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*Source / Izvornik:* **Collegium Antropologicum, 2009, 33, 37 - 42**

**Journal article, Published version**

**Rad u časopisu, Objavljena verzija rada (izdavačev PDF)**

*Permanent link / Trajna poveznica:* <https://urn.nsk.hr/urn:nbn:hr:105:018075>

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*Download date / Datum preuzimanja:* **2025-03-20**



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# The Occurrence of Hemorrhagic Fever with Renal Syndrome in Southern Parts of Bosnia and Herzegovina

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## ABSTRACT

*Bosnia and Herzegovina (B&H) has been known as an endemic region for hemorrhagic fever with renal syndrome (HFRS) for over 50 years. Multiple epidemics of this disease have been registered so far, especially in endemic parts of Central and Northeastern Bosnia, as well as the Sarajevo region. Seroepidemiological investigations demonstrate naturalization of Hantaviruses and their wide spread in B&H. However, there are no studies from the southern areas of B&H, and endemic foci of this disease are unknown. The aim of this study was to determine the distribution and serologic prevalence of Hantavirus infections by testing for specific IgG antibodies against hantaviruses in the population of Herzegovina. This study included two groups of participants. The target group consisted of 300 participants from exposed professional and population groups, and control group included 100 educators with lower exposure to HFRS. Identification of specific IgG antibodies against hantaviruses in 16 participants confirmed an initial assumption about the presence of Hantavirus infections in the region of interest. Seroprevalence of 5% was registered in the »exposed« and 1% in the »unexposed« group. Simultaneous circulation of Puumala (PUU) and Dobrava (DOB) viruses was discovered. The frequency of positive antibody results was higher in the population above 50 years of age, and three times more prevalent in men than at women. The highest proportion of exposed participants (80%) was registered in the municipalities which geographically belong to high or mountainous Herzegovina.*

**Key words:** hemorrhagic fever with renal syndrome, seroepidemiological investigation, Hantaviruses, Bosnia and Herzegovina.

## Introduction

HVBS is worldwide spread natural focus zoonosis caused by viruses of the genus Hantavirus<sup>1</sup>. Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) in Eurasia and Hantavirus pulmonary syndrome (HPS) in North and South America. In Europe, HFRS is caused by two types of hantaviruses – Puumala (PUU) and Dobrava (DOB)<sup>2-4</sup>. The disease appears in different clinical forms sporadically or in the form of less or larger epidemics.

The main reservoirs of HFRS are small rodents which excrete great amount of viruses by saliva, urine and feces. Humans have been infected by inhalation of infected aerosol in natural biotope of rodents, and probably by ingestion of infected food and water. Especially exposed

groups for acquirement of infection are soldiers, forestry workers, farmers, housewives and all those who are coming in contact with natural habitats of small rodents because of place of residence, profession or hobby (picnickers, hunters). Men are more often exposed to infection than women, so they get sick more often. The highest incidence of disease is at the age of 18–50 years<sup>5,6</sup>. It is quite difficult to accomplish diagnosis of HFRS based on clinical presentation only, so it should be confirmed by laboratory diagnostic. Serologic testing is the most frequent used diagnostic in clinical practise<sup>7</sup>. It is possible to establish the occurrence of disease in population by seroepidemiological investigations on particular area<sup>1,5,8,9</sup>.

Bosnia and Herzegovina (B&H) has been known as an endemic region for HFRS for over 50 years<sup>10,11</sup>. The first patient with HFRS was recognized in 1952, based on clinical observing<sup>12</sup>. Since then, the disease has been regularly occurring in our country and repeated epidemics have been registered, especially in the endemic parts of Central and Northeast Bosnia and in Sarajevo region. The first large epidemic of HFRS, and the largest one registered in Europe till then, appeared in 1967 near the town of Fojnica. There were 182 sick people registered out of which five people died<sup>18,10,13</sup>. The new large epidemic appeared in 1989, and the most cases were registered in Sarajevo region. A total of 152 sick people were involved, and nine died<sup>10,11</sup>. During the war in 1995, the largest epidemic of HFRS till then broke, and 367 sick persons were registered, out of which one died<sup>9,11,14,15</sup>. The last big epidemic of HFRS happened in 2002 with 98 sick people<sup>11,16</sup>. However, there are no studies from the southern areas of B&H, and endemic foci of this disease are unknown. Knowing that there were geomorphologic, climatologic, ecologic and epidemiologic characteristics in this area that were similar to known regions with natural foci of HFRS in B&H and neighboring countries, we assumed the natural occurrence of this zoonosis in Herzegovina region. The aim of this study was to determine the distribution and serologic prevalence of Hantavirus infections by testing for specific IgG antibodies against Hantaviruses in the population of southern regions of B&H.

## Subjects and Methods

Serologic and epidemiologic studies included two groups of participants. The target group consisted of 300 participants with the higher likelihood of exposure and acquisition of the infection (»exposed«) and included those who were likely to be exposed to HFRS through profession (forestry workers, soldiers) or residence (inhabitants of rural regions). This group included exposed inhabitants of several areas suspected to be foci of HFRS: Nevesinje (49 participants), Trebinje (54 participants), Konjic (67 participants), Jablanica (34 participants), Prozor-Rama (49 participants) and Livno (47 participants). The control group included participants with lower likelihood exposure to HFRS (»unexposed«). This group consisted of 100 educators from city of Mostar.

This investigation is cross-sectional, comparable to the control group. Epidemiologic methods (survey and epidemiologic inspection) were implemented and serologic testing of participants was performed. Epidemiologic inspection and survey were performed on all six localities with exposed population from June until October of 2006. An original survey instrument included the following questions: age, gender, profession, place of birth and residence, hobbies and chronic diseases. Participants were interviewed using direct personal questioning, while providing a blood sample was voluntary after each participant was provided with a detailed explanation of the

concept, logistics and meaning of the study. A control group was treated identically.

Blood samples were collected by vein puncture, and extracted sera were kept on -20°C till testing. Serologic testing was performed using the gold standard method – enzyme linked immunosorbent assay (ELISA) at the Department for Cellular Immunity, University Hospital for Infectious Diseases »Dr. Fran Mihaljević« (Zagreb, Croatia). First, participant sera were tested by commercial tests Hantavirus IgG DX Select (Focus Diagnostics, INC., California, USA) with a group antigen for hantaviruses which cause HFRS and HPS<sup>7,17</sup>. Testing was performed in accordance to the producer's instructions. This test was used for detection of specific antibodies to hantaviruses, but it couldn't give the answer which virus caused infection at our participants. Participant sera with detected specific antibodies by the first testing were tested by ELISA IgG test with antigen Hantaan 76–118 and Puumala CG 18–20. Hantaan 76–118 antigen gives clear cross reactions with Dobrava virus, and it is used for proving infection by this virus in Europe.

Symmetry of variables was tested by Kolmogorov-Smirnov test. Because of lack of symmetry of data, while comparing continued variables, Mann-Whitney U-test for independent samples was used.  $\chi^2$ -test was used for difference in division of nominal and ordinal variables, and Fisher exact test was used when expected frequencies were less. SPSS program system for Windows and Microsoft Excel were used for statistical analysis<sup>18</sup>.

## Results

Using commercial ELISA test, specific IgG antibodies to Hantaviruses were found at 15 (5.0%) out of 300 participants, and at one participant out of 100 participants in the control group. Positive sera of participants were tested by ELISA IgG test with the aim to define virus serotype. List of seropositive participants from the target group is shown in Table 1.

In Table 2, seropositive participants are shown by age. The highest infectiousness is at people from 51–60 years old (9.1%). There is no significant difference among positive and negative participants by age ( $\chi^2=5.39$ ;  $p=0.495$ ).

Out of 15 positive participants in target group, more than two thirds (11 or 73.3%) were males, but there is not statistically significant difference between male and female participants ( $\chi^2=3.27$ ;  $p=0.071$ ). Seroprevalance was 5.6% at male and 3.8% at female participants.

In semi-group with highly exposed professions, there were 13/15 seropositive participants of target group with seroprevalance of 6.6%, while in semi-group with less exposed professions, only 2/15 positive participants were found. That means that seroprevalance is significantly less and it is 2.0% (Tables 3 and 4). But, there is no statistically significant difference in number of positive participants related to exposition of profession ( $\chi^2=3.01$ ;  $p=0.083$ ).

**TABLE 1**  
LIST OF 15 SEROPOSITIVE PARTICIPANTS BY THE AGE, GENDER, PROFESSION, PLACE OF LIVING AND TYPE OF HANTAVIRUS INFECTION

No.	Age (years)	Gender	Profession	Municipality	Virus type
1.	42	Male	Forester	Prozor-Rama	Puumala
2.	67	Female	Housewife	Prozor-Rama	Dobrava
3.	22	Female	Student	Prozor-Rama	Dobrava
4.	36	Female	Housewife	Prozor-Rama	Dobrava/Puumala
5.	32	Male	Forester	Konjic	Puumala
6.	51	Male	Forester	Konjic	Puumala
7.	55	Male	Farmer	Konjic	Puumala
8.	35	Male	Farmer	Konjic	Puumala
9.	24	Male	Farmer	Konjic	Dobrava/Puumala
10.	65	Male	Pensioner	Trebinje	Dobrava
11.	57	Female	Housewife	Trebinje	Dobrava
12.	39	Male	Soldier	Livno	Puumala
13.	52	Male	Farmer	Jablanica	Puumala
14.	54	Male	Farmer	Jablanica	Dobrava
15.	72	Male	Farmer	Jablanica	Dobrava

Concerning locality, the highest infectiousness was recorded among participants in municipality of Jablanica (8.8%), and almost the same in Prozor-Rama (8.2%) and Konjic (7.5%). In municipality of Trebinje there were 3.7% of participants positive to Hantaviruses, and 2.1% of participants in municipality of Livno. There were no seropositive participants in Nevesinje (Figure 1). There is no statistically significant difference of seropositive participants concerning municipalities in which they live ( $\chi^2=6.52$ ,  $p=0.258$ ). Cartography of researched area with municipalities and number of seropositive participants is shown in Figure 1.

Specific IgG antibodies to PUU virus were found at 7 (46.7%) positive participants. DOB virus was serologically confirmed at 6 (40.0%) participants, and 2 (13.3%) participants had specific IgG antibodies also to PUU and

DOB virus (Table 1). There is no statistical difference in distribution of serotypes of Hantaviruses ( $\chi^2=6.52$ ,  $p=0.258$ ).

### Discussion

Appearance and clinical recognition of HFRS in B&H initiated seroepidemiological researches which confirmed naturalization of hantaviruses and their wide distribution<sup>8,15,16</sup>. It is supposed that there are significantly more people affected by HFRS than it is clinically diagnosed, as the disease is not often recognized because of different specter of clinical manifestations, and the disease is often mild and atypical. So, it is very possible that a number of unrecognized natural foci of the disease still exist.

So far, neither epizootiological, serological nor epidemiological investigations about manifestation and char-



Fig. 1. Areas under study, with municipalities and number of seropositive participants.

**TABLE 2**  
DISTRIBUTION OF SEROPOSITIVE PARTICIPANTS ACCORDING TO THE AGE

Age (years)	Participants (N)	Positive participants (N)	Percentage (%)
<21	6	0	0.0
21–30	24	2	8.3
31–40	81	4	4.9
41–50	82	1	1.2
51–60	55	5	9.1
61–70	32	2	6.3
>70	20	1	5.0
Total	300	15	5.0

**TABLE 3**  
DISTRIBUTION OF SEROPOSITIVE PARTICIPANTS WITH  
HIGHLY EXPOSED PROFESSIONS

Profession	Participants (N)	Positive participants (N)	Percentage (%)
Farmer	45	6	13.3
Forester	44	3	6.8
Soldier	49	1	2.0
Housewife	60	3	5.0
Total	198	13	6.6

acteristics of this disease in southern parts of B&H has been done. In this region, HFRS has been registered very seldom and sporadically, and there are some clinical and epidemiological remarks only incidentally noted<sup>10,19</sup>. Assuming that this natural focus zoonosis is also common in southern parts of B&H (especially in high forest parts), and with the aim to know the disease better, we made seroepidemiologic investigation, determining prevalence of antibodies to Hantaviruses in exposed population. Serologic finding of specific IgG antibodies against Hantaviruses confirmed an initial assumption about presence of Hantavirus infections in the region of interest. IgG specific antibodies persist for years after infection, so they are considered as very good indicators of population infectiousness<sup>20,21</sup>.

There were 15 (5%) positive sera among 300 participants in the target group what can be result of clinically manifested or unapparent infections which exist as sub-clinical or asymptomatic ones. Seroprevalence in control group was 1%, as specific IgG antibodies to Hantaviruses were detected at one participant. Although statistically difference in seroprevalence between target group (exposed) and control group (unexposed) was not determined, participants in exposed group were more often infected, and that proves our assumption that they are more exposed and with higher possibility to be infected. After we had taken more detailed epidemiologic anamnesis at serologically positive participant from control group, we found out that she spends a lot of free time in nature for recreation. Concerning stated results, there was a possibility and risk to get hantaviral infection, which the participant probably got over unapparently.

**TABLE 4**  
DISTRIBUTION OF SEROPOSITIVE PARTICIPANTS WITH LESS  
EXPOSED PROFESSIONS

Profession	Participants (N)	Positive participants (N)	Percentage (%)
Teacher	6	0	0.0
Worker	40	0	0.0
Services and administrative jobs	33	1	3.0
Pensioner	23	1	4.3
Total	102	2	2.0

Similar researches with the aim to determine seropositivity at different groups were made in other areas in B&H, neighboring countries, as well as in those European countries in which hantaviral infections are registered. According to World Health Organization (WHO) report, it is taught that approximately 4% of inhabitants of endemic parts are unapparently infected by hantaviruses<sup>22</sup>. In Sarajevo region, specific IgG antibodies to Hantaviruses were found at 2.51% of healthy examinees<sup>10</sup>. In Croatia, 1.6% of infected examinees were registered by seroepidemiologic screening of a group of foresters, and 5.6% of positive persons were found in general population<sup>23</sup>. During the epidemic of HFRS in Croatia in 1995, there were 5.6% of unapparently infected soldiers on Dinara mountain<sup>24</sup>. We can see that seroprevalence of our participants is similar and comparable to the statement of WHO and neighboring regions.

In our research, PUU and DOB viruses were confirmed at almost same numbers of participant, although PUU virus is much more often registered than DOB virus<sup>8,15,16</sup>.

Men are more often exposed to hantaviral infections, so they get sick more often, and that is confirmed by our research<sup>25</sup>. The most infected male participants are at the age of 51–60 with seroprevalence of 10.0%. In the groups of participants older than 60 years, we also find higher infectiousness than average for male participants. In total, a bit more than half of our positive participants are older than 50 years, and it proves that risk of hantaviral infection increases with age, and possibility to get infection increases with length of exposition.

HFRS appears at places where people come in contact with rodents during farming and works related to village households, during camping, mountaineering, hunting and in wars<sup>26,27</sup>. Majority of our participants took risk to get hantaviral infection because of the nature of their profession and life conditions in rural region.

Although there is no statistically important difference in geographical distribution of our seropositive participants, still 80% of them were registered in municipalities of Prozor-Rama, Jablanica and Konjic, which are parts of high or mountainous Herzegovina. These municipalities are adjacent to Sarajevo region and Central Bosnia region, which are well known as endemic regions and natural foci of HFRS. This fact confirms our real hypothesis that Hantaviruses are also common in southern parts of B&H, because of geological, climatological and ecological similarities of these areas.

## Conclusion

Initial hypothesis of this research was confirmed by seroepidemiologic testing and finding of antibodies to Hantaviruses, and the most important conclusions are the following:

- hantaviral infections and HFRS also appears in the southern parts of B&H;
- there were 5% of positive participants in the target group and 1% in the control group;

- PUU and DOB types of Hantaviruses circulate in this area;
- statistically important difference of infectiousness was not determined neither at certain localities, nor because of gender, profession and age of participants;
- but, hantaviral infectiousness was registered more often in high, mountainous areas of Herzegovina region, and at males and older population;
- it is very important to encourage further comprehensive epidemiological and epizootiological researches of hantaviruses in this region.

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## Acknowledgements

The paper was done within the project: Immunoreactions on hantaviruses and leptospirosis (143-1430115-0103), Centre for emergent and re-emergent infective diseases, and the program Hantaviruses and Leptospiroses (Ministry of science, education and sport, Republic of Croatia; Head: Alemka Markotić, MD, PhD.). Reagents for serological testing were given by Thomas G. Ksiazek, MD, PhD, Special Pathogens Branch Division of Viral and Rickettsial Diseases, Centers for Disease Control, CDS, Atlanta, USA

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## POJAVNOST HEMORAGIJSKE VRUĆICE S BUBREŽNIM SINDROMOM U JUŽNIM PODRUČJIMA BOSNE I HERCEGOVINE

### SAŽETAK

Bosna i Hercegovina (BiH) je više od 50 godina poznata kao endemska regija hemoragijske vrućice s bubrežnim sindromom (HVBS). Do sada je registrirano više epidemija ove bolesti, poglavito u endemskim dijelovima središnje i sjeveroistočne Bosne, te u sarajevskoj regiji. Seroepidemiološka istraživanja potvrđuju udomaćenost hantavirusa i njihovu distribuciju u BiH. Međutim, do sada nisu provedena nikakva seroepidemiološka istraživanja u južnim područjima BiH, niti su poznata prirodna žarišta ove bolesti. Cilj ovog istraživanja bio je seroepidemiološkim istraživanjem i nalazom specifičnih IgG protutijela na hantaviruse utvrditi rasprostranjenost i seroprevalenciju hantavirusnih infekcija u Hercegovini. U istraživanje su uključene dvije skupine ispitanika. Ciljana skupina obuhvaća 300 ispitanika iz izloženih profesionalnih i populacijskih skupina, a kontrolna skupina 100 prosvjetnih djelatnika, koji imaju manju izloženost HVBS-u. Nalazom specifičnih IgG protutijela na hantaviruse kod 16 ispitanika potvrđena je polazišna pretpostavka o

pojavnosti hantavirusnih infekcija i u istraživanom području. Kod ispitanika u izloženoj skupini bilježi se seroprevalencija od 5%, a u neizloženoj skupini 1%. Otkrivena je istodobna cirkulacija Puumala i Dobrava virusa. Češća prokuženost registrira se u starijih od 50 godina, a triput je veća kod muškaraca nego u žena. Najviše prokuženih ispitanika (80%) registrira se u općinama koje zemljopisno pripadaju visokoj ili planinskoj Hercegovini.