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Original article

Prevalence of Kemerovo virus in ixodid ticks from the Russian Federation

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ABSTRACT

The prevalence of Kemerovo virus in ixodid ticks collected in 2008–2012 from 11 regions of the Russian Federation was investigated by real-time reverse-transcription PCR (RT-PCR). The presence of Kemerovo virus in *Ixodes persulcatus*, *Ixodes ricinus*, and *Dermacentor reticulatus* was confirmed. Virus prevalence depended on the region and varied from zero to 10.1%.

Introduction

The biodiversity of tick-transmitted infections in the temperate latitudes of Eurasia remains unclear. A lot of investigations have addressed tick-borne bacterial infections, including those caused by *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia muris*, and members of the spotted fever group of Rickettsiales. The number of investigations which have addressed viral tick-transmitted agents are significantly smaller, and these studies focused mainly on tick-borne encephalitis virus (TBEV). However, approximately 30 tick-transmitted viruses have been detected in Eurasia (Hubálek and Rudolf, 2012). In addition to TBEV, several other viruses, such as Crimean-Congo hemorrhagic fever virus (CCHFV), Bhanja virus (BHAV), Eyach virus (EYAV), and Tribec virus (TRBV) are known to cause human disease.

A united expedition of Soviet and Czechoslovakian scientists in the Kemerovo region, headed by the Academician M.P. Chumakov, took place in 1962. Forty-eight Soviet and 6 Czechoslovakian scientists participated in this expedition. The basic mission of the expedition was a virological screening of *Ixodes persulcatus*, a vector of TBEV in Western Siberia, and collection of blood samples from tick-bitten individuals.

The samples were studied in vivo in newborn mice and chicken embryos and in vitro using porcine embryonic cells. As a result of the expedition, new information regarding TBEV was obtained. Also a novel virus was isolated. This virus was subsequently named Kemerovo virus (KEMV) following the suggestion of Chumakov et al. (1963).

The first strains of KEMV were isolated from homogenized suspensions of questing *I. persulcatus* females by Czechoslovakian scientists, headed by Libikova et al. (1970); 1035 adult ticks were collected near the Romanovka and Kuchum villages in the Kemerovo region and were pooled into 93 samples. Eight strains of KEMV were isolated and studied. Additionally, 2 strains of KEMV from patients with encephalitis were successfully isolated (Shapoval et al., 1964). More than 20 similar strains were isolated from *I. persulcatus* by other scientific groups from patients with encephalitis and from healthy tick-bitten individuals (Semashko, 1971).

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The differences between the KEMV and TBEV have been established in previous studies. Furthermore, the antigenic independence of KEMV and its affiliation with the Orbivirus genus within the Reoviridae family were shown (Borden et al., 1971).

In 1963, Libikova and colleagues collected 6319 adult *Ixodes ricinus* ticks in Czechoslovakia and isolated 22 virus strains that were closely related to KEMV (Libikova et al., 1964). The novel viruses were named Lipovnik virus (LIPV) and Tribec virus (TRBV) according to their geographic origin (Libikova et al., 1965; Gresikova et al., 1965).

These species were combined in a new classification group, the Kemerovo serological group, based on their antigenic relationships (Casals, 1965). In subsequent years, viruses with similar antigenic properties were isolated in different continents from various natural sources, mainly from ticks. Currently, KEMV, LIPV, and TRBV are included in the Great Island virus (GIV) group, which includes 36 members (King et al., 2012). However, genetic characteristics are known only for GIV, KEMV, TRBV, LIPV, and the Broadhaven virus (BRDV). Information regarding other members of the GIV is limited.

There is consensus that KEMV is a human pathogen and causes Kemerovo fever, a tick-borne infection that is characterized by fever, rash, and neurological disorders (Dilcher et al., 2012). Cases of Kemerovo fever were registered in Western Siberia in the 1960s and 1970s. Neutralization test results provided the basis for diagnosis. However, the interest in KEMV and Kemerovo fever decreased, and the diagnosis of Kemerovo fever has fallen out of practice in the last 40 years. Hence, KEMV has been classified as a forgotten infectious agent.

The virological features of KEMV have been studied (Semashko, 1971; Mikhailova, 1974). However, its basic ecological and epidemiological characteristics remain unknown.

In this study, we describe the prevalence of a well-known, but poorly studied member of the Orbivirus genus, Kemerovo virus (KEMV), in several regions of the Russian Federation.

We identified some endemic regions for KEMV in Russia using a specially designed real-time RT-PCR assay and assessed the prevalence of KEMV in ixodid ticks.

Materials and methods

Sample and nucleic acid extraction

We studied 3560 questing ixodid ticks (*I. persulcatus*, *Ixodes ricinus*, *Dermacentor reticulatus*, and *Dermacentor nuttalli*) collected in 11 regions of the Russian Federation from 2008 to 2012. All ticks were collected during field visits by flagging (flag size: 0.7 m × 1.1 m) from vegetation, as described in Bugmyrin et al. (2013). Each tick was frozen and stored at −70 °C until evaluation.

The ixodid species identity (excluding *D. nuttalli*) was validated by specific ITS2-targeting PCR (Rumer et al., 2011). *Dermacentor nuttalli* were determined based on morphological characters following Filippova (1977).

Total nucleic acids were extracted from the complete tick body by homogenization using a RIBO-prep DNA/RNA extraction kit (AmpliSens). Briefly, an individual tick was placed into the lysis tube, 100 μl of phosphate buffered saline pH 7.0 (Dako) was added, and homogenization was performed in a TissueLyser LT (Qiagen) for 5 min. The total nucleic acids were extracted and purified using the RIBO-prep DNA/RNA extraction kit according to the recommendations of the manufacturer. DNA/RNA was eluted with 50 μl of the RNase free elution buffer (AmpliSens) and stored at −70 °C.

Table 1
Design of the Kemerovo virus assay.

Primer/probe	Sequences (5′–3′)	Probe type	Product size
rt_Kem4f	tccgccaccctggaatgagac	TaqMan	116 bp
rt_Kem4r	tcaggatcggtcaaggccattc		
Kem_prb4	R6G-agccgtcttctgtccacgcagacg-BQ1		

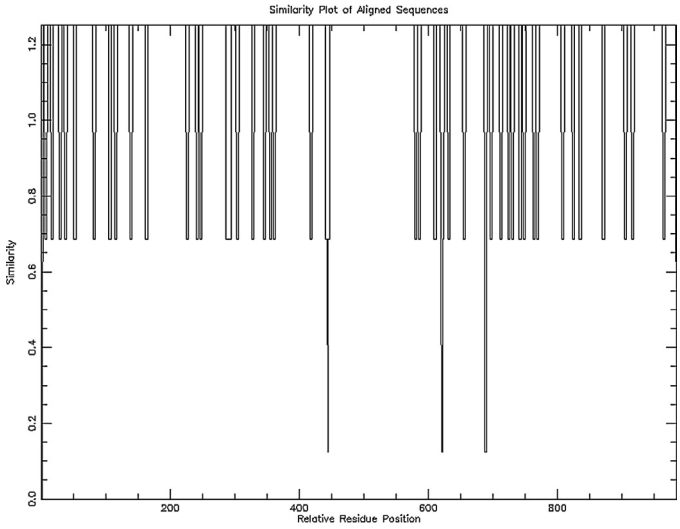

Fig. 1. Similarity of reference sequences (the region shown is 2000–3000 nt in the reference sequences HM543481 and KC288130). The area between 450 and 650 nt contains the target region for the KEMV assay.

Pol-gene real-time reverse-transcription PCR assay

For KEMV detection, a special real-time reverse-transcription (RT-PCR) assay was designed (Table 1) (Dedkov et al., 2012). A 116-nt fragment of the polymerase gene (VP1) (positions 2861–2976 nt in the reference sequences of KEMV, GenBank NCBI accession numbers HM543481 and KC288130) was selected as the target for amplification using PLOTCON (http://emboss.bioinformatics.nl/cgi-bin/emboss/plotcon) (Fig. 1).

The VP1 gene fragment was amplified and quantified in a 25-μl reaction containing 10 μl of RNA, 1 μl of rt_Kem4f (5 pmol per μl), 1 μl of rt_Kem4r (5 pmol per μl), 1 μl of Kem_prb4f (3 pmol per μl), 2.5 μl of dNTPs (1.76 mM, AmpliSens), 5 μl one-step RT-PCR mix2 FEP/FRT (AmpliSens), 0.25 μl of TM-revertase (AmpliSens), and 0.5 μl of TaqF polymerase (AmpliSens).

Thermal cycling parameters were 55 °C for 30 min, followed by 95 °C for 10 min, and then 45 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 10 s. Fluorescence was observed at 60 °C using a Rotor-Gene 6000 (Qiagen).

Recombinant dsRNA control

The PCR fragment covering the target region and some additional flanking nucleotides were generated using 5′-aggacggttacacattgtgccgactg-3′ and 5′-tcaaattagcgctcgggacgcgcc-3′ primers. The PCR product was ligated into the pGEM-T plasmid vector (Promega) under the control of T7 RNA polymerase promoter and transformed into *Escherichia coli* (strain XL1 blue) (Maniatis et al., 2003). Recombinant plasmids from individual clones were purified using Plasmid Miniprep kit (Axygen) and examined for the orientation and absence of mutations in the cloned PCR fragment by means of Sanger sequencing using an ABI-Prism 3500 XL device (Applied Biosystems). Plasmids with direct and reverse-oriented inserts were chosen. Two types of vector inserts were reamplified using plasmid-specific primers to obtain VP1 fragments under the control of T7 RNA polymerase promoter in both directions. The products were transcribed into RNA using T7 RNA polymerase (Fermentas) in the presence of NTPs and other buffer components