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Serological Survey for Viral Pathogens in Turkish Rodents

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ABSTRACT: Wild rodents ($n=330$) were trapped around the villages of Altındere and Coşandere (Maçka, Trabzon Province), Ayder, Ortan, and Yolkiyi (Çamlıhemşin, Rize Province), and Bozdağ (Ödemiş, İzmir Province) in northeastern and western Turkey during April 2004. Samples were tested for arenavirus, hantavirus, and cowpox virus (family *Poxviridae*, genus *Orthopoxvirus*, CPXV) antibodies by using immunofluorescence assays (IFAs). Antibodies against arenaviruses were found in eight of 330 (2.4%) rodents. Arenavirus-seropositive animals were found from all study sites. Antibodies to Puumala virus (family *Bunyaviridae*, genus *Hantavirus*, PUUV) were detected in four of 65 *Microtus* voles tested. Of the PUUV-IFA-positive voles, one *Microtus guentheri lydius* was caught from Izmir, and one *Microtus roberti* and two *Microtus rossiaemeridionalis* were captured near Trabzon. All 264 *Apodemus* spp. mice tested negative for antibodies to Saaremaa virus (family *Bunyaviridae*, genus *Hantavirus*, SAAV); the single *Dryomys nitedula* tested negative for both PUUV and SAAV antibodies. Only one (0.3%) of the rodents, an *Apodemus sylvaticus* from Trabzon area, tested seropositive to CPXV. This is the first serologic survey for rodent-borne viruses in their natural hosts in Turkey. Although these preliminary results support presence of several virus groups with zoonotic potential, additional studies are needed to identify the specific viruses that are present in these populations.

Key words: Arenaviruses, hantaviruses, poxviruses, rodent-borne viruses, rodents, seroprevalence.

Although many rodent-borne viruses are known to occur in Europe and adjoining areas (Kallio-Kokko et al., 2005), little is known about the diversity, distribution, or host range of rodent-borne viruses in Turkey (Kavukcu et al., 1997). Hantavirus-specific IgG antibodies, as detected by immunofluorescence assay (IFA), have been reported in 4.3% of patients from Izmir, Turkey, with acute and chronic renal failure (Kavukcu et al., 1997). Human cases of hemorrhagic fever also have occurred in the Giresun and Trabzon areas. As part of our ongoing project on viral zoonoses in Europe and adjoining areas, wild rodents trapped from several sites in northeastern and western Turkey were tested for arenavirus, hantavirus, and poxvirus antibodies. Human infections with hantaviruses and arenaviruses are incidental to the natural cycle of the viruses; transmission to humans usually occurs by inhalation of aerosolized rodent excreta. Cowpox virus (family *Poxviridae*, genus *Orthopoxvirus*, CPXV) transmission generally is associated with skin abrasions. Wild rodents rarely transmit poxviruses directly to humans, and transmission usually occurs through contact with infected free-roaming cats, or other pets.

Arenaviruses (family *Arenaviridae*, genus *Arenavirus*) and hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are enveloped negative-stranded RNA viruses. These viruses are usually associated with specific rodent reservoirs. The only arenavirus reported from Europe is lymphocytic choriomeningitis virus (LCMV), which is associated with the common house mouse, *Mus musculus* (Armstrong and Sweet, 1963). Five hantaviruses, Puumala virus (PUUV), Dobrava-Belgrade virus (DOBV), Saaremaa virus (SAAV), Seoul virus, and Tula virus (TULV), are known from rodents in Europe; with the exception of TULV, these viruses may cause hemorrhagic fever with renal syndrome in humans (Kallio-

TABLE 1. Serologic results for rodents tested for rodent-borne viruses in Turkey, April 2004.

Province	Species	LCMV-IFA No. positive/no. examined (% pos)	PUUV/SAAV-IFA No. positive/no. examined (% pos)	CPXV-IFA No. positive/no. examined (% pos)
Trabzon	*Apodemus flavicollis*	0/3	0/3	0/3
	Apodemus mystacinus	0/10	0/10	0/10
	Apodemus sylvaticus s.l.	2/72 (3)	0/72	1/72 (1)
	Microtus roberti	0/4	1/4 (25)	0/4
	Microtus rossiaemeridionalis	1/8 (13)	2/8 (25)	0/8
Rize	*Apodemus flavicollis*	1/28 (4)	0/28	0/28
	Apodemus mystacinus	1/10 (10)	0/10	0/10
	Apodemus sylvaticus s.l.	1/82 (1)	0/82	0/82
	Dryomys nitedula	0/1	0/1	0/1
	Microtus roberti	1/11 (9)	0/11	0/11
	Microtus rossiaemeridionalis	0/7	0/7	0/7
Izmir	*Apodemus mystacinus*	0/7	0/7	0/7
	Apodemus sylvaticus s.l.	1/52 (2)	0/52	0/52
	Microtus guentheri lydius	0/25	1/25 (4)	0/25
	Microtus roberti	0/1	0/1	0/1
	Microtus rossiaemeridionalis	0/9	0/9	0/9
	Total	8/330 (2.4)	4/330 (1.2)	1/330 (0.3)

Kokko et al., 2005). Orthopoxviruses (family *Poxviridae*, genus *Orthopoxvirus*) are large DNA viruses found in a variety of vertebrates (Kallio-Kokko et al., 2005); rodents are the reservoir hosts of CPXV (Chantrey et al., 1999). The objective of this study was to serologically test wild rodents for antibodies against these rodent-borne virus antibodies to provide preliminary information on the diversity, distribution, and prevalence of these viruses in Turkey. Efforts also were made to confirm the presence of these viruses by using polymerase chain reaction (PCR).

Rodents ($n=330$; Table 1) were trapped with snap-traps (Finnish type) in several locations around the villages of Altındere and Coşandere (40°45′N, 39°37′E; 848 m above sea level, Maçka, Trabzon Province), Ayder (41°00′N, 41°03′E; 932 m) and Ortan and Yolkiyi (41°01′N, 40°59′E; 443 m, Çamlıhemşin, Rize Province) in northeastern Turkey; and in Bozdağ (38°21′N, 28°05′E; 1,438 m, Ödemiş, İzmir Province) in western Turkey during April 2004. Each site was trapped for 2 to 3 days. Traps ($n=300$) were placed in areas with signs of rodent activity and checked every 8 hr. Necropsies were done in a field laboratory, and samples of heart, lung, kidney, and spleen were collected; these samples were stored in sterile vials on dry ice in the field. In the laboratory, samples were stored at −70 C or in RNAlater® (Ambion Inc., Huntingdon, UK) at 4 C. Several sets of instruments were used for dissection, and they were sterilized immediately after use. Protective masks and gloves were used during sample collection. Samples were transported to the Department of Virology (Haartman Institute, University of Helsinki, Helsinki, Finland), where all testing was done.

In the laboratory, one half of the frozen heart (with blood) was placed in phosphate-buffered saline, 1% Triton X-100. This diluted blood was tested for antibodies to PUUV (voles) or SAAV (mice) by IFAs (Kallio-Kokko et al., in press). One *Dryomys nitedula* was tested for antibodies to both PUUV and SAAV. All rodents were tested for antibodies to CPXV and LCMV by IFA (Kallio-Kokko et al., in press). Seropositive human sera were used as positive controls for the PUUV, SAAV, and CPXV IFAs; an LCMV mouse monoclonal antibody (Progen, Heidelberg, Ger-

many) was used as a positive control in the LCMV-IFA.

For hantaviruses, RNA isolation and reverse transcription (RT)-PCR were done as described previously (Plyusnin et al., 1995). Briefly, total RNA was isolated from samples of lung tissue from four seropositive *Microtus* by using TriPure reagent (Roche Diagnostics, Mannheim, Germany). Attempts to synthesize full-length S segment cDNAs were made using primer 5′-TAGTAGTAGAC-3′ and random hexamers. PCR was done with a single primer 5′-TTCTGCAGTAGTAG-TAGACTCCTTGAAAAG-3′. Because no PCR products corresponding to the full-length S segment (~1,800 base pairs) were observed using this technique, a nested PCR with primers based on TULV S segment sequence also was performed (Plyusnin et al., 1995).

For poxviruses, lung samples stored in RNAlater® were extracted for DNA as described previously (Bown et al., 2003). DNA was screened using a nested PCR targeting the fusion protein gene (adapted from Chantrey et al., 1999). DNA was screened for orthopoxvirus-specific DNA, by using a nested PCR targeting the fusion protein gene (Chantrey et al., 1999). Cycle conditions were adapted from Chantrey et al. (1999) and consisted of 94 C for 6 min (one cycle), 94 C for 30 sec, 50 C for 30 sec, and 72 C for 1 min (35 cycles), and 72 C for 10 min (one cycle).

Nucleic acid extraction from LCMV-IFA-positive animals (kidney and spleen tissue samples) and RT-PCR were done as described above for hantaviruses. DNA amplification was done using forward primers Lassa 2 (5′-GCC ACA CGT GGC AAA ATT GT) and Lassa 3 (5′-CCCACAGGTGGCAAAATTGT) and reverse primer Lassa 1 (5′-GCA CCG GGG ATC CTA GGC AT). The PCRs were performed in a total volume of 50 µl of AmpliTaq reaction buffer (Applied Biosystems, Foster City, California, USA) containing 2.5 units of Taq polymerase, 200 µM each dNTP, 100 ng of forward and reverse primers, and 1.5 µl template from RT-PCR. After an initial denaturation stage of 95 C for 5 min, samples were run through 35 cycles at 95 C for 1 min, 55 C for 30 sec and 72 C for 1 min, followed by a final extension stage at 72 C for 5 min. The PCR products were analyzed by electrophoresis in 1–1.4% agarose gels.

The prevalence of antibodies to hantavirus PUUV among the 65 *Microtus* voles was 6% (Table 1). Of the IFA-positive voles, one *Microtus guentheri lydius* was found from the Izmir area, and one *Microtus roberti* and two *Microtus rossiaemeridionalis* from the Trabzon area (Table 1). The 264 *Apodemus* spp. screened for SAAV antibodies and the one *D. nitedula* screened for PUUV and SAAV antibodies tested negative. All rodents were screened for CPXV antibodies, but only one CPXV-IFA-positive *Apodemus sylvaticus* (prevalence in this host species 1%) was detected from Trabzon (Table 1). Antibodies against arenaviruses were found in eight (2.4%) of the tested 330 rodents (Table 1) and from all study sites. PCR results from rodents testing positive for antibodies to hantavirus ($n=4$) and arenavirus ($n=8$) were negative. Both CPXV ($n=330$) antibody-positive and -negative samples were tested by PCR with negative results.

The presence of hantavirus antibodies in rodents from Turkey was demonstrated for the first time. Attempts to further characterize the hantavirus from the three species of *Microtus* voles by PCR were not successful. In Eurasia, hantaviruses are carried by several, but not all, *Microtus* species (Nemirov et al., 2004). In central and eastern Europe, TULV is widespread in *Microtus arvalis*, but this virus has not been unequivocally associated with human disease (Vapalahti et al., 2003). The antibody-positive rodents were trapped from regions where human cases have been reported (Kavukcu et al., 1997), and this association may warrant additional study. In contrast to reported results from

wild mice from eastern and central Europe (Vapalahti et al., 2003), none of the *Apodemus* in this study were hantavirus seropositive. The reason for this is not known.

Only one CPXV-IFA-positive rodent (*A. sylvaticus*) was detected in this study, even though all samples were tested by IFA and PCR. There is no previous information on the occurrence of poxviruses in Turkish rodents. Elsewhere, a high prevalence of CPXV has been detected in voles, especially during fall (Chantrey et al., 1999; Pelkonen et al., 2003). The timing of our rodent collections (spring) as well as the low number of voles ($n=65$) tested may explain the low prevalence of antibodies and infection observed in this study.

An intriguing finding in our recent surveys of rodents in different parts of Europe (Kallio-Kokko et al., 2005; Laakkonen et al., 2006) has been the relatively high prevalence of LCMV positive rodents. In this study, arenavirus-seropositive animals were found from all study sites (Table 1). In Europe, LCMV prevalence rates (based on antibody detection) found in the primary reservoir species, *M. musculus*, have ranged from 3.6% to 11.7% (Ackermann et al., 1964; Lledó et al., 2003); outside of Europe prevalence rates ranging from 2.5% to 9% have been reported (Morita et al., 1991; Childs et al., 1992). *Mus musculus* were not captured in this study, but the prevalence of antibodies to LCMV in *Apodemus* spp. ranged from 1% to 10%, and it was even higher in voles (1–13%). There are no previous studies on arenaviruses of rodents from Turkey. Our finding of arenavirus antibodies in voles raises the question whether members of the arvicoline subfamily carry a unique arenaviruses or whether these infections represent a spillover of arenaviruses normally associated with mice. Unfortunately, our attempts to characterize the viruses were unsuccessful; the identity of arenaviruses infections in wild rodents in Turkey awaits isolation of these viruses from the various host species.

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