

Original Article

Investigation of *Orthohantavirus* Seroprevalence in Northern Rural Areas of Denizli Province, Turkey

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SUMMARY: Orthohantaviruses infect humans via inhalation of the viral particles in the excreta of infected rodents or direct contact with infected rodents. The infections caused by *Puumala orthohantavirus* (PUUV) and *Dobrava-Belgrade orthohantavirus* (DOBV) have been reported in Turkey. Serum samples of 346 healthy volunteers who are in the high-risk group of *Orthohantavirus* infections among the residents of Çal, Baklan, Çivril, and Bekilli counties, located in the northeast part of Denizli province, were used in this study. The samples were screened and confirmed using commercial ELISA and immunoblot tests, which detect IgG antibodies against DOBV, PUUV, and *Hantaan orthohantavirus*. IgG antibodies against PUUV were detected in the samples of 2 volunteers (2/346, 0.6%). One was a veterinarian and the other a farmer and they live in the Baklan and Çal counties, respectively. Both of them have a high probability of exposure to the virus, based on their occupation and living conditions. However, no symptoms were found in the clinical findings of both cases. This study is the first publication of reported PUUV seropositivities from the southwestern part of Turkey.

INTRODUCTION

Orthohantavirus is a single-stranded, enveloped RNA virus. It is transmitted to humans through virus-containing aerosolized excreta of rodents or some insectivores or rarely direct contact with them (1). It causes hemorrhagic fever with renal syndrome (HFRS) in Eurasia and Hantavirus pulmonary syndrome in the Americas (2).

Diagnosis of the infection is difficult because of the non-specific clinical manifestation and flu-like symptoms in the early period (3). Therefore, it is important to acquire epidemiological data from the local communities and know the baseline of seropositivity rates against orthohantaviruses in the regions.

The infections caused by *Puumala orthohantavirus* (PUUV) and *Dobrava-Belgrade orthohantavirus* (DOBV) in humans and rodents, and *Tula orthohantavirus* in rodents have been reported in Turkey (4–16). However, there is no data on the infections caused by *orthohantaviruses* in the Denizli province of Turkey. Thus, the serum samples of 346 volunteers who are in the high-risk group of *Orthohantavirus* infections

among the residents of the northern villages of Denizli province were evaluated for the presence of antibodies against orthohantaviruses.

MATERIALS AND METHODS

Samples group: This study was approved by the Non-Invasive Clinical Research Ethics Committee of the Pamukkale University (No: 06/2017). The serum samples of the 346 healthy volunteers, who are residents of the villages of Çal ($n = 220$, 63.6%), Baklan ($n = 68$, 19.6%), Çivril ($n = 54$, 15.6%), and Bekilli ($n = 4$, 1.2%) counties in the northeast part of Denizli province, were collected (Fig. 1). All of the volunteers were at high risk of *Orthohantavirus* infections because of their social and occupational conditions as identified by Jonsson et al., such as living in rural areas, being veterinarians, farmers, animal husbandmen, foresters, and soldiers (2).

Enzyme-Linked Immunosorbent Assay (ELISA) and Immunoblot Assay: The serum samples of the volunteers were screened for the presence of antibodies against the agents that cause HFRS in Europe and Asia. The commercial anti-Hantavirus Pool 1 Eurasia ELISA IgG (Euroimmun, Luebeck, Germany) and EUROLINE Hanta Profile 1 IgG (Euroimmun) kits were used for the detection of IgG antibodies against the DOBV, PUUV, and *Hantaan orthohantavirus* (HTNV) recombinant nucleocapsid antigens. The sensitivities were 78% for ELISA and 93% for immunoblot. The specificities were 100% for ELISA and 89% for immunoblot. The tests were performed according to the manufacturer's instructions. The results of both tests were evaluated together.

Received August 21, 2019. Accepted November 28, 2019.

J-STAGE Advance Publication December 25, 2019.

DOI: 10.7883/yoken.JJID.2019.330

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Fig. 1. Bekilli, Çal, Çivril, and Baklan districts of Denizli province where the volunteers reside, are indicated on the map.

RESULTS

Of the 346 volunteers, 164 (47%) were females and 182 (53%) were males. The mean age of the volunteers was 43 ± 13 (16–84) years. The occupations, living conditions, and daily activities of the volunteers are presented in Table 1.

Table 1. Distribution of the occupations and daily activities of the volunteers in the study

	n	%
Having animals in the barn/garden	319	92.2
Farmer	278	80.3
Outdoor accommodation (staying sometimes in the shelter, tent, field, etc.)	245	70.8
Touching a wild animal	91	26.3
Hunting	54	15.6
Veterinary/veterinary technician	27	7.8
Shepherd	13	3.8

Fifteen serum samples were positive with ELISA (4.3%). However, only 2 of them (2/346, 0.6%) were confirmed with immunoblot, and the specific IgG antibodies against PUUV recombinant nucleocapsid antigen were detected in these 2 individuals (Fig. 2). The ELISA-negative samples were also negative with immunoblot (Fig 3).

According to the demographic data, one of the PUUV infected individuals was a veterinarian and the other was a farmer (Table 2). Both of them were in their 30's and males.

DISCUSSION

The infections caused by *Orthohantavirus* species, which are known as HFRS agents, have been reported in Turkey (4,6,8–11,16). Kavukcu et al. reported that the seropositivity rate was 4.3% in nephropathy cases and 2.6% in healthy individuals (4). In 2009, 52.2% of the patients had antibodies against PUUV in the outbreak in



Fig. 2. Immunoblot strips which shows PUUV IgG positivity belonging to the individual number 2 and 125 and positive control.

the Bartın and Zonguldak provinces, which are located in the Western Black Sea Region (6,17). Sarıgüzel et al. reported that DOBV infection was detected in one patient who had tenderness in the lymph nodes and pharyngeal infection. The infection was confirmed using both serological and molecular tests (8). IgM positivity against DOBV was detected in another patient who had complaints of fatigue, pain, nausea, and vomiting. The patient died after being moved to the intensive care unit (9). Kaya et al. investigated the presence of antibodies in 100 patients diagnosed with *Orthohantavirus* between 2009 and 2012. The positivity was detected in 20 of these patients (10). The presence of IgG antibodies against *Orthohantavirus* in 626 healthy individuals was investigated and the seroprevalence was 3.2% in the Giresun province, which is located in the Eastern Black Sea Region (11).

While the seroprevalence was 0.6% in the individuals who are in the high-risk group of *Orthohantavirus* infections among the residents of the northern villages of Denizli province, the rate was higher in the studies conducted in different areas. This may be due to the differences in rodent populations depending on the



Fig. 3. Some of the immunoblot strips which shows negative results.

Table 2. Detailed data of PUUV seropositive individuals

Sample number	County	Village	Occupation	Age	Gender	Contact with wild animal	Bite by a wild animal	Outdoor accommodation	Hunting	Having animals in the barn/garden	Altitude
2	Baklan	İcikli	Veterinary	34	Male	Yes	No	Barrack	No	Yes	850 m Çivril and Baklan plain villages
125	Çal	Yukarıseyit	Farmer	35	Male	No	No	Field	No	Yes	1,840 m Büyük Çökelez mountain villages

Information on the PUUV seropositive individuals

climatic and geographic characteristics of the areas or the differences in *Orthohantavirus* prevalence at the sampling times.

The serological tests are the preferred methods for diagnosis and/or seroepidemiological monitoring of *Orthohantavirus* infections in humans. Generally, the molecular methods have lower sensitivity than the serological tests and they are insufficient in detecting viremia (18). In this study, the serum samples were screened using ELISA and typed with immunoblot for the detection of IgG antibodies against the recombinant nucleocapsid antigens of PUUV, DOBV, and HTNV. It was determined that both cases were PUUV infection.

While *Myodes glareolus* is known as the carrier of PUUV, there is no record of *M. glareolus* existence in the Denizli province. PUUV outbreaks and cases have been reported from Zonguldak, Bartın, and Düzce provinces in the Western Black Sea Region of Turkey (6,16,17).

PUUV infections were reported from the southwestern part of Turkey in this study, which is the first report of PUUV infections in this area. According to the demographic data, both PUUV seropositive individuals lived at an altitude of 850 m or more. The occupations of these 2 volunteers are veterinarian and farmer. Both of them have a high probability of exposure to the virus due to their working and living conditions. They stated that they have outdoor accommodation conditions but do not go hunting. In addition, the veterinarian stated that he had contact with a wild animal (Table 2).

Therefore, it is necessary to screen potential rodent hosts where seropositive individuals are exposed and at risk. Further studies in rodents in high-risk areas are important in terms of providing information about the situation and the prevalence of *Orthohantavirus* infections in the region.

Individuals who live in the rural areas, or are veterinarians, farmers, animal husbandmen, foresters, and soldiers are at high risk of *Orthohantavirus* infections (2). The occupational and social risks factors identified by Jonsson et al. were also detected in the seropositive individuals in this study (2). However, there were no clinical signs of *Orthohantavirus* infection in both cases. A mild form of HFRS called epidemic nephropathy is seen in PUUV infections. It is a well-known situation that, recovery without any or mild clinical findings in many PUUV cases may occur and the mortality rate of PUUV cases is very low compared to the cases caused by other HFRS agents (19). It is thought that the results of this study show subclinical and/or mild findings for both cases.

This study provided the first data on the existence of orthohantavirus seropositivity in the Denizli province. There are many provinces in Turkey where the prevalence of orthohantaviruses has not yet been detected. With similar studies, the high-risk areas where the virus can cause infection might be identified, and the

health management authorities can be better informed about possible outbreaks.

Conflict of interest None to declare.

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