Occurrence of Anti-*Rickettsia* spp. Antibodies in Hospitalized Patients with Undifferentiated Febrile Illness in the Southern Region of Kazakhstan

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Abstract. Undifferentiated febrile illness still represents a demanding medical problem all over the world, but primarily in low- and middle-income countries. Scientific and clinical investigations related to undifferentiated febrile illness and rickettsial diseases in Kazakhstan are lacking. This study reflects the investigation of antibodies against spotted fever group (SFG) and typhus group (TG) rickettsiae in patients with undifferentiated febrile illness in the southern region of Kazakhstan (Almaty and Kyzylorda oblasts). Paired serum samples were gathered from 13 hospitals in these two oblasts and explored for the presence of IgM and IgG antibodies against typhus group and IgG antibodies against spotted fever group rickettsiae using ELISA. Patient's questionnaires were statistically analyzed. In total, 802 inpatients from Almaty ($N = 9$) and Kyzylorda ($N = 4$) hospitals were included in this research. Based on ELISA results, 250 patients out of 802 (31.2%) from both oblasts had IgG antibodies against SFG rickettsiae. Results from 11 (1.4%) patients indicated acute infection with tick-borne rickettsiosis. Regarding TG rickettsiae (*R. typhi*), a past infection was detected in 248 (30.9%) febrile patients and acute infection in 22 (2.7%) patients in the two selected oblasts. The data indicated that SFG and TG rickettsioses are present in Kazakhstan. Kazakh physicians should be aware of these emerging diseases in both investigated oblasts because the occurrence of these diseases is not suspected during day-to-day clinical practice. The identification of rickettsial pathogens and implementation of modern laboratory methods for the diagnostics of rickettsioses are in need throughout Kazakhstan.

INTRODUCTION

Despite the advances achieved in differentiating diagnosis and treatment in recent decades, undifferentiated febrile illness remains a challenging clinical problem all over the world.^{1,2} To date, there are more than 200 diseases that have been associated with undifferentiated febrile illness.³ In Kazakhstan there are limited studies on patients with undifferentiated febrile illness. In this study we focused on investigating antibodies against rickettsiae in patients with diagnosed undifferentiated febrile illness. *Rickettsiae* are well known as arthropod-transmitted pathogens of vertebrate hosts and can cause a number of severe infectious diseases.^{4–6} According to novel data, *Rickettsia* spp. are divided into two major groups with several subgroups.^{7–9}

Rickettsia prowazekii, as a member of the typhus group (typhus group rickettsiae [TGR]), is transmitted by the human body louse and is a causative agent of epidemic typhus. Outbreaks of epidemic typhus often occur among the representatives of the poor strata of the population during the cold season.¹⁰ Further, according to WHO, recent outbreaks of epidemic typhus have been reported in eastern Africa (Burundi, Ethiopia, Rwanda), Central and South America, and Asia.¹¹ *Rickettsia typhi*, another member of the typhus group, is transmitted by fleas and causes murine typhus (endemic typhus, flea-borne typhus). Mostly, persons who come into

contact with flea-infested domestic and wild animals (rodents) are at high risk for endemic typhus.¹⁰ Flea-borne typhus is widely distributed around the world, mainly in tropical and subtropical areas where rats are present.¹²

Rickettsiae of the spotted fever group (SFG) are pathogens that induce a number of tick-transmitted rickettsioses, including Rocky Mountain spotted fever, Mediterranean spotted fever, Far Eastern spotted fever, North Asian tick typhus, and Astrakhan spotted fever.¹⁰ Tick-borne rickettsioses are distinguished by their natural focality and endemicity, which are often reflected in the name of a rickettsiosis.

Many types of rickettsial diseases (louse, fleas, tick bite) are characterized by similar clinical characteristics, such as an incubation period of 1–2 weeks, and include symptoms such as fever, headache, malaise, rash (maculopapular, vesicular, or petechial), nausea, and vomiting.¹⁰ In this regard, the clinical diagnosis of rickettsioses presents certain difficulties for physicians. Some tick-bite rickettsioses are characterized by typical signs that facilitate the diagnosis by clinicians in this group of patients.^{10–15} It is well known that *R. slovaca* and *R. raoultii* are the causative agents of tick-borne lymphadenopathy and that they are common in southern and eastern Europe and Asia.^{10,13} Recently, it was described that also *R. massiliae* is responsible for the onset of such disease.^{14,15}

In Kazakhstan, patients with typical symptoms of tick-borne rickettsiosis were clinically described for the first time in the early 1950s in Almaty oblast.¹⁶ The first tick *Rickettsia* was identified and described about 10 years later from the same area of Kazakhstan.¹⁷ The official registration of tick-borne

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rickettsiosis incidence in humans in Kazakhstan started in 1995 and is still based on clinical case definition criteria and complement fixation test with *R. sibirica* as the antigen.¹⁸ Human cases are annually reported from four oblasts in Kazakhstan (North Kazakhstan, Pavlodar, East Kazakhstan, and Kyzylorda oblasts), which are considered as endemic for tick-borne rickettsioses. More than 4,000 cases of rickettsioses have been officially registered in Kazakhstan during the last 23 years (from 1995 to 2018), demonstrating a reliable increase in the incidence from 0.41 to 1.19 (per 100,000 inhabitants per year), especially in Kyzylorda (1.64–11.1 per 100,000 inhabitants per year) and Pavlodar (1.07–7.0 per 100,000 inhabitants per year) oblasts.¹⁹

There is a molecular biological confirmation of the circulation of the following *Rickettsia* species in Kazakhstan: tick-borne *R. raoultii*,^{20–25} *R. conorii* subsp. *caspia*, *R. aeschlimannii*,^{22,23,26} *R. asembonensis*, and flea-borne *R. felis/Candidatus R. senegalensis*.^{27,28} Recently, *R. slovaca* and two new rickettsial species with undetermined human pathogenicity (*Candidatus R. yembekshikazakhstanensis* and genotype *R. talgarensis*) have been identified in Almaty oblast, which is still considered as non-endemic for rickettsioses.¹⁸

Kazakhstan has no official registry and no appropriate diagnostic tests available for epidemic and murine typhus. Kazakh physicians often diagnose typhus on the basis of epidemiological data and typical clinical constellations.

Until now, in Kazakhstan no large-scale serological study on the prevalence of antibodies against *Rickettsia* has been conducted. The aim of this study was to investigate antibodies against SFG and TG rickettsiae to determine acute rickettsioses, based on the 4-fold change titer of IgG antibodies in conjunction with IgM antibodies in hospitalized patients with undifferentiated febrile illness in the Southern region of Kazakhstan (Almaty and Kyzylorda oblasts).

MATERIALS AND METHODS

Study areas. The study areas are Almaty and Kyzylorda oblasts in the Southern part of Kazakhstan. Almaty oblast (45°0' N, 78°0' E) is located in the south-eastern part of Kazakhstan and occupies an area of 224,000 km² where 777 settlements are located. The population of Almaty oblast and Almaty city is 3.9 million (2020). Almaty oblast is primarily agricultural.²⁹

Kyzylorda oblast (45°0' N, 64°0' E) is located in the south-western part of the country and occupies 226,000 km², with 411 settlements. The population of the Kyzylorda oblast is 803,500 (2020), and this oblast is also agricultural.³⁰

Sampling. Paired sera (day 0, day 10–14) were collected from adolescent (15–18 years old) and adult (≥ 18 years old) hospitalized patients with undifferentiated febrile illness during the warm season in 2015–2016 (April–October each year) from 13 hospitals in the Almaty (*N* = 9) and Kyzylorda (*N* = 4) oblasts of Kazakhstan to investigate several agents as the causes of fever. Undifferentiated febrile illness was defined as fever for more than 3 days with tympanic temperature of ≥ 37.5°C and exclusion of meningitis, pneumonia, tuberculosis, and rheumatologic disease (autoimmune conditions). All enrollees completed a specially designed questionnaire with 56 questions concerning socio-demographic, living, livestock, and vector habitat factors and clinical signs. Signed informed consent was obtained at the time of sampling. Patients meeting all inclusion criteria (i.e., those who had

completed questionnaires and had signed consent forms and for whom both sera available) were included in this study. Ethical clearance was awarded by the national Kazakh Ethical Committee (Almaty, #3, April 1, 2015) and in Germany by the ethical committee from the Ludwig-Maximilians-Universität (Munich, 176-16, April 16, 2016), respectively. For details see References 31 and 32.

Serology. Collected blood was centrifuged, and sera were frozen in four aliquots at –20°C. Serum samples were tested for the presence of IgG and IgM antibodies against TG rickettsiae (*R. typhi*) and IgG antibodies against SFG rickettsiae (*R. rickettsia*, *R. conorii*, *R. sibirica*, *R. australis*, *R. akari*, etc.) using commercial ELISA kits (Fuller Company, Fullerton, CA) according to the standard operating procedures and the manufacturer's guide concerning the conduction, cutoff calibrators, and indices for evaluation.

During the first step, all collected second serum samples were screened for IgG (TG and SFG rickettsiae) with appropriate ELISA kits. At the second step, the first and the second sera of all positive second samples were retested for IgG (TG and SFG rickettsiae), and the samples with a difference in index ≥ 0.4 were titrated (1:100, 1:200, 1:400, up to 1:12,800) to identify acute and exposed infection using the ELISA kits. For all three kits the cutoff calibrator is set at an index of 1.0. By dividing the absorbance values of the test sera by the mean absorbance value of the cutoff calibrator, an index value for each serum is derived. For TG rickettsiae IgG, an index > 1.2 was accepted as positive; an index < 0.9 was considered negative. For SFG rickettsiae, an index > 1.1 was considered as positive; an index < 0.9 was considered negative. For TG IgM, indices from 0.9 to 1.1 may be considered equivocal. Indices > 1.1 are considered positive, and those < 0.9 are considered negative. We used the negative and positive controls supplied in the kit. In a third step, the first IgG-negative sera of all IgG-positive second samples with supposed acute infection (TG rickettsiae) were screened with IgM ELISA kits.

Statistical analysis. For statistical processing, the data derived from questionnaires, and the R statistical environment (version 3.5.3) with RStudio graphical front-end (version 1.1.463) were used.^{33,34}

We tested the influence of different demographical, environmental, and occupational factors (predictors) on the positive results of SFG rickettsiosis or TG rickettsiosis (outcome variables) in the binomial univariate regression model using the *glm* function of the R *stats* package.

At the next step, we evaluated odds ratios (ORs) predicting the relationship between outcomes (SFG rickettsiosis or TG rickettsiosis) and factor variables in the binomial multivariate model, in which factor variables were adjusted by confounders (sex and age).

The Fisher's exact test was applied to evaluate the difference of clinical symptoms between groups positive and negative for rickettsiosis. *P* values < 0.05 were considered statistically significant.

RESULTS

In total, 950 patients with undifferentiated febrile illness from 13 hospitals of the two selected regions were gathered in this study in 2015 and 2016. Of these, 802 (84.4%) patients met all inclusion criteria and were included in this study. In most patients the fever lasted at least for 7 days.

Out of 802 paired serum samples, IgG antibodies to SFG rickettsiae were detected in 99 of 378 (26.2%; 95% confidence interval [CI]: 22–30.9) patients from Almaty oblast, in 151 of 424 (35.6%; 95% CI: 31.2–40.3) patients from Kyzylorda oblast. In total, IgG antibodies to SFG rickettsiae were detected in 250 of 802 (31.2%; 95% CI: 28.1–34.5) patients from both oblasts (Table 1).

Forty (5%; 95% CI: 4.7–6.7) collected paired serum samples were titrated for IgG to distinguish previous and acute infections with SFG rickettsiae. In summary, 16 serum pairs had a low titer, eight serum pairs had a medium titer, and 16 serum pairs had a high titer (for details see Supplemental Table 1). In 11 (1.4%; 95% CI: 0.8–2.4) of the patients from the two selected oblasts, a 4-fold increase in IgG titer between first and second serum was detected by ELISA, indicating acute infection with SFG rickettsiae (Table 2).

All 11 patients with evidence of an acute spotted fever group rickettsiosis had fever, headache, and weakness ($P = 0.19$) (Supplemental Table 2). Nine of the 11 patients (81%) complained of exanthema. Six febrile inpatients (54.5%) reported lymphadenitis ($P = 0.36$). Four enrollees (36.3%) had muscle pain ($P = 0.24$), and one had neck pain ($P = 0.19$) (Supplemental Table 4).

IgM antibodies against TG rickettsiae in the first sera with parallel evidence of IgG antibodies in the second sera were identified in 15 (1.9%; 95% CI: 1.1–3.1) serum samples collected from Almaty and Kyzylorda oblasts (Table 2). The 4-fold titer increase in IgG antibodies by ELISA between first and second sera was detected in seven (0.9%; 95% CI: 0.4–1.8) patients from both oblasts, indicating the presence of acute infection with TG rickettsiae (Supplemental Table 4). A previous infection with TG rickettsiae was distinguished by ELISA in 248 (30.9%; 95% CI: 27.8–34.2) febrile cases from the two selected oblasts (Table 2).

Also, 49 (6.1%; 95% CI: 4.7–8) sera from both regions were titrated for the presence of TG rickettsiae IgG antibodies. Further, 33 paired sera had a low titer, 14 paired sera had a medium titer, and two paired sera had a high titer (Supplemental Table 3).

All patients with acute typhus had nonspecific clinical symptoms, such as fever (100%), weakness (90.9%, $P = 0.03$),

headache (86.4%, $P > 0.05$), muscle pain (31.8%, $P = 0.18$), lymphadenitis (31.8%, $P = 0.51$), and neck pain (27.3%, $P = 0.81$). Exanthema, which is a key symptom of TG rickettsioses, was observed in 40.9% of acute cases ($P = 0.06$) (Table 5).

Among enrollees with acute SFG rickettsiosis from Almaty and Kyzylorda oblasts ($N = 11$), patients aged 46–75 years predominated (Table 3). The interquartile range of the age of these inpatients from both oblasts was 6. Table 3 gives an overview on demographic, epidemiological, and clinical characteristics of the patients with acute tick-borne rickettsiosis in the two oblasts of Kazakhstan.

There was a significant difference in age groups in tick-borne rickettsiosis-positive scores. Logistic regression analysis revealed an age older than 55 years as an independent predictor for this disease (OR for 56–65 years age group: 6.05; 95% CI: 1.55–20.56; $P < 0.05$; OR for 66–75 years age group: 7.07; 95% CI: 1.04–29.36; $P < 0.05$).

A positive tick-borne rickettsiosis in the group infested by a tick was 4.27 times more frequent than in the group of noninfested persons (95% CI: 1.27–14.96; $P < 0.05$). However, the association between the disease and a tick bite is strongly confounded by age because the prevalence of a *Rickettsia*-positive result is much higher in older age groups. Therefore, after adjustment for age, the association between a tick bite and disease is no longer significant (OR: 2.9; 95% CI 95: 0.84–10.03; $P > 0.05$).

Our analyses revealed a statistically significant association between work in forestry and a chance of positive results for *Rickettsia* antibodies (OR: 11.16; 95% CI: 0.57–71.7; $P < 0.05$). Moreover, the risk group was unskilled laborers (OR: 5.42; 95% CI: 1.16–19.38; $P < 0.05$). The chance of a tick-borne rickettsiosis in the group of unskilled laborers was 8.1 times higher in the group of skilled workers after adjustment for sex, tick bite, and age (95% CI: 1.84–35.69; $P < 0.05$).

Of the febrile participants showing an acute SFG rickettsiosis, 45% ($N = 5$) were male. The same pattern as for TG *Rickettsia* was found with place of residence: five patients were living in a rural area and six patients in an urban area. The majority of inpatients with acute SFG rickettsiosis (81.8%, $N = 9$) did not report any trip into nature during the last month,

TABLE 1

Prevalence of IgG antibodies against spotted fever group rickettsiae in patients presenting with undifferentiated febrile illness in two oblasts of Kazakhstan, 2015–2016

Localities (oblasts/hospitals)	Acute infection*	Previous infection†	Negative samples	Number of samples
Almaty oblast				
Almaty	0	8 (6%)	125 (94%)	133
Taldykorgan	0	23 (52.3%)	21 (47.7%)	44
Tekeli	1 (1.2%)	30 (35.7%)	53 (63.1%)	84
Usharal	0	14 (58.3%)	10 (41.7%)	24
Yessyk	0	15 (25.0%)	45 (75.0%)	60
Kaskelen	0	8 (29.6%)	19 (70.4%)	27
Shelek	0	0	4	4
Kabanbay	0	0	1	1
Kapshagay	0	0	1	1
Subtotal	1 (0.3%)	98 (25.9%)	279 (73.8%)	378
Kyzylorda oblast				
Kyzylorda	6 (2.3%)	90 (34.8%)	163 (62.9%)	259
Syrdarya	1 (2.2%)	9 (20.0%)	35 (77.8%)	45
Shieli	1 (2.0%)	27 (55.1%)	21 (42.9%)	49
Zhanakorgan	2 (2.8%)	15 (21.1%)	54 (76.1%)	71
Subtotal	10 (2.4%)	141 (33.2%)	273 (64.4%)	424
Total	11 (1.4%)	239 (29.8%)	552 (68.8%)	802

* Shown by 4-fold titer change in IgG.

† IgG-positive sera samples including titrated samples.

TABLE 2

Prevalence of IgM and IgG antibodies against typhus group rickettsiae in patients presenting with undifferentiated febrile illness in two oblasts of Kazakhstan, 2015–2016

Localities (oblasts/hospitals)	Previous infection*	Four-fold titer increase in IgG	IgM-positive samples	Negative samples	Number of samples
Almaty oblast					
Almaty	29 (21.8%)	0	2 (1.5%)	102 (76.7%)	133
Taldykorgan	29 (65.9%)	2 (4.6%)	3 (6.8%)	10 (22.7%)	44
Tekeli	42 (50.0%)	0	1 (1.2%)	41 (48.8%)	84
Usharal	16 (66.7%)	0	0	8 (33.3%)	24
Yessyk	28 (46.7%)	1 (1.7%)	0	31 (51.6%)	60
Kaskelen	14 (51.9%)	0	2 (7.4%)	11 (40.7%)	27
Shelek	2 (50.0%)	0	0	2 (50.0%)	4
Kabanbay	1 (100%)	0	0	0	1
Kapshagay	0	0	0	1 (100%)	1
Subtotal	161 (42.6%)	3 (0.8%)	8 (2.1%)	206 (54.5%)	378
Kyzylorda oblast					
Kyzylorda	45 (17.4%)	1 (0.4%)	5 (1.9%)	208 (80.3%)	259
Syrdarya	7 (15.6%)	0	0	38 (84.4%)	45
Shieli	14 (28.6%)	1 (2%)	1 (2%)	33 (67.4%)	49
Zhanakorgan	21 (29.6%)	2 (2.8%)	1 (1.4%)	47 (66.2%)	71
Subtotal	87 (20.5%)	4 (0.9%)	7 (1.7%)	326 (76.9%)	424
Total	248 (30.9%)	7 (0.9%)	15 (1.9%)	532 (66.3%)	802

* IgG-positive sera samples including titrated samples with a titer difference lower than 4-fold.

although most of them (72.7%, $N = 9$) reported daily garden work. Of the 11 enrollees, six (54.5%) had a tick bite during the past month. In 63.6% of acute cases ($N = 7$), daily contact with livestock was observed (Table 3).

Patients with acute infection ($N = 11$) in addition to unspecific mild clinical symptoms such as fever (100%), headache (100%), weakness (81.8%; $P > 0.05$) developed distinctive signs, such as lymphadenitis (54.5%; $P > 0.05$) and exanthema (81.8%; $P < 0.05$) (Supplemental Table 4).

The demographic, epidemiological, and clinical characteristics of the acute cases with TG rickettsiosis in two oblasts of Kazakhstan are presented in Table 4. Patients with acute infections with rickettsiae of the typhus group ($N = 22$) were between 15 and 35 years old ($N = 12$). The interquartile range of the age of these inpatients from both oblasts was 30.

As for spotted fever group rickettsioses, it was revealed that a risk of infection with TG rickettsia of the typhus group, after adjustment for sex and contact with rodents, was 3.37 times higher in the group of people aged 56–65 years (95% CI: 1.19–9.58; $P < 0.05$). The majority of febrile patients with acute TG rickettsia infection (81.8%, $N = 18$; $P > 0.05$) are male. Less than half of these enrollees (45.5%, $N = 10$; $P > 0.05$) were living in a rural area. In many acute cases no contacts with wild animals (95.6%, $N = 21$; $P > 0.05$), with rodents (81.8%, $N = 18$; $P > 0.05$), or stays in the nature (68.2%, $N = 15$; $P > 0.05$) was reported. Further, the 59.1% of acute patients ($N = 13$; $P > 0.05$) had daily contact with livestock (Table 4).

Also, an increase in the occurrence of antibodies of the tick-borne and TG rickettsioses was found in relation to age of inpatients with undifferentiated febrile illness (Figure 1).

DISCUSSION

In this study, undifferentiated febrile illness was defined by temperature $> 37.5^{\circ}\text{C}$ with duration > 3 days. Undifferentiated febrile illness still poses a serious challenge to public health systems in developed countries.¹ Due to the implementation of serological and molecular biological methods (ELISA, polymerase chain reaction), the definition of undifferentiated febrile illness was revised, and its classification was represented. In

recent studies, undifferentiated febrile illness occurs in 30–50% of cases an infectious etiology.²

This cross-sectional study of inpatients with undifferentiated febrile illness from two selected oblasts in Southern Kazakhstan is the first research on rickettsiae inducing undifferentiated febrile illness in Kazakhstan.^{33,34} We found that in both oblasts 1.4% of enrolled patients had acute SFG rickettsioses and that 2.7% had acute TG rickettsioses, respectively. Further, we detected previous infection with SFG rickettsiae in 29.8% and with TG rickettsiae in 30.9% of patients. Historically, the investigation of tick-borne rickettsioses in Kazakhstan started in Almaty oblast.^{16–18} At present, the natural foci of SFG rickettsioses are reported to be located in four areas of Kazakhstan, in North-Kazakhstan, Pavlodar, East-Kazakhstan, and Kyzylorda oblasts.¹⁸ In these oblasts, the incidence of the disease is under surveillance, and cases are annually reported.¹⁹ However, data on the serological prevalence of rickettsioses in humans in Kazakhstan do not exist. Regarding typhus rickettsioses, during the last decades no relevant research on this disease have been conducted in Kazakhstan. Moreover, until now this group of diseases has not been included in the list of officially registered diseases in the territory of the Republic of Kazakhstan.

The microimmunofluorescent antibody test (micro-IFAT) remains the method of choice in the diagnosis of rickettsial diseases.^{35–37} Unfortunately, the micro-IFAT has not yet been introduced in Kazakhstan as a routine method for the diagnosis of rickettsioses. In spite of this, ELISA is more widely accepted compared with micro-IFAT and has widespread use due to its low cost and ease and speed of implementation. It is also suitable for seroepidemiological studies.^{36,38–40}

This serological investigation for both rickettsial groups had a number of challenges. Paired serum sample and complete questionnaires were available from only 84.4% ($N = 802$) of febrile patients ($N = 950$). The prevalence of acute SFG rickettsiosis among febrile inpatients was diagnosed by a detection of the 4-fold increase of IgG titer in paired serum samples. This study was intended for both rickettsial diseases—SFG and TG rickettsioses—as a cause of acute fever. During our study diagnosed 11 cases with acute SFG rickettsioses and 22 cases

TABLE 3
Revealed features of inpatients with acute infection* of the spotted fever group rickettsiae in two oblasts of Kazakhstan, 2015–2016

Factor	Number of samples (N = 799)	Positive samples (N = 11)	Logistic regression model			
			Univariate analysis		Multivariate analysis	
			OR (95% CI)	P value	ORa (95% CI)	P value
Sex						
Female	302	6	Reference	–	–	–
Male	497	5	0.5 (0.14–1.68)	0.26	0.58 (0.17–1.98)	0.39
Age, yr						
15–25	291	0	NAP	NAP	NAP	NAP
26–35	199	2	0.67 (0.1–2.61)	0.61	0.73 (0.15–3.45)	0.69
36–45	121	0	NAP	NAP	NAP	NAP
46–55	87	3	3.14 (0.68–11.1)	0.1	2.51 (0.64–9.83)	0.19
56–65	72	4	6.05 (1.55–20.56)	0.005	5.24 (1.47–18.73)	0.01
66–75	26	2	7.07 (1.04–29.36)	0.02	4.39 (0.84–22.94)	0.08
> 75	3	0	NAP	NAP	NAP	NAP
Tick bite						
No	620	5	Reference	–	–	–
Yes	179	6	4.27 (1.27–14.96)	0.02	2.9 (0.84–10.03)	0.09
Place of residence						
Urban area	422	6	Reference	–	–	–
Rural area	377	5	0.93 (0.27–3.12)	0.91	0.75 (0.22–2.57)	0.65
Contacts with wild animals						
No	788	10	Reference	–	–	–
Yes	11	1	7.78 (0.4–46.92)	0.06	4.71 (0.46–48.28)	0.19
Nature trip						
No	594	9	Reference	–	–	–
Yes	205	2	0.64 (0.1–2.51)	0.57	0.6 (0.12–2.98)	0.53
Garden work						
No	273	2	Reference	–	–	–
Yes	526	9	2.36 (0.6–15.54)	0.27	1.4 (0.29–6.91)	0.68
Livestock availability						
No	440	4	Reference	–	–	–
Yes	359	7	2.17 (0.65–8.33)	0.22	1.71 (0.48–6.13)	0.41
Current occupation						
Student	113	0	NAP	NAP	NAP	NAP
Plant farmer	13	1	6.47 (0.34–38.06)	0.09	4.8 (0.5–45.95)	0.17
Animal farmer	15	0	NAP	NAP	NAP	NAP
Forestry farmer	8	1	11.16 (0.57–71.7)	0.03	7.79 (0.63–96.62)	0.11
Keeping the house	32	0	NAP	NAP	NAP	NAP
Unskilled laborer	54	3	5.42 (1.16–19.38)	0.01	8.1 (1.84–35.69)	0.06
Skilled laborer	132	1	0.49 (0.03–2.59)	0.49	0.74 (0.09–6.16)	0.78
Driver	27	1	3.33 (0.18–18.49)	0.26	4.93 (0.52–46.35)	0.16
Adm/acad. professional	63	11	1.17 (0.06–6.26)	0.88	1.74 (0.2–15.05)	0.61
Businessman/woman	25	0	NAP	NAP	NAP	NAP
Nurse/physician/pharmacist	20	1	4.05 (0.22–22.79)	0.19	4.33 (0.47–39.6)	0.19
Unemployed	136	1	0.39 (0.02–2.04)	0.37	0.39 (0.05–3.12)	0.37
Retiree	38	1	2.03 (0.11–11.02)	0.51	0.17 (0.02–1.64)	0.12
Military person	50	0	NAP	NAP	NAP	NAP
Declined to answer	26	0	NAP	NAP	NAP	NAP
NA	23	0	NAP	NAP	NAP	NAP

CI = confidence interval; NA = not available; NAP = not applicable; OR = odds ratio; ORa = odds ratio adjusted for sex and tick bite rate; bold values = significant.

* Shown by 4-fold titer change in IgG.

with acute typhus rickettsioses using ELISA. The prevalence of acute SFG rickettsiosis in Almaty oblast was much lower than that observed among febrile volunteers from Kyzylorda oblast. The gained data once again prove a high endemicity of Kyzylorda oblast for this rickettsial disease in comparison with Almaty oblast.¹⁸ This study showed that the rate of seropositivity of spotted fever group rickettsiosis was significantly higher in humans aged 55–75 years, with tick bite and for unskilled laborers.

Our results show that TG rickettsiae, in comparison to SFG rickettsiae, may be twice as often the cause of the undifferentiated febrile illness in patients admitted to the hospitals from Almaty and Kyzylorda oblasts of Kazakhstan. The dynamic change in antibody response to acute TG rickettsiae in Almaty oblast was similar to that observed

among hospitalized patients with unexplained fever in Kyzylorda oblast.

In a similar study, Indian researchers tested 432 blood samples collected from patients with fever of unknown origin that might resemble undifferentiated febrile illness and detected 115 (26.6%) samples positive for SFG rickettsiosis.⁴⁰ Most positive samples were found in the group aged 0–15 years. Most of their patients were from rural areas and were farmers and self-employed, as in our study. In contrast, we detected more antibodies in older patients and in unskilled laborers. The common symptoms in their study (excluding fever) were rashes (exanthema) and lymphadenopathy.⁴¹ The same results were found in our study. The obtained data also overlap with the data of other studies performed in India, where the seroprevalence of antibodies to SFG rickettsiae

TABLE 4
Characteristics of inpatients with acute infection* of the typhus group rickettsiae in two oblasts of Kazakhstan, 2015–2016

Factor	Number of samples (N = 799)	Positive samples (N = 22)	Logistic regression model			
			Univariate analysis		Multivariate analysis	
			OR (95% CI)	P value	OR a (95% CI)	P value
Sex						
Female	302	4	Reference	–	–	–
Male	497	18	2.8 (1.03–9.76)	0.06	3.05 (1.02–9.16)	0.05
Age, yr						
15–25	291	5	0.5 (0.16–1.29)	0.18	0.46 (0.16–1.27)	0.13
26–35	199	7	1.42 (0.54–3.42)	0.45	1.49 (0.59–3.71)	0.4
36–45	121	3	0.88 (0.2–2.64)	0.84	0.85 (0.25–2.92)	0.79
46–55	87	0	NAP	NAP	NAP	NAP
56–65	72	5	3.12 (1–8.17)	0.03	3.37 (1.19–9.58)	0.02
66–75	26	2	3.14 (0.48–11.62)	0.14	3.58 (0.78–16.46)	0.1
> 75	3	0	NAP	NAP	NAP	NAP
Contact with rodents						
No	696	18	Reference	–	–	–
Yes	103	4	1.07 (0.25–3.21)	0.92	0.87 (0.25–3.04)	0.83
Place of residence						
Urban area	422	12	Reference	–	–	–
Rural area	377	10	0.93 (0.39–2.18)	0.87	0.89 (0.37–2.13)	0.79
Contacts with wild animals						
No	788	21	Reference	–	–	–
Yes	11	1	3.65 (0.19–20.42)	0.23	2.98 (0.34–26.39)	0.33
Nature trip						
No	594	15	Reference	–	–	–
Yes	205	7	1.36 (0.51–3.29)	0.5	1.26 (0.5–3.17)	0.62
Garden work						
No	273	9	Reference	–	–	–
Yes	526	13	0.74 (0.32–1.82)	0.5	0.64 (0.27–1.54)	0.32
Livestock availability						
No	440	9	Reference	–	–	–
Yes	359	13	1.49 (0.63–3.56)	0.36	1.43 (0.6–3.39)	0.41
Current occupation						
Student	113	0	NAP	NAP	NAP	NAP
Plant farmer	13	1	3.04 (0.16–16.53)	0.3	1.96 (0.23–16.43)	0.54
Animal farmer	15	0	NAP	NAP	NAP	NAP
Forestry farmer	8	0	NAP	NAP	NAP	NAP
Keeping the house	32	0	NAP	NAP	NAP	NAP
Unskilled laborer	54	1	0.65 (0.04–3.18)	0.68	0.6 (0.08–4.54)	0.62
Skilled laborer	132	3	0.79 (0.18–2.37)	0.71	0.76 (0.22–2.65)	0.67
Driver	27	2	3.01 (0.46–11.1)	0.15	2.06 (0.44–9.59)	0.36
Adm/acad. professional	63	3	1.89 (0.43–5.74)	0.32	2.39 (0.67–8.52)	0.18
Businessman/woman	25	0	NAP	NAP	NAP	NAP
Nurse/physician/ pharmacist	20	0	NAP	NAP	NAP	NAP
Unemployed	163	6	1.48 (0.52–3.67)	0.42	1.57 (0.6–4.13)	0.36
Retiree	38	3	3.35 (0.76–10.42)	0.06	2.42 (0.46–12.64)	0.3
Military person	50	2	1.52 (0.24–5.41)	0.58	1.87 (0.38–9.29)	0.44
Declined to answer	26	1	1.43 (0.08–7.29)	0.73	0.89 (0.11–7.09)	0.91
NA	23	0	NAP	NAP	NAP	NAP

CI = confidence interval; NA = not available; NAP = not applicable; OR = odds ratio; ORa = odds ratio adjusted for sex and contact with rodents; bold values = significant.

* Shown by IgM and 4-fold titer change in IgG.

ranged between 13.6% and 44.38%.^{42,43} Similar investigations have been conducted in other countries around the world. Colombian researchers defined the seroprevalence of IgG antibodies to SFG rickettsiae (*R. rickettsii*) in patients with acute undifferentiated febrile syndrome as 2.9%, based on indirect immunofluorescence assay (IFA).⁴⁴

In our study, the occurrence for TG rickettsiae was 2.7% and comparable to investigations in other countries. The seroprevalence of murine typhus in other countries range from 3.3% in Peru to 7.1% in India.^{43,45} The seroprevalence of antibodies to TG rickettsiae in China, a bordering country to Kazakhstan, is 4.1%;⁴⁶ seroprevalence was in Bhutan 3.5%³⁶ and in Malaysia 3.6%.⁴⁷

Vietnamese colleagues studied a healthy population of people living in North Vietnam for the presence of antibodies against SFG rickettsiae (*R. conorii*) and TG (*R. typhi*) based on ELISA. The IgG antibody prevalence against *R. conorii* and *R. typhi* was detected as 1.7% and 6.5% respectively, with predominance of urban residents only for TG rickettsiae.⁴⁸ Bangladesh scientists, during their investigation of hospital-based febrile patients, found the seroprevalence of antibodies to SFG and TG rickettsiae, based on immunofluorescence antibody test, as 18% and 1%, respectively.⁴⁹ Research conducted in Bhutan demonstrated the seroprevalence, based on micro-IFAT, of 15.7% healthy participants against SFG rickettsiae and 3.5% against TG rickettsiae.³⁷

FIGURE 1. The proportion of patients with previous¹ infection of SFG and TG rickettsiae among enrolled inpatients with undifferentiated febrile illness. ¹ IgG-positive sera samples including titrated samples with a titer difference lower than 4-fold. * Calculated in relation to the number of samples by age groups.

A few recently published articles reported the occurrence of antibodies against SFG rickettsiae at the risk group population as herders, foresters, or farmers. Polish investigators focused on the study of risk group population as foresters and farmers, bitten by ticks, for the presence of antibodies against SFG rickettsiae, based on ELISA. Among 164 foresters and farmers, 39.02% (64/164) enrollees seroconverted to anti-*Rickettsia* IgG antibodies.⁵⁰ Mongolian scientists reported the seroprevalence of IgG antibodies against SFG rickettsiae (*R. rickettsii*), based on indirect IFA, in 19.5% herders, which are at high risk for tick-borne rickettsiosis.⁵¹ Malaysian colleagues detected the SFG rickettsiae (*R. conorii*) exposure in 50% indigenous people and in 13.8% farm workers based on indirect IFA.⁵²

The occurrence of antibodies usually increases with age; this was found in our study.³³ An increasing occurrence of antibodies from a previous infections across age groups is a sign of cumulative exposure over life time. There are no data on the duration of the stability of SFG and TG rickettsiae antibodies after infection.

Serological reaction can be related to infection, for example to *R. raoultii*, *R. slovaca*, and one of two new recently described *Rickettsia* spp.¹⁸

We could not find a significant difference between urban and rural residents infected with tick-borne rickettsiosis and murine typhus, whereas scientists from India and New

Zealand indicated that people living in rural areas are more at risk of murine typhus infection than urban residents.⁴³

This study had several limitations. For example, we could not collect enough samples in several hospitals and both sera from several patients, and the differentiation of *Rickettsia* spp. was not done with micro-IFAT.

CONCLUSIONS

This is the first comprehensive study on both SFG and TG rickettsiae in Kazakhstan. The obtained data show that tick-borne and TG rickettsiosis are present in Kazakhstan. Most of the Kazakh general clinicians are not familiar with the symptoms caused by rickettsial diseases, and therefore many cases probably remain undiagnosed in the country. Therefore, awareness on these emerging diseases should be raised in Kazakhstan.

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Disclaimer: All ethical issues were reviewed and approved by the national Kazakh Ethical Committee from Kazakh National Medical

TABLE 5

Clinical manifestations in patients with acute infection* of the typhus group rickettsiae†

Symptoms	OR (95% CI)	P value
Neck pain	1.12 (0.35–3.06)	0.81
Weakness	4.4 (1.05–39.11)	0.03
Muscle pain	1.9 (0.64–5.06)	0.18
Lymphadenopathy	0.7 (0.24–1.84)	0.51
Exanthema	2.47 (0.91–6.36)	0.06

CI = confidence interval; OR = odds ratio; bold values = significant.

* Shown by IgM and 4-fold titer change in IgG.

† Data based on Fisher's exact test.

University and by the ethical committee from the Ludwig-Maximilians-Universität in Germany. The authors declare that they have no competing interests. The opinions expressed by the authors contributing to this study do not necessarily reflect the opinions of the involved institutes.

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