Epidemiological dynamics of nephropathia epidemica in the Republic of Tatarstan, Russia, during the period of 1997–2013

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SUMMARY

This report summarizes epidemiological data on nephropathia epidemica (NE) in the Republic of Tatarstan, Russia. NE cases identified in the period 1997–2013 were investigated in parallel with the hantavirus antigen prevalence in small rodents in the study area. A total of 13,930 NE cases were documented in all but one district of Tatarstan, with most cases located in the central and southeastern districts. The NE annual incidence rate exhibited a cyclical pattern, with the highest numbers of cases being registered once in every 3–5 years. The numbers of NE cases rose gradually from July to November, with the highest morbidity in adult males. The highest annual disease incidence rate, 64·4 cases/100,000 population, was observed in 1997, with a total of 2,431 NE cases registered. NE cases were mostly associated with visiting forests and agricultural activities. The analysis revealed that the bank vole Myodes glareolus not only comprises the majority of the small rodent communities in the region, but also consistently displays the highest hantavirus prevalence compared to other small rodent species.

Key words: Epidemiology, haemorrhagic fever, hantavirus, infectious disease epidemiology zoonoses.

INTRODUCTION

Hantaviruses are tri-segmented, single-stranded negative sense RNA viruses that are naturally maintained in the populations of their rodent and insectivore hosts [1]. Most of the currently known hantaviruses (also referred to as ‘hantavirus species’) preferably infect their specific natural host causing asymptomatic infection in that particular small mammal species [2]. Phylogenetic analysis of the genetic relationship of the known hantaviruses has revealed three separate groups of viruses harboured by murine, arvicoline, and sigmodontine rodents [3, 4]. Hantavirus transmission generally does not involve any arthropod vectors.
Humans become infected while inhaling virus-contaminated aerosols and in most cases develop acute disease [5]. Clinical manifestations of the illness may vary depending on the host’s affiliation of the corresponding virus. Among rodent-borne hantaviruses, Murinae-borne viruses usually cause haemorrhagic fever with renal syndrome (HFRS), while infection with Sigmodontinae-borne viruses usually manifests as hantavirus pulmonary syndrome (HPS) [6–9]. The third group includes Arvicolinae-borne hantaviruses. These viruses are either non-pathogenic for humans or cause a mild form of HFRS, often referred to as nephropathia epidemica (NE) [6, 10–12].

The main cause of NE is Puumala virus (PUUV) circulating in nature in populations of the bank vole Myodes glareolus (formerly Clethrionomys glareolus). Mirroring the geographical distribution of the PUUV-specific host, NE is well-recognized in Scandinavia, many countries of Western and Central Europe, Russia (both European and Asian parts), and some Asian countries [3, 8, 11, 13]. It has been shown that PUUV infection is a main cause of hantavirus disease in the European part of the Russian Federation, while sporadic cases HFRS caused by the Dobrava-Belgrade virus and related murine-borne virus strains are identified less frequently [6, 14, 15]. In European Russia, the majority of NE cases occur in the Volga Federal District, particularly, in the Republics of Tatarstan, Udmurtia, and Bashkortostan, as well as in the Samara and Orenburg regions [7, 16–18].

In Tatarstan, the first NE cases were diagnosed in 1958 [19]. The disease is characterized by the sudden onset of fever, headache, back pain, and microvascular bleeding symptoms [20–23] and clinical presentation is mainly associated with disturbed kidney function and bleeding syndrome of various degrees. Recovery is most often complete and post-morbid complications are rarely documented [22, 23]. Immunity post-infection is lifelong, and no cases of recurring NE have been recorded [24]. NE outbreaks are seasonal, with the highest number of cases registered during summer and autumn, and are often associated with occupational activities such as farming, landscaping, fishing and hunting [25, 26]. Migration of the hantavirus natural hosts to grain harvest and storage sites increases the opportunities for contact with humans and the frequency of contacts between infected rodents and humans can be linked to annual variation in demographics of the host rodent populations [27, 28]. As the bank vole M. glareolus is the main natural carrier for PUUV in Tatarstan [29], rodent control and annual monitoring of this species population are essential for developing measures aimed at prevention of hantavirus infection and prediction of future outbreaks. As a consequence, these measures have been conducted routinely in Tatarstan for several decades. This report summarizes data on the spatial and temporal distribution of NE in the Republic of Tatarstan, Russia, during the extended period from 1997 to 2013.

METHODS

Study area

The Republic of Tatarstan is located in the centre of the East European Plain, about 800 km east of Moscow, at the confluence of the Volga and Kama rivers. The landscape is mostly low-lying plain (not more than 200 m above sea level) comprising over 68 000 km² of territory. The republic lies in the natural forest and forest-steppe zones, with about 16-2% of its territory covered by forest which varies from predominantly coniferous and mixed vegetation in the northern part to deciduous forest further south. The majority of the land is used for agricultural purposes, with the main crops being wheat, corn, legumes, etc.

Collection and evaluation of epidemiological data on NE

In Tatarstan, all cases of NE are required to be reported to the Centre for Hygiene and Epidemiology situated in Kazan. The preliminary diagnosis of NE is based on clinical observations combined with epidemiological data and is confirmed by demonstration of a fourfold increase in serum titre of anti-hantavirus IgG antibodies in paired patient sera. Analysis of the NE morbidity and mortality rates presented here is based on the raw data collected by the authors for the Annual Reports of the Office for Consumer Rights Protection and Human Health Control Services (‘RosPotrebNadzor’) in the Republic of Tatarstan. This surveillance programme has been in effect in its present form since 1997. All personal data and publicly available secondary data were anonymized.

In order better to evaluate the dynamics of NE outbreaks in the republic, the case annual incidence rate was calculated for two time periods, 1997–2006 and
2007–2013. These time periods were chosen for two main reasons. First, a large time-frame is definitely required to obtain a better estimate of the average annual incidence rate of the disease that displays a natural 3–5 years’ cyclical pattern. Second, there has been significant increase in agricultural activities and rural/semi-rural construction in Tatarstan in the last 7 years, so it was of a particular interest to find out if such human-induced environmental changes affected NE morbidity.

Animal data collection
The most recent government-commissioned comprehensive investigation of hantavirus prevalence in the wild rodent populations in Tatarstan was conducted in 1995–2000. This 5-year-long study provided a basis for further routine rodent surveillance; the data obtained are presented here. Currently, annual surveys of the small rodent population are conducted according to ‘The Protocol for Capture, Analysis and Prognosis of the Small Rodent and Bird Population Sizes in the Natural Zoonotic Foci’ MU 3.1.1029-01, approved by The Ministry of Health of The Russian Federation in 2001. Since adoption of this protocol, small animals were routinely trapped in the various locations in the different administrative districts of the republic. The trapping sites usually included forests, fields, and settings around residential areas; most of the sites were used continuously over 10 years. The traps were typically set during the evening hours, and animals were collected in the morning. Lung tissues were collected after returning to the campsite and immediately placed into liquid nitrogen. After returning to the institution, the samples were kept under refrigeration at −50 °C until further processing, but for no longer than 1 month. For hantavirus detection, lung samples from each animal were homogenized in sterile phosphate-buffered saline (pH 7.0). ‘Hantagnost’ Diagnostic ELISA kit (Institute of Poliomyelitis and Viral Encephalitides, Russia) was used for detection of hantavirus antigen; anti-hantavirus antibodies were detected using an indirect immunofluorescence assay (IFA) (‘Diagnostikum GLPS’ IFA kit, Institute of Poliomyelitis and Viral Encephalitides, Russia).

Statistical analysis
The standard t test was used to determine level of significance. A P value of <0.05 was considered statistically significant. Stata software v. 11.0 (StataCorp, USA) was used for all statistical calculations.

RESULTS
NE in the Republic of Tatarstan: temporal and spatial patterns
In the 17 years of surveillance, 1997–2013, 13930 NE cases were recorded in 42 of the 43 districts of the republic. The highest annual disease incidence rate (64.4 cases/100 000 population) was observed in 1997, with a total of 2431 NE cases registered. The disease appeared to exhibit a cyclical pattern, with the highest and the lowest annual numbers of human cases being recorded every 3–5 years (Fig. 1). For example, the highest annual incidence rate of 1997 was followed by a steady decline to its lowest rate 5 years later, in 2002 (10.3 cases/100 000). The next 4 years, 2003–2006, were characterized by an increased annual incidence rate reaching 22.2/100 000 and 20.3/100 000 in the years 2005 and 2006, respectively, declining sharply in 2007 (6.7/100 000) followed by a rise in the ensuing 2 years, with the last highest annual incidence rate in 2009 (30.6/100 000). During the next 4 years, the observed annual incidence rate was significantly lower, with only 5.3 NE cases/100 000 registered in 2013. Nevertheless, even with this decline, the annual NE incidence rate in Tatarstan still remained 2.5–5.0 times higher than the overall rate in the Russian Federation [30].

Although NE cases were registered throughout Tatarstan, the majority were documented in the central regions along the Kama River and the southeastern regions bordering the Republic of Bashkortostan, which is another well-known hantavirus zoonotic focus [16, 31]. These regions of Tatarstan are covered by coniferous or mixed forest in the northern part of the republic and by deciduous trees further south. The seeds of the latter trees constitute the principal food source for the large populations of bank-vole species that serve as the natural reservoir for hantaviruses, thus maintaining the reservoir of these viruses.

A gradual increase in NE cases was observed from July to November, when the number of cases peaked, followed by decline until next January (Table 1). Only sporadic cases were identified between February and June, emphasizing the summer–autumn pattern of the disease. The majority of cases (85%) were male and most were individuals of productive age (20–49
The average NE case mortality over the study period was 0.43%, with fatal cases distributed across nine districts and two cities.

Four groups were defined with respect to NE morbidity across districts of Tatarstan. The first, high-risk group, comprised districts with an annual incidence rate of >20 cases/100 000; the second group, moderate risk, 10–20/100 000, the third, low risk, <10/100 000, and the fourth, no or minimal risk, recorded no NE cases within the study period.

Figure 2a shows that for the first time period (1997–2006), 22 districts were categorized as high-risk groups. In particular, the highest incidence rate was registered in Muslyumovsky district where 123.6 cases/100 000 population were recorded, followed by Almetyevsky and Bavlinsky districts with incidence rates of 97.3/100 000 and 93.3/100 000, respectively. Twelve districts had moderate risk rates and eight other districts had the lowest risk rate for the disease. All but two of the latter districts are located in the western part of the Republic of Tatarstan, bordering the Mari-El Republic and the Chuvash Republic.

No case of NE was registered in Drozhzhanovsky district, which is also located in the southwestern corner of the republic, bordering the Chuvash Republic and the Ulyanovsk Oblast.

During the years 2007–2013, there were fewer districts with a high risk of NE compared to the previous period, 17 vs. 22 (Fig. 2b). For this period, the highest incidence rate (62.5/100 000) was observed in Alexeevsky district and the rates in Muslyumovsky, Almetyevsky and Bavlinsky districts were lower compared to the previous period when they had the highest incidence rates across all districts in Tatarstan. The number of districts with moderate risk rates remained similar (13 vs. 12) over the two periods and low-risk years). The average NE case mortality over the study period was 0.43%, with fatal cases distributed across nine districts and two cities.

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districts rose from eight to 12. Drozhzhanovsky district remained free of NE cases. It appeared that more districts with moderate risk of NE infection were located in the eastern and northeastern parts of the republic during 2007–2013, as previously. Similarly, lower NE incidence was detected in the western part of Tatarstan. Therefore, despite the decreasing NE incidence rate to the East and to the West, the Central part remained the most active endemic region for NE in the Republic of Tatarstan.

Hantavirus prevalence in rodent populations

On a regular basis, rodent captures in the enzootic loci in Tatarstan were initiated during 1995–2000. In the course of the government-commissioned investigation 1669 small rodents were captured, and their species and hantavirus infection status determined (Table 2). Bank voles (M. glareolus) represented the majority of captured animals and had higher hantavirus antigen prevalence compared to other small rodents. Other hantavirus antigen-positive rodent species, with much lower hantavirus antigen prevalence included pygmy wood mice [Apodemus (Sylvaemus) uralensis], red-backed voles (Myodes rutilus), and common voles (Microtus arvalis). No hantavirus antigen-positive animals were found in field mice (A. agrarius) and yellow-necked mice (A. flavicollis). These data suggest that M. glareolus serves as the main natural host reservoir for hantavirus in Tatarstan, as well as consistently displaying the highest hantavirus antigen titre. In M. glareolus samples titres ranged from 1:8 to 1:256, and were generally <1:64 for Microtus arvalis and <1:8 for A. uralensis.

Since 2001, investigation of the hantavirus antigen prevalence in small rodent populations in the Republic of Tatarstan was performed according to the ‘Protocol for capture, analysis and prognosis of the small rodent and small bird population sizes in the natural zoonotic foci’ approved by the Ministry of Health of the Russian Federation, 2001. Small rodents were captured in the various districts of Tatarstan, and their lung tissues were used to determine presence of the hantavirus antigen. Rodent trapings were conducted annually, with the exception of 2003, 2007, and 2008. However, rodent species determination was not required by the official investigation protocol until 2013, and hence data on hantavirus prevalence in particular rodent species are not available for this entire period. The average hantavirus antigen prevalence in small rodents captured in 2000–2013 was calculated to be 9.6% but varied markedly over the investigation period, in particular, dropping markedly from 19.9% in 2004 to 1.1% in 2005 (Table 3).

DISCUSSION

The Republic of Tatarstan represents one of the most active endemic regions for NE in the Russian Federation [32]. Annually, over 1000 cases of NE are recorded, with an average mortality rate of 0.43%. The majority of cases (35–7%) is associated with visiting forest and includes recreational activities such as hiking and camping, as well as professional activities of the forestry and nature conservation workers. Another large group (28–8%) represented residential NE cases, with infection acquired around the house; usually, such cases occur during winter. Finally, up to 24.4% of NE cases are associated with agricultural activities, e.g. farming and gardening [32].

Our data demonstrated that the bank vole M. glareolus is the primary natural hantavirus reservoir in the Republic of Tatarstan. This species predominated (78.5%) in small rodents captured in 1995–2000, and was the most frequent (13.7%) hantavirus antigen-positive animal possibly suggesting adaptation of hantavirus strains circulating in the republic. In addition, hantavirus antigen titres, reaching 1:256, were the highest in these animals compared to other species.

Considering the fact that ‘Hantagnost’ kit is based on the cell culture-grown PUUV, a hantavirus known to be naturally maintained in bank-vole populations and causing NE in Scandinavia, Western Europe and some other enzootic foci in European Russia, the high virus antigen titres in bank voles is a good indication that PUUV plays a primary role in hantavirus activity in Tatarstan. Although a systematic molecular genetic study has not, as yet, been conducted, our preliminary investigation indicates the existence of local strains of PUUV that are genetically similar, but not identical, to the strains previously described in adjacent regions of the Russian Federation such as Udmurtia and Bashkortostan [16, 31]. Interestingly, no hantavirus antigen was detected in field mice and yellow-necked mice, while low titres were found in pygmy wood mice, red-backed voles and common voles. This raises the possibility that activity of the hantaviruses carried by field mice (e.g. Dobrava-Belgrade, Saaremaa, Kurkino viruses, etc.) is low or absent in Tatarstan, while vole-borne hantaviruses are more prevalent. Besides PUUV, it is likely that...
Fig. 2. Incidence rate of nephropathia epidemica (NE) in the administrative districts of Tatarstan. (a) Annual NE incidence rate in the administrative districts of Tatarstan calculated for the period of 1997–2006. (b) Annual NE incidence rate in the administrative districts of Tatarstan calculated for the period of 2007–2013. The administrative districts are numbered as follows: 1, Agryzsky; 2, Aznakayevsky; 3, Aksubaevsky; 4, Aktanyshsky; 5, Alekseevsky; 6, Alkeyevsky; 7, Almetyevsky; 8, Apastovsky; 9, Arsky; 10, Atinsksky; 11, Bavlinsky; 12, Baltasinsky; 13, Bugulminsky; 14, Buinsky; 15,
Tula virus associated with common vole *Microtus arvalis* [33] is present in the study area.

Forests of mixed and deciduous trees cover about 24% of Central and Southeastern regions of Tatarstan where the majority of NE cases occurred; this level of forestation is higher than the average (16%) for the republic. Seeds of oaks, linden trees and aspen trees can serve as the main food source for voles. Moreover, >50% of the territory is covered by grasslands and crop fields (wheat, rye, barley, oat, pea, corn) which produce 5% of the Russian Federation’s agricultural output. The boundaries of the crop fields are often marked by hedge-rows which also represent a known common habitat for the bank vole [34, 35]. This proximity of hedge-rows to crops provides a favourable environment for the bank vole to maintain its colonies. Thus environmental factors such as ample food sources in the forests and close proximity to crop fields play an important role in supporting bank-vole populations in the Central and Southeastern regions of Tatarstan. Since the majority (51.4%) of NE cases occurred in forest workers, farmers and gardeners, these habitats most likely constitute ‘infection hot spots’ where hantaviruses are maintained in the bank-vole populations.

There was only one district, where no NE cases were registered in the period 1997–2013. Drozhzhanovsky district is located in the Southwest of the Republic of Tatarstan bordering the Chuvash Republic and the Ulyanovsk Oblast. This is a mainly agricultural district together with cattle breeding and dairy farms. Little is known about small rodent community composition in this district, and hantavirus prevalence in small rodents has never been investigated. The lack of NE cases and data on hantavirus circulation in small rodents in Drozhzhanovsky district possibly explains why this area has hitherto not been

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### Table 2. Prevalence of hantavirus infection in rodents of six species captured in the Republic of Tatarstan during 1995–2000

<table>
<thead>
<tr>
<th>Animal species</th>
<th>No. of animals analysed</th>
<th>Antigen-positive animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pygmy wood mouse <em>Apodemus (Sylvaemus) uralensis</em></td>
<td>198</td>
<td>2 (1.0 ± 0.2)</td>
</tr>
<tr>
<td>Yellow-necked mouse <em>Apodemus flavicolli</em></td>
<td>26</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Field mouse <em>Apodemus agrarius</em></td>
<td>22</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bank vole <em>Myodes (Clethrionomys) glareolus</em></td>
<td>1283</td>
<td>177 (13.7 ± 0.7)</td>
</tr>
<tr>
<td>Red-backed vole <em>Myodes (Clethrionomys) rutilus</em></td>
<td>35</td>
<td>1 (2.8 ± 0.8)</td>
</tr>
<tr>
<td>Common vole <em>Microtus arvalis</em></td>
<td>105</td>
<td>7 (6.7 ± 0.9)</td>
</tr>
</tbody>
</table>

* Mean ± s.d., n = 6, P < 0.05.

### Table 3. Prevalence of hantavirus infection in rodents captured in the Republic of Tatarstan during 2000–2013, and January–June, 2014

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of animals analysed</th>
<th>Antigen-positive animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>328</td>
<td>54 (16.4 ± 0.5)</td>
</tr>
<tr>
<td>2001</td>
<td>143</td>
<td>9 (6.2 ± 0.7)</td>
</tr>
<tr>
<td>2002</td>
<td>54</td>
<td>7 (13.0 ± 1.2)</td>
</tr>
<tr>
<td>2003</td>
<td>n.d.</td>
<td>—</td>
</tr>
<tr>
<td>2004</td>
<td>276</td>
<td>55 (19.9 ± 1.8)</td>
</tr>
<tr>
<td>2005</td>
<td>722</td>
<td>8 (1.1 ± 0.2)</td>
</tr>
<tr>
<td>2006</td>
<td>60</td>
<td>5 (0.3 ± 1.1)</td>
</tr>
<tr>
<td>2007</td>
<td>n.d.</td>
<td>—</td>
</tr>
<tr>
<td>2008</td>
<td>n.d.</td>
<td>—</td>
</tr>
<tr>
<td>2009</td>
<td>294</td>
<td>35 (11.9 ± 1.2)</td>
</tr>
<tr>
<td>2010</td>
<td>273</td>
<td>20 (7.3 ± 0.9)</td>
</tr>
<tr>
<td>2011</td>
<td>155</td>
<td>16 (10.3 ± 1.0)</td>
</tr>
<tr>
<td>2012</td>
<td>226</td>
<td>19 (8.4 ± 0.8)</td>
</tr>
<tr>
<td>2013</td>
<td>178</td>
<td>10 (5.6 ± 0.5)</td>
</tr>
<tr>
<td>2014†</td>
<td>95</td>
<td>6 (6.3 ± 0.7)</td>
</tr>
</tbody>
</table>

**Average (%)** (9.6 ± 1.5)

n.d., Not done.

* Values given as mean ± s.d. (n = 15, P < 0.05).
† For January–June 2014.
specifically targeted for investigation due to its presumed lack of epidemiological significance.

During the last 5 years (2009–2013) of the study, the overall NE incidence rate in Tatarstan has been declining. It could be explained by extrapolating from the cyclical pattern of NE morbidity observed during the previous decade, when peaks of infection were registered every 3–5 years. If this pattern is repeated, it could be anticipated that the NE rate will once again increase significantly within the next 2 years. Close monitoring of the population dynamics and hantavirus prevalence in small rodent populations is essential for reliably predicting future disease outbreaks. It is particularly important for those regions which are considered to be 'the hotspots' for NE, i.e. Central and Southeastern regions of the Republic of Tatarstan.

In conclusion, our data demonstrate that NE is endemic in the Republic of Tatarstan, Russia, and the main reservoir for hantavirus appears to be the bank vole *M. glareolus* which represents the major part of the small rodent communities in the region. These data strongly suggest that PUUV that is generally associated with this vole species is the primary infectious agent causing NE in the study area. The NE annual incidence rate exhibits a cyclical pattern, with the highest numbers of cases occurring every 3–5 years, and in November of each year. Infection is most frequent in adult males. This gender bias is likely to be the result of the higher probability/frequency of occupational exposure to potential source of virus due to specific occupational and recreational activities of men. One district in Tatarstan remained disease free for the entire 17-year study but it is unclear whether this is due to low hantavirus prevalence in small rodents or low numbers of *M. glareolus* in the area. Further investigations will be needed to clarify distribution of the vole- and field mice-borne hantaviruses in Tatarstan and to characterize these viruses by molecular genetic techniques.

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DECLARATION OF INTEREST

None.

REFERENCES


