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Original Article

Clinical and virological characteristics of hantavirus infections in a 2014 Croatian outbreak

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Abstract

Introduction: Croatia is endemic for hemorrhagic fever with renal syndrome (HFRS), with both Puumala (PUUV) and Dobrava virus (DOBV) documented. Several large outbreaks were recorded in 1995, 2002, and 2012. We analyzed demographic, clinical, laboratory, and virological characteristics of HFRS cases detected in three geographically close natural foci (Ogulin, Slunj, and the Plitvice Lakes surroundings) during the 2014 outbreak.

Methodology: From January to December 2014, 122 patients with suspected HFRS were tested for hantavirus IgM/IgG antibodies using an indirect immunofluorescence assay (IFA). Cross-reactive samples were further tested using a western blot (WB). For hospitalized patients from Ogulin area, clinical and laboratory data were analyzed.

Results: Acute infection was documented in 57 (46.7%) patients, of whom 75.4% were hospitalized. Ten (8.2%) patients were found to be IgG seropositive. Patients were 15–69 years of age and predominantly male (74.5%). The outbreak started in winter months, with most cases recorded from May to July (80.7%). The most frequently reported symptoms were fever (96.3%), chills/shivering (62.9%), and lumbar pain (48.1%). Mild clinical form was found in 66.7% patients, moderate in 18.5%, and severe in 14.8% patients (all but one infected with PUUV). One patient died. Using IFA, 48.8% patients showed monotypic antibody response, while in 51.2%, cross-reactive antibodies were found. PUUV was confirmed in 94.7% and DOBV in 5.3% HFRS cases by WB.

Conclusions: Central mountainous Croatian regions are still highly endemic areas for HFRS. A higher percentage of severe PUUV infections could be at least partly associated with a patient's immune status.

Key words: hantaviruses; epidemiology; cross-reactivity; Croatia

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Introduction

Hantaviruses are diverse group of rodent-borne viruses that belong to the genus *Hantavirus* of the family *Bunyaviridae*. In humans, hantaviruses cause two clinical forms of disease: hemorrhagic fever with renal syndrome (HFRS), which occurs primarily in Europe and Asia, and the hantavirus pulmonary syndrome (HPS), which occurs in the Americas [1,2]. Puumala (PUUV), Dobrava (DOBV), and Saaremaa virus (SAAV) are known to cause HFRS in Europe. Hantaan virus (HTNV) is widely distributed in Asia, while Seoul virus (SEOV) is found worldwide [3]. The clinical presentation of HFRS varies from subclinical or mild to severe, sometimes fatal infection, depending in part on the causative virus. In general, PUUV and SAAV cause a mild form of disease with mortality below 1%. In contrast, DOBV causes a more severe clinical form, with mortality rates from 5% to 15% [4].

In Croatia, HFRS was described for the first time in 1952 [5]. Since then, sporadic cases as well as outbreaks have been continuously reported. Several small outbreaks were reported in the Plitvice Lakes (1967), Velika Gorica (1989), and Novska (1991) [6-8]. The first large outbreak, which included 125 cases, was recorded in 1995 in several well-known endemic foci (Gorski Kotar, Lika, Slunj, Mala Kapela, and west Slavonia) [9]. In addition, new natural focus of HFRS was described on the Dinara mountain [10]. During the

2002 Croatian outbreak, 401 cases of HFRS were recorded. Except for the coastal region and islands, the whole country was found to be an area endemic for HFRS [11-13]. In 2012, 154 patients were described, of whom 69.5% acquired the infection on the Medvednica mountain near the Croatian capital of Zagreb [14]. In the interepidemic years, approximately 10–20 cases were diagnosed annually [15]. Both PUUV and, to a lesser extent, DOBV have been found to circulate among humans in Croatia [12]. Besides PUUV and DOBV, SAAV is also detected in rodents [16-18]. In the 1990s, 1.6% of forest workers and 5.4% of the general population from the endemic territory of Lika and Gorski Kotar were seropositive for hantaviruses. In addition, 4.9%–5.5% of rodents from the same area were found to be positive for the hantavirus antigen [19].

We analyzed epidemiological, clinical, laboratory, and virological characteristics of HFRS cases detected in three geographically close natural foci during the 2014 Croatian outbreak. The data obtained in this study will contribute to the epidemiology of hantavirus infections.

Methodology

From January to December 2014, a total of 122 patients from three well-known natural foci for HFRS in Croatia (Ogulin and Slunj surroundings, the Plitvice Lakes) were tested at the National Reference Laboratory for Arboviruses, Croatian National Institute of Public Health. All of them presented with clinical symptoms suggestive of hantavirus infection, and the majority reported risk activities, mainly exposure to rodents. The analyzed area is located in the central mountainous part of Croatia, which represents the transition between the continental mainland and the Adriatic coast. The climate in this area is sub-mountainous and mountainous, with cool summers and harsh winters. Summer temperatures range from 22°C to 26°C in the continental and 15°C to 20°C in the mountain region. The mean winter temperature is 4°C to 0°C [20]. Serologic tests were performed using a commercial indirect immunofluorescence assay (IFA) based on BioChip Mosaic (Hantavirus mosaic; Euroimmun, Lübeck, Germany) for the simultaneous detection of IgM and IgG antibodies to the most important hantaviruses: PUUV, DOBV, HTNV, SEOV, and SAAV. Cross-reactive samples were further tested using a western blot (WB) method (Euroline Hantavirus; Euroimmun, Lübeck, Germany). For hospitalized patients from the Ogulin area, demographic data and clinical and laboratory parameters obtained from medical reports were analyzed. To classify disease severity, the following parameters were used: shock/hypotension, bleeding, fever ≥ 40°C, visual impairment, urea/creatinine levels, platelet count, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels, and chest X-ray [11].

Statistical analysis

The data were analyzed by the percentage of patients with symptoms/clinical signs or abnormal laboratory findings. Chi-square (χ^2) and analysis of variance (ANOVA) tests were used to compare differences between groups (MedCalc for Windows version 7.0). P value < 0.05 was considered to be statistically significant.

Table 1. Demographic characteristics, clinical symptoms, and laboratory parameters of 27 hospitalized patients with hemorrhagic fever with renal syndrome.

Characteristic	N	%
Gender		
Male	22	81.5
Female	5	18.5
Age distribution (years)		
20–29	4	14.8
30–39	8	29.6
40–49	7	25.9
50–59	5	18.6
60+	3	11.1
Underlying disease		
Diabetes	5	18.5
Hypertension	4	14.8
Cardiomyopathy	4	14.8
Malignant disease	1	3.7
Epilepsy	1	3.7
Clinical symptoms/signs		
Fever	26	96.3
Chills/shivering	17	62.9
Lumbar/back pain	13	48.1
Rash/petechiae	4	14.8
Respiratory symptoms	3	11.1
Renal findings		
Proteinuria	26	96.3
Hematuria	19	70.4
Oliguria	2	7.4
Polyuria	6	22.2
Laboratory findings		
Thrombocytopenia	23	81.5
Elevated urea/creatinine	20	74.1
Elevated transaminases	7	25.9
Outcome		
Recovered	26	96.3
Died	1	3.7

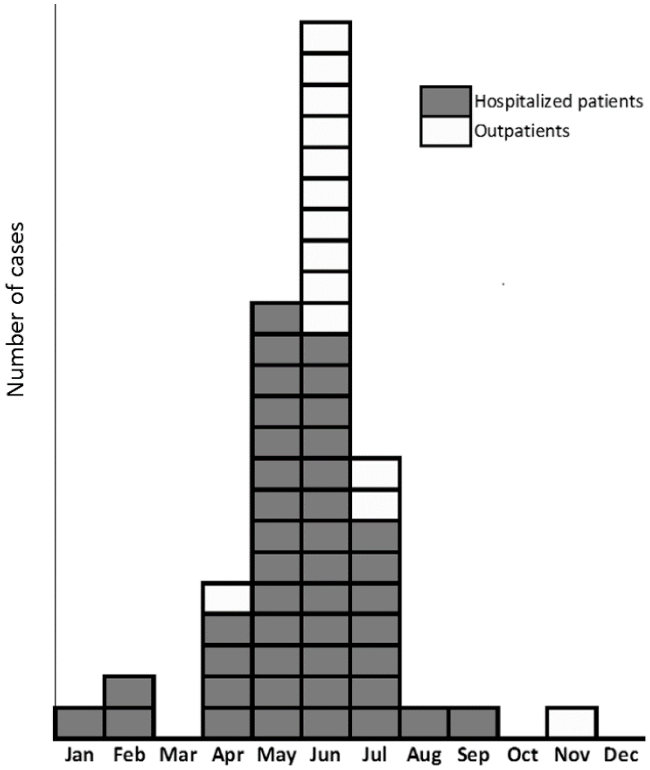
Results

Epidemiological data

Acute hantavirus infection (detection of IgM/IgG antibodies) was documented in 57/122 (46.7%) patients, while 10 (8.2%) patients showed previous exposure to hantaviruses (IgG antibodies). Of 57 serologically confirmed HFRS cases, 43 (75.4 %) were hospitalized and 14 (24.6%) were treated as outpatients. Among hospitalized patients, the majority (27; 62.8%) were treated at the General Hospital Ogulin from January to August 2014.

Males were infected more commonly than females (43; 75.4%), with a male-to-female ratio of 3:1. The mean age was 37.9 years (range, 15–69 years). Contact with rodents was noted in 16/27 (59.3%) of hospitalized patients: 9 (33.3%) reported professional exposure (4 forestry workers, 5 agriculture workers), 5 (18.5%) reported the presence of rodents surrounding their house/resting house, and 2 (7.4%) reported frequent stays in the forest area (Table 1). The outbreak started in the winter months, with the majority patients recorded from May to July (46; 80.7%). However, sporadic cases were reported until November (Figure 1). Geographical distribution of HFRS cases and IgG-seropositive subjects is presented in Figure 2. More than half of the patients were from Ogulin surroundings (32; 55.1%), followed by Slunj surroundings (13; 22.8%) and the Plitvice Lakes (8; 14.0%).

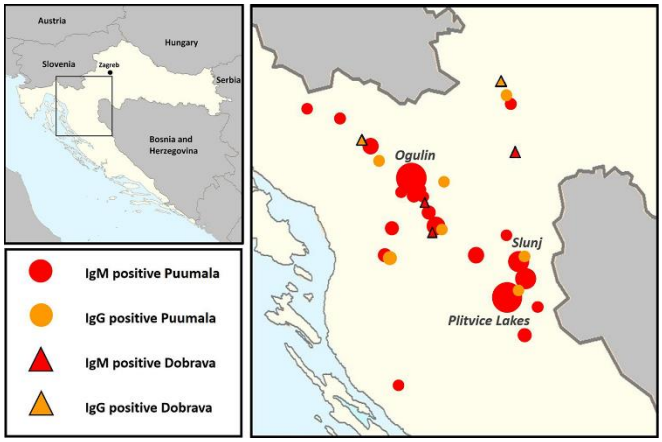
Figure 1. Seasonal distribution of hemorrhagic fever with renal syndrome cases in 2014 Croatian outbreak.



Virology results

Of 67 patients with detected hantavirus IgM and/or IgG antibodies, 32 (48.8%) showed monotypic antibody response, while in 35 (51.2%) patients, cross-reactive IgM and/or IgG antibodies were found by IFA. Using a WB, 59 (88.1%) samples were confirmed as PUUV infection and 8 (11.9%) samples as DOBV infection. Cross-reactivity was higher for IgG than for IgM antibodies (35/67; 51.2%, and 10/57; 17.5%, respectively). PUUV IgG-positive sera reacted most commonly with HTNV (21; 70.0%) and DOBV (18; 60.0%). A lower degree of cross-reactivity was also found with SEOV (9; 30.0%) and SAAV (8; 26.6%). DOBV-positive sera cross-reacted broadly with all tested hantaviruses (PUUV 80.0%; SEOV 80.0%; HTNV 100%; SAAV 100%). All tested samples with a monotypic response found by IFA also showed a monotypic serologic response by WB. Among cross-reactive samples, a low/moderate level of cross-reactivity was also found on WB, mainly for DOBV (Table 2). However, based on signal intensity, very strong band intensity to homologous virus antigen was found compared to weak/medium bands of related hantavirus antigens (Figure 3). Only two samples showed a very strong band to PUUV/DOBV/HTNV IgG antibodies. Detection of monotypic IgM response by IFA to PUUV in one sample and DOBV in the other

Figure 2. Geographical distribution of hemorrhagic fever with renal syndrome cases and IgG seropositive subjects. The size of circles/triangles is proportional to a number of positive samples (1-8).



Source: https://commons.wikimedia.org/wiki/Category:SVG_maps_of_Croatia#/media/File:Croatia_map_blank_standard_colors.svg

sample was suggestive of acute PUUV and DOBV infection.

Clinical presentation and laboratory results

Clinical symptoms and main laboratory findings of 27 hospitalized patients are presented in Table 1. The mean duration of symptoms before hospitalization was 4.2 (range, 2–8) days. The most common clinical symptoms on admission were high fever 39°C–40°C (26; 96.3%), chills/shivering (17; 62.9%), and lumbar pain (13; 48.1%). Three patients (11.1%) reported respiratory symptoms with interstitial inflammatory infiltrates on X-ray. Febrile phase was noted in 26 (96.3%) patients, with a mean duration of 6.7 (range, 3–10) days. Hypotensive phase was noted in 10 (37.0%) patients, oliguric phase in 2 (7.4%), and polyuric phase in 6 (22.2%) patients. Acute renal failure was noted in 20 (74.1%) patients, but only 1 patient (3.7%) required hemodialysis. More than half of the patients (15; 55.5%) reported an underlying disease: 5 (18.5%) diabetes, 4 (14.8%) hypertension, 4 (14.8%) cardiomyopathy, 1 (3.7%) received chemotherapy for malignant disease, and 1 had (3.7%) epilepsy. The most common abnormal laboratory findings were albuminuria (26; 96.3%) and thrombocytopenia (23; 81.5%). Other laboratory abnormalities included elevated urea and/or creatinine (20; 74.1%), hematuria (19; 70.4%), and elevated transaminases (7; 25.9%). Based on disease severity [11], a mild clinical form of HFRS was detected in 18 (66.7%) patients, moderate in 5 (18.5%), and severe in 4 (14.8%) patients. There was no difference in disease severity between genders (χ^2 = 2.812, p = 0.945) and age groups (p = 0.681).

All patients received symptomatic and supportive therapy (antipyretics and rehydration). Antibiotics were administered to patients with a strong inflammatory response. One patient was treated with hemodialysis to manage renal failure. The mean duration of hospitalization was 9.6 (range, 4–19) days. Twenty-six

Figure 3. Western blot showing signal intensity of hantavirus indirect immunofluorescence assay cross-reactive samples.

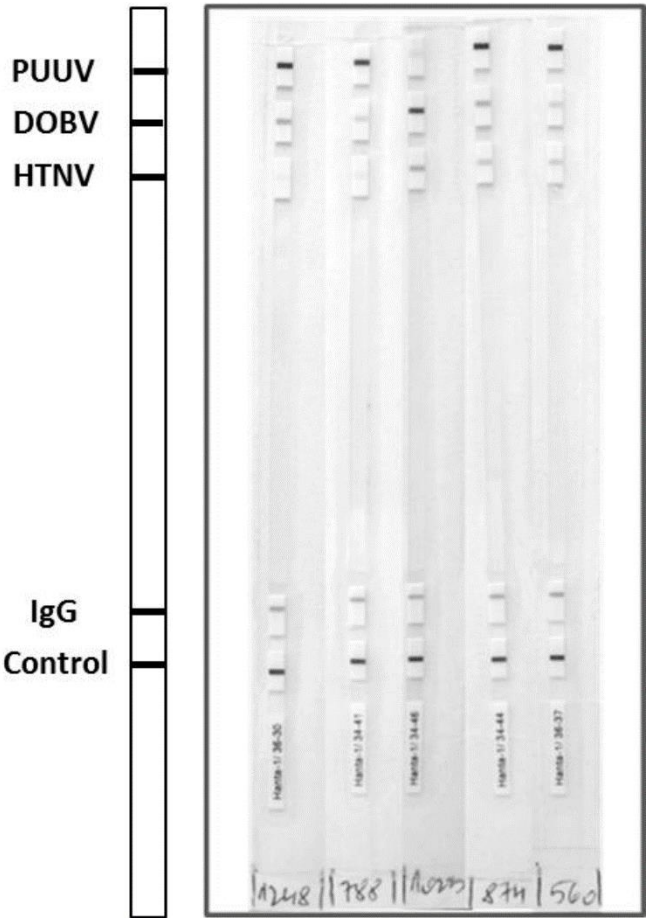


Table 2. IgG cross-reactivity patterns among hantaviruses by IFA and western blot.

	IFA	Western blot		
		Strong positive (+++)[a]	Positive (+, ++)[b]	Borderline (+/-)[c]
PUUV cross-reactive response (n = 30)				
HTNV	21 (70.0%)	1 (3.3%)	2 (6.6%)	2 (6.6%)
DOBV	18 (60.0%)	1 (3.3%)	3 (9.9%)	1 (3.3%)
SEOV	9 (30.0%)	NT	NT	NT
SAAV	8 (26.6%)	NT	NT	NT
PUUV monotypic response (n = 29)		29 (100)	0	0
DOBV cross-reactive response (n = 5)				
PUUV	4 (80.0%)	1 (20.0%)	2 (40.0%)	0
HTNV	5 (100%)	1 (20.0%)	1 (20.0%)	2 (40.0%)
SEOV	4 (80.0%)	NT	NT	NT
SAAV	5 (100%)	NT	NT	NT
DOBV monotypic response (n = 3)		3 (100%)	0	0

IFA: indirect immunofluorescence assay; PUUV: Puumala virus; HTNV: Hantaan virus; DOBV: Dobrava; SEOV: Seoul virus; SAAV: Saaremaa virus; NT: not tested; [a] Very strong band; [b] Medium to strong band; [c] Very weak band.

patients (96.3%) recovered, and one PUUV-infected patient (3.7%) died.

Three patients with a severe form of the disease were infected with PUUV (cases 1, 3, 4) and one with DOBV (case 2). Comorbidity was noted in three patients. Case 1 (M, 52 years) reported hypertension; during hospitalization, he developed massive polyuria. Case 2 (M, 20 years) reported diabetes and cardiomyopathy; he developed polyuria and prolonged lumbar pain. Case 3 (M, 35 years) was previously healthy; during hospitalization, he developed pericarditis and cardiac failure. Patients were hospitalized for 19, 16, and 14 days, respectively, and fully recovered. Case 4 (M, 48 years) was admitted to the hospital with high fever, severe intraorbital pain, and visual impairment. His previous medical history revealed myocardial infarction that occurred 7 years ago, when he was resuscitated and required hemodialysis thereafter for one month. During hospitalization, he developed multiorgan failure and died two months after disease onset.

Discussion

During the 2014 outbreak, a total of 209 HFRS cases were reported in Croatia (data from the Reference Epidemiology Centre, Croatian National Institute of Public Health). More than one-third of cases were from the central mountainous Croatian regions (Ogulin, Slunj, Plitvice Lakes).

Demographic characteristics of HFRS patients in this study (males 75.4%, mean age 37.9 years) were similar to previous Croatian outbreaks (males 76.6%–79%, mean age 36.6–40 years) [11,14] as well as to outbreaks in other European countries [21].

Although cases were reported throughout the year, this study detected the majority of HFRS cases from May to July, with a peak in June (40.3%). During the 2013/2014 winter season, the average monthly air temperature was above the multi-annual average, with corresponding temperature anomalies within the range of 2.4°C to 6°C [20], which could explain the earlier occurrence of an HFRS outbreak. A similar seasonal distribution was observed in 2002 [11], while in 1995, outbreak peak incidence shifted two months earlier (*i.e.*, in April [46%]) [9]. In addition to climate factors, environmental conditions favor the occurrence of HFRS. Several hantavirus outbreaks following years of beech mast tree, which promotes reproduction of bank vole, were recently reported [22,23]. Similarly, in autumn 2013, beech trees produced large quantities of seed (masts) in Croatia [24].

The most common clinical symptoms reported in our patients (fever, chills, malaise, headache, and back and abdominal pain) were similar to those previously reported in Slovenia, Germany, and Croatia [12,25,26]. However, there are some differences in the frequency of symptoms as well as in disease severity compared to previous Croatian outbreaks. Lumbar pain was reported less frequently (48.1%) compared to 50%–91.7% in previous outbreaks. Although respiratory symptoms appear frequently, especially in PUUV infections [4], in 2014, respiratory symptoms were present less frequently (11.1%) compared to 2002 and 1995 outbreaks (35.4% and 30%, respectively). Based on disease severity, 66.7% patients presented with a mild form of the disease in 2014, which is similar to the 2002 outbreak (65%) [11], while in 2012, a mild form was observed in 76.5% patients [14]. The highest frequency of severe disease was noted in 1995 (50%) [9], compared to 5.3% in 2002 [12], 4.7% in 2012 [14], and 14.8% in 2014.

Several studies showed that the severity of clinical symptoms depends on the patient's characteristics. In Sweden and Finland, gender-specific differences in symptoms as well as mortality rates were identified for epidemic nephropathy [27,28]. A similar observation was reported for HFRS in China [29]. Our results showed no association between gender and severity of disease ($p = 0.945$), which is similar to results reported in German patients [30]. In addition, the disease severity in Croatian patients differed among outbreaks and the interepidemic period (86.2% patients had a mild clinical form) [15]. A recently published German study showed that polyuria in HFRS reflected disease severity and is associated with prolonged hospital stay [31]. The duration of hospital stay for two patients with severe HFRS who developed polyuria was 19 and 16 days, respectively, compared to the average of 9.6 days.

Although a higher frequency of the severe form was observed in DOBV-infected patients [12], severe cases may also occur during PUUV infection [9]. In this study, three of four hospitalized patients who presented with severe hantavirus infection were infected with PUUV, of whom one died. In other Balkan countries, severe forms and fatal cases were associated mostly with DOBV infection [4,32]. In 1995, 20.5% HFRS cases were caused by HTNV, which could explain the high frequency of the severe form of the disease. Since the proportion of PUUV-infected Croatian patients was similar in 2012 and 2014 (95.3% and 94.7%, respectively), a higher frequency of severe infections in 2014 could be at least partly related to the patient's immune status, including comorbidities.

Due to the hazardous nature of hantaviruses and a short-term viremia in hantavirus-infected individuals, diagnosis of HFRS is usually based on serology [33]. The main limitation of serology is cross-reactivity, which often complicates interpretation of results, especially in areas where multiple hantaviruses co-circulate [34]. The results of this study indicated broadly serological cross-reactivity among hantaviruses. Using IFA, 17.5% IgM and 51.2% IgG positive samples cross-reacted with different hantavirus antigens. Cross-reactivity was found to be much higher for DOBV compared to PUUV. A high/very high cross-reactivity of DOBV IgG antibodies was observed with SEOV, HTNV, and SAAV antigens (80.0%–100%). These results are in accordance with the phylogenetic relatedness of hantaviruses. However, a high cross-reactivity (80.0%) was also found with PUUV, although PUUV is phylogenetically distantly from DOBV. A recently published multicenter study on the simultaneous detection of hantaviruses using IFA showed a high cross-reactivity of sera from DOBV-infected patients with SAAV, HTNV, and SEOV (60%–100%) and a moderate cross-reactivity with PUUV (up to 43%) [35].

In this study, PUUV IgG antibodies reacted highly/moderately with HTNV and DOBV (70.0% and 60.0%, respectively) and lower with SEOV and SAAV (30.0% and 26.6%, respectively). In the study of Lederer *et al*. [35], cross-reactivity between PUUV and HTNV IgG was somewhat higher (79%) compared to our results, while the reactivity to other tested hantaviruses was similar. Using WB, serotype determination was successful in most cross-reactive samples. In two samples that cross-reacted in both IFA and WB, a monotypic IgM response indicated the type of the causative virus.

In addition to acute HFRS cases, this study showed that 8.2% patients were IgG seropositive to hantaviruses. A study from 1991 conducted in the same geographic area showed a wide range of seroprevalence rates of 1.85%–3.26% among forest workers and 4.39%–8.89% in the general population [19]. A limited number of tested participants might be the cause of differences in seropositivity in these groups.

In Croatia, the bank vole (*Myodes glareolus*) is considered to be the principal vector for PUUV, with yellow-necked field mice (*Apodemus flavicolis*), long-tailed field mice (*Apodemus sylvaticus*), and striped field mice (*Apodemus agrarius*) playing a minor role in hantavirus transmission [12,19]. Several studies addressed the prevalence of hantaviruses in rodent reservoirs in Croatia. Between the 1980s and 2000s, hantavirus antigen was detected in 5.4% of rodents (*Clethrionomys glareolus*, *Apodemus flavicolis*) captured in the Plitvice region [19] and in 11.9% rodents captured on the Dinara mountain [10]. A study conducted in 2007–2008 revealed specific antibodies against hantaviruses in 25.5% of rodents from the mountainous area, while 29% of rodents from the lowland area were positive for either PUUV or DOBV RNA [36]. In addition, SAAV and DOBV-Kurkino genome sequences were also found in rodents, but so far, no human cases have been associated with these viruses in Croatia [18,37]. Moreover, a high infection rate of bank voles with PUUV (77.4%) was documented on the Medvednica mountain near Zagreb during the 2012 outbreak [17].

Conclusions

Our results indicate that central mountainous Croatian regions are still highly endemic areas for hantavirus infections, with co-circulation of PUUV and DOBV. Although the number of patients included in this study is limited, our results suggest that PUUV also caused a severe form of HFRS, with one fatal case. The respiratory symptoms that are frequently reported in PUUV infection were reported less frequently in our study. Since PUUV infection is generally mild to moderately severe, a higher percentage of the severe form recorded in this study indicates that disease severity could be at least partly associated with the patient's immune status.

References

1. Bi Z, Formenty PB, Roth CE (2008) Hantavirus infection: a review and global update. J Infect Dev Ctries 2: 3-23. doi:10.3855/jidc.317.
2. Sargianou M, Watson DC, Chra P, Papa A, Starakis I, Gogos C, Panos G (2012) Hantavirus infections for the clinician: from case presentation to diagnosis and treatment. Crit Rev Microbiol 38: 317-329.
3. Jonsson CB, Figueiredo LT, Vapalahti O (2010) A global perspective on hantavirus ecology, epidemiology, and disease. Clin Microbiol Rev 23: 412-441.
4. Avšič Županc T, Korva M, Markotić A (2014) HFRS and hantaviruses in the Balkans/South-East Europe. Virus Res 187: 27-33.
5. Radosevic Z, Mohacek I (1954) The problem of nephropathia epidemica Myhrman-Zetterholm in relation to acute interstitial nephritis. Acta Med Scand 149: 221-228.
6. Vesenjak-Hirjan J, Hrabar A, Vince-Ribaric V, Borcic B, Brudnjak Z (1971) An outbreak of hemorrhagic fever with a renal syndrome in the Plitvice Lakes area (preliminary report). Folia Parasitol (Praha) 18: 275-279.
7. Ropac D, Popović V, Baltić J, Kobetić T, Komatina G, Gligić A (1991) Epidemiological and clinical data on the outbreak of

hemorrhagic fever with renal syndrome. Med Jad 21: 107-114. [Article in Croatian.]
8. Borčić B, Puntarić D, Turković B, Aleraj B, Tvrtković N (1996) New natural focus of hemorrhagic fever with renal syndrome in Novska. Croat Med J 37: 115-118.
9. Kuzman I, Markotic A, Turcinov D, Beus I (1997) An epidemic of hemorrhagic fever with renal syndrome in Croatia in 1995. Lijec Vjesn 119: 311-315. [Article in Croatian.]
10. Ledina D, Bradarić N, Borcić B, Turković B, Ivić I, Bakić J, Erceg M, Tvrtković N (2002) Dinara - new natural focus of hemorrhagic fever with renal syndrome in Croatia. Croat Med J 43: 576-580.
11. Kuzman I, Puljiz I, Turčinov D, Markotić A, Turković B, Aleraj B, Andrić Z, Petković D, Tutek V, Herendić B, Iskra M, Pandak N, Misetić Z, Perić L, Jelaska D, Majetić-Sekovanić M, Ledina D, Mišić-Majerus L, Radonić R (2003) The biggest epidemic of hemorrhagic fever with renal syndrome in Croatia. Acta Med Croatica 57: 337-346.
12. Markotić A, Nichol ST, Kuzman I, Sanchez AJ, Ksiazek TG, Gagro A, Rabatić S, Zgorelec R, Avsic-Zupanc T, Beus I, Dekaris D (2002) Characteristics of Puumala and Dobrava infections in Croatia. J Med Virol 66: 542-551.
13. Mulić R, Ropac D, Gizdić Z, Sikić N (2003) What is new in the epidemiologic characteristics of hemorrhagic fever with renal syndrome in Croatia? Acta Med Croatica 57: 399-405.
14. Čivljak R, Papić N, Kuzman I, Topić A, Đaković Rode O, Tadin A, Cvetko Krajinović L, Vargović M, Svoboda P, Kurolt IC, Markotić A (2013) Epidemiology of hemorrhagic fever with renal syndrome in Croatia in 2012. In Book of abstracts, 10th Croatian Congress of Clinical Microbiology and 7th Croatian Congress of Infectious Diseases. 116 p.
15. Džepina G, Cvetko Krajinović L, Markotić A (2009) Clinical picture of HFRS during the nonepidemic period. Croat J Infect 29: 13-18.
16. Cvetko L, Markotić A, Plyusnina A, Margaletić J, Miletić-Medved M, Turk N, Milas Z, Avsic-Zupanc T, Plyusnin A (2005) Puumala virus in Croatia in the 2002 HFRS outbreak. J Med Virol 77: 290-294.
17. Tadin A, Bjedov L, Margaletic J, Zibrat B, Krajinovic LC, Svoboda P, Kurolt IC, Majetic ZS, Turk N, Rode OD, Civljak R, Kuzman I, Markotic A (2014) High infection rate of bank voles (Myodes glareolus) with Puumala virus is associated with a winter outbreak of haemorrhagic fever with renal syndrome in Croatia. Epidemiol Infect 142: 1945-1951.
18. Plyusnina A, Krajinović LC, Margaletić J, Niemimaa J, Nemirov K, Lundkvist Å, Markotić A, Miletić-Medved M, Avšič-Županc T, Henttonen H, Plyusnin A (2011) Genetic evidence for the presence of two distinct hantaviruses associated with Apodemus mice in Croatia and analysis of local strains. J Med Virol 83: 108-114.
19. Borčić B, Turković B, Aleraj B, Tvrtković N (1991) Hemorrhagic fever with renal syndrome in Croatia: incidence of infection in human and wild animal reservoirs. Lijec Vjesn 113: 320-323.
20. Meteorological and Hydrological Service, Croatia (2014) Climate seasonal reports. Available: http://meteo.hr/index_en.php. Accessed 24 January 2016.
21. Mailles A, Vaillant V, Haeghebaert S, Fradet MR, Capek I, Zeller H (2005) Increase of Hantavirus infections in France, 2003. Med Mal Infect 35: 68-72.
22. Ettinger J, Hofmann J, Enders M, Tewald F, Oehme RM, Rosenfeld UM, Ali HS, Schlegel M, Essbauer S, Osterberg A, Jacob J, Reil D, Klempa B, Ulrich RG, Kruger DH (2012) Multiple synchronous outbreaks of Puumala virus, Germany, 2010. Emerg Infect Dis 18: 1461-1464.
23. Reil D, Imholt C, Drewes S, Ulrich RG, Eccard JA, Jacob J (2015) Environmental conditions in favour of a hantavirus outbreak in 2015 in Germany? Zoonoses Public Health 63: 83-88.
24. Glavaš M (2013) Croatian forests. Available: http://casopis.hrsume.hr/pdf/202.pdf. Accessed 24 January 2016. [Article in Croatian.]
25. Pal E, Strle F, Avsic-Zupanc T (2005) Hemorrhagic fever with renal syndrome in the Pomurje region of Slovenia-an 18-year survey. Wien Klin Wochenschr 117: 398-405.
26. Latus J, Schwab M, Tacconelli E, Pieper FM, Wegener D, Dippon J, Müller S, Zakim D, Segerer S, Kitterer D, Priwitzer M, Mezger B, Walter-Frank B, Corea A, Wiedenmann A, Brockmann S, Pöhlmann C, Alscher MD, Braun N (2015) Clinical course and long-term outcome of hantavirus-associated nephropathia epidemica, Germany. Emerg Infect Dis 21: 76-83.
27. Klingström J, Lindgren T, Ahlm C (2008) Sex-dependent differences in plasma cytokine responses to hantavirus infection. Clin Vaccine Immunol 15: 885-887.
28. Hautala T, Hautala N, Mähönen SM, Sironen T, Pääkkö E, Karttunen A, Salmela PI, Vainio O, Rytky S, Plyusnin A, Vaheri A, Vapalahti O, Kauma H (2011) Young male patients are at elevated risk of developing serious central nervous system complications during acute Puumala hantavirus infection. BMC Infect Dis 11: 217.
29. Klein SL, Marks MA, Li W, Glass GE, Fang LQ, Ma JQ, Cao WC (2011) Sex differences in the incidence and case fatality rates from hemorrhagic fever with renal syndrome in China, 2004-2008. Clin Infect Dis 52: 1414-1421.
30. Krautkrämer E, Grouls S, Urban E, Schnitzler P, Zeier M (2013) No gender-related differences in the severity of nephropathia epidemica, Germany. BMC Infect Dis 13: 457.
31. Latus J, Kitterer D, Dippon J, Müller S, Artunc F, Segerer S, Alscher MD, Braun N (2014) Polyuria in hantavirus infection reflects disease severity and is associated with prolonged hospital stay: A systematic analysis of 335 patients from Southern Germany. Nephron Exp Nephrol 128: 111-115.
32. Tulumovic D, Imamovic G, Mesic E, Hukic M, Tulumovic A, Imamovic A, Zerem E (2010) Comparison of the effects of Puumala and Dobrava viruses on early and long-term renal outcomes in patients with haemorrhagic fever with renal syndrome. Nephrology (Carlton) 15: 340-343.
33. Meisel H, Wolbert A, Razanskiene A, Marg A, Kazaks A, Sasnauskas K, Pauli G, Ulrich R, Krüger DH (2006) Development of novel immunoglobulin G (IgG), IgA, and IgM enzyme immunoassays based on recombinant Puumala and Dobrava hantavirus nucleocapsid proteins. Clin Vaccine Immunol 13: 1349-1357.
34. Lindkvist M, Näslund J, Ahlm C, Bucht G (2008) Cross-reactive and serospecific epitopes of nucleocapsid proteins of three hantaviruses: prospects for new diagnostic tools. Virus Res 137: 97-105.
35. Lederer S, Lattwein E, Hanke M, Sonnenberg K, Stoecker W, Lundkvist Å, Vaheri A, Vapalahti O, Chan PK, Feldmann H, Dick D, Schmidt-Chanasit J, Padula P, Vial PA, Panculescu-Gatej R, Ceianu C, Heyman P, Avšič-Županc T, Niedrig M (2013) Indirect immunofluorescence assay for the simultaneous detection of antibodies against clinically important old and new world hantaviruses. PLoS Negl Trop Dis 7: e2157.

36. Svoboda P, Dobler G, Markotić A, Kurolt IC, Speck S, Habuš J, Vucelja M, Krajinović LC, Tadin A, Margaletić J, Essbauer S (2014) Survey for hantaviruses, tick-borne encephalitis virus, and Rickettsia spp. in small rodents in Croatia. Vector Borne Zoonotic Dis 14: 523-530.
37. Németh V, Oldal M, Madai M, Horváth G, Kemenesi G, Dallos B, Bányai K, Jakab F (2013) Molecular characterization of Dobrava and Kurkino genotypes of Dobrava-Belgrade hantavirus detected in Hungary and Northern Croatia. Virus Genes 47: 546-549.

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