EPIDEMIOLOGICAL DYNAMICS OF NEPHROPATIA EPIDEMICA IN THE REPUBLIC OF TATARSTAN, RUSSIA, DURING THE PERIOD OF 1997-2013

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EPIDEMIOLOGICAL DYNAMICS OF NEPHROPATIA EPIDEMICA IN THE REPUBLIC OF TATARSTAN, RUSSIA, DURING THE PERIOD OF 1997-2013

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Running title: NE epidemiology in Tatarstan, Russia

Summary

Current report summarizes epidemiological data on nephropathia epidemica (NE) in the Republic of Tatarstan, Russia. NE cases registered over the extended period of 1997-2013 have been scrutinized in parallel with investigation of hantavirus serological prevalence in small rodents in the study area. A total of 13,930 NE cases were documented in all but one district of Tatarstan. Analysis of NE morbidity over the 17-years period revealed that most of the NE cases were registered in the central and southeastern districts. NE incidence rate exhibits a cyclical pattern, with the highest numbers of the NE cases being registered once in 3-5 years. Every year, the numbers of NE cases show gradual rise from July to November, with higher morbidity observed in adult males. The highest annual disease prevalence, 64.4 cases per 100,000 of population, was observed in 1997, with a total of 2,431 NE cases registered. NE cases were mostly associated with visiting forests and with agricultural activities. 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 as compared to other small rodent species.

INTRODUCTION

Hantaviruses are tri-segmented single stranded negative sense RNA viruses naturally maintained in the populations of the 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 revealed three separate groups of viruses harbored 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 affiliation of the corresponding virus. Among rodent-borne hantaviruses, Murinae-borne viruses usually cause Hemorrhagic 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 circulating in nature in the populations of the bank vole Myodes glareolus (formerly known as Clethrionomys glareolus). Mirroring geographic distribution of the Puumala virus specific host, NE is well-known 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 Puumala virus 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 and related murine-borne virus strains are registered less frequently [6, 14, 15]. In European Russia, the majority of NE cases are registered 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, first NE cases were diagnosed in 1958 [19]. Disease is characterized by the sudden onset of fever, headache, back pain, and microvascular bleeding symptoms [20-23]. Clinical presentation is mainly associated with disturbed kidney function and bleeding syndrome of various degrees. Recovery is complete; post morbid complications are rarely documented [22, 23]. Post infectious immunity lasts for lifetime, with no cases of reoccurring NE recorded [24]. NE outbreaks are seasonal, with the highest number of cases registered during summer and fall, and often associated with human occupational activities such as farming, landscaping, fishing and hunting [25, 26]. Migration of the hantavirus natural hosts to the sites of grain harvest and storage increases chance for contact with humans. Additionally, frequency of contacts between infected rodents and humans can be influenced by annual variation in demographics of the host rodent populations [27, 28]. Bank vole Myodes glareolus (previously known as Clethrionomys glareolus) is the main natural carrier for Puumala virus in Tatarstan [29]. Therefore, rodent control and annual monitoring of M. glareolus population are essential for developing measures aimed on prevention of hantavirus infection and prediction of future outbreaks. In Tatarstan, disease control and monitoring of the host rodent populations have been conducted on a routine basis for several decades. Current 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 center of East European Plain, approximately 800 km east of Moscow, around the confluence of the Volga and Kama rivers. Tatarstan landscape is mostly a low plain (not more than 200 m above the sea level) comprising over 68 thousand km^2 territory. The republic lies in the natural forest and forest-steppe zones, with about 16.2% of its territory being actually covered by the forest. Forest composition varies from the predominantly coniferous or mixed forests in the northern part of the Republic to the deciduous forest further south. The majority of the land is used for agricultural purposes, with the main crops being wheat, corn, legumes, etc.

Rodent Data Collection. In Tatarstan, 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. Animals are routinely captured in the various locations across Tatarstan and used to collect lung tissues for subsequent detection of hantavirus antigen using "Hantagnost" Diagnostic ELISA Kit (Institute of Poliomyelitis and Viral Encephalitides, Russia) or anti-hantavirus antibody using indirect immunofluorescence assay (IFA) ("Diagnostikum GLPS" IFA Kit, Institute of Poliomyelitis and Viral Encephalitides, Russia).

Patient Data Collection. In Tatarstan, all cases of NE are to be reported to the Center for Disease Control and Prevention of the Republic of Tatarstan. Preliminary diagnosis of NE is based on the clinical observations combined with epidemiological data. Each diagnosis is confirmed by ELISA detection of the anti-hantavirus antibody in the patient sera ("Hantagnost" Diagnostic ELISA Kit, Institute of Poliomyelitis and Viral Encephalitides, Russia), with a 4-fold increase in serum titer of anti-hantavirus antibodies being considered as a clear evidence of hantavirus infection. Analysis of the NE morbidity and mortality rates presented here is based on the raw data collected for the Annual Reports of the Office for Consumer Rights Protection and Human Health Control Services ("RosPotrebNadzor") in the Republic of Tatarstan, Russia. All personal data were anonymous, as were publicly available secondary data.

RESULTS

NE Prevalence in the Republic of Tatarstan. NE morbidity in Tatarstan was analyzed on the basis of data encompassing 17 years of surveillance, from 1997 to 2013. During this period, a total of 13,930 NE cases were recorded in 42 out of 43 districts of the Republic. The highest annual disease prevalence (64.4 cases per 100,000 of population) was observed in 1997, with a total of 2,431 NE cases registered. Overall, NE morbidity in Tatarstan seems 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 prevalence rate of 1997 was followed by a steady decline reaching the lowest annual incidence 5 years later, in 2002 (10.3 cases per 100,000). The next 3 years, 2003 – 2006, were characterized by increased annual incidence reaching 22.2 and 20.3 in the years 2005 and 2006, respectively. Sharp drop of 2007 (6.7 NE cases per 100,000) was followed by another 2 years of elevated NE morbidity, with the last highest annual incidence registered in 2009 (30.6 NE cases per 100,000). During the next four years, observed annual incidence was significantly lower, with only 5.3 NE cases per 100,000 registered in 2013. Nevertheless, even when considering this significant decline over the last few years, annual NE morbidity rate in Tatarstan still remained 2.5 - 5.0 times higher than overall in Russian Federation [30].

Although NE cases were registered all over the Republic of Tatarstan, the majority of those were documented in the central regions along the Kama River and the southwest regions bordering the Republic of Bashkortostan, 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 such as aspens, birches, oaks, and linden trees, further south. Seeds of the deciduous trees constitute the principal food source for the vole species that serve as the natural reservoir for hantaviruses. Therefore, it seems natural that in the regions mentioned above abundant food sources support large and continuous populations of the bank vole which in turn provide favorable environment for continuously maintaining hantavirus.

Analysis of the seasonal distribution of NE cases revealed a gradual increase from July to November, when the NE usually reaches its peak, followed by decline till next January (Fig 2). Only sporadic cases are registered between February and June. Therefore, we conclude that NE in the Republic of Tatarstan is characterized by a Summer-Fall pattern. Analysis of the NE cases registered during 1997 – 2013 demonstrated higher numbers of cases in males versus females (85% vs 15%, respectively). In addition, the majority of NE cases were individuals of the productive age, between 20 to 49 years old. During the period investigated, average NE mortality was 0.43%, with fatal cases having been registered in nine districts and two cities.

With respect to NE morbidity observed, all districts of the Republic of Tatarstan could be divided into four groups. The first, high risk group included the districts with the annual incidence rate of NE over 20 cases per 100,000. The second, moderate risk group included districts where annual NE incidence varied from 10 to 20 cases per 100,000. The districts where the NE incidence was found to be less than 10 cases per 100,000 of population were assigned into the third group with low risk for NE. Finally, the remaining single district where no NE cases were registered within the time frame investigated was classified as the fourth group with no or minimal risk for NE. In order to better evaluate dynamics of NE outbreaks in the Republic of Tatarstan, NE case prevalence was calculated separately for two subsequent time frames, specifically, for the 10 years period of 1997 – 2006, and for the seven years period of 2007 - 2013.

For the period of 1997-2006, 22 districts were placed into the high risk group (Fig. 3). In particular, the highest NE incidence rate was registered in the Muslyumovsky district where 123.6 NE cases per 100,000 of population were recorded. It was followed by Almetyevsky and Bavlinsky districts where NE incidence rate was 97.3 and 93.3, respectively. Twelve districts had moderate NE incidence, with morbidity ranging between 10 and 20 cases per 100,000 of population. Eight other districts had lower NE incidence rates, forming a low risk group for NE. Most of these latter districts, with exception of two, are located in the western part of the Republic of Tatarstan, bordering the Mary-El Republic and the Chuvash Republic. No cases of NE were 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 period of 2007-2013, there were fewer districts with the high NE incidence as compared to the previous period, 17 versus 22 (Fig 4). For this period, the highest incidence rate of 62.5 was observed in Alexeevsky district. Interestingly, NE incidence rate in the Muslyumovsky, Almetyevsky and Bavlinsky districts was lower as compared to the previous period when those had the highest NE incidence among all districts in Tatarstan. Number of districts with moderate risk of NE infection remained similar to that in the previous period, 13 versus 12. Number of districts in the group with low risk of NE increased from 8 to 12. Drozhzhanovsky district still remained NE free, as in the years 1997-2006. It appeared that more districts with moderate risk of NE infection were located in the eastern and northeastern parts of the Republic of Tatarstan during 2007 -

2013 as compared to the previous period studied. Similarly, lower NE incidence was detected in the western part of Tatarstan as well. Therefore, it could be concluded that, with the decreasing NE incidence rate to the east and to the west, the central part of the Republic of Tatarstan still continued to represent the most active endemic region for NE.

Hantavirus antigen prevalence in small rodent populations in the Republic of Tatarstan. 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.

On the regular basis, rodent captures in the enzootic loci in Tatarstan were initiated during 1995 – 2000. A total of 1669 small rodents were captured, and their species and infection status determined (Table 1). Bank voles (Myodes glareolus) represented the majority of captured animals and had higher hantavirus antigen prevalence compared to other small rodents. Other hantavirus antigen positive rodent species that had much lower hantavirus antigen prevalence, included pygmy wood mice Apodemus (Sylvaemus) uralensis, red-backed voles Myodes (Clethrionomys) rutilus, and common voles Microtus arvalis. No hantavirus antigen positive animals were found among field mice (Apodemus agrarius) and yellow-necked mice (Apodemus flavicollis). Apparently, Myodes glareolus serves as the main natural host reservoir for hantavirus in the Republic of Tatarstan. Besides having the highest antigen prevalence, this rodent species also consistently displayed higher hantavirus antigen titer. Specifically, in

Myodes glareolus it varied between 1:8 to 1:256, while it was generally less than 1:8 in Apodemus (Sylvaemus) uralensis, and less than 1:64 in Microtus arvalis.

During 2000 - 2013, rodent captures were conducted annually, with exception of 2003, 2007, and 2008; however, rodent species determination was not required by the official investigation protocol until 2013. Thus, data on the hantavirus prevalence in the particular rodent species are not available for this period. Average hantavirus antigen prevalence among small rodents captured in 2000–2013 was calculated to be 15.8%. It is worth to mention that hantavirus antigen prevalence varied significantly between different years of investigation, in particular, showing dramatic increase from 1.1% in 2005 to 83.3% in 2006 (Table 2).

DISCUSSION

The Republic of Tatarstan represents one of the most active endemic regions for NE in the Russian Federation [32]. Annually, over 1,000 cases of NE are registered, with average mortality rate of 0.43%. The majority of NE cases (35.7%) is associated with visiting forest and includes such recreational activities 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 are registered during the winter time. Finally, up to 24.4% of NE cases are associated with agricultural activities, such as farming and gardening.

Our data demonstrated that the bank vole Myodes glareolus is the primary natural hantavirus reservoir in the Republic of Tatarstan. The bank vole was the predominant species (78.5%) among small rodents captured in 1995 - 2000, suggesting that this species is indeed dominating in Tatarstan. Among all the animals captured, the bank voles had the highest percentage of the hantavirus positive animals (13.7%). Therefore, it could be concluded that the hantavirus strain(s) circulating in the Republic of Tatarstan are adapted to the bank vole Myodes glareolus. In addition, hantavirus antigen titer was the highest in these animals as compared to other species, reaching 1:256. Considering the fact that “Hantagnost” Kit is based on the cell culture grown Puumala virus, a hantavirus known to be naturally maintained in the bank vole populations and causing NE in Scandinavia, Western Europe and some other enzootic foci in European Russia, the highest virus titer in bank voles is a good indication of the Puumala virus playing a primary role in the hantavirus activity in Tatarstan. Although no systematic molecular genetic study has been conducted yet, our preliminary investigation indicated existence of the local strains of Puumala virus that are genetically similar, but not identical, to the strains previously described in such adjacent regions of the Russian Federation as Udmurtia and Bashkortostan [16, 31]. Interestingly, no hantavirus antigen was detected in field mice and yellow-necked mice, while low hantavirus antigen titer (up to 1:8) was observed in pygmy wood mice, red-backed voles and common voles. This allows suggesting that activity of the hantaviruses carried by field mice (Dobrava-Belgrade, Saaremaa, Kurkino viruses, etc.) is low or absent in Tatarstan, while vole-borne hantaviruses are more prevalent. Besides the Puumala virus discussed above, it is likely that Tula virus associated with common vole Microtus arvalis [33] is present in the study area.

The majority of NE cases were registered in the Central and Southeastern regions of the Republic of Tatarstan. Mixed and deciduous tree forests are covering 24% of this territory, which is higher than the republic average (16%). Seeds of oaks, linden trees and aspen trees can serve as main food source for voles. Still, more than 50% of this territory is covered by grasslands and crop fields that produce 5% of the Russia's agricultural products such as wheat, rye, barley, oat, pea, and corn. The boundaries of the crop-fields are often marked by hedgerows which also represent a known common habitat for the bank vole [34, 35]. Such close proximity of hedgerows to crops provide favorable environment for the bank vole to maintain its colonies. Therefore, it could be concluded that environmental factors such as ample food sources in the forests and close proximity of crop fields to the natural habitats play important role in supporting flourishing bank vole populations in the central and southeastern regions of Tatarstan. Since the majority of NE cases (51.4%) are registered among forest workers, farmers and gardeners, forests and hedgerow habitats are most likely to represent the "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 of 1997-2013. Drozhzhanovsky district is located in the southwestern part of the Republic of Tatarstan bordering the Chuvash Republic and the Ulyanovsk Oblast. This is a mainly agricultural district producing wheat, rye, barley, oat, pea and corn. Cattle breading and dairy farms are also prominent in this district. Little is known about small rodent community composition in this district. Besides, hantavirus prevalence among small rodents in this district has never been investigated. This lack of data on hantavirus circulation in small rodents in Drozhzhanovsky district could be explained by the fact that no NE cases were registered there, so this district was never specifically targeted for investigation due to its presumed lack of epidemiological significance.

During the last 4 years (2009 - 2013), overall NE incidence rate in the Republic of 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 NE incidence were registered every 3-5 years. Therefore, it could be anticipated that NE incidence rate will once again experience significant increase within the next two 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 hot spots” for NE incidence, i.e., central and southeastern regions of the Republic of Tatarstan.

Taken together, our data demonstrate that NE is endemic in the Republic of Tatarstan, Russia. The main reservoir for hantavirus in Tatarstan appears to be the bank vole Myodes glareolus which represents the major part of the small rodent communities in the region. These data strongly suggest that Puumala virus that is generally associated with this vole species is the main infectious agent causing NE in the study area. Our limited preliminary sequencing data seem to confirm this hypothesis. The NE incidence rate exhibits a cyclical pattern, with the highest numbers of NE cases being registered every 3-5 years. Every year, the highest numbers of NE cases are registered in November, with higher morbidity observed in adult males. Interestingly, one district in Tatarstan have been disease free for the entire period from 1997 to 2013. It remains not clear whether lack of NE cases in this district is due to low hantavirus prevalence among small rodents or low numbers of Myodes glareolus in the area. Further investigations will be needed to clarify distribution of the vole and field mice borne hantaviruses in Tatarstan and to genetically characterize those.

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CONFLICT OF INTEREST

None

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Table 1. Hantavirus antigen prevalence among small rodents captured in the Republic of Tatarstan during 1995 – 2000.

Animal species	Number of animals analyzed	Number of animals serologically positive	% of seropositive animals
pygmy wood mouse *Apodemus (Sylvaemus) uralensis*	198	2	1,0±0,2
yellow-necked mouse *Apodemus flavicollis*	26	0	0
field mouse *Apodemus agrarius*	22	0	0
bank vole *Myodes (Clethrionomys) glareolus*	1283	177	13,7±0,7
red-backed vole *Myodes (Clethrionomys) rutilus*	35	1	2,8±0,8
common vole *Microtus arvalis*	105	7	6,7±0,9

Table 2. Hantavirus seroprevalence among small rodents captured in the Republic of Tatarstan during the period of 2001 - 2013 and in January – June of 2014.

Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014 [x]	*Average*
Sero positive (%)	16.4	6.2	13.0	ND	19.9	1.1	83.3	ND	ND	11.9	7.3	10.3	8.4	5.6	6.3	15.8± 6.3

ND – not determined

[x] - for January – July 2014

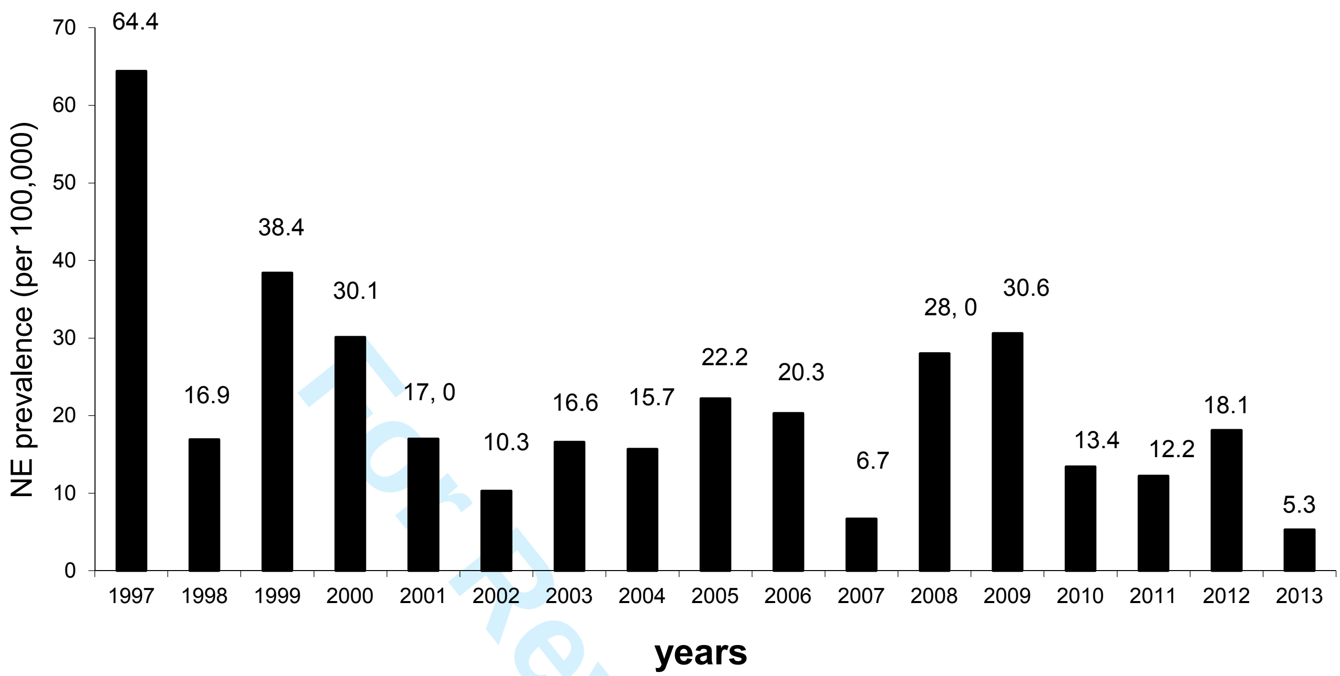

Figure 1. NE morbidity in the Republic of Tatarstan during the period of 1997 – 2013.

NE morbidity in Tatarstan was analyzed on the based on the raw data collected for the Annual Reports of the Office for Consumer Rights Protection and Human Health Control Services ("RosPotrebNadzor") in the Republic of Tatarstan, Russia.

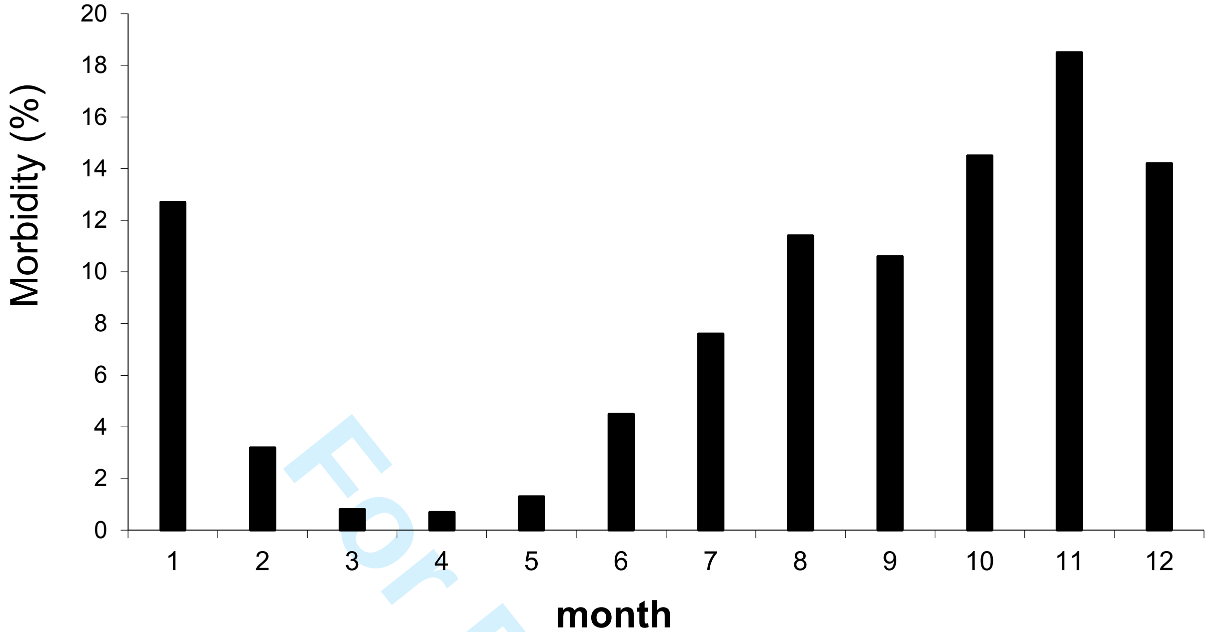

Figure 2. Seasonal distribution of NE morbidity in the Republic of Tatarstan calculated for 1997 – 2013.

Seasonal analysis of NE morbidity was performed based on the Annual Reports of the Office for Consumer Rights Protection and Human Health Control Services (“RosPotrebNadzor”) in the Republic of Tatarstan, Russia.

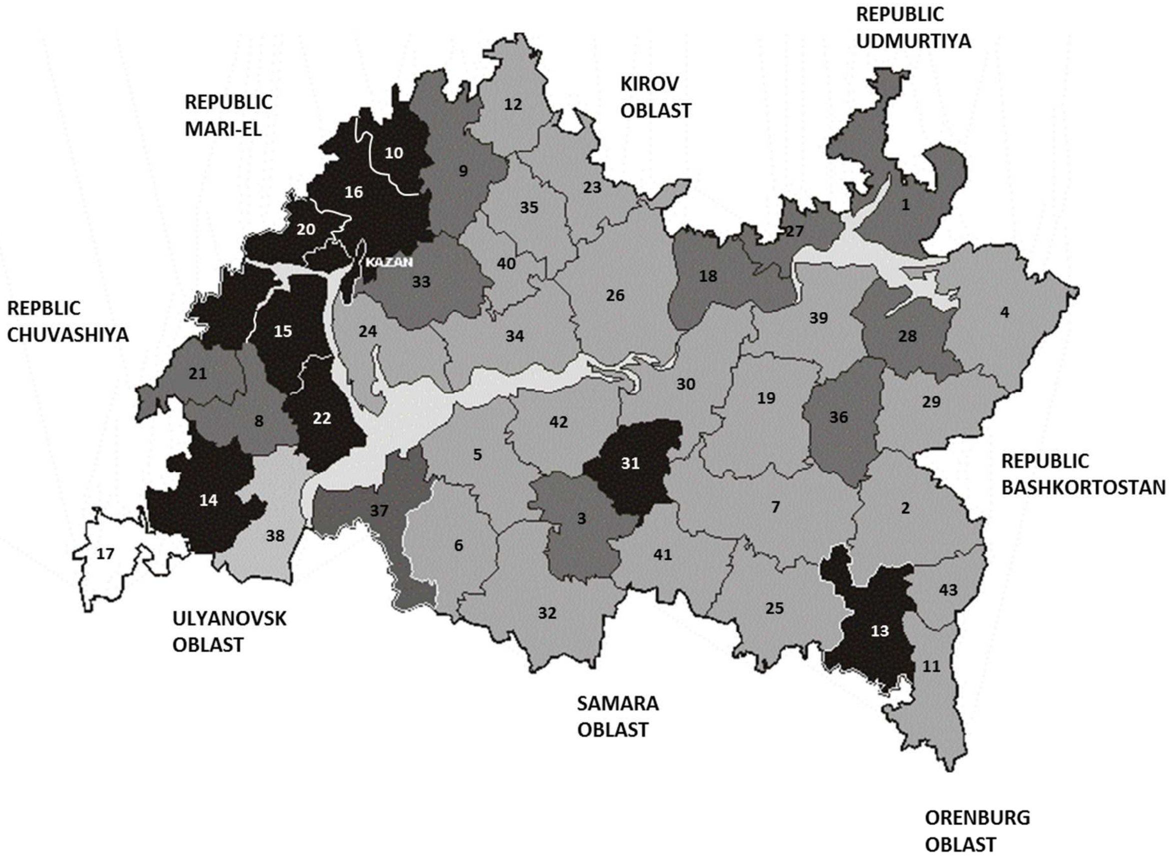

Figure 3. NE incidence in the Republic of Tatarstan (1997 – 2006)

NE morbidity > 20 per 100,000

NE morbidity 10 – 20 per 100,000

NE morbidity < 10 per 100,000

No cases of NE registered

Districts are numbered as follows:

1. Agryzsky	33. Pestrechinsky
2. Aznakayevsky	34. Rybno-Slobodsky
3. Aksubaevsky	35. Sabinsky
4. Aktanyshsky	36. Sarmanovsky
5. Alekseevsky	37. Spassky
6. Alkeyevsky	38. Tetyushsky

7. Almetyevsky
8. Apastovsky
9. Arsky
10. Atninsky
11. Bavlinsky
12. Baltasinsky
13. Bugulminsky
14. Buinsky
15. Verhneuslonsky
16. Vysokogorsky
17. Drozhzhanovsky
18. Yelabuzhsky
19. Zainsky
20. Zelenodolsky
21. Kaybizky
22. Kamsko-Ustyinsky
23. Kukmorsky
24. Laishevsky
25. Leninogorsky
26. Mamadyshsky
27. Mendeleyevsky
28. Menzelinsky
29. Muslyumovsky
30. Nizhnekamsky
31. Novosheshminsky

39. Tukayevsky
40. Tyulyachinsky
41. Cheremshansky
42. Chistopolsky
43. Yutazinsky

39 32. Oktyabrsky

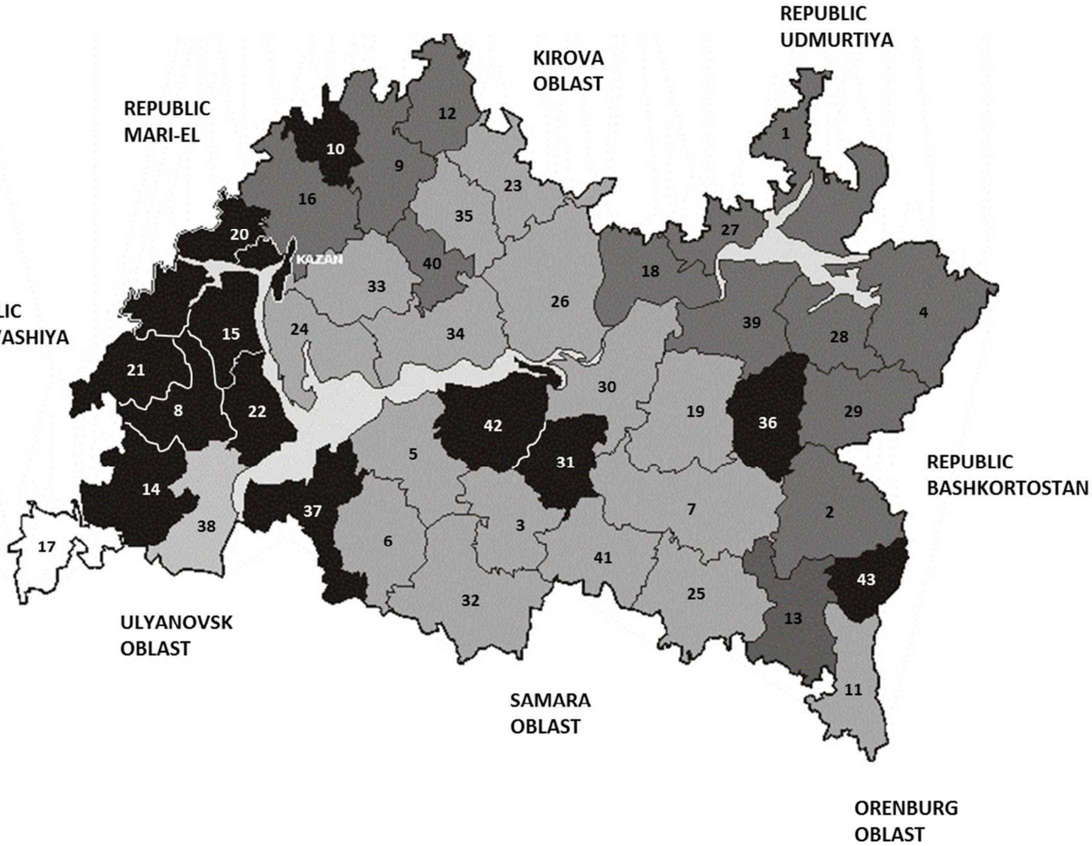

Figure 4. NE incidence in the Republic of Tatarstan (2007 – 2013)

NE morbidity > 20 per 100,000

NE morbidity 10 – 20 per 100,000

NE morbidity < 10 per 100,000

No cases of NE registered

Districts are numbered as follows:

1. Agryzsky	32. Oktyabrsky
2. Aznakayevsky	33. Pestrechinsky
3. Aksubaevsky	34. Rybno-Slobodsky
4. Aktanyshsky	35. Sabinsky
5. Alekseevsky	36. Sarmanovsky
6. Alkeyevsky	37. Spassky
7. Almetyevsky	38. Tetyushsky

8. Apastovsky
9. Arsky
10. Atninsky
11. Bavlinsky
12. Baltasinsky
13. Bugulminsky
14. Buinsky
15. Verhneuslonsky
16. Vysokogorsky
17. Drozhzhanovsky
18. Yelabuzhsky
19. Zainsky
20. Zelenodolsky
21. Kaybizky
22. Kamsko-Ustyinsky
23. Kukmorsky
24. Laishevsky
25. Leninogorsky
26. Mamadyshsky
27. Mendeleyevsky
28. Menzelinsky
29. Muslyumovsky
30. Nizhnekamsky
31. Novosheshminsky

39. Tukayevsky
40. Tyulyachinsky
41. Cheremshansky
42. Chistopolsky
43. Yutazinsky