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### ABSTRACT

From 2006 to 2011, in the Tomsk region (south of Western Siberia), eight species of pathogens were detected in birds and the ticks feeding on them: Tick-borne encephalitis virus (TBEV), West Nile virus (WNV), *Borrelia* spp., *Rickettsia* spp., *Bartonella* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Babesia* spp. The identification of a number of strains of viruses and bacterial genovariants related geographically with the Russian Far East, Eastern Siberia, China and Japan and confirms the possibility of the role of birds in the spread of pathogens in the direction of Western Siberia and back. Most of the species that breed and migrate in Western Siberia are of Eastern origin and mostly fly for wintering to South-East Asia. Among these species in our samples, *Phylloscopus proregulus* was a carrier of both TBEV and *Bartonella* spp.; *Luscinia calliope* were infected with both TBEV and *Borrelia*, while *Tarsiger cyanurus* were infected with WNV. © 2015 The Authors. Hosting by Elsevier B.V. on behalf of Far Eastern Federal University. This is an

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## Introduction

The role of birds as reservoirs for the maintenance and transmission of arthropod-borne infections has long attracted the interest of ornithologists and other experts, such as virologists, microbiologists, and physicians. The role of migratory birds in the spread of arboviruses has been confirmed by a series of All-Union interdisciplinary conferences held in the second half of the XX century in Novosibirsk (1969, 1972, 1978). Currently, interest in this issue has decreased, intensifying only in times of disease outbreaks associated with migratory birds in Russia as well as the rest of the world (avian influenza virus, West Nile virus, etc.). However, new methods and approaches allow for the identification of pathogens more accurately and rapidly to determine their diversity at the genetic level. Such identification could contribute to the early diagnosis and treatment in the case of human suction of ixodic tick carriers of the pathogen. Unfortunately, there is low interest for these types of studies on the part of medical facilities, even though a significant portion of infectious diseases transmitted by ticks are not cured or diagnosed.

Studies were conducted by a team of authors from 2006 to 2011 in the Tomsk region (south of Western Siberia). Eight infections in ixodic ticks and birds were found: tick-borne encephalitis virus (TBEV), West Nile virus (WNV), *Borrelia* spp., *Rickettsia* spp., *Bartonella* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Babesia* spp. (Moskvitina et al. 2008, 2014; Chausov et al., 2009; Mikryukova et al., 2014). In

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Western Siberia, the study of the natural infection foci is particularly relevant because the incidence of tick-borne encephalitis here is particularly high in Russia (Zhukova et al., 2002). In addition, since the beginning of the XXI century in the Tomsk region, the ornithocophilous tick *Ixodes pavlovskyi* has been registered (Romanenko and Chekalkin, 2004), which currently prevails in urban habitats over *Ixodes persulcatus*. The natural habitat of *I. pavlovskyi* is located in the territories of Altai, Gornaya Shoriya and Primorye (Filippova and Ushakova, 1967; Sapegina, 1969; Ushakova et al., 1969).

In total, 736 individual birds were caught from which 804 ticks were removed — larvae, nymphs and imagos of the genus *lxodes*. Organs (liver, spleen, brain) from 443 birds were analyzed for pathogens. The individuals belonged to 60 species of the following nine orders: Passeriformes, Anseriformes, Charadriiformes, Columbiformes, Galliformes, Piciformes, Apodiformes, Cuculiformes, and Falconiformes. A total of 378 larvae, nymphs and adult ticks were removed from the birds. All samples were analyzed for the presence of TBEV and WNV by polymerase chain reaction with reverse transcription (RT-PCR) and enzyme immunoassays (EIA). Furthermore, from 2009 to 2011, samples were analyzed using PCR for bacterial infection (*Borrelia, Rickettsia, Anaplasma, Ehrlichia, Bartonella*) and protozoa (*Babesia*).

In the Tomsk region, 333 species of birds were recorded, among which 30 species are sedentary, 48 are vagrant, 6 appear only in winter, and 216 are nesting. The migrants (migratory and passing) include more than 250 species. Except for the vagrant birds and birds appearing only in winter, a potential role in the transmission of contagious matter and maintenance of natural loci can be attributed to 279 species. The position of the Tomsk region in the center of Eurasia stipulates a wide dispersion of birds flying toward wintering sites (Moskvitin and Dubovik, 1969, 1977; Moskvitin and Strelkov, 1977; Moskvitin et al., 2010). There are five main flyway directions of the "Tomsk" birds: African – toward wintering sites in Western, Eastern and tropical Africa; European – toward the Mediterranean and Western Europe; Arabic – toward the Caspian Sea, the Arabian Peninsula, Syria, and Egypt; Indian – toward India, Pakistan, and Afghanistan; and East Asian (Indo-China, Philippines, Malaysia). Separately, the Australian way can be identified as the destination of the oriental cuckoo, Pacific swift and needletail swift (Ryabitsev, 2008). The main direction of flight for wintering is Southern. For "Tomsk" birds, of particular importance is the Indian way, which is followed by 124 species of birds (most species of ducks, waders, and rails as well as many species of raptors and passerines). In Africa, 84 species stay for wintering (including a number of duck species, ruff, marsh terns, nightjar, garden warbler, willow warbler, pied flycatcher, redstart, thrush nightingale and many others) and in Europe, 74 species stay for wintering (loons, grebes, bean goose, whooper, long-tailed duck, black scoter, scaup, fieldfare, and redwing). The Caspian Sea and the Arab world receive 66 species (mallard, goldeneye, lapwing, common terns, and gulls). A total of 62 species (Pacific golden plover, marsh snipe, pintail snipe, a range of warblers, greenish warbler, yellow-browed warbler, Pallas' warbler, dusky warbler, Radde's willow, nightingales - Siberian rubythroat, Siberian blue robin, whistler, bluestart, yellowhammer, pine bunting, little bunting and others) are associated with Southeast Asia. Most species (136) have only one wintering site, and 95 species fly away for the winter in two directions. Three wintering sites are known for 25 species. It is also known that the wintering of pochards and garganeys is located in four separate sites.

The list of countries with which the "Tomsk birds" are connected through migration routes includes: India, Syria, Iran, Egypt, Sudan, Israel, the Netherlands, Switzerland, Italy, Spain, Norway, Denmark, Sweden, Germany, Belgium, France, England, Japan and others. At the present moment, there is no information about the wintering of birds of the respective region in China. The wide spread of birds for wintering and their gathering in nesting areas allow for the introduction and emergence of various pathogens from geographically remote areas, which creates an additional risk factor in the form of recombination exposure.

The infestation of the studied birds with various pathogens consisted of: TBEV - 42.4%, WNV - 32.9%, *Rickettsia* - 18.8%, *Bartonella* - 15.7%, *Anaplasma* - 13.7%, *Babesia* - 12.7%, *Borrelia* - 12.2%, and *Ehrlichia* - 6.9%. Mixed infections are registered in more than half of the total number of infected birds - 67.6% of the cases. Most of the mixed infected birds (87.2%) carried two or three pathogens. A maximum of six markers of infections in urban areas were detected in an individual fieldfare, and seven markers were found in the common redstart (Moskvitina et al., 2014; Mikryukova et al., 2014).

Data on the phylogeography of pathogens showed that ticks of the region carried both the Siberian (72.2%) and Far Eastern (27.8%) strains of TBEV. Far eastern strains are more common in urban areas than in the suburbs (42.9 and 15%, respectively), suggesting that the genotype of Siberian TBEV is endemic for the Tomsk region, and the Far Eastern strains appeared afterward (Chausov et al., 2009). The same is supported by the smaller variety of options of TBEV of the Far Eastern genotype — two genogroups, one of which turned out to be close to the prototype strain 205 TBE.

Phylogenetic analysis of WNV found in ticks from the territory of the Tomsk region allowed referring it to genogroup Ia, which is identical to that previously identified in the Volgograd region (Lvov et al., 2004) and afterward in the Novosibirsk region (Ternovoy et al., 2004, 2007; Kononova et al., 2006) as well as in Primorsky Krai (Ternovoy et al., 2006).

The identified *Borrelia* were attributed to *Borrelia garinii* that is widespread in Eurasia. Rickettsiae belonged to *Rickettsia tarasevichiae* and *Rickettsia raoultii* or perhaps to a new, not yet described, subspecies of the latter. In addition, rickettsial strains were similar to those from North-eastern China. *Ehrlichia* were associated with *Ehrlichia muris* (Chausov et al., 2009; Mikryukova et al., 2013), which was first described in Japan (Korenberg, 1999).

Thus, the identification of a number of strains of viruses and bacterial pathogen genovariations related geographically to the Russian Far East, Eastern Siberia, China and Japan, which confirms the possibility of the role of birds in the spread of pathogens in Western Siberia and the opposite direction. Many of the species of birds nesting and migrating in Western Siberia are of Eastern origin and are representative of the species that fly for wintering mainly in Southeast Asia. These species include: Pallas' warbler (carried TBEV and *Bartonella* at the same time), Siberian rubythroat (several individuals were infected with TBEV and *Borrelia*), and bluestart (WNV).

These findings demonstrate the urgent need for the development of inter-regional research in this area, increased public awareness, and heightened academic and governmental attention to this problem.

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