# Hantavirus infections in forestry workers

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#### Abstract

Forestry workers are exposed to wild animals, vegetation and soil, which may contain pathogens harmful to humans. Infection can have different pathways from inhalation, direct contact and vector-borne inoculation through the skin injuries. The aim of our study was to determine infection rates in forestry workers caused by different rodent-borne hantaviruses (HTV) in the central Posavina region, Croatia. We tested sera of 295 forestry workers for the presence of specific IgG antibodies for Puumala, Dobrava-Belgrade and Hantaan. Additionally at two different locations also in Posavina region 105 wild rodents were trapped and tested for HTV using direct fluorescence antibody test. Only bank voles were screened for presence of PUU N antigen using Western blot method. Nova Gradiška and Slavonski Brod are known as endemic regions for HTV. However, the high HTV seroprevalence of foresters (16.3%) was unexpected. 11.2 % of forestry workers were infected with Puumala hantavirus (PUUV) and 4.4% with Dobrava-Belgrade hantavirus (DOBV) and rest of 0.7% were positive for both viruses. Overall 14% of rodents trapped on two locations were tested positive for HTV. In lungs of 26.3% of bank voles virus antigen was detected. Such high infection rates in forestry workers show how important is to monitor rodent populations and to analyze prevalence of particular rodent born zoonoses.

Keywords: bank vole, Dobrava-Belgrade hantavirus, forestry workers, Puumala hantavirus, rodents

#### Introduction

Hantaviruses, which are members of a genus within the family *Bunyaviridae*, can cause either of two human zoonoses: hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS) (Schmaljohn and Hjelle, 1997). Hantaviruses are primarily rodent-borne and their transmission from reservoir hosts to humans may occur through inhalation of aerosols of dried excreta, inoculation through the conjunctiva, or entry through broken skin or rodent bites (Hjelle et al., 1995). Forestry workers as well as farmers, soldiers, hunters, campers, hikers, veterinarians, biologists and laboratory workers have the highest risk of infection. The aim this study was to determine infection rates in forestry workers caused by rodent-borne hantaviruses (HTV) in the central Posavina region, Croatia.

## Materials and methods

In the central Posavina region, Croatia, sera of 295 forestry workers and 60 controls (volunteers from the same region) was tested in seven different administrative forestry units for the presence of specific Ig antibodies. The determination of specific Ig antibodys for Puumala and Dobrava-Belgrade virus was done using ELISA IgG test with antigen Hantaan 76-118, Puumala CG 18-20 and Dobrava 907/5. At two different locations also in Posavina region 105 wild rodents were trapped and tested for HTV. Rodents were captured during September using snap traps at two different locations: Okučani (45°22′28″N, 17°17′05″E) and Nova Gradiška (45°18′30″N, 17°17′10″E) (approximately 20 km apart). Sampling of small rodents was done at a hillside on Psunj mountain, 400 meters above sea level, in forests where common beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) predominate. Captured rodents were tested for HTV using direct fluorescence antibody test from kidney and lung tissue. Only bank voles were screened for presence of PUU N antigen using Western blot method described previously (Plyusnin et al. 1995). Total RNA isolated from rodent lung tissues was reverse transcribed followed by PCR amplification with primers specific for PUUV medium (M) or small (S) genome segments (data previously published and described in Cvetko et al. 2005).

## Results

High percentage of forestry workers (16.3%) was found to contain HTNV-specific antibodies. From total of 295 forestry workers in 11.2 % Puumala hantavirus (PUUV) and in 4.4%Dobrava-Belgrade hantavirus (DOBV) reactive antibodies were detected. The rest of 0.7% was positive for both viruses.

Rodents captured included bank voles (*Myodes glareolus*, n=57), yellow-necked mice (*Apodemus flavicollis*, n=35), and wood mice (*A. sylvaticus*, n=13). Overall 14.3% of rodents trapped on two locations were tested positive for hantaviruses. According to the direct fluorescence antibody test wood mouse (*A. sylvaticus*) had 23.1% positive individuals. Within bank voles (*M. glareolus*) there was 14.0% of infected animals and 11.4% within captured yellow-necked mice (*A. flavicollis*). Western blot method showed that *M. glareolus* as the most abundant species had antigen presence in lungs of 15 individuals (26.3%). Positive *M. glareolus* were found at both locations (see detailed results in Cvetko et al. 2005).

## Discussion

Forestry workers are exposed to wild animals, vegetation and soil, which may contain eukaryotic parasites, fungi, bacteria and viruses harmful to humans. Rodent borne HTV are widely endemic in Europe and at least two different human - pathogenic species, PUUV and DOBV have been reported (Krüger et al., 2011). Our data shows high hantavirus seroprevalence in forestry workers, most like due to PUUV and DOBV infections. The natural reservoirs of hantaviruses are small rodents, and each of the various virus species is associated primarily with a single host species. Rodents captured in the same part of Croatia also show high infection rates. In Croatia the bank vole is considered to be the principal reservoir of PUUV, with yellow-necked field mice, long-tailed field mice, and striped field mice (*Apodemus agrarius*) playing minor roles in maintenance and transmission of other hantaviruses (Borčić et al., 1991; Markotić et al., 2002). The high percentage of infections with hantaviruses in forestry workers and rodents indicated that additional epidemiological measures as well as education of forestry workers about the risk of HTV transmission should be established. Further studies should be conducted, to address the area-, hantavirus- and rodent species specific modes of transmission, as well as mapping of endemic and hyperendemic foci.

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