (Ternovoi et al. 2007). Briefly, the viral antigen was captured on the surface of 96-well polystyrene plates from 100 µl of the homogenates of ticks and internal organs (spleen, liver and brain from individual bird) using monoclonal antibodies 10H10 against protein E of TBEV that was preliminarily immobilised on the plates. Immune complexes were detected using EB1 monoclonal antibody against protein E of TBEV labeled with biotin, which was identified by avidin-peroxidase. WNV antigens were detected using a previously described

Fig. 1. Scheme of the wintering grounds of wild birds from Western Siberia.
Fig. 2. Prevalence of TBEV and WNV markers (total, viral antigen, and RNA) in wild birds.

PCR analysis

RNA extraction, reverse transcription, and detection of TBEV and WNV cDNA using RT-PCR, as well as the detection of other bacterial and protozoan pathogens using PCR, were performed as described earlier (Chausov et al. 2010). Briefly, a combined RNA/DNA mixture was extracted from 100 μl of the homogenate of ticks and organs of birds using a RIBOSorb (AmpliSens, Moscow, Russia) set and a DNA/RNA Extraction Kit (NPF Litekh, Moscow, Russia), according to the manufacturer’s instructions. Synthesis of cDNA was performed using a REVERTA-L kit (AmpliSens, Moscow, Russia) according to the manufacturer’s protocol. We calculated and synthesised the oligonucleotide primers for detecting the RNA and DNA of TBEV, WNV, and species of Borrelia, Rickettsia, Ehrlichia, Babesia, and Bartonella based on the comparison of the nucleotide sequences of their different strains deposited in GenBank. Table 1 shows the primer pairs for the amplification of the sequences of viral, bacterial, and protozoan agent markers.

Comparison of pathogens in birds and ticks

In 45 cases, we conducted pairwise comparisons of the pathogens in birds and ticks that feed on them. All analysed ticks were engorged with blood. Among all the cases of pairwise comparisons of pathogens between birds and ticks feeding on them, we made the following statement: If the infected birds were carriers of uninfected ticks (irrespective of how many uninfected ticks were on the bird and what development stages and species they were), these cases were considered as one event of comparison. The same situation was considered if ticks on one bird were in-
Pathogens detected in wild birds

A total of 60 species of wild birds were examined for the presence of the above-mentioned tick-borne pathogens. We found that 43 species (71.7%) were the carriers of some pathogens (Table 2). Some species among individually analysed birds had one to eight infection agents, which were detected in their samples. The number of pathogens in some species of birds did not always depend on the number of analysed samples of that species of birds. Sometimes, the number of pathogen markers was higher in species with a small sample (the common willow (n = 2) had four pathogens and the Eurasian nuthatch (n = 3) had five pathogens; scientific names of birds are in Table 2) than in species with a larger sample (the great tit (n = 11) had only three pathogens and the garden warbler (n = 5) had only one pathogen).

We assumed that some species of birds were more or less susceptible to pathogens and, consequently, played a different role as reservoirs. To illustrate this assumption, we estimated the percentage of birds of different species infected with a pathogen common for all ticks that fed on this bird. If the infected or uninfected bird was a carrier of several ticks each of which was infected with a different set of pathogens, every case of comparison of the pathogens in this bird and each tick was considered as an independent event. By the term “event” we mean one pairwise comparison of pathogens in a bird and in a tick (or ticks) feeding on it. We excluded from the analysis those cases when both the bird and the tick were free of pathogens.

Statistical analysis

Statistica 6.0 and Microsoft Office Excel 2007 were used to perform statistical analysis. The F-test was used to confirm the differences in the rates of infected animals. The Spearman method was used to evaluate the correlations of some parameters.

RESULTS

Pathogens detected in wild birds

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We assumed that some species of birds were more or less susceptible to pathogens and, consequently, played a different role as reservoirs. To illustrate this assumption, we estimated the percentage of birds of different species infected by TBEV and WNV (Fig. 2). We confirmed that the species had a different percentage of prevalence of viral infections. For some species, but not all, the percentage of birds infected with viruses was associated with the frequency of their contact with ticks. For instance, the fieldfare, whose mean abundance of parasites was 5.7 ticks per bird, had one of the highest percentages of viral infection markers of approximately 50% (Fig. 2). In contrast, birds rarely attacked by ticks, such as the rock pigeon, the common rosefinch, and the magpie, whose mean abundance were 0, 0.3, and 0.5 ticks per bird, respectively, had a low prevalence of viruses. However, there were also such species (the hooded crow, the great tit and the brambling) that were 0, 0.3, and 0.5 ticks per bird, respectively, had a low prevalence of infections, probably due to their susceptibility.

Regarding the methods of detecting viral infections, the viral antigen, compared with the viral RNA, was detected more often for TBEV markers by 1.6 times and for WNV markers by 4.6 times. In some species, RNA could be detected more often than the antigen. Simultaneously, both methods confirmed the presence of TBEV markers in birds in 7.3% of cases and WNV markers in 6.3% of cases. The number of pathogens was almost similar in the groups of sedentary and migratory birds. There were also no significant differences in the percentage of birds infected with certain pathogens in both groups, except that of species Borrelia and Bartonella (Table 3). The mass departure of Siberian birds from nest sites to the wintering areas after tick parasitisation can result in the distribution and exchange of pathogens between the territories.

Number of pathogens in birds against number of ticks

We attempted to verify whether the number of pathogens in birds that play the major role in sustaining the tick population (the tree pipit, the fieldfare and the redwing) was in fact higher than that in birds that have less contact with ticks (the European pied flycatcher and the lesser whiteth-
Table 2. Species of wild birds with infections markers in Western Siberia and places of their wintering ground

| Species of birds | TBEV | WNV | Bartonella spp | Rickettsia spp | Babesia spp | Ehrlichia spp | Anaplasma spp |
|----------------|------|-----|----------------|--------------|-------------|--------------|--------------|
| Fieldfare Turdus pilaris Linnaeus | 41(37/9)/80 | 37(34/5)/80 | 8/80 | 7/80 | 5/22 | 2/22 | 2/22 |
| Blathy's reed warbler Acrocephalus dumetorum Blyth | 15(5/0)/39 | 7(3/3)/39 | 4/39 | 8/39 | 1/13 | 1/13 | 3/13 |
| Common reed warbler Phoenicurus phoenicurus (Linnaeus) | 8(3/6)/15 | 5(5)/0/15 | 2/15 | 3/15 | 1/4 | 2/4 | 1/4 |
| Common shalfinch Fringilla coelebs Linnaeus | 5(5)/1/22 | 8(7/2)/22 | 2/22 | 1/22 | 2/12 | 2/12 | 1/12 |
| European pied flycatcher Ficedula hypoleuca (Pallas) | 4(1)/3/9 | 2(0)/2/9 | 1/9 | 1/9 | 2/4 | 1/4 | 0/4 |
| Brambling Fringilla montifringilla Linnaeus | 7(7)/10/11 | 5(5)/0/11 | 0/11 | 2/11 | 0/3 | 1/3 | 0/3 |
| Eurasian nuthatch Sitta europaea Linnaeus | 1(1)/0/3 | 2(2)/0/3 | 0/3 | 1/3 | 0/1 | 1/1 | 0/1 |
| Lesser whitethroat Sylvia curruca (Linnaeus) | 4(3)/2/9 | 2(2)/0/9 | 1/9 | 0/9 | 1/4 | 0/4 | 0/4 |
| Sand martin Riparia riparia (Linnaeus) | 10(3)/7/48 | 4(0)/4/0/8 | 9/21 | 3/21 | 0/21 | 0/21 | 2/21 |
| Hooded crow Corvus cornix Linnaeus | 3(3)/0/10 | 5(5)/2/10 | 1/10 | 1/10 | n.a. | n.a. | n.a. |
| Willow warbler Phylloscopus trochilus (Linnaeus) | 2(2)/1/2 | 2(2)/0/2 | 1/2 | 1/2 | 0/1 | 0/1 | 0/1 |
| Common reed warbler Carpodacus erythrinus (Pallas) | 1(1)/0/7 | 1(1)/0/7 | 1/7 | 1/7 | 0/2 | 0/2 | 0/2 |
| Rock-pigeon Columba livia Gmelin | 1(0)/1/7 | 0/7 | 0/7 | 4/7 | 0/7 | 1/7 | 0/7 |
| Redwing Turdus iliacus Linnaeus | 3(3)/2/8 | 3(2)/1/8 | 0/8 | 1/8 | 2/4 | 0/4 | 0/4 |
| Common woodcock Scolopax rusticola Linnaeus | 0/5 | 1(1)/0/5 | 0/5 | 1/5 | 1/4 | 0/4 | 0/4 |
| Chiffchaff Phylloscopus collybita (Vieillot) | 1(3)/1/2/6 | 1(1)/1/6 | 0/6 | 1/6 | 0/4 | 0/4 | 0/4 |
| Tree pipit Anthus trivialis (Linnaeus) | 3(0)/3/3 | 0/3 | 2/3 | 1/3 | 0/1 | 0/1 | 0/1 |
| European greenfinch Chloris chloris (Linnaeus) | 1(1)/0/2 | 2(1)/1/2 | 0/2 | 1/2 | n.a. | n.a. | n.a. |
| Great tit Parus major Linnaeus | 5/5/0/11 | 6/5/1/11 | 0/11 | 2/11 | 0/3 | 0/3 | 0/3 |
| Common bullfinch Pyrrhula pyrrhula (Linnaeus) | 2(2)/0/4 | 1(1)/0/4 | 0/4 | 1/4 | 0/2 | 0/2 | 0/2 |
| Starling Sturnus vulgaris Linnaeus | 2(2)/0/8 | 2(2)/0/8 | 0/8 | 1/8 | n.a. | n.a. | n.a. |
| Eurasian tree sparrow Passer montanus (Linnaeus) | 3(3)/0/3 | 3(3)/0/3 | 0/3 | 0/3 | n.a. | n.a. | n.a. |
| Yellow hammer Emberiza citrinella Linnaeus | 2/2/0/4 | 2/2/0/4 | 0/4 | 0/4 | n.a. | n.a. | n.a. |
| Jackdaw Corvus monedula Linnaeus | 1(1)/0/4 | 3/1/1/4 | 0/4 | 0/4 | n.a. | n.a. | n.a. |
| Lapwing Vanellus vanellus (Linnaeus) | 2(0)/2/8 | 2/0/2/8 | 0/8 | 0/8 | n.a. | n.a. | n.a. |
| Mallard Anas platyrhynchos (Linnaeus) | 4/0/4/35 | 0/35 | 1/35 | 0/35 | n.a. | n.a. | n.a. |
| Pall’s warbler Phylloscopus proroguula (Pallas) | 1(0)/1/1 | 1/0/1 | 0/1 | 0/1 | 1/1 | 0/1 | 1/1 |
| Siberian rubythroat Luscinia calliope (Pallas) | 2(1)/2/3 | 0/3 | 1/3 | 0/3 | 0/1 | 0/1 | 0/1 |
| Goldfinch Carduelis carduelis (Linnaeus) | 2(0)/2/4 | 0/4 | 0/4 | 1/4 | 0/2 | 0/2 | 0/2 |
| Willow tit Parus montanus Baldeinstein | 2(2)/0/4 | 2/0/2/4 | 0/4 | 0/4 | n.a. | n.a. | n.a. |
| Common magpie Pica pica (Linnaeus) | 1(0)/1/7 | 2(2)/0/7 | 0/7 | 0/7 | n.a. | n.a. | n.a. |
| Hazel en Tetrao bonasia (Linnaeus) | 1(0)/1/5 | 0/5 | 0/5 | 0/5 | n.a. | n.a. | n.a. |
| Thrush nightingale Luscinia svecica (Linnaeus) | 1(0)/1/1 | 0/1 | 0/1 | 0/1 | n.a. | n.a. | n.a. |
| Eurasian siskin Spinus spinus (Linnaeus) | 1(1)/0/3 | 0/3 | 0/3 | 0/3 | 0/1 | 0/1 | 0/1 |
| Greater spotted woodpecker Dendrocopos major (Linnaeus) | 1(0)/1/2 | 0/2 | 0/2 | 0/2 | 0/1 | 0/1 | 0/1 |
| Garden warbler Sylvia borin (Boddart) | 0/5 | 0/5 | 0/5 | 0/5 | 0/3 | 1/3 | 0/3 |
| Blaestart Tarsiger cyanurus (Pallas) | 0/2 | 1(0)/1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| European wigeon Anas penelope Linnaeus | 0/5 | 0/5 | 1/5 | 0/5 | n.a. | n.a. | n.a. |
| Green-winged teal Anas crecca Linnaeus | 1(0)/1/3 | 0/3 | 0/3 | n.a. | n.a. | n.a. | n.a. |
| Common merganser Mergus merganser Linnaeus | 1(0)/1/3 | 0/3 | 0/3 | n.a. | n.a. | n.a. | n.a. |
| Waxwing Bombycilla garrulus (Linnaeus) | 1(0)/1/0 | 0/1 | 0/1 | 0/1 | n.a. | n.a. | n.a. |
| Coal tit Parus ater Linnaeus | 1(1)/0/1 | 0/1 | 0/1 | 0/1 | n.a. | n.a. | n.a. |
| Spoonbill Anas clypeata Linnaeus | 1(1)/0/3 | 0/3 | 0/3 | n.a. | n.a. | n.a. | n.a. |

aSpecies are listed according to the number of revealed pathogens; bThis species belongs to specific parasitic system including tick *Ixodes ricinus* Koch, 1844, inhabiting sand martins caves; cnot analyzed

Comparison of infection markers in birds and ticks

We revealed the tendency of simultaneous infections (co-infections) in birds and ticks. Coinfections (two or seven pathogens simultaneously in one bird) were highly common. Some individuals were positive for the markers from collected from the birds that were in close contact with ticks were more often considered to be carriers of the pathogens than the ticks collected from the birds that served as secondary feeders, for example, the family Sylviidae.
Infections in birds were not found, but the ticks contained different infections, and their combinations are listed below. Both birds and ticks were infected by one to four infection markers in individual ticks. Anaplasma spp. and Babesia spp., the common chaffinch had four pathogens (TBEV, WNV, Rickettsia spp., and Ehrlichia spp. and Anaplasma spp.), the common chaffinch had four pathogens (TBEV, WNV, Rickettsia spp., and Ehrlichia spp. and Anaplasma spp.), and the common redstart had seven pathogens (all except WNV). We also found up to four infection markers in individual ticks.

In the present study, we focused on the comparison of pathogens in individual birds and the ticks that fed strictly on them. As we had data on the pairwise comparison of infected birds and ticks captured from these individuals, we compared the number and composition of pathogens in these pairs (n = 45). Consequently, we identified several variants of pairwise combinations (Table 4) as follows:

- Birds had one or more markers of infections, but the ticks collected from these birds were free of pathogens (53.3%).
- Infections in birds were not found, but the ticks collected from them had one infection agent (11.1%).
- Complete match by the type of pathogens was observed in the infected birds and the ticks collected from them (8.9% of cases).
- Both birds and ticks were infected by one to four pathogens, one or more of them being the same. In general, a partial match was detected in 15.6% of cases.
- Birds and ticks contain different infections, and their mismatch was observed in 11.1% of cases.

**DISCUSSION**

Of the 43 species of birds examined in the Tomsk region in which the pathogens were identified, the proportions of birds associated with wintering in different regions were as follows: 23.3% in Europe, 25.6% in India, 18.6% in Africa, 16.3% in the Caspian and the Middle East, 9.3% in Southeast Asia, and 6.9% in Central Asia, and 32.6% of birds were sedentary. It is certain that the emergence of the Far Eastern strain of TBEV, as well as WNV and the tick *Ixodes pavo*skyi, in our region was directly caused by birds (Ternovoi et al. 2004, Mikryukova et al. 2014, Moskvitina et al. 2014). Similarly, owing to birds, the tick *Hyalomma marginatum* Koch, 1844 has extended its habitat range in Europe, potentially spreading the pathogens associated with it (Poupon et al. 2006, Molin et al. 2011, Diakou et al. 2016, Klaus et al. 2016). This tick was found on the migrating birds *Phoenicurus phoenicurus* (Linnaeus) and *Anthus trivialis* (Linnaeus) in the westernmost region of Russia, i.e., the Kaliningrad region, where one of the ticks was found to have an exotic species, *Rickettsia aeshlimannii* (see Movila et al. 2013). We can expect the discovery of the same species of pathogens that have been identified in the wintering areas of our birds (Cazorla et al. 2008, Tonetti et al. 2009, Ghosh and Nagar 2014, Sparagano et al. 2015, Yang et al. 2015, Kuo et al. 2017).

It is obvious that birds often attacked by ticks, primarily those from the family Turdidae, play an important role in maintaining the foci of natural infections. This was confirmed by the number of infected ticks that were more common on ground-foraging birds, which are the major tick hosts. A correlational analysis between the number of pathogens detected in birds and the number of ticks parasitising on them revealed that the number of pathogens was equal in birds with frequent and rare contact with ticks. On the one hand, it was logical to assume that the more often the ticks attack, the more infections the birds have. In contrast, we may expect the result indicating that more frequent attacks by ticks lead to fewer pathogens. The latter result may be associated with the formation of immune mechanisms of pathogen suppression during prolonged and/or multiple parasitisation of ticks, as demonstrated in several studies (Wakelin 1996, Wikel et al. 1997, Kislenko and Korotkov 1998, Heylen et al. 2010).

The finding that the number of pathogens in birds did not directly depend on the number of parasitic ticks can be explained by the fact that the birds get infected over a long period, and thus the number of pathogens does not necessarily depend on the number of ticks found on them at the time of capture.

Quite unexpectedly, we found a significant number of birds (32.9%) with WNV markers far from the territories endemic for this infection. Furthermore, the obtained indicator of prevalence significantly exceeded that in several other regions (Balança et al. 2009, Jourdain et al. 2011, López et al. 2011, Murata et al. 2011, Czank et al. 2016). The prevalence of infections in different species of ticks by WNV markers also confirmed to be quite high at 3.4–5.1%. In contrast, in Italy and Greece, which are located closer to the areas of WNV distribution, this virus was not detected in ticks parasitising on migratory birds (Hagman et al. 2014).

The suggestion that tropical arboviruses can be disseminated and settled in temperate latitudes in ticks was made more than 30 years ago (Nekipelov 1978). Despite the fact that WNV is traditionally considered as a mosquito infection, it is known that argasid and ixodid ticks can be involved in the circulation of this pathogen (Mumcuoglu 2005, Reiter 2010). In some cases, in the absence of mosquitoes, circu-

| Group of birds | TBEV | WNV | Borrelia spp. | Rickettsia spp. | Bartonella spp. | Babesia spp. | Ehrlichia spp. | Anaplasma spp. |
|---------------|------|-----|---------------|----------------|----------------|--------------|--------------|---------------|
| Sedentary     | 37.7 | 34.4| 1.63          | 16.4           | 0.7            | 12.5         | 0            | 25.0          |
| Migrants     | 35.8 | 25.4| 10.4          | 10.1           | 14.9           | 10.3         | 6.5          | 11.2          |
| F – test      | F = 0.28 | F = 1.43 | F = 2.89* | F = 1.34 | F = 2.96* | F = 0.261 | F = 1.93 | F = 1.35 |
| p-value       | P > 0.05 | P > 0.05 | P > 0.01 | P > 0.05 | P > 0.01 | P > 0.05 | P > 0.05 | P > 0.05 |

*Differences are statistically significant*
Table 4. Comparison of infection markers in wild birds and attached ticks

| Variants of combinations | Pathogens in birds | Pathogens in ticks |
|--------------------------|-------------------|-------------------|
| Species of birds         | Pathogens         | Species, number and development stages of ticks (F- Adult female; N- Nymph; L- Larvae) |
| Fieldfare                | TBEV (antigen)    | 2 N. I. persulcatus |
| Fieldfare                | TBEV (antigen)    | 1 N. I. persulcatus, 4 L. I. persulcatus, 3 L. I. pavlovskyi |
| Redwing                  | TBEV (antigen+RNA)| 2 N. I. pavlovskyi |
| Lesser whitethroat       | TBEV (antigen)    | 1 N. I. pavlovskyi |
| Common redstart          | TBEV (RNA)        | 2 N. I. pavlovskyi, 1 L. I. pavlovskyi, 1 L. I. persulcatus |
| Fieldfare                | WNV (antigen)     | 1 F. I. persulcatus, 1 F. I. pavlovskyi |
| Redwing                  | WNV (RNA)         | 2 F. I. pavlovskyi, 4 N. and 8 L. I. pavlovskyi |
| Common redstart          | WNV (antigen)     | 1 N. I. persulcatus, 6 N. I. pavlovskyi, 2 L. I. pavlovskyi |
| Hooded crow              | WNV (antigen+RNA) | 6 F. I. persulcatus, 3 F. I. pavlovskyi |
| 1. Birds are infected with one or more pathogens, but ticks collected from them are not infected |
| Blyth’s reed warbler     | Borrelia spp.     | 2 L. I. persulcatus, 1 L. I. pavlovskyi |
| Blyth’s reed warbler     | Rickettsia spp.   | 1 L. I. persulcatus |
| Common chaffinch         | Babesia spp.      | Not found |
| Fieldfare                | Batronella spp.   | 2 L. I. pavlovskyi |
| Fieldfare                | TBEV+WNV (both antigen) | 1 N. I. pavlovskyi |
| Fieldfare                | TBEV+WNV (both antigen) | Not found |
| Eurasian tree sparrow    | TBEV+WNV (both antigen) | 1 F. I. persulcatus, 2 F. I. pavlovskyi |
| Eurasian tree sparrow    | TBEV+WNV (both antigen) | Not found |
| Eurasian tree sparrow    | TBEV+WNV (both antigen) | Not found |
| Common redstart          | TBEV (antigen+RNA) + +WNV (antigen) | 4 N. I. pavlovskyi, 4 L. I. pavlovskyi |
| Fieldfare                | TBEV (antigen)+Rickettsia spp. | 1 F. I. pavlovskyi |
| Common redstart          | Rickettsia spp.+Babesia spp. +Anaplasma spp. | Not found |
| Fieldfare                | WNV (antigen+RNA) ++Erlichia spp. + Babesia spp.+ Rickettsia spp. | 1 F. I. pavlovskyi |
| Brambling                | Not found         | 1 N. I. persulcatus |
| Fieldfare                | Not found         | 3 N. I. pavlovskyi |
| Fieldfare                | Not found         | 6 N. I. pavlovskyi |
| Fieldfare                | Not found         | 1 F. I. persulcatus |
| Common chaffinch         | Not found         | 3 N. I. persulcatus |
| Fieldfare                | TBEV (antigen+RNA) +WNV (antigen)3 N. I. pavlovskyi | TBEV+WNV (antigen+RNA) |
| 2. Birds are free of pathogens, but ticks feeding on them are infected with one infection |
| Common redstart          | TBEV+RN (both antigen) | 2 L. I. persulcatus |
| Fieldfare                | TBEV+RN (both antigen) | 2 L. I. persulcatus |
| Fieldfare                | TBEV+RN (both antigen) | 1 F. I. persulcatus |
| Fieldfare                | TBEV+RN (both antigen) | 1 F. I. persulcatus |
| Fieldfare                | TBEV+RN (both antigen) | 1 N. I. pavlovskyi |
| Fieldfare                | TBEV+RN (both antigen) | 2 F. I. pavlovskyi |
| Tree pipit               | TBEV (RNA) ++Borrelia spp. | 1 N. I. persulcatus |
| Redwing                  | Bartonella spp.   | 1 N. I. pavlovskyi |
| Fieldfare                | TBEV (antigen+RNA) | 2 F. I. pavlovskyi |
| Fieldfare                | TBEV (antigen)    | 2 F., 2 N., 3 L. I. persulcatus |
| Fieldfare                | Borrelia spp.     | Rickettsia spp. |
| Redwing                  | Bartonella spp.   | TBEV (RNA) |
| Fieldfare                | TBEV+WN (both antigen) | 1 N. I. pavlovskyi |

1+4 Birds under the same number represent the same individual

On the basis of the above-described data, WNV can persist and winter in ticks, thereby hypothetically supporting its circulation in nature. This scenario is probably in action in our research area. Earlier, WNV was found here in
questing adult ticks that were sampled using a flag. Moreover, it was found in small mammals and birds and the sub-adult ticks that fed on them (Moskvitina et al. 2008). The transmission of this infection by ticks was confirmed by WNV markers (antigens and RT-PCR detection) in sedentary birds (great tit, hooded crow, Eurasian nuthatch, common bullfinch, and willow tit) and in ticks collected from them. WNV was found in sedentary birds as frequently as in migratory birds. WNV was also detected in the nestlings of migratory birds that had never migrated yet. Furthermore, the highest rate of infection by WNV was exhibited by the great tit, the sedentary or vagrant bird, which does not migrate to WNV-endemic areas (Fig. 2). The second most infected bird was the hooded crow, the bird belonging to the family Corvidae and well-known as a WNV reservoir (Eidson et al. 2001).

Notwithstanding that several facts indicate that ticks are involved in the transmission of WNV, the level of viremia in birds after a tick bite remains unknown. Attempts to isolate WNV from ticks and bird organs have been unsuccessful, although this was possible for TBEV (Mikryukova et al. 2014). According to experimental data (Ciota et al. 2015), WNV in the tick cell line culture underwent genetic modification and actually became unable to infect vertebrates. However, there are contrasting data that have been obtained experimentally (Azarova and Mishaeva 2002). The authors confirmed in the laboratory the transmission of WNV from infected mice to uninfected ticks (Ixodes ricinus) and from infected ticks to uninfected mice through the developmental stages of ticks and the transovarial transfer of pathogen with titres sufficient for circulation.

Therefore, laboratory experiments confirm the possibility of the participation of ticks of the genus Ixodes in the circulation of the virus. However, the question of the participation of ticks in the transmission of WNV in nature remains open. We cannot deny that some birds could be infected in wintering areas and through mosquito bites, especially considering the duration of WNV viremia in birds (Wheeler 2012).

The data obtained in the present study on the infection of birds and ticks by various pathogens have similarities with both different territories and their regional specificities. Hence, the presence of Borrelia spp. in ticks collected from birds in Western Siberia (Moskvitina et al. 2014) confirmed to be quite similar to that found in some countries of Europe such as Germany (Franke et al. 2010), Czech Republic (Dubska et al. 2011), Latvia (Capligina et al. 2014), Switzerland (Lommano et al. 2014), but it was lower than that in Italy (Toma et al. 2014), Great Britain (Kurtenbach et al. 1998), the USA (Hamer et al. 2012), and the western part of Russia (Alekseev et al. 2001).

The prevalence of tick infection with Rickettsia spp. was lower than that in the majority of studies (Śpitalska et al. 2011, Hornok et al. 2014, Toma et al. 2014, Diakou et al. 2016), and that of Ehrlichia spp. was also lower (Alekseev et al. 2001, Toma et al. 2014). At the same time, the level of infection was higher than that reported in several studies on Bartonella spp. (Molin et al. 2011, Movila et al. 2014) and on Anaplasma spp. (Dubska et al. 2012, Palomar et al. 2012, Geller et al. 2013, Lommano et al. 2014).

Most of the studies conducted in recent years were aimed primarily at identifying pathogens in ticks collected from birds, with the results being considered a priori as facts confirming the circulation of certain infections in ticks and birds (Dubska et al. 2012, Capligina et al. 2014, Lommano et al. 2014, Michelet et al. 2016). In our opinion, the detection of pathogens only in ticks found on some species of birds, without identifying the pathogens in the bird itself, cannot be unequivocal evidence of the role of birds in the circulation of pathogens.

For instance, in several studies, the great tit was reported as a carrier of ticks infected with Borrelia spp. (Comstedt et al. 2006, Dubska et al. 2009, Geller et al. 2013, Heylen et al. 2013, Hornok et al. 2013), as well as with Anaplasma spp. (Śpitalska et al. 2011) and Babesia spp. (Hildebrandt et al. 2010). However, none of the birds of this species were analysed or harboured markers of these pathogens. On the one hand, this may be a random coincidence or regional specificity, because the great tit in our biotopes is not often infected with ticks. At the same time, as mentioned earlier, the great tit ranked the topmost in terms of the level of infection with WNV and TBEV and was also a carrier of Rickettsia spp. The latter finding corresponds to the fact that ticks in the great tit were infected by this species of pathogen as reported in several studies (Hildebrandt et al. 2010, Movila et al. 2011, Śpitalska et al. 2011, Hornok et al. 2013).

A similar situation was observed with the redwing, one of the important tick hosts. Like the great tit, several authors have mentioned it as a carrier of ticks infected with Anaplasma spp., Babesia spp., and Borrelia spp. (Ölsén 1995, Comstedt et al. 2006, Geller et al. 2013, Capligina et al. 2014). Our studies did not identify infection by these pathogens in the birds themselves. At the same time, this species was again a carrier of WNV, TBEV, Rickettsia spp., and Bartonella spp. The absence of Borrelia spp. is perhaps accidental, because a species similar to the redwing, the fieldfare, was infected with all pathogens. These results illustrate the need to evaluate the infection of not only the ticks that feed on birds but also the birds themselves, as the role of the latter may not coincide with their role assumed solely on the basis of the presence of infected ticks.

In recent years, several studies have analysed the infection of the birds themselves (de la Fuente et al. 2005, Stańczak et al. 2009, Yang et al. 2015, Ebani et al. 2016), including the infection of birds and their ectoparasites (Norte et al. 2013, Hornok et al. 2014, Sándor et al. 2016). These studies show both cases of coincidence and mismatch of pathogens between birds and ticks parasitising on them, which confirms the need to analyse the infection with pathogens in the bird–tick system, because this allows us to better understand the mechanism of pathogen circulation in ecosystems.

Our pairwise comparisons of pathogens in birds and ticks showed that in 75.5% of cases, there were discrepancies in the identified pathogens. One of the reasons was that the exchange of pathogens between birds and ticks had
not occurred yet. On the other hand, there is a possibility of some barriers in the tick–bird system that prevents the transmission of pathogens. Therefore, some researchers (Alekeev and Dubinin 2007) have put forward an idea of the antagonistic relationship between pathogens, which prevents their coexistence in the same tick or vertebrate. However, from these viewpoints, it is impossible to explain the presence of mixed infections in birds if, for example, four to seven pathogens in one bird or four pathogens in one tick are detected.

Hence, the redstart had seven pathogens, except for WNV; one nymph of *I. pavlovskyi* collected from a redwing had WNV, *Babesia* spp., *Bartonella* spp., and *Anaplasma* spp., whereas the redwing itself was infected with *Bartonella* spp. The latter fact indicates the possible involvement of birds in the circulation of *Bartonella* spp.

*Borrelia* spp. can also be used to illustrate a different spectrum of variants of infection with this pathogen in the bird–tick system with the participation of various bird species (Table 4). Therefore, the garden warbler was infected with *Borrelia* spp., whereas the ticks on it were free of pathogens. The common redstart was infected with *Borrelia* spp. and *Rickettsia* spp., whereas the ticks were uninfected. In contrast, two cases, a brambling and a fieldfare that were free of pathogens, were carriers of nymphs infected with *Borrelia* spp. There was a case of a fieldfare infected with *Borrelia* spp., and three adult ticks (females) on it were infected with TBEV. In two fieldfaires infected with TBEV + WNV, the ticks were infected with, apart from viruses, *Borrelia* spp.; in one of these birds, the tick was infected only with *Borrelia* spp. This finding may indicate either the trans-stadial transmission of *Borrelia* spp. by these ticks or that cofeeding with bacteremia has not yet begun in these birds.

Finally, a case was observed when in a tree pipit infected with TBEV + WNV + *Borrelia* spp., one nymph with TBEV and two larvae infected with TBEV + *Borrelia* spp. were found, which confirmed the transmission of this pathogen in the bird–tick system.

Of all cases of pairwise-compared infections in the bird–tick system, we detected a coincidence indicating the possibility of transmission for four pathogens, the above-mentioned *Bartonella* spp. and *Borrelia* spp. as well as TBEV and WNV. The coincidence of infections in the bird–tick system was not detected for *Ehrlichia* spp., *Babesia* spp., *Anaplasma* spp., and *Rickettsia* spp., although all these pathogens were found in birds and ticks separately.

Although a study by Sándor et al. (2016) provides evidence of infection in birds and ticks with *Anaplasma* spp. and *Rickettsia* spp., it is not clear whether the infected ticks were found on the infected birds. The study conducted by Hornok et al. (2014) reported examples of synchronous infection with *Rickettsia* spp. of birds and ticks collected from them, which confirms the transmission of this bacterium, and there is also evidence of mismatch in birds and ticks infected with *Anaplasma* spp. However, it is believed that birds play an important role in maintaining *Anaplasma* spp. (de la Fuente et al. 2005, Ioannou et al. 2009, Franke et al. 2010).

Regarding *Babesia* spp., no consensus exists on the role of birds in the circulation of these pathogens. We detected markers of this infection in 13% (13/109) of birds, as well as in two ticks collected from an uninfected bird, which may indicate a possible transmission through the cofeeding mechanism. The same possibility has been suggested previously (Kuo et al. 2017). However, in another study (Yabsley et al. 2013), it was observed that birds are an unlikely reservoir of *Babesia* spp.

On the basis of the data of the synchronous infection of birds and ticks, we attempted to understand the relationship in the tick–bird–pathogen system. The presence of the pathogen in birds and ticks may indirectly indicate a possible transmission of infection, but the level of bacteremia and viremia in birds and ticks may not be sufficient to transmit the infection. Considering the facts we have disclosed about a high proportion of mismatches in pathogen species between birds and ticks parasitising on them, it can be assumed that the relationships between the components of the parasitic bird–tick–pathogen system are not so straightforward, depend on the multiplicity of manifestations of these connections, and require more in-depth research.

On the basis of our study results, the following conclusions can be made:

1. In 43 species of birds, one to eight infection agents transmitted through tick bites were detected. Comparison of the number of infected birds among migratory and sedentary species did not reveal significant differences between them in the prevalence of pathogens. This finding may indicate that most of the pathogens could have been transmitted through tick bites at our research sites, rather than being introduced from outside.

2. Different species of birds exhibited differences in the prevalence of pathogens, in particular viral infections (TBEV and WNV). For example, the highest prevalence of WNV infection was found in the sedentary hooded crow *Corvus cornix* and the great tit *Parus major*. Although the former species is known as a typical natural reservoir of WNV, the great tit probably proved to be nonspecific, but susceptible, to the pathogen species of birds.

3. The number of detected pathogens in birds on which ticks frequently feed did not differ from that in birds that have rare contact with ticks. In addition, the number of pathogens in individual birds did not correlate with the number of ticks parasitising on them.

4. Despite the significant prevalence of infection markers in birds and ticks, the pairwise infection comparison analysis in birds and ticks showed that complete or partial coincidence of infection markers was observed in only 24.5% of cases. It was observed for TBEV, WNV, *Borrelia* spp. and *Bartonella* spp. We are forced to admit and emphasise that this are only preliminary data because the most of pathogens were identified only up to genus level. Viruses (TBEV and WNV), as well as the combination of TBEV and *Borrelia* spp., were the most frequently co-occurring pathogens.
Authors’ contribution. Igor G. Korobitsyn wrote the manuscript, supervised ornithological work, data analysis. Nina S. Moskvitina initiated and supervised the study, critically revised the manuscript. Oleg Yu. Tyutenkov mist-netted birds and collected ticks, data analysis. Sergey I. Gashkov mist-netted birds and collected ticks. Yulia V. Kononova detected pathogens in ticks and birds by IEA, data analysis. Sergey S. Moskvitin mist-netted birds and collected ticks. Vladimir N. Romanenko identified ticks based on morphology. Tamara P. Mikryukova performed the molecular analyses. Elena V. Protopopova performed the molecular analyses. Mikhail Yu. Kartashov detected pathogens in ticks. Evgeny V. Chausov detected pathogens in ticks. Svetlana N. Konovalova detected pathogens in ticks. Natalia L. Tupota detected pathogens in ticks. Alexandra O. Sementsova detected pathogens in ticks. Vladimir A. Ternovoi performed the molecular analyses. Valery B. Loktev supervised parasitological and microbiological work.

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