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BRIEF COMMUNICATION

Hantavirus RNA was not detected in *Dermacentor reticulatus* ticks

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Abstract

A total of 190 *Dermacentor reticulatus* ticks (80 males, 110 females) collected on the territory of Ostrów Lubelski, Suchawa, Zalutyń and Kazimierz Dolny (Lublin Province, eastern Poland) were examined by reverse transcription PCR and nested PCR methods for the presence of hantavirus RNA. None of the examined *Dermacentor reticulatus* specimens showed the presence of the hantavirus-specific RNA in spite of using two pairs of primers and the clearly positive results obtained with the positive control. Thus, the hypothesis about the possible participation of ticks in the transmission of hantaviruses was not confirmed.

Key words

hantaviruses, epidemiology, *Dermacentor reticulatus*, PCR, Poland

INTRODUCTION

Hantaviruses (HDV) are enveloped single-stranded RNA viruses measuring 120–160 nm that belong to family Bunyaviridae. Their genome is tri-segmented into small (S), medium (M) and large (L) segments [1]. Infections caused by hantaviruses pose an increasing global health problem and are regarded as 'emerging infectious diseases' [2, 3, 4, 5]. Hantaviruses are distributed worldwide with over 150,000 HDV cases being registered annually [3]. In Eurasia, two forms of the disease are distinguished:

1) severe Haemorrhagic Fever with Renal Syndrome (HFRS) caused by Hantaan virus (HTNV), Dobrava virus (DOBV) and Seoul virus (SEOV), with the mortality rate amounting up to 20%;

2) considerably milder Nephropathia Epidemica (NE) caused mainly by the Puumala virus (PUUV) and Saaremaa virus (SAAV) [4, 5, 6, 7]. In America, there occurs the very severe Hantavirus Cardio-Pulmonary Syndrome (HCPS), caused mainly by the Sin Nombre virus (SNV) and Andes virus (ANDV).

Small mammals are both reservoir and vectors of the disease. PUUV is mainly transmitted by bank vole (*Myodes glareolus*), DOBV by yellow-necked mouse (*Apodemus flavicollis*), and SAAV by the striped field mouse (*Apodemus agrarius*) [2, 6]. A recent study carried out by our group on the territory of Lublin Province in eastern Poland revealed the presence of hantavirus RNA in 4 species of small mammals: *Apodemus agrarius, Microtus agrestis, Myodes glareolus, Sorex araneus* [8].

Human infection occurs mainly by inhalation of dust polluted with rodent excrements. Nevertheless, some other modes of infection are considered, including transmission by blood-sucking arthropods [9, 10, 11, 12, 13]. Houck et al. [9] detected RNA of the HCPS-causing Bayou hantavirus in trombiculid mites (chiggers) and an ixodid tick collected in Texas. Gamasid and chigger mites have been PCR positive for HTNV in the People's Republic of China [10]. The Chinese authors proved that HDV exists in the wild mites *Leptotrombidium scutellare* and could be transmitted by stinging [11, 12]. Thus, the role of mites in the transmission of the Old and New World hantaviruses is documented. Valiente Moro et al. [13] expressed an opinion that mites belonging to the super-family Dermanyssoidea should be considered as potential vectors of various pathogens, including hantaviruses.

Recently, the first focus of hantavirus disease (HDV) has been established in the Carpathian mountains in southeast Poland, close to foci described earlier in Slovakia [4, 7, 14]. A total of 13 serologically-confirmed clinical cases of HDV were described, of which 10 were HFRS cases caused by DOBV and 3 were NE cases caused by PUUMV [4, 7]. In seroepidemiological studies of forestry workers in eastern Poland, Knap et al. [15] and Grygorczuk et al. [16] found a positive response to hantaviruses in 2.5% and 8.7%, respectively, which indicates the possibility of occupational exposure.

As forestry workers in eastern Poland are often exposed to tick bite, an attempt was undertaken to screen *Dermacentor reticulatus* ticks collected in eastern Poland for the presence of hantavirus RNA.

MATERIALS AND METHODS

Collection of ticks. Unfed ticks were collected from spring to autumn seasons in 2011 and 2012 on the territory of Ostrów Lubelski, Suchawa, Zalutyń and Kazimierz Dolny (Lublin Province, eastern Poland). Ticks were collected by dragging a woolen flag over lower vegetation and litter along the paths and edges of coniferous and mixed forests, and on the meadows neighbouring the forests. After collection, the ticks were placed in plastic vials and stored at -80 °C for further investigation. A total of 190 *Dermacentor reticulatus* ticks (80 males, 110 females) were collected.

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RNA isolation. After thawing, the ticks were investigated individually. Each specimen was crushed separately in liquid nitrogen and then homogenized with a syringe needle and suspended in buffer containing guanidine isothiocyanate (GITC), inhibiting RNA-ase enzyme. Total RNA was extracted from homogenized ticks using the RNeasy Mini Kit (Qiagen, USA) according to the producer's instructions. The amounts of extracted RNA measured with NanoDrop ND1000 spectrophotometer (USA) were in the range of 30–80 ng.

Reverse Transcription Reverse transcription was carried out using the QuantiTect Reverse Transcription kit (Qiagen, USA) according to the producer's instructions.

PCR and nested PCR reaction. The reactions were performed according to Arai et al. [17].

To amplify hantavirus L-segment sequences, the polymerase chain reactions (PCR) were carried out with the following primers: For the region of the L-segment: outer HAN-L-F1: 5'-ATGTAYGTBAGTGCWGATGC-3' and HAN-L-R1: 5'-AACCADTCWGTYCCRTCATC-3'; inner HAN-L-F2: 5'-TGCWGATGCHACIAARTGGTC-3' and HAN-L-R2: 5'-GCRTCRTCWGARTGRTGDGCA-3'.

The first PCR reaction was carried out in a 50 µl reaction volume which contained the following mix of reagents: 2.5 U *Taq* DNA polymerase (Qiagen, USA), 1 × PCR buffer containing 15 mM $MgCl_2$, additional 1.25 mM $MgCl_2$ (all from Qiagen, USA), 250 mM each of dNTP (Fermentas, Lithuania), 0.25 µM of each of primer (Eurogentec, Seraing, Belgium), nuclease-free water (Applied Biosystems, USA) and 2 µl of cDNA (10 × diluted in nuclease-free water). The reaction was performed in a C1000 Thermal Cycler (BioRad, USA) under the following conditions: initial denaturation at 94°C for 30 sec, two-degree step-down annealing from 46°C –38°C for 40 sec, and elongation at 72°C for 1 min; then 30 cycles (denaturation 94°C for 30 sec, annealing at 42°C for 40 sec, elongation at 94°C for 30 sec); and final extension step at 72°C for 7 min.

Nested PCR reaction was carried out under the same conditions with 2 µl of the first amplification product. For detection of the specific reaction product of 347 bp, electrophoresis was performed in 2% agarose gel under standard conditions. After ethidium bromide staining, the strips were read under UV light.

As a positive control, the antigens of 6 hantaviruses (Hantaan, Sin Nombre, Puumala, Dobrava, Seoul, Saremaa) were retrieved from slides of the commercial kit for detection of anti-hantavirus IgG antibodies by immunofluorescence method (Euroimmune, Germany). RNA was isolated using Qiamp Viral RNA Mini Kit (Qiagen, USA) according to manufacturer's procedure. Nuclease-free water was used as a negative control.

The precautions to exclude contamination were as follows: One-use tips with filters (PCR-clean/dualfilter/sterile) and one-use Eppendorf test tubes (PCR-clean, free of detectable human DNA, DNase, RNase, PCR inhibitor) were used. The glass and plastic surfaces were treated with RNase Zap (Ambion, USA) which completely removes contamination with RNase.

RESULTS AND DISCUSSION

None of the examined *Dermacentor reticulatus* specimens showed the presence of the hantavirus-specific RNA, in spite of using 2 pairs of primers and the clearly positive results obtained with the positive control. This result is in line with our earlier study [18] in which we did not find hantavirus-specific RNA in 174 *Ixodes ricinus* ticks collected in the southeastern part of Lublin Province of eastern Poland. Thus, the hypothesis about the possible participation of ticks in the transmission of hantaviruses (put forward by one of the authors [J. D.]) [7, 15] repeatedly was not confirmed for an epidemiologically important tick species [19, 20], recently demonstrated as a new vector for different pathogens, such as tick-borne encephalitis virus [21], *Babesia microti* [22] and *Toxoplasma gondii* [23]. For a definitive solving of this problem, an examination of a greater number of tick specimens and species from various biotopes is needed.

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