SHORT REPORT: DUAL INFECTIONS WITH PUUMALA VIRUS AND LEPTOSPIRA INTERROGANS SEROVAR LORA IN A BANK VOLE (CLETHRIONOMYS GLAREOLUS)

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Abstract. Leptospirosis and hemorrhagic fever with renal syndrome are public health problems in Croatia. Diagnosis and epidemiology of these diseases are complicated because these two diseases are sympatric in certain areas. We describe a natural dual infection of Puumaala virus and a leptospire in a bank vole (Clethrionomys glareolus).

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).1 Leptospirosis, also a zoonotic infection, is caused by pathogenic members of the bacterial genus Leptospirosa.2 Both etiologic agents are rodent-borne and are maintained in persistently infected small rodents. Transmission from reservoir hosts to humans is possible through inhalation of virus-contaminated aerosol of rodent excreta (hantaviruses)3 or direct or indirect contact with contaminated environment (leptospira).3 Farmers, soldiers, hunters, campers, hikers, veterinarians, and laboratory workers have the highest risk of infection. The widespread geographic distributions of rodents harboring these pathogens indicate considerable disease-causing potential essentially worldwide. Dual infection of these two agents in humans has been described previously.4,5 We have reported dual infection with Dobrava virus (DOBV) and Leptospirosa interrogans serovar hardjo in one patient.5

During September 2002, we trapped rodents in northeastern Croatia at two sites: Okučani (45°22′28″N, 17°17′05″E) and Nova Gradiška (45°18′30″N, 17°17′10″E) (approximately 20 km apart) (Figure 1), which were recently shown to be natural foci of HFRS.6 The same region in northeastern Croatia is also known as an area where human and animal leptospirosis are common.7 Sampling of small rodents was done at a hillside on Psun mountain, ≤ 400 meters above sea level, in forests where common beech (Fagus sylvatica L.) and sessile oak (Quercus petraea (Matt.) Liebl.) dominate. Rodents were captured using Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) and trap nets. We followed animal experimentation guidelines approved by the American Society of Mammalogists.8 Rodents were identified to species, humanely killed, and dissected aseptically, with kidney and lung tissues collected for further studies. Small 50-kg pieces of frozen lung tissues were used for immunoblotting and kidney tissue was extracted for detection of hantaviral RNA. A Western blot was used to test for the presence of hantaviral nucleocapsid antigen. Homogenized specimens were sepa-
that this leptospire was most closely related to the genotype
*L. interrogans sensu stricto* serogroup *Australis* serovar lora.

Except for the Adriatic coastal and islands, all of Croatia
has been found to be endemic for HFRS. During HFRS
outbreaks in Croatia, small, focal, disease-endemic areas with
a high prevalence of antibody to hantaviral antigens were
reported at Mala Kapela mountain, Novska, and Dinara
mountain. In the last 2002 HFRS outbreak, a newly rec-
ognized disease-endemic area was confirmed near Nova
Gradiška (in northeast Croatia). In addition, a small survey
of rodents showed that the entire area of Posavina in north-
eastern Croatia was a wide natural focus of leptospires (Fig-
ure 1). Coincidentally, rodents most commonly found with
leptospires are these same four species. Serovar lora has
been isolated from yellow-necked field mice and genetically
characterized in Croatia.

We found one bank vole captured near Nova Gradiška with
a dual infection of PUUV and *L. interrogans* serovar lora
(Figure 1). Since these pathogens share the same reservoir
host, this suggests that their geographic distribution provides
opportunity for individual bank voles to be infected with both
pathogens.

Hantaviruses are principally associated with single rodent
hosts. There are no data on the prevalence hantavirus/leptospira
dual infections among rodents. It is also not known
what would be the risk for humans to encounter both infec-
tions at the same time. To the best of our knowledge, our
study is the first to demonstrate co-infection of a hantavirus
and a leptospira in a rodent reservoir host. From previous
studies it is known that northeastern Croatia has a status of a
combined focus of leptospirosis and hantavirus infection (Fig-
ure 1). We showed that dual infection with these two
pathogens may occur in bank voles under natural conditions.

Therefore, determining the presence and spread of these etio-
logic agents of human illnesses by testing local rodent popu-
lations might predict the potential for disease emergence. We
suggest that tests for both pathogens be used for diagnosis of
illnesses in patients suspected to have HFRS or leptospirosis
in Croatia and elsewhere in the region.

Co-infection of PUUV and *Leptospira* in a Bank Vole

**Figure 1.** Geographic distribution of hantaviral infections and leptospirosis in Croatia. *Clethrionomys glareolus* with dual infection was captured at trapping site Nova Gradiška (45°18′30″N, 17°17′10″E). HFRS = hemorrhagic fever with renal syndrome.
REFERENCES


