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Original article

Surveillance of tick-borne encephalitis virus in wild birds and ticks in Tomsk city and its suburbs (Western Siberia)

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ABSTRACT

To study the role of wild birds in the transmission of tick borne encephalitis virus (TBEV), we investigated randomly captured wild birds bearing ixodid ticks in a very highly endemic TBE region located in Tomsk city and its suburbs in the south of Western Siberia, Russia. The 779 wild birds representing 60 species were captured carrying a total of 841 ticks, *Ixodes pavlovskyi* Pom., 1946 ($n = 531$), *Ixodes persulcatus* P. Sch., 1930 ($n = 244$), and *Ixodes plumbeus* Leach. 1815 ($n = 66$). The highest average number of ticks per bird in a particular species was found for the fieldfare (*Turdus pilaris* Linnaeus, 1758) (5.60 ticks/bird) and the tree pipit (*Anthus trivialis* Linnaeus, 1758) (13.25 ticks/bird). Samples from wild birds and ticks collected in highly endemic periods from 2006 to 2011 were tested for the TBEV markers using monoclonal modified enzyme immunoassay (EIA) and RT-PCR. TBEV RNA and antigen were found in 9.7% and 22.8% samples collected from wild birds, respectively. TBEV markers were also detected in 14.1% *I. persulcatus* ticks, 5.2% *I. pavlovskyi*, and 4.2% *I. plumbeus* ticks collected from wild birds. Two TBEV strains were also isolated on PKE (pig kidney embryo) cells from fieldfare and Blyth's reed warbler (*Acrocephalus dumetorum* Blyth, 1849). Sequencing of 5′-NCR of TBEV revealed that all TBEV isolates belong to Far Eastern (dominate) and Siberian genotypes. Several phylogenetic subgroups included TBEV sequences novel for the Tomsk region. Our data suggest that wild birds are potential disseminators of TBEV, TBEV-infected ixodid ticks, and possibly other tick-borne infections.

Introduction

Tick-borne encephalitis virus (Flaviviridae, Flavivirus) is a causative infectious agent of tick-borne encephalitis, one of the most dangerous viral infectious diseases in the Russian Federation (Korenberg and Kovalevskii, 1999). A TBEV infection in humans is characterized by high fever and various lesions in the central nervous system. Mortality of TBEV infection is highly dependent on the involved genotype of the virus. Three genotypes, European, Siberian, and Far Eastern, have been described and named consistently after their highest territorial prevalence. Mortality is relatively low for European and Siberian genotypes (1–2%), but goes up to 30% for Far Eastern genotype (Gritsun et al., 2003a). The geographical distribution of different genotypes of TBEV is more complex than their names suggest and can be described as mixed and/or mosaic (Zlobin et al., 2007). For example, the Far Eastern TBEV genotype was detected in the Crimea, in the Baltic States, in the Ural Mountains, Western and Eastern Siberia (Kovalev et al., 2010; Iurchenko et al., 2012). The European genotype was recently found as far east as in the Republic of Korea (Yun et al., 2009).

The average incidence of TBE in Russia varied from 1.9 to 4.4 cases per 100,000 per year from 2001 to 2011 (State report Russian Federation, 2011c). Significant geographical variation in the incidence of TBE has been consistently observed across Russia. Higher than the national average incidence of TBE cases in humans has been reported in some regions in Siberia in the past years. Notably,

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in Tomsk region, the officially reported incidence of the disease was from 15.5 to 72.5 per 100,000 in the past decade (State report Tomsk, 2011b). The majority of these cases (74%) were within Tomsk City, the largest city in the region. Interestingly, the annual TBE incidence in the Novosibirsk region located only 200 km to the south of Tomsk has been much lower with 4.80–6.48 cases in the past 3 years (State report Novosibirsk, 2010). An even lower annual incidence (0.36–1.0 cases per 100,000) has been reported for the Far Eastern region, where TBEV was originally discovered in 1937 (State report Khabarovsk, 2011a). These data suggest unique eco-epidemiological features of the TBEV foci in Tomsk region.

Previously, we isolated viruses of both Siberian and Far Eastern genotypes from ticks in Tomsk and its suburbs. Far Eastern TBEV genotype was more prevalent in the urban foci, whereas the Siberian genotype was dominant in suburban foci (Chausov et al., 2009, 2010). The principal vectors and hosts for TBEV in Tomsk and other regions in Siberia are *I. persulcatus* ticks and small rodents, respectively. However, in the urban landscapes, it has recently been shown that *I. pavlovskyi* ticks are predominant and may support TBEV circulation in the city (Romanenko, 2011; Romanenko and Kondrat'eva, 2011). Interestingly, *I. pavlovskyi* ticks feed mainly on wild birds at all stages of their life cycle suggesting that birds may be involved in the eco-epidemiology of TBEV (Bolotin et al., 1977).

To determine the role of wild birds in the circulation and spread of TBEV in urban and suburban foci in Tomsk, we studied samples from wild birds and individual ticks collected from 2006 to 2011 for the presence of TBEV markers (viral RNA and antigen).

Materials and methods

Wild bird and tick sampling

In May to August in 2006–2011, wild birds were captured in 3 types of locations: urban landscapes (Stadium, University Grove, Southern graveyard); suburban settings (TNHZ, Kolarovo); rural and forest areas (Haldeevo, Maloyksinskyi), as previously described (Moskvitina et al., 2008). The capture of and experiments with wild birds were done in accordance with the permits 70 no. 024401 and 70 no. 024399 from the local government and regulations for working with wild animals. The ticks (adults, nymphs, and larvae) were collected from captured wild birds. The identification of tick and bird species was carried out as recommended by zoological taxonomic guides (Ryabitsev, 2001; Filippova, 1997). Ticks were stored at −70 °C until nucleic acid extraction and virus isolation. RT-PCR with primers specific to tick mitochondrial DNA was also used to identify tick species.

Detection of TBEV markers

TBEV antigens were detected by means of the immune-enzyme analysis as previously described (Ternovoi et al., 2007). Briefly, viral antigen was captured on the surface of 96-well polystyrene plates from 10% homogenates of ticks and internal organs of birds with monoclonal antibodies 10H10 against protein E of TBEV. Immune complexes were detected by EB1 monoclonal antibody against protein E of TBEV labeled with biotin, which was revealed by avidin-peroxidase. RNA extraction, reverse transcription, and detection of TBEV cDNA by RT-PCR was done as described previously (Chausov et al., 2009, 2010).

Virus isolation

Tick homogenates diluted 1:10 were used to infect PKE (pig kidney embryo) cells. The infected cells were incubated at 37 °C for 4 days in Eagle MEM medium supplemented with 2% fetal bovine serum. The cell culture supernatant was used to infect the next passage of PKE cells. The viral RNA for sequencing was purified from infected PKE cells after the 5th passage.

Sequencing of PCR products, structural and phylogenetic analysis

RT-PCR products were separated in 1.5% agarose gel and gel-purified using Wizard SV Gel and PCR Clean-Up System kit (Promega, USA) according to the manufacturer's instructions. Sequencing was performed using CEQ DTCS Kit (Beckman-Coulter, Cat# 608000, USA) on Beckman Coulter CEQ2000 XL genetic analyzer according to the manufacturer's instructions. All samples were analyzed twice in independent experiments. Multiple sequence alignment and phylogenetic studies were performed using Vector NTI Suite 10.0 (www.invitrogen.com) and MEGA 5 (Tamura et al., 2011).

Results

Ticks collected from wild birds

In the course of the study, we captured 779 wild birds and collected 841 larval, nymphal, and adult ixodid ticks from these birds, 244 *I. persulcatus*, 531 *I. pavlovskyi*, and 66 *I. plumbeus*. The maximum number of collected ticks from one fieldfare (*Turdus pilaris* Linnaeus, 1758) was 70 ticks including 19 larvae, 33 nymphs, and 18 adults. The list of captured bird species with all stages of ixodid ticks is presented in Table 1. In summary, we collected 3 species of ticks from captured wild birds: *I. persulcatus*, *I. pavlovskyi*, and *I. plumbeus*. The birds we captured in suburban locations were predominantly carrying *I. persulcatus*, whereas the urban birds mostly carried *I. pavlovskyi*. *Ixodes plumbeus* were found only on the sand martins. Ticks (all stages) were found from early May until mid August on birds with maximum numbers in June and July. Most frequently, we found larvae and nymphs – 47.9% and 37.7%, respectively. Adults constituted only 14.4%. Different stages of ticks (in various combinations) on a single bird were found in 46.2% of birds.

Detection of TBEV markers

To determine the prevalence of TBEV in wild birds, we analyzed 489 wild-bird samples, collected from May until mid August, for TBEV infection, for viral antigen and RNA. We found TBEV markers in 40 bird species out of 60 species tested. The prevalence of TBEV markers (RNA and antigen) in TBEV-positive bird species ranged from 14% to 63% with a very significant month-to-month and year-to-year fluctuation. On average, among TBEV-positive bird species, 9.7% of the individuals were found to be positive for viral RNA and 22.8% for viral antigen. The highest prevalences (>50%) of TBEV markers were found in fieldfares, common redstarts, and brambling birds, which could be explained by their relatively frequent contact with infected ticks and a more or less high individual susceptibility of these birds to TBEV.

To determinate the prevalence of TBEV markers in ixodid ticks collected from wild birds in TBEV foci, we studied 466 individual ticks (larvae, nymphs, adults). Specifically, we analyzed 71 *I. persulcatus*, 324 *I. pavlovskyi*, and 71 *I. plumbeus* ticks. Both TBEV RNA and antigen were found in 14.1% of *I. persulcatus*, 5.2% of *I. pavlovskyi*, and 4.2% of *I. plumbeus* ticks. We found TBEV-positive *I. persulcatus* larvae on the common redstart and the tree pipit and *I. pavlovskyi* larvae only on the fieldfare. TBEV-positive *I. persulcatus* nymphs were found on the fieldfare, the redwing, the Eurasian tree sparrow, and the tree pipit, and *I. pavlovskyi* nymphs on the fieldfare, the redwing, the Eurasian tree sparrow, and the tree pipit. Most of TBEV-positive ticks (larvae and nymphs) were collected from fieldfares and redwings (11 from 17). However, no infected *I. plumbeus*

Table 1
List of wild bird species infested by ticks in Tomsk TBEV foci in 2006–2011.

Species[a]	Captured birds/birds with ticks/ATB[a]	Ticks								TBEV markers	
		I. persulcatus				*I. pavlovskyi*				In wild birds with ticks	In ticks collected from birds
		Collected ticks	ATB[a]			Collected ticks	ATB[a]				
			Larvae	Nymphs	Adults		Larvae	Nymphs	Adults		
Tree pipit (*Anthus trivialis* Linnaeus, 1758)	4/4/13.25	34	4.50	4.00	0	19	3.75	1.00	0	+	+
Fieldfare (*Turdus pilaris* Linnaeus, 1758)	91/57/5.60	146	0.68	0.85	0.08	364	1.55	1.40	1.05	+	+
Redwing (*Turdus iliacus* Linnaeus, 1766)	9/8/3.34	5	0	0.56	0	25	0.89	1.11	0.78	+	+
Hooded crow (*Corvus cornix* Linnaeus, 1758)	5/2/2.2	8	0	0	1.60	3	0	0	0.60	+	–
Siberian rubythroat (*Luscinia calliope* Pallas, 1776)	9/6/1.44	9	0.33	0.67	0	4	0.33	0.11	0	+	–
Thrush nightingale (*Luscinia luscinia* Linnaeus, 1758)	2/1/1.5	1	0	0.50	0	2	1.00	0	0	+	–
Eurasian tree sparrow (*Passer montanus* Linnaeus, 1758)	3/3/1.33	1	0	0.33	0	3	0.67	0.33	0	+	+
Common redstart (*Phoenicurus phoenicurus* Linnaeus, 1758)	49/13/1.32	10	0.14	0.06	0	55	0.69	0.43	0	+	+
Chaffinch (*Fringilla coelebs* Linnaeus, 1758)	46/16/0.96	14	0.20	0.11	0	30	0.37	0.28	0	+	–
Brambling (*Fringilla montifringilla* Linnaeus, 1758)	11/3/0.54	5	0.18	0.27	0	1	0	0.09	0	+	–
Eurasian magpie (*Pica pica* Linnaeus, 1758)	2/1/0.5	0	0	0	0	1	0	0	0.50	+	–
Eurasian nuthatch (*Sitta europaea* Linnaeus, 1758)	6/3/0.68	1	0	0.17	0	3	0.17	0.17	0.17	+	+
Common rosefinch (*Carpodacus erythrinus* Pallas, 1770)	7/2/0.28	1	0	0.14	0	1	0.14	0	0	+	–
Blyth's reed warbler (*Acrocephalus dumetorum* Blyth, 1849)	64/12/0.22	5	0.06	0.02	0	9	0.06	0.06	0.02	+	–
European greenfinch (*Chloris chloris* Linnaeus, 1758)	6/1/0.17	0	0	0	0	1	0.17	0	0	+	NT
Great tit (*Parus major* Linnaeus, 1758)	121/10/0.09	2	0.02	0	0	8	0.02	0.05	0	+	+
Lesser whitethroat (*Sylvia curruca* Linnaeus, 1758)	21/1/0.05	1	0	0.05	0	0	0	0	0	+	–
European pied flycatcher (*Ficedula hypoleuca* Pallas, 1764)	111/4/0.03	1	0	0.01	0	2	0.02	0	0	+	+
Sand martin[b] (*Riparia riparia* Linnaeus, 1758)	106/12/0.63	66	0.40	0.10	0.12	0	0	0	0	+	+

The following species birds were negative for ixodid ticks, but positive for TBEV markers (total number of birds: $n = 106$): willow warbler (*Phylloscopus trochilus* Linnaeus, 1758); rock pigeon (*Columba livia* J.F.Gmelin, 1789); brown willow warbler (*Phylloscopus collybita* Vieillot, 1817); starling (*Sturnus vulgaris* Linnaeus, 1758); Pallas' warbler (*Phylloscopus proregulus* Pallas, 1811); thistle finch (*Spinus spinus* Linnaeus, 1758); common bullfinch (*Pyrrhula pyrrhula* Linnaeus, 1758); yellow hammer (*Emberiza citronella* Linnaeus, 1758); daw (*Corvus monedula* Linnaeus, 1758); pewit (*Vanellus vanellus* Linnaeus, 1758); goldfinch (*Carduelis carduelis* Linnaeus, 1758); willow tit (*Parus montanus* Baldenstein, 1827); greater spotted woodpecker (*Dendrocopos major* Linnaeus, 1758); waxwing (*Bombycilla garrulous* Linnaeus, 1758); coal tit (*Parus ater* Linnaeus, 1758); mallard *(Anas platyrhynchos* Linnaeus, 1758); shoveler *(Anas clypeata* Linnaeus, 1758); merganser (*Mergus merganser* Linnaeus, 1758); European teal (*Anas crecca* Linnaeus, 1758); hazel hen (*Tetrastes bonasia* Linnaeus, 1758).

[a] ATB, average number of ticks per bird.

[b] Only *I. plumbeus* ticks were collected. + any TBEV marker found in the bird species; none of the TBEV markers detected; NT, not tested.

larvae or nymphs were found. Generally, the prevalence of TBEV markers in *I. plumbeus* ticks was lower than in other ixodid ticks.

Characterization of the TBEV isolated from wild birds and ixodid ticks

We isolated 2 strains of TBEV designated as Tms B08-12 and Tms B08-14 (GenBank KC602117 and KC602118) on PKE cells from fieldfare and Blyth's reed warbler. All other attempts to isolate any viral strains from TBEV-positive samples on PKE cells failed. We used total viral RNA from Tms B08-12 and Tms B08-14 virus as well as viral cDNA fragments from 20 RT-PCR-positive individual ticks and 10 birds to determine viral sequences of the 5′-NCR of the viral genome for phylogenetic analysis as described previously (Chausov et al., 2009, 2010). Eleven TBEV sequences were obtained from *I. pavlovskyi* ticks and 9 sequences from *I. persulcatus* ticks.

The phylogenetic analysis of 5′-NCR sequences is presented in Fig. 1. We found 23 isolates of the Far Eastern genotype and 9 of the Siberian genotype. Both isolated TBEV strains Tms B08-12 and Tms B08-14 from birds belonged to the Far Eastern genotype. The Far Eastern sequences from birds and ticks segregate into several putative subclusters. Two subclusters grouped with the well-known prototype strains Glubinnoe/2004 and 205. We found Glubinnoe-like isolates in samples from ticks and wild birds. The sequences of the Siberian genotype isolates segregated into 3 putative subclusters: Zausaev-like, Kolarovo-like, and a third unique subcluster. The Zausaev-like sequences isolated from ticks and wild birds were very similar to the human isolate Zausaev (1983) from a patient with lethal chronic TBEV infection (Gritsun et al., 2003b). The Kolarovo-like sequences in this study were isolated from wild birds, and the TBEV strain Kolarovo-2008 was isolated from an *I. pavlovskyi* tick in the study site Kolarovo (Chausov et al., 2011). The c37-2 and 220308 sequences from *I. persulcatus* ticks were unique and did not cluster with any other Siberian strains of TBEV. The same is true for the Tms 10–18 isolate (from *I. pavlovskyi*). This isolate is a possible candidate for a new subcluster within the Siberian genotype.

In summary, we discovered a high genetic biodiversity of TBEV and novel TBEV sequences in wild birds and ixodid ticks in Tomsk region for 2 main genotypes of TBEV. We propose that circulation of TBEV between wild birds and ixodid ticks is a possible driving factor for the often high TBEV variability in highly endemic TBEV foci.

Discussion

The role of wild birds in the transmission of flaviviruses is well documented for several mosquito-borne flaviviruses such as West Nile, Japanese encephalitis, and Usutu viruses (Hubálek, 2004; Tsiodras et al., 2008; Lelli et al., 2008). TBEV as a tick-borne flavivirus is most often transmitted by *I. ricinus* ticks in Europe and *I. persulcatus* in northern Asia (Gritsun et al., 2003a). Previously, 6 species of ixodid ticks such as *Dermacentor reticulatus* Fabr., 1794 (*D. pictus*); the taiga tick, *I. persulcatus* P. Sch., 1930; *I. pavlovskyi* Pom., 1946; *I. trianguliceps* Bir., 1901; *I. plumbeus* Leach. 1815; and *Haemaphysalis concinna* Koch 1844 were reported as vectors for TBEV in Tomsk foci (Romanenko, 2004, 2009). Usually, only *I. pavlovskyi*, *I. persulcatus*, and *D. reticulatus* ticks attack humans. The other species of ixodid ticks (*I. trianguliceps*, *I. plumbeus*, *H. concinna*) have not been reported to attack humans probably because of their low abundance and close association with their main hosts.

The TBEV usually circulates in natural foci between ticks and small mammals (Gritsun et al., 2003a). The role of wild birds in the spread and circulation of TBEV is not well defined. It was suggested that wild birds participate in TBEV circulation in the natural foci of Tomsk region 50–60 years ago (Fedorov, 1958). Since then, dissemination of ticks infected with TBEV and/or other tick-borne pathogens by wild birds has been described in several other regions (Hubálek, 2004; Waldenström et al., 2007; Kempf et al., 2011; Dietrich et al., 2011; Movila et al., 2013). Moreover, TBEV viremia was demonstrated in mallards after experimental infection and thereby also their potential involvement in TBEV circulation in nature (van Tongeren, 1983). TBEV spread by migratory birds was also hypothesized by Suzuki et al. (2007) based on the phylogenetic analysis of TBEV sequences. They proposed that Far Eastern TBEV was exported to Japanese islands by migratory birds from continental regions of Far Eastern Russia.

The prevalence of TBEV markers in ixodid ticks varied broadly depending on the month of tick collection, the life stage of ticks, and weather conditions. Prevalence was found to range from 5.7% to 7.5% in *I. pavlovskyi* and *I. persulcatus* ticks (all stages) randomly collected in Tomsk and its suburbs (Chausov et al., 2010). These figures are generally consistent with our data on the finding of TBEV markers in ticks collected from wild birds. The high incidence of tick-borne infections in Tomsk region correlates with the very high frequency of tick attacks on humans. The cardinal feature of the current eco-epidemiological situation in Tomsk is the high abundance of *I. pavlovskyi* ticks and their apparent spread supported by wild birds living in suburban and urban environments. A similar situation was recently reported for Novosibirsk and its suburbs (Livanova et al., 2012; Malkova et al., 2012). Our study complements these reports confirming the importance of wild birds for the spread of *I. pavlovskyi* ticks in and around large cities in Siberia.

We used RT-PCR for the detection of TBEV RNA and EIA for the detection of TBEV antigen in tick and wild-bird samples. Viral antigen was detected more frequently than viral RNA. The high efficiency of antigen-capture EIA for detection of flaviviruses in field samples was previously demonstrated for dengue and Japanese encephalitis virus in swine, human, and mosquito samples (Duong et al., 2011; Voge et al., 2013; Mei et al., 2012). We, too, found that antigen-capture EIA is suitable for analysing tick homogenates (Chausov et al., 2009, 2010).

The genetic markers of several infections such as West Nile virus, *Borrelia* spp., *Rickettsia* spp., *Ehrlichia* spp. were also previously detected in ixodid ticks collected in Tomsk foci (Chausov et al., 2009). Other studies suggested that migratory birds are involved in the spreading of tick-borne infections such as *Rickettsia* spp., *Borrelia* spp., *Babesia* spp., and possibly also the European genotype of TBEV (Hamer et al., 2012a,b; Dietrich et al., 2011; Marsot et al., 2012; Movila et al., 2011, 2013). Together with our data, these studies suggest a possible role of wild birds in the dissemination of TBEV and other tick-borne infections that warrant further investigation.

We surveyed 779 captured wild birds and found ixodid ticks on 19 species of birds. Multiple contacts of fieldfares with *I. persulcatus* and/or *I. pavlovskyi* ticks were found with captured birds bearing 6 ixodid ticks on average. Taking into account the period of activity of ixodid ticks in this region and their feeding time on birds, we can estimate that one fieldfare may be in contact with approximately 60–120 ticks within one endemic season. Thus, the probability of multiple contacts between fieldfares and TBEV-infected *I. persulcatus* and/or *I. pavlovskyi* ticks within one endemic season appears very high. The relatively long lifespan of these birds (approximately 18 years) together with the high population size both in urban and suburban environments makes the contact of the birds with the virus virtually inevitable (Ravkin and Ravkin, 2005). The finding of TBEV markers in other species of birds suggests that several species of birds are involved in the circulation of TBEV during the high tick activity season. The high density of fieldfares in suburban areas and large cities supports the hypothesis that this bird species

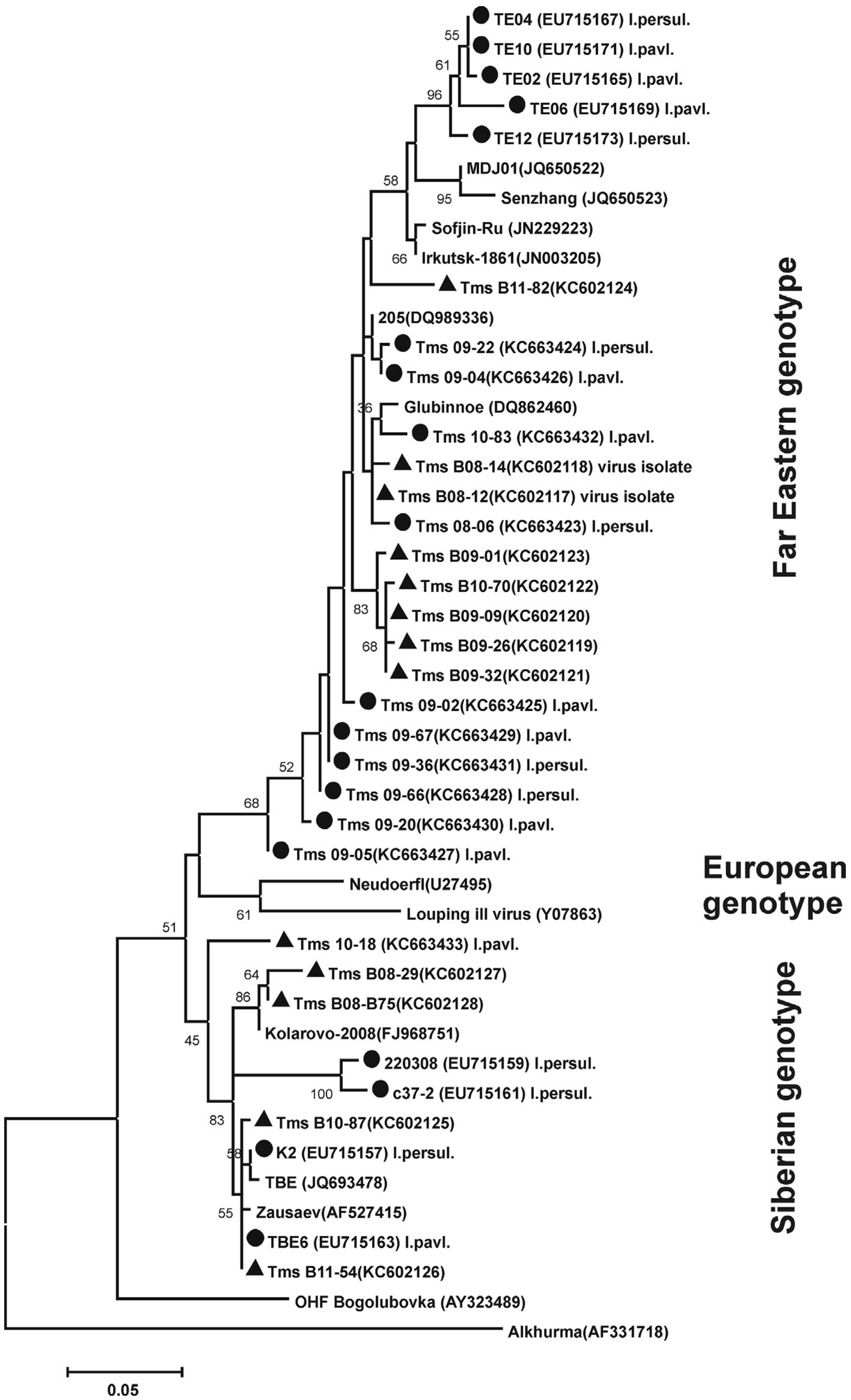

Fig. 1. The phylogenetic tree (neighbor-joining, Kimura two-parameter distances) of TBEV sequences from wild birds, ixodid ticks, and viral strains from TBEV foci in Tomsk. Bootstrap is shown when >60%. Alkhurma virus (AF331718) was used as an outgroup. • TBEV sequences isolated from ixodid ticks. ▲ TBEV sequences from wild birds. The sequences TE 12 (EU715173), 220308 (EU715159), TE04 (EU715167), c37-2 (EU715161), K2 (EU715157), Tms 08-06 (KC663423), Tms 09-22 (KC663424), Tms 09-66(KC663428), and Tms 09-36(KC663431) were isolated from *I. persulcatus* ticks. The sequences TE06 (EU715169), TE02 (EU715165), TE10 (EU715171), TBE6 (EU715163), Tms 09-02(KC663425), Tms 09-04(KC663426), Tms 09-05(KC663427), Tms 09-67(KC663429), Tms 09-20(KC663430), Tms 10-83 (KC663432), and Tms 10-18 (KC663433) were isolated from *I. pavlovskyi* ticks.

may be the principal disseminator for rapid short and possible also long-distance spread of TBEV-infected ticks throughout Siberia and possibly also other regions of Russia and beyond.

Fieldfares typically migrate from Siberia to southern regions of Europe for wintering. Tagged bird rings came back to Tomsk from Italy, France, and Belgium (Moskvitin, 1992). The finding of the Far Eastern genotype of TBEV in Crimea (Iurchenko et al., 2012) could be linked to the migration of infected wild birds (fieldfares) from Western Siberia to the basin of the Black Sea. The sand martin and the garden warbler are other species of migratory wild birds found with TBEV-infected ixodid ticks. The sand martin winters in Africa, whereas the garden warbler migrates to India (Moskvitin, 1992; Ravkin and Ravkin, 2005). The nomadic and migratory activities of the birds throughout Europe and Asia, their high densities in suburban areas and large cities suggests that wild birds may be implicated in (i) spreading of TBEV and other tick-borne infections by carrying infected ticks; (ii) transportation of ixodid ticks (over short and long distances) to new biotopes; (iii) establishing tick-borne infection foci in and around large cities; (iv) supporting TBEV biodiversity in highly endemic TBEV foci.

Sequencing of 5′-NCR of TBEV and phylogenetic analyses showed also that the Far Eastern genotype was dominant in studied birds and tick samples. Similar results were obtained previously when the Far Eastern genotype was predominantly found in ticks collected in Tomsk city, whereas the Siberian genotype was predominantly found in ticks in suburban foci (Chausov et al., 2009, 2010). We were able to isolate and sequence genomic fragments of the 2 newly isolated TBEV strains Tms B08-12 and Tms B08-14 from wild birds. Notably, viral isolates and RNA isolates from wild birds clustered with TBEV strain Glubinnoe/2004 in phylogenetic analyses. This strain was isolated from fulminant lethal human cases in the Far Eastern region of Russia. Rapid replication in PKE cells and a high level of infectious virion production are the major differences between Glubinnoe/2004 and other TBEV strains (Ternovoi et al., 2007). The high level of homology between 5′-NCR of the TBEV variants from wild birds and Glubinnoe/2004 suggests that the highly effective replication is typical for the isolated TBEV strains. Importantly, this is the first isolation of Glubinnoe/2004-like TBEV strains outside the Far Eastern region of Russia. TBEV strains isolated in China Senzhang and MDJ01 as well as other Chinese strains belong to the Far-Eastern subtype (Zhang et al., 2012; Wu et al., 2013). These strains cluster with common Russian Far Eastern strains such as Glubinnoe/2004, Kavalerovo, Svetlogorie, Primorye-52 in cluster III, Senzhang-like TBEV strains (Leonova et al., 2013). These molecular epidemiological data suggest that Far Eastern, Chinese, and Siberian TBEV have a similar coevolution and dissemination in these geographic regions. The role of wild birds in TBEV dissemination, even though appealing, remains hypothetical. Unfortunately, we could find only fragmentary reports (bird ring messages) of possible seasonal migration of birds in the triangle Far East–China–Siberia. The fast dissemination of avian influenza (H5N1) from China to Siberia by wild birds (Shestopalov et al., 2006) has shown that transmission of TBEV (Far Eastern genotype) by wild bird is possible.

The isolation of TBEV strains from fieldfares indicates that a persistent presence of the virus is possible in this species of birds. This is also indirectly supported by the following: (a) detection of TBEV antigen and RNA as well as live virus in these birds; (b) detection of TBEV markers and virus in ixodid ticks collected from birds; (c) the large size of populations of wild birds and ixodid ticks in Western Siberia. Additionally, TBEV persistence in wild birds is indirectly supported by proliferation of TBEV in different cell cultures and its potential to cause chronic forms of TBEV infection (Gritsun et al., 2003b). The duration of TBEV persistence in wild birds remains unknown. However, the closely related West Nile virus (WNV) is capable of persisting for more than 12 weeks in birds (Wheeler et al., 2012). If the time of TBEV persistence in wild birds is similar to that of WNV, the distance of spreading TBEV and possibly also other tick-borne infections by birds could be long.

In this study, we confirm the involvement of wild birds in the formation of natural foci of TBEV in Tomsk in 2006–2011. We propose that wild birds, particularly fieldfares, are potential disseminators for quick dissemination of Far Eastern and Siberian genotypes of TBEV, for TBEV-infected ixodid ticks, and possibly other tick-borne infections over short and perhaps also long distances.

Disclosure statement

The authors have no commercial associations that might create a conflict of interest in connection with the manuscript entitled "Surveillance of tick-borne encephalitis virus in wild birds and ticks in Tomsk City and its suburbs (Western Siberia)". All authors are working in non-profit federal organizations such as State Research Center of Virology and Biotechnology "VECTOR", Koltsovo, Novosibirsk Region, and Tomsk State University, Tomsk, Russia. No competing financial interests exist.

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