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

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Running head: Orthohantavirus seroprevalence in Denizli
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 from Turkey.

Serum samples of 346 healthy volunteers who are in risk group with regards to *Orthohantavirus* infections among the residents of Çal, Baklan, Çivril and Bekilli counties located in northeast part of Denizli province were used in this study. The samples were screened and confirmed with commercial ELISA and immunoblot tests which detect IgG antibodies against DOBV, PUUV and *Hantaan orthohantavirus* (HTNV).

IgG antibodies against PUUV were detected in the samples of two volunteers (2/346, 0.6%). They are veterinary and farmer and they live in Baklan and Çal counties, respectively. Both of them have a high probability of exposure to the virus, with respect to either occupation or living conditions. But any symptoms were not found in the clinical findings of both cases.

This study has the feature of the first publication is reported PUUV seropositivities from the southwestern part of Turkey.

Introduction

*Orthohantavirus* is a single-stranded, enveloped RNA virus. It is transmitted to humans by 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 (HPS) in Americas (2).

Diagnosis of the infection is difficult because of non-specific clinical manifestation and flu-like symptoms in 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 about the infections caused by orthohantaviruses in Denizli province of Turkey. Thus, the serum samples of 346 volunteers who are in risk group with regards to 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 Pamukkale University, Non-Invasive Clinical Research Ethics Committee (No: 06/2017).

The serum samples of the 346 healthy volunteers, who are the 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 under risk with regards to orthohantavirus infections because of the social and occupational conditions which identified by Jonsson et al. like living in
the rural areas, being veterinarians, farmers, animal husbandries, foresters and soldiers
(2).

**Enzyme-Linked Immunosorbent Assay (ELISA) and Immunoblot Assay (IBA)**

The serum samples of the volunteers were screened for the presence of antibodies against the agents which causes to HFRS in Europe and Asia. The commercial Anti-Hantavirus Pool 1 Eurasia ELISA IgG (Euroimmun, Germany) and EUROLINE Hanta Profile 1 IgG (Euroimmun, Germany) kits were used for the detection of IgG antibodies against the DOBV, PUUV and *Hantaan orthohantavirus* (HTNV) recombinant nucleocapsid antigens. The sensitivities are 78% for ELISA and 93% for immunoblot. The specificities are 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.

**Results**

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

Fifteen serum samples were positive with ELISA (4.3%). However, only two of them (2/346, 0.6%) were confirmed by immunoblot and the specific IgG antibodies against PUUV recombinant nucleocapsid antigen were detected in these two 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 male.

Discussion

The infections caused by orthohantavirus species, which are known as HFRS agents, have been seen 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 of Bartın and Zonguldak provinces which are located in Western Black Sea Region (6, 17). Sarıgül et al. reported that DOBV infection detected in one patient who has tenderness in lymph nodes and pharyngeal infection. The infection was confirmed by both serological and molecular tests (8). IgM positivity against DOBV was detected in an another patient who has complaints of fatigue, pain, nausea and vomiting. The patient died after being removed 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 were investigated and seroprevalence was 3.2% in Giresun province which is located in Eastern Black Sea Region (11).

While the seroprevalence was 0.6% in the individuals who are in risk group with regards to orthohantavirus infections among the residents of the northern villages of Denizli province, the rate is higher in the studies conducted in different areas. This may be due to the differences in rodent populations depending on the climatic and
geographic characteristics of the areas or the differences of *Orthohantavirus* prevalences at sampling times.

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

While *Myodes glareolus* is known as the carrier of PUUV, there is no any record of *M. glareolus* existence from 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 have been 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 of PUUV seropositive individuals lived at an altitude of 850 meters or more. Occupations of these two volunteers are veterinary and farmer. Both of them have a high probability of exposure to the virus, with respect to working and living conditions. They stated that they have outdoor accommodation conditions but do not go hunting. Also, the individual who is the veterinary stated that he contacted with a wild animal (Table 2).

Therefore, it is necessary to screen potential rodent hosts where seropositive individuals are exposed to be at risk. Further studies to be performed in rodents in risky 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, veterinarians, farmers, animal husbandries, foresters and soldiers are at risk for Orthohantavirus infections (2). The occupational and social risks which 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 well-known situation that, the recovery without any clinical findings 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 to have a subclinical and/or mild findings of both cases reported with this study.

This study provided the first data on the existence of orthohantavirus seropositivity in Denizli province. There are many provinces have not been detected the prevalence of orthohantavirus in Turkey yet. With similar studies, the risky areas where the virus can cause infection might be identified and the health management authorities can be informed against possible outbreaks.

Conflict of interest

None to declare.

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

| Activity                                                   | 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 |
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 | İçikli  | Veterinary | 34  | Male   | Yes                      | No                     | Barrack              | No      | Yes                             | 850 meter Çivril and Baklan plain villages |
| 125           | Çal    | Yukariseyit | Farmer     | 35  | Male   | No                       | No                     | Field                | No      | Yes                             | 1840 meter Büyük Çökelez mountain villages |
Figure 1. Bekilli, Çal, Çivril and Baklan districts of Denizli province where the volunteers reside, are indicated on the map.
Figure 2. Immunoblot strips which shows PUUV IgG positivity belonging to the individual number 2 and 125 and positive control.
Figure 1. Bekilli, Çal, Çivril and Baklan districts of Denizli province where the volunteers reside, are indicated on the map.

Figure 2. Immunoblot strips which shows PUUV IgG positivity belonging to the individual number 12 and 125 and positive control.

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