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Detection of IgG against Rickettsia typhi: a population-based study in southern Kazakhstan

Detekcija IgG protutijela na Rickettsia typhi: populacijska studija u južnom Kazahstanu

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Abstract

Background. *Rickettsia typhi* belongs to the typhus group of rickettsiae and causes endemic typhus. Cases of endemic typhus and seropositivity to *R. typhi* have been reported in the neighbouring China and Russia. However, little is known of the endemic typhus in Kazakhstan. The purpose of this study was to evaluate the prevalence of IgG antibodies to *R. typhi* in the population of southern region of Kazakhstan.

Methods. A total of 253 individuals (142 women, 111 men) aged from 1 to 71 years were recruited into the study. Detection of serum IgG antibodies against *R. typhi* was performed by enzyme-linked immunosorbent assay (ELISA).

Results. The overall *R. typhi* seropositivity has reached 34.4%. The highest seroprevalence of 91.8% was recorded in the Turkestan Region. The lowest seropositivity of 6.1% was detected in the village Lepsinsk, Almaty Region. The seroprevalence did not differ significantly between genders. Seropositivity in adult individuals was not significantly associated with age, but positive results were not detected in the age group of children under 14 years.

Conclusion. The obtained results confirm active circulation of *R. typhi* in the Turkestan and Almaty Regions of Kazakhstan. The data indicate an urgent need for further studies aimed to evaluate the clinical impact caused by *R. typhi* in the southern region of Kazakhstan.

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Sažetak

Uvod. *Rickettsia typhi* svrstava se u skupinu pjegavih tifusa i uzrokuje endemski tifus. Slučajevi endemskog tifusa i seropozitivnosti na *R. typhi* zabilježeni su u susjednoj Kini i Rusiji. Međutim, o endemskom tifusu u Kazahstanu se malo zna. Svrha ove studije bila je procijeniti prevalenciju IgG protutijela na *R. typhi* u populaciji južne regije Kazahstana.

Metode. U istraživanje je uključeno ukupno 253 osoba (142 žena, 111 muškaraca) u dobi od 1 do 71 godine. Detekcija serumskih IgG protutijela na *R. typhi* provedena je imunoenzimskim ELISA testom.

Rezultati. Ukupna seropozitivnost na *R. typhi* iznosila je 34,4%. Najveća seroprevalencija od 91,8% zabilježena je u regiji Turkestan. Najniža seropozitivnost od 6,1% otkrivena je u selu Lepsinsk, regija Almaty. Seroprevalencija se nije značajno razlikovala prema spolu. Seropozitivnost kod odraslih pojedinaca nije bila značajno povezana s dobi, ali pozitivni rezultati nisu otkriveni u dobnoj skupini djece mlađe od 14 godina.

Zaključak. Dobiveni rezultati potvrđuju aktivnu cirkulaciju *R. typhij* u regijama Turkestan i Almaty u Kazahstanu. Podaci ukazuju na hitnu potrebu za daljnjim istraživanjima čiji je cilj procijeniti klinički učinak *R. typhi* u južnoj regiji Kazahstana.

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Introduction

Rickettsioses are febrile diseases caused by obligate intracellular bacteria of the genus *Rickettsia* (family *Rickettsiaceae*)[1]. *Rickettsia* spp. is closely related to blood-feeding arthropods and two groups are known: the typhus group (TG) and the spotted fever group. Spotted fever rickettsia are transmitted by ticks, while rickettsia of the TG are transmitted by fleas and lice[2]. *Rickettsia typhi (R. typhi)* belongs to the TG and is the causative agent of murine or endemic typhus. The main zoonotic reservoirs of *R. typhi* are rats of the genus *Rattus*. The oriental rat flea (*Xenopsylla cheopis*) and the cat flea (*Ctenocephalides felis*) are classic vectors transmitting murine typhus to humans[3]. Rats and fleas do not suffer from *R. typhi* infection, but the latter remain infected throughout life. A human infection can occur alimentary (consumption of food contaminated with rat faeces), through rat fleas (infected flea bites, inhalation of dried flea faeces), as well as ticks that parasitize on rats (infection occurs when a person is bitten by an infected tick) [4]. The incidence of endemic typhus is sporadic, it is often recorded in summer and early autumn and usually associated with the presence of rats[4].

Endothelial cells are the primary target cells of *R. typhi*[5]. Damaged blood vessels, altered vascular permeability and vascular inflammation/dysfunction result in a number of clinical symptoms observed in patients with murine typhus. Thus, the clinical symptoms of murine typhus include high fever, persistent headache, and skin rash prevailing on the body, as well as vomiting and myalgia. The rash is usually less present than in epidemic typhus, and is often absent. The clinical symptoms are different in children. Headache, malaise, anorexia, chills and rashes are recorded in half of children. Abdominal pain, diarrhoea and sore throat are also often observed[6]. Since the clinical symptoms of endemic typhus are not pathognomonic, the disease is often misdiagnosed or overlooked[7].

According to a systematic review of 33 studies on murine typhus available in the Pubmed MEDLINE database from 1980 to 2017, the death rate from murine typhus is low (0.33%). However, complications have been observed in 26% of patients[6]. The most commonly reported complications were pulmonary, central nervous system complications, kidney injures, and ophthalmologic complications[6-13]. Complications often develop in elderly patients and patients with transplants[14, 15]. A recent experimental study has shown persistence and neurotropism of *R. typhi*, which suggests that these bacteria should be taken into account in cases of undifferentiated inflammatory diseases of the central nervous system[16].

Although murine typhus is considered to be a mild disease, delayed antibiotic therapy is a major factor of poor prognosis in patients[17]. Considering that flea-borne rickettsiae are endemic to Kazakhstan and the fact that fevers of unknown origin are often registered in southern Kazakhstan, the purpose of this study was to evaluate the prevalence of IgG antibodies to *R. typhi* in a population of southern Kazakhstan[18].

Materials and Methods

Serum samples were collected from 253 residents of Turkestan and Almaty Region from January to December 2018 (Fig. 1). Written informed consent was obtained from all participants or their parents in case of minor participants. Serum samples were stored in 2 aliquots at -20°C. Before examination, serum samples were thawed and inactivated at 56°C for 20 minutes.

IgG antibodies against *R. typhi* were detected using a *Rickettsia typhi* IgG ELISA Kit (Fuller Laboratories, USA), which uses the species-specific rOmp B protein isolated from *R. typhi*. The analysis was performed according to the manufacturer's instructions. Discrimination of positive and negative results was performed by dividing the optical density (OD) values of test serum samples by the OD values of the Cutoff Calibrator. Indicators above 1.2 were considered positive, below 0.8 - negative, from 0.8 to 1.2 - borderline.

Biostatistical analysis was performed by using the EpiInfo 7 software (CDC, USA). The Fisher's exact test was used to establish associations between different variables. The results were presented as mean ± standard deviation. Significance was determined at the level of $p<0.05$.

Results

The average age of the population subject to research was 36.9 years (range 1-71 years), of which 142 were women (56.1%) and 111 men (43.9%). The collection of serum samples took place in hospitals, clinics, as well as during visits to local residents in the cities and administrative Centres of southern Kazakhstan (Table 1).

Out of the 253 analysed samples, 87 (34.4%) were positive for IgG *R. typhi*, of which 15 samples (17.2%) had a very high antibody titter (the OD value of the sample exceeded the OD value of the Cutoff Calibrator more than 4 times), 44 samples (50.6%) had a high antibody titer (OD of the sample exceeded the OD of the Cutoff Calibrator 2-4 times) and 28 samples (32.2%) had a low antibody titer (the OD of the sample exceeded the OD of the Cutoff Calibrator less than 2 times). The highest percentage of seropositive samples was

found in the Turkestan, Zhalandy and Almaty Regions (Table 1).

The odds of exposure to *R. typhi* were similar for males and females (Table 2). The mean age of seropositive individuals was 37.3±14.7, while the mean age of seronegative donors was 35.9±16.2. Performed univariate logistic regression analysis revealed no statistically significant associations between seropositivity and age (data not shown). When stratified by age groups, seroprevalence was also not significantly associated with age, however, positive results were not detected in the age group of children under 14 years (Table 2). Rural residence was not associated with seropositivity for IgG *R. typhi* (Table 2).

Table 1. Results of IgG *R. typhi* detection according to geographic location

Regions of the south Kazakhstan	City/administrative Centre	Number of samples	Positive (%)	Negative (%)	Borderline (%)
Turkestan Region	Sholakkorgan	15	14 (93.3%)	0	1 (6.7%)
	Taukent	15	15 (100%)	0	0
	Zhetysay	16	13 (81.3%)	2(12.5%)	1 (6.2%)
	Turkestan	15	14 (93.3%)	0	1 (6.7%)
	Total in the oblast:	**61**	**56 (91.8%)**	**2 (3.3%)**	**3 (4.9%)**
Almaty Region	Esik	11	2 (18.2%)	9 (81.8%)	0
	Talgar	2	0	1 (50%)	1 (50%)
	Almaty	56	4 (7.1%)	50 (89.3%)	2 (3.6%)
	Kokzhar	5	0	4 (80%)	1 (20%)
	Zhalandy	69	22 (31.9%)	32 (46.4%)	15 (21.7%)
	Lepsinsk	49	3 (6.1%)	46 (93.9%)	0
	Total in the oblast:	**192**	**31 (16.1%)**	**142 (74.0%)**	**19 (9.9%)**
Total in the region:		**253**	**87 (34.4%)**	**144 (56.9%)**	**22 (8.7%)**

Table 2 Distribution of seroprevalence of antibodies to R. typhi by gender, age groups and residence of participants

	Total (%)	Positive (%)	Negative	OR (95% CI)
Sex:				
Male	111 (43.9%)	32 (28.8%)	68	1
Female	142 (56.1%)	55 (38.7%)	76	1.5 (0.9; 2.7)
Age group:				
≤14	11 (4.3%)	0	10	0
15-24	53 (20.9%)	18 (34.0%)	32	1
25-34	60 (23.7%)	25 (41.7%)	32	1.4 (0.6; 3.0)
35-44	47 (18.6%)	18 (38.3%)	24	1.3 (0.6; 3.1)
45-54	36 (14.2%)	11 (30.6%)	22	1.1 (0.4; 2.8)
55-64	31 (12.3%)	8 (25.8%)	18	1.3 (0.5; 3.5)
≥ 65	13 (5.1%)	6 (46.2%)	5	1.8 (0.5; 6.3)
Data not known	2 (0.8%)	1	0	0
Residence:				
Urban (Zhetysay, Turkestan, Esik, Talgar, Almaty)	100 (39.5%)	33 (33.0%)	62	1
Rural (Sholakkorgan, Taukent, Kokzhar, Zhalandy, Lepsinsk)	153 (60.5%)	54 (35.3%)	82	1.2 (0.7; 2.1)

OD – odds ratio, 95% CI – 95% confidence interval

Discussion

The cases of endemic typhus and seropositivity to *R. typhi* have been reported in the territories of neighbouring China and Russia[19,20]. However, the incidence statistics for murine typhus in Kazakhstan is currently unavailable. The results of our study suggest that southern Kazakhstan, particularly Turkestan and Almaty Regions are an area of active *R. typhi* circulation. Evidence of past exposure to *R. typhi* was observed in one third of the studied population representing the region.

In the current study, detection of serum IgG antibodies against *R. typhi* was performed using *Rickettsia typhi* IgG ELISA Kit test system (Fuller Laboratories, USA). It should be noted that, despite the high specificity (97%) of this test system, manufacturers note the likelihood of cross-reactivity with IgG against *Rickettsia prowazekii*. Therefore, we cannot rule out the possibility that high seropositivity in individuals older than 65 years was associated with the epidemic typhus, outbreaks of which had been officially registered in Kazakhstan in the 1940s.

The obtained results demonstrated that Turkestan Region, where the percentage of seropositive samples reached 91.8%, may be considered an endemic territory for *R. typhi*. A significantly lower percentage of seropositive samples (an average of 16.1% in the Region) was found among residents of Almaty Region ($p<0.001$). Out of the 6 studied areas in Almaty Region, the highest level of seropositive results was shown in Zhalandy. Such differences may be attributed to the climate in the studied areas, contributing to the spread of rats and other rodents transmitting the disease, limited rodent control measures, as well as housing in adobe buildings made from clay and straw with crevices in the walls. In addition, previous studies have shown that endemic typhus is usually associated with poor sanitary and hygienic living conditions, which could be a case in the aforementioned areas[21]. Another possible reason for the observed high prevalence may be cluster collection of sera from the nearest buildings located in the zone of active circulation of rats. Therefore, a study with wider territory coverage is recommended for Turkestan Region.

Since this study did not reveal any significant differences between urban and rural residents, we can assume that both populations are equally exposed to risk factors. Nevertheless, it should be noted that private farming is significantly developed in such cities as Talgar, Esik, Zhetysay, which increases the risk of contacts with rodents and domestic animals.

We have also shown that both women and men were equally exposed to risk factors for *R. typhi* infection. The results are consistent with earlier studies conducted in other countries[22-24]. Seropositivity in adult individuals was not associated with age, which suggests that the individuals did not retain antibodies of prior or repeated exposure. Interestingly, the analysis of age-adjusted seroprevalence demonstrated the absence of positive samples in the age group of children under 14 years. These results may be explained by the small sample size obtained in this age group (11 people, 4.3% of the studied population), they may also indicate that children under 14 years are not exposed to *R. typhi* possibly due to the fact that they are not usually engaged in outdoor work activity, which has been reported to be a risk factor for *R. typhi* infection[23].

This study was limited by the unavailability of information about the occupation of the participants, making it impossible to evaluate farming as a risk factor for *R. typhi* infection. Also, the medical histories were unavailable, therefore, it is unclear if participants had clinical manifestations of the disease in the past.

Conclusion

In summary, the results of our study suggest that infections with *R. typhi* may be common in the southern region of Kazakhstan. Consequently, there is an urgent need for further prospective studies to evaluate the role of flea-born rickettsial infections as potential causes of febrile illness in the region.

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Conflict of interest

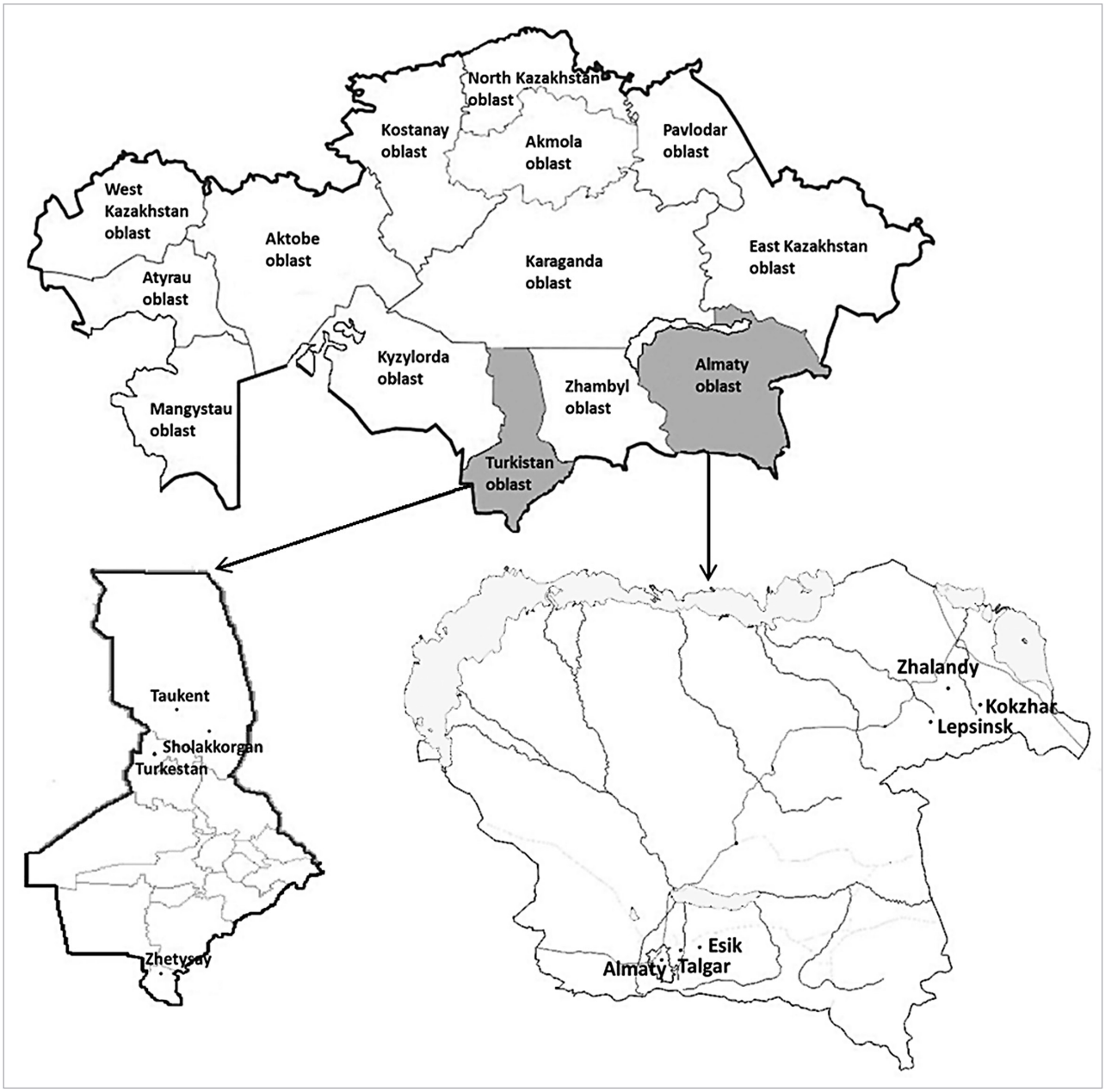
The authors have no conflicts of interest to declare.

Author contributions

AD conceived the study; ASN, EM and DN designed the study protocols; AN, YO and TK carried out the laboratory assays; ZB and AB designed the figures, prepared the manuscript for publishing; YP carried out statistical analysis of the data and drafted the

manuscript; TS, GA, and RY helped with sample collection; AD and SM critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Fig. 1. Study area. Administrative units of Kazakhstan (regions) and the study area are depicted. Areas colored with gray color indicate selected regions for the study.



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