

Serological investigation of orthohantaviruses in patients with fever of unknown origin in Kazakhstan

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Abstract

Objective: Orthohantaviruses are geographically widely distributed and present various clinical manifestations from mild symptoms to the severe form of haemorrhagic fever with renal syndrome (HFRS) in Eurasia. Official registration of HFRS in Kazakhstan started in the year 2000. However, the true prevalence of human infections by orthohantaviruses within Kazakhstan is unknown. The aim of this study was to investigate of the seroprevalence of orthohantavirus infections in patients with fever of unknown origin (FUO) in two regions, Almaty and Kyzylorda region.

Methods: Paired serum samples from 802 patients with FUO were screened for the presence of orthohantavirus IgG and IgM antibodies by ELISA. Positive samples were further tested by immunoblotting and indirect immunofluorescence tests (IIFT) to determine the respective orthohantavirus serotypes. Suspected acute serum samples were additionally checked by a RT-PCR to identify viral RNA.

Results: In total 178/802 (22.2%) serum samples reacted with orthohantavirus IgG antibodies and 4/802 (0.5%) with IgM antibodies. All positive samples were tested by immunoblotting which resulted in 2.9% positive samples with IgG antibodies against Puumala (PUUV), Hantaan (HTNV) and Dobrava (DOBV) virus serotypes in Almaty region and 5.4% to PUUV and DOBV serotypes in Kyzylorda region, respectively. In the IFFT, 1.9% positive samples from Almaty and 3.1% from Kyzylorda were confirmed for PUUV and DOBV serotypes. Out of four IgM ELISA positive samples only three were positive against PUUV in the immunoblot and showed weak positive reactivity for the Saaremaa (SAAV), PUUV and HTNV serotypes in the IFFT.

Conclusions: This study demonstrates the presence of orthohantavirus infections among patients with FUO in Kazakh regions that were so far considered as non-endemic. The healthcare system needs to be prepared accordingly in order to be capable of detecting cases and providing adequate management of patients.

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KEYWORDS

fever of unknown origin, Kazakhstan, orthohantavirus, serology

1 | INTRODUCTION

Orthohantaviruses (family *Hantaviridae*, order *Bunyavirales*) are RNA viruses, dispose of a lipid envelope and form spherical or oval virions of 80 to 120 nm. The virus genome consists of three segments of a single-stranded negative orientated RNA (Vaehri, Henttonen & Voutilainen, 2013; Vaehri et al., 2013). Presently, according to the actual report of the International Committee on Taxonomy of Viruses there are at least 41 species of orthohantaviruses (ICTV, 2018).

Orthohantaviruses are detected in many species of small mammals throughout the world. The viruses are mainly circulating in rodents such as *Arvicolinae* and *Murinae*, but are sometimes also found in bats or shrews (Essbauer & Krautkrämer, 2015; Krautkrämer, Zeier, & Plyusnin, 2013). Humans become infected by contact with rodents or their products: urine, saliva and faeces and by inhalation of aerosols containing virus (Hart & Bennett, 1999; Johnson, 2001; Lednicky, 2003). In general, orthohantaviruses can induce two distinct types of diseases: hantavirus cardiopulmonary syndrome (HCPS) in the Americas and haemorrhagic fever with renal syndrome (HFRS) in Europe and Asia (Essbauer & Krautkrämer, 2015; Schmaljohn & Hjelle, 1997).

Haemorrhagic fever with renal syndrome is caused by the strains Hantaan orthohantavirus (HTNV), Seoul orthohantavirus (SEOV), Puumala orthohantavirus (PUUV), Dobrava-Belgrade orthohantavirus (DOBV), Tula orthohantavirus (TULV) and Amur orthohantavirus (AMRV), (Papa et al., 2016; Schmaljohn & Hjelle, 1997; Vapalahti et al., 2003; Vaehri et al., 2013). Clinical and epidemiological features of infection may be different for various orthohantavirus strains. TULV infection is mostly manifested in mild clinical forms, only two cases were reported from Germany, also with no fatality (Klempa et al., 2003; Schultze, Lundkvist, Blauenstein, & Heyman, 2002). PUUV and SEOV both cause mild clinical manifestations including renal symptoms (PUUV: *nephropathia epidemica*) and mortality is low, between 1% and 2%. Four genotypes have been identified in DOBV: For the Dobrava genotype, clinical manifestations range from mild to severe with a case fatality rate of 10%–12%. For the Kurkino genotype, clinical manifestations are mild to moderate and the case fatality rate is 0.3%–0.9%. For the Saaremaa (SAAV) genotype asymptomatic infections are known, and data on lethality are not available. Infections with the Sochi virus genotype are moderate to severe with a case fatality rate of more than 6%. Finally, HTNV induces the most severe clinical manifestations in the spectrum of HFRS and goes on with a higher lethality rate of 10%–15% (Krautkrämer et al., 2013; Essbauer & Krautkrämer, 2015).

The vastness of the territory of Kazakhstan harbours many natural foci of zoonotic diseases. Only few zoonotic diseases

IMPACTS

- FOU can be caused by a broad variability of zoonotic infectious agents such as orthohantaviruses. There exist no data on orthohantaviruses in patients with FOU in Kazakhstan.
- We demonstrate a high seroprevalence against orthohantaviruses in two regions of Kazakhstan. Additionally, we showed acute infections and that the present virus type might be Puumala orthohantavirus.
- Physicians in Kazakhstan should be aware that clinical symptoms starting with mild fever could be caused by orthohantaviruses. As rodents are a reservoir for orthohantaviruses further studies on these reservoir animals should be initiated.

have been studied and for some there are only indications based on clinical symptoms. Besides, haemorrhagic fever can be caused by orthohantaviruses, which is of interest for the health surveillance system in Kazakhstan. The first human cases of HFRS were detected and laboratory–confirmed (IIFT, ELISA IgG paired serum samples) in the village of Zharsuat in the Burlinskiy district in the West Kazakhstan region in the year 2000. From 2000 to 2018, 245 cases of HFRS were clinically registered and serologically confirmed by IIFT/ELISA in the West Kazakhstan region (Bekmukhambetov, 2012; Zakharov, Grazhdanov, Zakharov, & Nazhimova, 2010). Investigations of rodents as reservoir host are limited in Kazakhstan. Only one report describes TULV found in tissue samples of *Microtus arvalis* in the Almaty region (Taldykorgan and Bakanas), but TULV is usually not pathogenic in humans or causes only mild diseases (Plyusnina, Laakkonen, Niemimaa, Henttonen, & Plyusnin, 2008). From 2001 to 2011, 49,676 small mammals were screened for the orthohantavirus antigen by ELISA in West Kazakhstan region. In four rodent species, 758 positive results were obtained (Grazhdanov et al., 2014). Nowadays the West Kazakhstan region is designated as an endemic area for orthohantaviruses. Unfortunately, no information concerning other regions of Kazakhstan is available. Orthohantavirus infections are expected to be underdiagnosed as these do not uncommonly lead to atypical or mild illness and diagnostic testing is difficult (Bi, Formently, & Roth, 2008; Sevensan et al., 2015). The exact prevalence of orthohantavirus infections in cases of FOU within Kazakhstan is unknown. The aim of the study was to investigate the seroprevalence and serotype of orthohantavirus infections in patients with FOU in two regions of Kazakhstan.

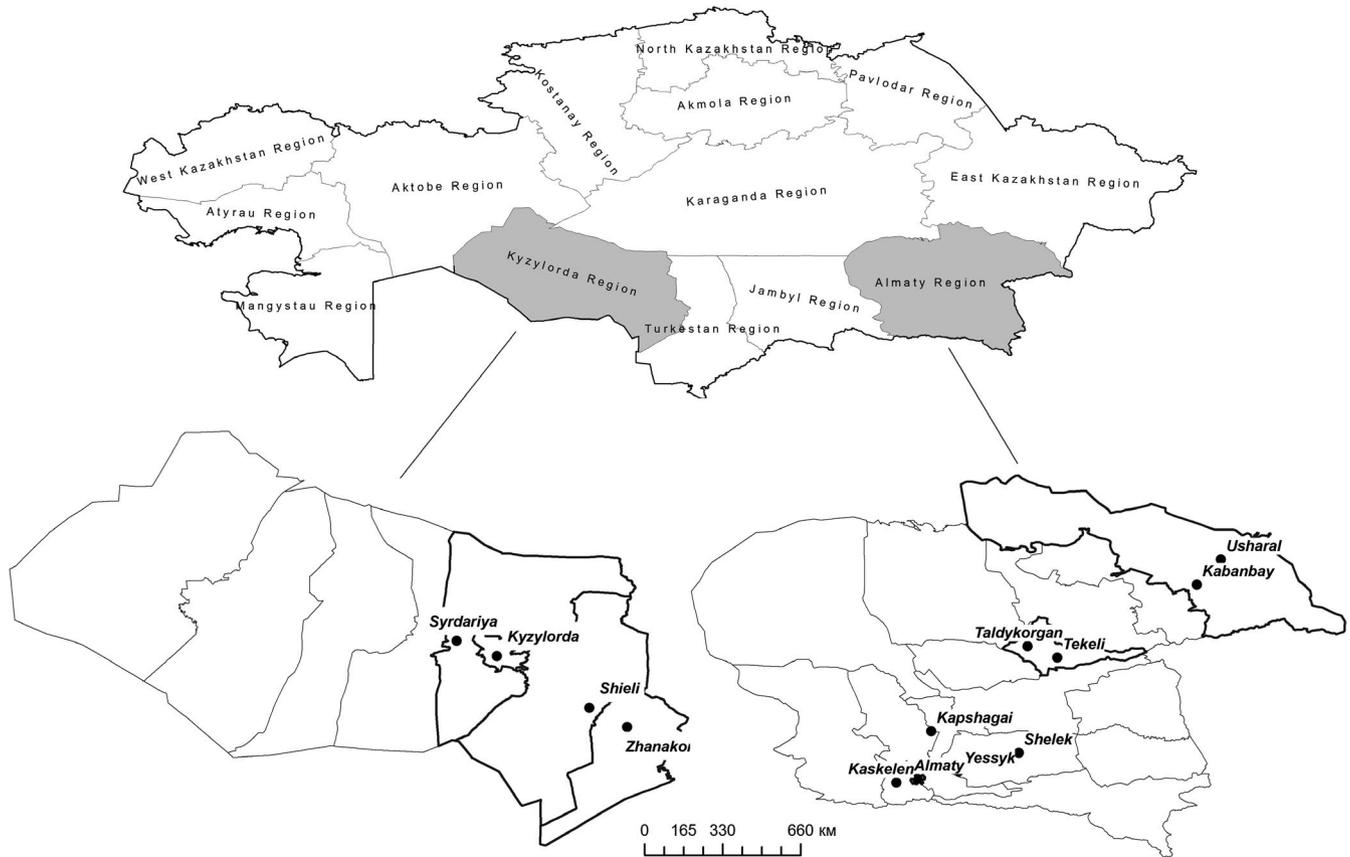


FIGURE 1 Geographical location of sampling points in two regions of Kazakhstan: Almaty and Kyzylorda

2 | MATERIALS AND METHODS

2.1 | Study design

A cross-sectional descriptive study was set up in 2015–2016 among patients with FUO in Kazakhstan in the Almaty and the Kyzylorda region (Figure 1). In these two regions, 13 hospitals were selected to conduct various studies in patients with FUO with a focus on rodent- and arthropod-borne infections (Abdiyeva et al., 2019).

2.2 | Ethics approval

This study was performed in accordance with the Kazakhstan local ethics committee at the Kazakh National Medical University in Almaty, Kazakhstan (opinion numbers 194–15, 564–18) and Ludwig-Maximilians-Universität in Munich, Germany (opinion numbers 16–175, 18–631). Blood sampling was conducted after signed informed consent. From participants under 18 years of age, the signed informed consent was taken from both parents or guardians and the underage participant.

2.3 | Sample collection

Responsible doctors identified hospitalized patients with FUO at the 13 hospitals included in the Almaty and Kyzylorda region. FUO was

defined as presenting with sub-febrile or febrile temperatures. Fever was defined by taking the temperature via tympanic measurement and lasting at least for three days. Rhinitis or any other laboratory-confirmed diseases represented exclusion criteria. Participants of both sexes and of age ≥ 15 years were included in the study. All participants signed an informed consent form. A standardized questionnaire was completed using a face-to-face interview method. The questionnaire included 47 questions with sociodemographic, living and housing, livestock, vector habitat and clinical symptoms modules.

Blood sampling was performed twice: the first serum sample was taken on the first day of hospitalization; the second serum sample was taken 10–14 days later. Paired blood samples were centrifuged, and sera were split into aliquots and conserved at -20°C for further serological testing. The required amount of serum was heat-inactivated (56°C , 60 min) before further being processed in the serological study.

2.4 | ELISA-screening

All serum samples were tested for the presence of orthohantavirus IgG and IgM by a commercial ELISA (Novatec Immunodiagnostica). The ELISA plates were read by optical density (OD) with an ELISA plate reader (Infinite F50, Tecan). OD values were measured at 450 nm with 620 nm as a reference (Novatec, Immunodiagnostica GmbH, Novalisa HANG0670 Manual). Results were calculated in Novatec Units (NTU) as the patients mean absorbance value multiplied with ten and divided through the mean

cut-off. Patients with a NTU < 8 were negative, patients with NTU > 11 were designated as serum samples from patients that had contacts with the antigen and therefore as positive. Serum samples with a NTU between 9 and 11 were judged as equivocal and repeated. If the result was equivocal again the sample was judged as negative.

All second serum samples were screened for IgG antibodies. To find out if it was an acute or a previous infection, all IgG positive second serum and the corresponding first serum samples were further tested for IgG antibodies and gained NTU were compared. If the first serum was negative for IgG antibodies, the first serum was tested against IgM antibodies. In the case that the first serum was negative for IgM antibodies, this first serum was further tested by molecular methods as well as all IgM-positive first serum samples. If both paired serum was positive (NTU > 11) for IgG antibodies and if the difference was ≤ 2 , it was declared as being negative for an acute infection. In the case the difference was >2 a titration with serial dilution was performed (1:101, 1:201, 1:401, 1:801). A 4-fold and higher titre difference between second and first serum was estimated as an acute infection.

2.5 | Serotyping

To verify the orthohantavirus serotypes of positive serum samples, IgG and IgM were further investigated by IgG and IgM immunoblotting tests (Microgen recomLine HantaPlus) and IgG and IgM IIFT (Euroimmun) according to the manufacturers' protocols.

The immunoblotting test provides a strip assay for the detection of human antibodies of the IgG and IgM classes for five different orthohantavirus serotypes and one phlebovirus: PUUV, HTNV, DOBV, Seoul virus (SEOV) and Sandfly virus. The test strips were visually evaluated from (-) to (+++). Low intensity (+) to strongly (++)/+++ coloured bands were interpreted to indicate positively.

Anti-orthohantavirus IIFT for the determination of antibodies class IgG and IgM were performed by using commercial slides of the Hantavirus Mosaic 2 Eurasia (Euroimmun) for HTNV, PUUV, SEOV, SAAV, DOBV serotypes with 1:10 and 1:100 dilutions. Results were evaluated independently by two persons using a fluorescence microscope (MicroOptix MX 300).

2.6 | Molecular investigations

RNA was extracted from 140 μ l serum sample using the commercial kit QiAmp Viral RNA Mini Kit (Qiagen) according to the manufacturer's

instructions. Presence of RNA was examined by a panHanta reverse transcriptase qPCR (Mossbrugger, Felder, Gramsamer, & Wölfel, 2013) in a Qiagen One-Step RT-PCR mix on a Rotor-Gene Q cycler (Qiagen).

2.7 | Data analysis

The statistical analysis of the results was performed using STATA (R) 15.1 (StataCorp, 2017). Chi-square test was calculated for the estimation of the association between risk factors and seropositivity. *p*-values of $\leq .05$ were considered as statistically significant. Univariate analysis was conducted to calculate odds ratio (OR) and 95% confidence interval (CI) to identify possible risk factors.

3 | RESULTS

During the study period 2015 and 2016, 950 patients with FUO presented in the 13 hospitals of the two regions in Kazakhstan. In summary, 148 patients had to be excluded per protocol for not providing paired serum samples or completing the study questionnaires. Out of the remaining 802 paired serum samples, orthohantavirus specific IgG antibodies were found by ELISA in 22.2% (178/802) of the study subjects. In four serum samples, 0.5% (4/802) positive orthohantavirus IgM antibodies were detected indicating the suspicion of an acute infection (Table 1).

All 178 IgG-positive serum samples were further checked for titration. In 130 from 178 serum pairs (73.0%) OD was ≤ 2 units and therefore these were not titrated. Out of 178 serum pairs, 31 (17.4%) showed low titres (1:101) and 17 serum pairs (9.5%) showed medium titres (1:201, 1:401) by titration and were evaluated as having had already previous exposure. There were no samples with high titres (Table 2).

All orthohantavirus ELISA IgG-reactive ($n = 178$) and IgM-reactive ($n = 4$) samples were further tested by immunoblotting assay (IgG and IgM) and IIFT to identify circulating serotypes of orthohantaviruses. Among 178 ELISA IgG-positive serum samples the reactivity for PUUV, HTNV, DOBV was confirmed by IgG immunoblotting test in 20 serum samples (11.2%) and by IgG IIFT for PUUV, DOBV serotypes in 34 serum samples (19.1%, 5 positive in 1:10, 15 positive in 1:100 dilution). Three of four tested serum samples were positive for PUUV serotype by IgM immunoblotting

TABLE 1 Results of the orthohantavirus serology study by ELISA IgG and IgM among patients with FUO in the Almaty and Kyzylorda region 2015–2016

Regions	Total number of tested serum samples	Number of IgG positive orthohantavirus samples 2nd/1st serum (%)	Number of IgM positive orthohantavirus samples 1st serum (%)	Number of negative serum samples (%)
Almaty	378	80 (21.2)	4 (1.0)	294 (77.8)
Kyzylorda	424	98 (23.1)	0	326 (76.9)
Total	802	178 (22.2)	4 (0.5)	620 (77.3)

TABLE 2 Results of tested anti-orthohantavirus IgG positive paired serum samples on ELISA

ELISA IgG result (2nd/1st serum)	Number of serum samples (%)
Low titre (1:101/1:101) ^a	130 (73.0%)
Low titre (1:101/1:101)	31 (17.4%)
Moderate titre (1:201-1:401/1:201-1:401)	17 (9.5%)
High titre (1:801/1:801)	0
Total	178

^aIf the optical density between second and first serum was \leq OD units, these were not titrated.

TABLE 3 Results of orthohantavirus immunoblotting and IIFT IgG and IgM among patients with FUO in the Almaty and the Kyzylorda region 2015–2016

Regions	Serotype	IgG (n = 178)		IgM (n = 4)	
		Immunoblot test	IIFT	Immunoblot test	IIFT
Almaty	PUUV	7	6	3	3 ^a
	HTNV	3	0	0	0
	DOBV	1	1 ^a	0	0
	(%)	2.9	1.9	1.0	1.0
Kyzylorda	PUUV	15	9	0	0
	DOBV	8	4	0	0
	(%)	5.4	3.1	0	0
Total (%)		34 (19.1)	20 (11.2)	3 (1.0)	3 (1.0)

Abbreviations: DOBV, Dobrava orthohantavirus; HNTV, Hantaan orthohantavirus; PUUV, Puumala orthohantavirus.

^aReactivity with Saaremaa, Puumala, Dobrava, Seoul and Hantaan serotypes.

TABLE 4 Results of ELISA, immunoblotting test, IIFT and RT-PCR positive orthohantavirus IgM serum samples^a

Serum samples		YEN1 200–050	YEN1 200–059	ALM 800–108	ESK 600–004
ELISA IgM	1st serum	+	+	+	+
Immunoblotting IgM	PUUV	+	+	+	–
	SINV	±	±	–	–
	HNTV	±	±	–	–
	DOBV	±	±	±	–
	SEOV	–	–	–	–
	SFV	–	–	–	–
IIFT IgM (1:10, 1:100)	HNTV	±	–	–	–
	PUUV	±	±	±	–
	SEOV	±	–	–	–
	SAAR	–	±	±	–
	DOBV	–	–	±	–
	Non infected cells	–	–	–	–
Reverse transcriptase qPCR	1st serum	Negative	Negative	Negative	Negative

Abbreviations: DOBV, Dobrava orthohantavirus; HNTV, Hantaan orthohantavirus; PUUV, Puumala orthohantavirus; SAAR, Saaremaa orthohantavirus; SEOV, Seoul orthohantavirus; SFV, Sandfly virus; SINV, Sin Nombre orthohantavirus.

^a+ positive (low intensity), +/- weak positive (very low intensity).

testing. In one case no serotype identification could be seen. IIFT showed in three serum samples a weak positive reactivity in 1:10 and 1:100 dilution with SAAV, PUUV, DOBV, SEOV and HTNV serotypes (Table 3).

The four serum samples indicating an acute orthohantavirus infection originated from the Almaty region from three hospitals (Yessyk hospital: 2 positive patients (YEN1-200 50, YEN1-200 59), Almaty hospital: one positive patient (ALM-800 108), Tekeli hospital: 1 positive patient (ESK-600 004)). Of the four positive participants, three were female with ages of ages 22, 33 and 51 and one male at the age of 19. Of the IgM-positive participants, two individuals lived in rural and two in urban areas ($p = 1.000$).

Concerning the daily activities investigated half of the participants did garden and fieldwork ($p = .864$), and three of them had seen rodents ($p = .213$). The clinical manifestations of positive IgM subjects showed fever ($n = 4$), headache ($n = 3$), weakness ($n = 2$), arthralgia ($n = 2$), back pain ($n = 1$) and nose congestion ($n = 1$). In total three of the four IgM positive ELISA serum samples were confirmed by Immunoblotting tests for the PUUV serotype (YEN1-200 50, YEN1-200 59, ALM-800 108) with low intensity (+) coloured bands. All these three samples showed weak positive result in the IIFT with 1:10 and 1:100 dilution to SAAV, PUUV, DOBV, SEOV and HTNV serotypes. All IgM-positive serum samples were additionally tested by RT-PCR to detect RNA of orthohantaviruses. In none of these samples orthohantavirus RNA was detected (Table 4).

To assess the potential risk factors for orthohantavirus infections, a univariate logistic regression was performed on in the ELISA IgG-positive serum samples. No significant association could be identified between risk factors such as sex, last nature trip, house location in urban or rural area or the fact that the person had seen rodents with seropositivity. Working in a garden and in the field, as often 1.7 and as always 2.9, increased risk of seropositivity but it was not significant ($p = .05$). By the way, patients with age ≤ 50 had 2.26 times more seropositivity compared with the age > 50 and it was statistically significant. On the other hand, there were no risk factors identified on positive immunoblot IgG serum and IIFT IgG serum samples.

4 | DISCUSSION

Orthohantavirus infections are globally wide-spread and during the last two decades are receiving more attention as a relevant public health problem. In Kazakhstan, the investigation of orthohantaviruses has been focusing so far on the West Kazakhstan region as there were previous human cases recognized by clinical patterns which were also laboratory confirmed. Nevertheless, some rodent investigations revealed that the natural foci of orthohantaviruses are located between the West Kazakhstan region and Orenburg, the Samara regions of the Russian Federation (Alexeyev, Elgh, Zhestkov, Wadell, & Juto, 1996; Aminev, Korneev, Slobodenyuk, & Solovich, 2014; Grazhdanov et al., 2013). Annual registrations of HFRS in the West Kazakhstan region began in 2000, and a high incidence rate of 16 per 100,000 inhabitants was described in 2005 (Grazhdanov et al., 2014). In the West Kazakhstan region from 2001 onwards, the investigation of reservoirs started. These showed the orthohantavirus antigen by ELISA in different species of rodents: bank voles, common voles, forest mice and house mice (Grazhdanov et al., 2014). Another report demonstrated that rodent tissue suspensions collected in the Almaty region Dzungarian, in the Alatau mountains in 2010–2016, 2.2% (15/684) were positive for orthohantavirus antigens using ELISA (Test system: Hantagnost, Russia), (Sutyagin, Belyaev, Kim, & Berdibekov, 2017).

However, there exist no systematic data on the seroprevalence of orthohantaviruses in humans in Kazakhstan. Some studies showed that the orthohantavirus seroprevalence in Asian countries, for example China, Korea, Thailand and Singapore prevailed between 0.5% and 33.3%, and in European countries between 0% and 24%. (Bi et al., 2008; Mertens et al., 2011; Jiang, Zhang, et al., 2016; Xiao et al., 2018; Zou, Chen, & Sun, 2016).

In Kazakhstan, various zoonotic agents have been suspected to be endemic that can cause FUO with mild clinical presentations. Investigations of patients with FUO can provide adequate information for the public health priority setting. However, in resource-limited settings such as in Kazakhstan, the needed high-quality laboratory diagnostics are not or only insufficiently established. Parallel investigations of the same FUO samples used in this study for other arthropod-borne infectious showed that some serum samples with confirmed orthohantavirus IgG antibodies were reactive also for other agents: for Crimean-Congo haemorrhagic fever virus (CCHFV), six IgG serum samples, for *Rickettsia spotted fever* group ELISA (IgG), 13 serum samples and for *Rickettsia typhus* group ELISA, 15 serum samples. However, none of the patients that were orthohantavirus IgM positive had simultaneously antibodies against CCHFV, *Rickettsia of spotted fever* group and *Rickettsia of typhus* group (Abdiyeva et al., 2019).

This study presents the first seroprevalence study of orthohantavirus infection among patients with FUO in two regions of Kazakhstan using a combination of serological assays. Our study identified an acute orthohantavirus infection in four serum samples on ELISA and three of them reacted with PUUV serotype by immunoblotting and showed a weak positive reaction for PUUV, HTNV, SAAV, DOBV, SEOV serotypes by IIFT. However, IgM titres against orthohantaviruses can stay positive for several months after the onset of disease, which relativizes our assumptions on acute cases in our patient group (Krüger, Figueiredo, Song, & Klempa, 2015; Meisel et al., 2006). In this study, we could not type the patient's serum by FRNT as such tests are currently not available in Kazakhstan. RT-PCR has been done for the four suspected acute serum samples. However, viremia phases during orthohantavirus infections in humans are short and present before IgM antibodies are present, which could also be the case in this study (Krautkrämer et al., 2013; Krüger et al., 2015). Clinical manifestations of HFRS are characterized by acute renal failure followed by haemorrhage and flu-like symptoms such as fever, headache, abdominal/back pain and range from subclinical or mild to severe symptoms (Krautkrämer et al., 2013). In the present study, patients with IgM-positive serum samples developed unspecific clinical signs that can also be attributed to a mild form of the disease (Golovljova et al., 2007; Jiang, Du, Wang, Wang, & Bai, 2016). Moreover, orthohantavirus IgM levels were investigated instead to determine suspected acute cases among patients with FUO. Generally, this study showed that IgM-positive patients were more females than males, but this was not statistically significant as given by the small case numbers (Latronico et al., 2018; Sevcenkan et al., 2015). We did not find a relationship with some risk factors such as living place, garden or

fieldwork or the observation of rodents with IgM-positive cases (Botros et al., 2004).

The most practical approach of orthohantavirus infections are based on ELISA IgG antibodies as it was also used for seroepidemiological studies. Likewise, in some seroepidemiological studies initial screening was also done by ELISA followed by further analyses using Immunoblot, IIFT and FRNT assays (Hukic et al., 2010; Zou et al., 2016). In this study, screened serum samples showed 22.2% positive results for IgG antibodies to orthohantaviruses by ELISA. Immunoblotting and IIFT confirmed all samples considered as positive. According to the results of the immunoblotting, the orthohantavirus IgG exposure among people with FUO was estimated to be 2.9% in the Almaty region and 5.4% in the Kyzylorda region with different serotypes (PUUV, HTNV and DOBV), by IIFT 1.9% in Almaty and 3.1% in Kyzylorda regions (PUUV and DOBV), respectively. In our study, the high rate of positive IgG antibodies by ELISA shown here could be false-positive, originating from the sensitivity of the screening test. Moreover, the difference between ELISA and confirmatory assays has been shown in several orthohantavirus seroprevalence studies (Engler et al., 2013; Sevancan et al., 2015). The different results by immunoblotting and IIFT can be explained by sensitivity (immunoblotting—96.1%, IIFT—99%) and specificity (immunoblotting—100%, IIFT—98%) of the used assays (mikrogen.de, euroimmun.de). However, immunoblotting assay is used as more suitable diagnostic and confirmatory test (Engler et al., 2013; Escadafal et al., 2012). In the Almaty region some rodent studies were conducted, in which in some areas rodents were found to be positive for orthohantaviruses, but no clinical case of HFRS has officially been registered in this region so far (Plyusnina et al., 2008; Sutyagin et al., 2017). Notable is that at some parts of the border between the Almaty region and China the orthohantavirus seroprevalence has been reported to range between 1% and 12% (Avšič Županc & Korva, 2014; Bi et al., 2008). So far in the Kyzylorda region, orthohantaviruses have not been studied in human cases. We are therefore the first to promote that orthohantaviruses seem to circulate in this region.

In agreement with previous studies in the present study, no significant association was identified between risk factors concerning sex, last nature trip, house location in urban or rural area or the fact that the patient had seen rodents with the IgG ELISA seropositivity (Botros et al., 2004; Christova et al., 2017; Sin et al., 2007). In our study, garden fieldwork and the age ≤ 50 years was the risk factor associated with IgG seropositivity on ELISA. Similar data of outdoor activities were demonstrated in a study from Sweden (Gherasim et al., 2015). It is probable that such findings are due to having had contact with rodents or their excreta during gardening.

In conclusion, these data present the first seroprevalence study of orthohantavirus infections in humans with FUO in Kazakhstan. The data obtained show that the diagnostics of orthohantaviruses among individuals with FUO is important given the potential severe course of the presentation and the specific treatment options. However, in many cases, the initial presentation with mild forms of the disease with fever and flu-like symptoms may render the differential diagnosis a challenge. So far also data on orthohantaviruses in

rodents in Kazakhstan are limited. Additional studies in rodents and humans are necessary in order to be able to better characterize the circulation of virus strains in the region.

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CONFLICT OF INTEREST

None to declare.

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