

Original Article

Detection of Space-Time Clusters and Ambient Temperature Effects on Non-Toxigenic *Vibrio Cholerae* in Russia from 2005 To 2021

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ABSTRACT

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Introduction: The identification of climate temperature-sensitive pathogens and infectious diseases is essential in addressing health risks resulting from global warming. Such research is especially crucial in regions where climate change may have a more significant impact like Russia. Recent studies have reasoned that the abundance of non-toxigenic *V. cholerae* is environmentally driven and can be part of early global warming signals for Russian territory. The aim of the study is to investigate the spatial-temporal trends and thermo-climatic sensitivity of non-toxigenic *V. cholerae* abundance in Russia.

Methods: This study employed Kulldorff's space-time statistics to identify persistent clusters of the *V. cholerae* ctx- isolation and areas for exploring temperature-depended patterns of the vibrio distribution. Correlation analysis was used to identify regions with temperature-driven *Vibrio* abundance in water samples.

Results: The spatial analysis detected 16 persistent (7-8 year) clusters of *V. cholerae* ctx- across the study period 2005-2021. The number of clusters with RR >1 abandoning from the south to the north and the total number of persistent clusters (9) is greater in the period of 2014(5)-2021 compared with the period 2005-2013 (7). A distinct significant thermo-climatic effect on the abundance of *V. cholerae* ctx- in water basins was found in three Russian regions with temperate marine (the Kaliningrad region) and sharp continental climatic conditions (the Irkutsk region and the Republic of Sakha). The temperature and *Vibrio* prevalence trend curves are peaky (the Kaliningrad region and the Republic of Sakha) or bell-shaped (the Irkutsk region) changed and closely followed together.

Conclusion: The persistent clusters should become targeted areas to improve sanitation conditions. The study offers valuable outcomes to support simplified empirical evaluations of the potential hazards of vibrio abundance that might be useful locally for public health authorities and globally as a part of Russia's warning system of climate change effects.

Introduction

Strong dependence on temperature and salinity makes *Vibrio* spp. environmentally-driven

bacteria and climate change affect the spatial distribution and abundance of *Vibrio* species. Baker-Austin et al. showed that sea surface temperature >18°C is a notable risk factor,

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significantly increasing the quantity of *Vibrio* spp. in seawater and reported *Vibrio* cases in the Baltic region.¹⁻⁴ Taylor et al.⁵ reported the largest outbreak of *V. parahaemolyticus* in Canadian history in 2015 which was probably associated with the increased water temperature in British Columbia. In the US, the increased non-cholera *Vibrio* incidence rate between 1999 and 2014 was associated with the El Niño-Southern Oscillation conditions.⁶

During the past decades, European marine temperate regions have warmed at four- to sevenfold the global rate.⁷ The salinity of water basins is also likely to be changed under global warming conditions. Melting icebergs refreshes seawater⁸ which will affect the future abundance of the genus *Vibrio*.⁴ As a result, the prevalence of *Vibrio* spp. in aquatic systems is a subject of strong monitoring of outbreak-preventable systems in several countries in Europe and North America with a linkage to ambient temperature. Much less attention has been applied to studying the spatial distribution and abundance of *Vibrio* in Russia's global warming context. However, Russia is warming 2.5 times faster than the rest of the world due to a large territory and greenhouse gas emissions. If the impact of climate change continues over the next years, Russia will start to feel the consequences of climate change in relation to extreme weather effects such as heat waves, floods, and droughts by 2030.^{9,10} In Russia, which is not endemic territory to toxigenic strains (*ctx*⁺) of *V. cholerae*, but rarely O1/O139 *ctx*⁻ and, frequently non-O1/O139 *ctx*⁻ strains of *V. cholerae* are isolated in almost all administrative Russian regions every year. The non-toxigenic *V. cholerae* rarely cause vibriosis but their detection in water often is used as epidemiological markers of the sanitary

unfavourability of water reservoirs in Russia.¹¹ Currently, Russia has a 16-year available data set of *V. cholerae ctx*⁻ prevalence for different regions that can be used with relatively high resolution to analyze the spatial distribution and the abundance of *V. cholerae ctx*⁻ with respect to thermo-climatic changes at local levels to identify contaminated areas. Moreover, due to the large Russian territory, the analysis might be valuable for the indirect detection of global warming effects.

The aim of the study is to explore the possibility to form space-time clusters for non-toxigenic strains of *V. cholerae* in Russia and identify regions where the abundance of *V. cholerae ctx*⁻ is driven by ambient temperature.

Materials and Methods

Study design and data collection

We retrospectively analyzed surveillance data submitted by all the cholera-reporting areas to the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) between 2005 and 2021 (www.rospotrebнадзора.ru). Federal state sanitary and epidemiological supervision uses the detection of *V. cholerae* O1, O139, non-O1/O139 serogroups, and other vibrio species in environmental objects to characterize sanitary conditions of water reservoirs (rivers, lakes, sea, and sewage) that often are used for recreational purposes during the summer period. Monitoring of *V. cholerae ctx*⁻ is a part of the anti-epidemic measures to prevent the occurrence and spread of cholera. All samples are analyzed with classical cultivation methods of bacterial isolation according to the national recommendation.¹¹

Officially, zoning of the Russian territory according to the type of cholera epidemic manifestation is divided into three types and subtypes – I, II, and IIIa, IIIb, IIIc. Depending on climatic conditions and the current epidemiological situation and the type of zone the annual number of water sampling and cholera testing are changed for:

type I - test water and sewage samples from May to September 1 time per 7 days;

type II - test water and sewage samples from June to September 1 time per 7 days;

type IIIa and b - test water and sewage samples from July to August 1 time per 7 days;

type IIIc is not mandatory to test water and sewage samples but they might do it voluntarily during the summer.¹²

Administrative territories of type IIIb are territories where cholera cases and isolated *V. cholerae ctx+* in the environmental objects have never been reported. IIIc type territories will be requalified into another subtype, if any cholera case is registered.

Monthly data on surface air temperature in summer were obtained from local weather stations closer sited to monitored water estuaries (www.meteorf.gov.ru). Table 1 provides information about these weather stations with

Table 1. General information on Weather Stations that climate data used in the study

Weather Station Name	WMO Station Number	Latitude, °N	Longitude, °E	Altitude, m above Sea Level	Köppen-Geiger climate Classification*
Kaliningrad	26702	54.71°N	20.51°E	27	Dfb
Vologda	27037	59.22°N	39.88°E	131	Dfb
Yaroslavl	Levtsovo airport (UUBX)	57.61°N	39.88°E	91	Dfb
Chelyabinsk	28645	55.15°N	61.43°E	237	Dfb
Omsk	28698	54.93°N	73.37°E	123	Dfb
Kemerovo	29642	55.25°N	86.08°E	148	Dfb
Irkutsk	30719	52.3°N	104.3°E	469	Dwb/Dwc
Chita	airport Kadala	52.03°N	113.3°E	692	Dwc
Yakutsk	24959	62.03°N	129.73°E	98	Dfd
Khabarovsk	31735	48.48°N	135.08°E	72	Dwb/Dwa
Voronezh	34122	51.70°N	39.18°E	154	Dfb
Ryazan	airport Turlatovo	54.57°N	39.85°E	158	Dfb
Krasnodar	34929	45.04°N	38.98°E	28	Cfa
Volgograd	34560	48.72°N	44.50°E	134	Dfa
Rostov-on-Don	34730	47.23°N	39.72°E	75	Dfa
Stavropol	34949	45.04°N	41.97°E	453	Dfb
Elista	34861	46.30°N	44.26°E	197	Dfa

*Dfa, Hot summer humid continental climate; Dfb, Warm summer continental climate; Dfc, Subarctic climate without dry season; Dfd, Extremely cold continental subarctic climate; Dwc, Dry winters cold summers, and strong seasonality; Dwb/Dwa, Monsoon-influenced warm/hot summer humid continental climate; Cfa, Humid subtropical climate

World Meteorological Organization (WMO) Station Number, geographical coordinates, and climate characteristics according to the Köppen-Geiger classification.¹³ The weather stations' data used in the study, are shown as red dots on the map of Russia in supplementary material.

Data missing

The collected data were checked for the presence of errors in data entry including missing data and misspellings. Following this process, there was no error in misspelling; however, there was 16.9% of missing data of annual *V. cholerae ctx* isolates mostly for type III regions (e.g., Magadan region or Komi, Republic) in 14 out of 83 regions. There were no missing values in the used climatic data set.

Statistical analysis

The annual dynamics of *Vibrio* prevalence were studied using simple linear regression. We used Spearman (ρ) correlation test to find an association between the prevalence of *V. cholerae ctx* strains and the mean summer air temperature. These statistical analyses were performed in R (version 2023.03.1+446).¹⁴ Kulldorff's retrospective scan statistic was used to identify persistent space-time clusters of *V. cholerae ctx* isolates.¹⁵ The discrete Poisson probability model was chosen for scanning since the annual cases of laboratory-confirmed isolations of *V. cholerae ctx* were not very high in some areas.¹⁶ The area with the highest likelihood is designated as the most probable cluster, while other clusters with statistically significant log-likelihood ratios (LLR) are considered secondary potential clusters. The

LLR for a specific cluster is calculated using the formula developed by Kulldorff:¹⁵

$$LLR = \log(n / (E(n)))^n ((N - n) / (N - E(n)))^{(N-n)} I',$$

where N – is the total number of cases; n – is the observed number of positive water samples in the scanning window; $E(n)$ and $N - E(n)$ – are the expected number of positive water samples within and outside the scanning window under the null hypothesis (H_0): the spatiotemporal clustering of the study areas is caused by random factors, respectively; and I is an indicator function. It takes 1 when the window has more cases than expected under the null hypothesis; otherwise, 0. The p-values of *LLR* were estimated through 9999 Monte Carlo simulations.^{16,17} A p-value < 0.05 indicates a significantly high risk inside of the scan window, which might be a potential cluster of a high risk of vibrio isolation. The relative risk (RR) indicates whether the observed number of *V. cholerae ctx* isolates that occurred in a given category is larger than expected. $RR > 1$ represents a greater-than-expected number of bacterial isolates from water samples for each cluster.¹⁸

This study used a spatial and temporal scanning window size of 50% of the total number of water samples at investigation and the total study period. The time frame of the space-time scan analysis was set at 7-8 years with a restriction to the number of positive water samples by 50 isolates per cluster. The radius was considered optimal (250 km) for analysis if there were fewer overlaps between areas defined by the radius.¹⁹ Clusters were considered significant for p-values < 0.05 . Significant clusters according to these settings are persistent clusters defined as constant bacterial isolation

over periods ≥ 7 years. Adjustments for missing data were made according to the program's user manual to account for regions that did not report cases of *V. cholerae ctx* isolation.¹⁵

The SaTScan™ software v 10.1 was used for spatiotemporal clustering analysis. The QGIS software v 3.28.3 was used to visualize the *V. cholerae ctx* clusters. The source of the shapefile for Russia – <https://mydata.biz.ru>.

Results

As shown in the map of Russia (Fig. 1), SaTScan identified 16 persistent clusters that are unevenly distributed across Russia. The RR values for observed clusters ranged from 1.19 to 4.74. Based on the value of RR, all clusters

were divided into high- ($RR > 3$) and low-risk ($1 < RR \leq 3$) areas. The highest RR values were found in the Chelyabinsk, Kaliningrad, and Omsk regions. The largest LLR was 2839.38 and the smallest 24.45 ($p < 0.001$) indicates a statistical significance in both space and time in these areas. Considering the temporal clustering pattern, just five clusters are observed within 2005-2013 while the 2014-2021 - time window includes a greater number of clusters (one “most likely” cluster and nine “secondary likely” clusters). Two large likely secondary clusters partly covering three regions are located in North Caucasian District (223.8 km) with the center in the Voronezh region (51.70°N, 40.23°E), and Central Federal District (200.3 km) with the center in Kalmykia, Republic (46.43°N,

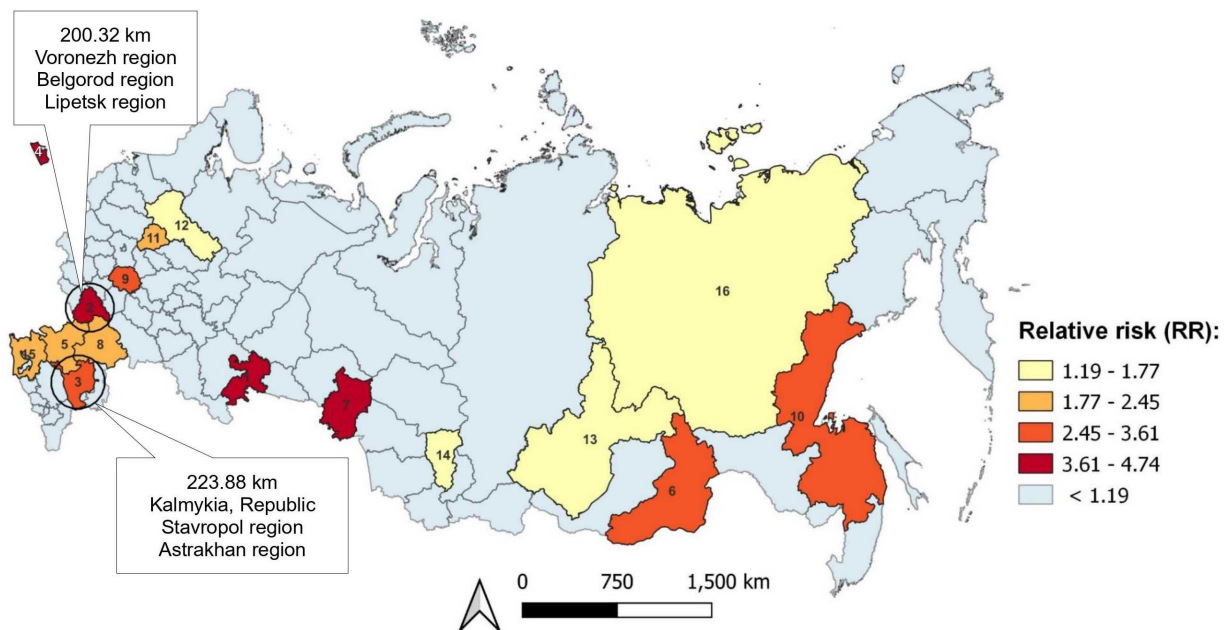


Figure 1. Spatio-temporal clustering of *V. cholerae ctx* isolates from environmental objects in Russia, 2005–2021. Map displays the results of the SaTScan space-time analysis (Poisson model). Labels on the map refer to the cluster's orders according to the RR values ($p < 0.05$). Circles represent the spatial extent of a given cluster in kilometers (km). Time periods indicate the duration of the cluster in years. 1 = Chelyabinsk region (2014-2021), 2 = Voronezh region (2005-2013), 3 = Kalmykia, Republic (2014-2021), 4 = Kaliningrad region (2014-2021), 5 = Rostov region (2014-2021), 6 = Zabaykalsky region (2014-2021), 7 = Omsk region (2005-2013), 8 = Volgograd region (2014-2021), 9 = Ryazan region (2005-2013), 10 = Khabarovsk region (2014-2021), 11 = Yaroslavl region (2005-2013), 12 = Vologda region (2014-2021), 13 = Irkutsk region (2014-2021), 14 = Kemerovo region (2014-2021), 15 = Krasnodar region (2005-2013), 16 = Sakha, Republic (2015-2021) ($p = 0.130$)

Table 2. Clusters of *Vibrio cholerae* ctx⁻ Russian regions 2005 to 2021 ($p < 0.001$)

Cluster	Observed cases	Expected cases	LLR	RR
1. Ural Federal District				
Chelyabinsk region (2014-2021), 0 km	3823	857	2839.36	4.74
2. Central Federal District				
Voronezh, Belgorod, and Lipetsk region (2005-2013), 200.32 km	5497	2349	16.28	2.50
Ryazan region (2005-2013), 0 km	662	224	281.39	2.98
Yaroslavl region (2005-2013), 0 km	410	223	62.88	1.84
3. North Caucasian Federal District				
Kalmykia, Republic and Stavropol region (2005-2013), 223.88 km	5497	3572	1628.23	2.50
4. Southern Federal District				
Rostov region (2014-2021), 0 km	4249	2039	960.04	2.18
Volgograd region (2014-2021), 0 km	1752	918	305.19	1.94
Krasnodar region (2005-2013), 0 km	84	35	24.45	2.40
5. Siberian Federal District				
Omsk region (2005-2013), 0 km	686	175	428.69	3.96
Kemerovo region (2005-2013), 0 km	1075	850	27.97	1.27
Irkutsk region (2014-2021), 0 km	394	252	34.32	1.57
6. Northwestern Federal District				
Kaliningrad region (2014-2021), 0 km	2049	452	1524.54	4.68
Vologda region (2014-2021), 0 km	536	346	45.12	1.56
7. Far Eastern Federal District				
Khabarovsk region (2014-2021), 0 km	384	109	208.49	3.53
Zabaykalsky region (2014-2021), 0 km	1172	431	436.83	2.76
Sakha, Republic (2015-2021), 0 km*	348	293	4.94	1.19

*The cluster is significant for the time aggregation of 7 years

45.30°E). The Southern and Siberia Federal Districts encompass two and three clusters (2014-2021) respectively (Table 2). Clusters in the Southern District are neighbored and had a high prevalence of *V. cholerae* ctx⁻ over the observed period of time. Clusters in the Siberia Federal District are spatially split by a hundred kilometers and situated far from countries' sea coastlines. The Far Eastern encompassed two significant clusters the Khabarovsk (48.48°N135.08°E) and Zabaykalsky (52.03°N, 113.3°E) regions but the SaTScan also defined the Sakha, Republic (66.70°N,

130.77°E) as a significant cluster ($p < 0.05$) if the time aggregation was 7 years in program settings (Table 2). This also indicates that there are differences in temporal trends in the *Vibrio* prevalence in some regions. The “most likely” spatio-temporal cluster area 2014-2021 is detected in the Ural Federal district (LLR = 2839.38, $p < 0.001$) (Table 2). The location of the cluster is the Chelyabinsk region (54.45°N, 60.39°E) covering 26 municipalities. A total of 3823 vibrio isolates were reported in this area during the study period, and the RR was 4.74. We found three split high-latitude clusters for

Table 3. Trend analysis of *Vibrio cholerae* ctx⁻ strains prevalence and mean summer air temperature for clustered regions in Russia, 2005-2021*

Clustered regions	The slope for trend lines:			
	V. cholerae ctx ⁻ prevalence-years	p-value	Mean summer air temperature-years	p-value
Khabarovsk region	3.86	0.0039	-0.08	0.0820
Chelyabinsk region	2.33	0.000006	0.04	0.4835
Irkutsk region	1.54	0.0248	0.06	0.0661
Kalmykia, Republic	1.32	0.6290	0.04	0.8934
Kaliningrad region	1.33	0.00001	0.06	0.0983
Zabaykalsky region	1.25	0.0154	-0.01	0.6789
Volgograd region	1.02	0.0007	0.05	0.4835
Vologda region	0.53	0.0733	0.02	0.6859
Omsk region	0.38	0.2880	0.02	0.5365
Rostov region	0.31	0.2060	0.03	0.5574
Sakha, Republic	0.20	0.2390	0.06	0.1886
Kemerovo region	0.19	0.2880	0.04	0.4141
Stavropol region	0.003	0.9830	0.02	0.6925
Yaroslavl region	-0.76	0.0008	0.02	0.7710
Voronezh region	-1.09	0.1810	0.07	0.2960
Ryazan region	-1.98	0.00007	0.03	0.7354
Krasnodar region	-2.06	0.0585	-0.003	0.9274

*Regions marked in italics have significant ($p < 0.05$) positive time trends of the *V. cholerae* ctx⁻ prevalence over the study period

the Northwestern Federal District the Vologda (59.96°N, 40.50°E) and Kaliningrad (54.71°N, 20.45°E) regions, and the Sakha, Republic (62.03°N, 129.73°E) in the Far Eastern Federal District. Volga Federal District does not include any statistically significant clusters.

The next step of the study was the detection of temperature effects on the distribution of *V. cholerae* ctx⁻ for each cluster to highlight temperature sensitivity and possible global warming effects in the future. In doing so, we used linear regression and correlation tests including precise analysis of epidemiological data by region based on the annual report of local public health agencies of the Rospotrebnadzor. The temperature temporal trend (16 years of observation) is not significant for all regions.

Investigations of large-scale data sets (at least 28-30 years of observation) for each region might be needed to reveal a significant temperature-temporal trend. Noticeable that for three regions Khabarovsk, Irkutsk, and Kaliningrad, the p-values of slopes essentially differ from other regions approaching the upper border of the significance of 0.05 (Table 3). We sift regions with significant positive trends for *Vibrio* prevalence during the study period such as Chelyabinsk, Irkutsk, Zabaykalsky, Khabarovsk, Volgograd, and Kaliningrad regions (Table 3). Intentionally, two clustered regions with large data sets (28 years) the Stavropol region (from the North Caucasian Federal District, Dfa - hot summer humid continental climate) and the Republic of Sakha

(from the Far Eastern Federal District, Dfd - extremely cold continental subarctic climate) were included in the correlation analysis to assess if temperature trends for *Vibrio* isolation are more pronounced in these territories. There were significant correlations between the mean summer temperature variable and the prevalence of *V. cholerae ctx* for the Irkutsk region, the Republic of Sakha, and the

Kaliningrad region (Fig. 2) other regions were excluded from further consideration. All three identified regions with positive temperature sensitivity belong to the IIIa-type territories according to the Russian cholera surveillance system which means they are mandatory to take water samples from stationary possible hot spots of *Vibrio* isolation places over the summer months.

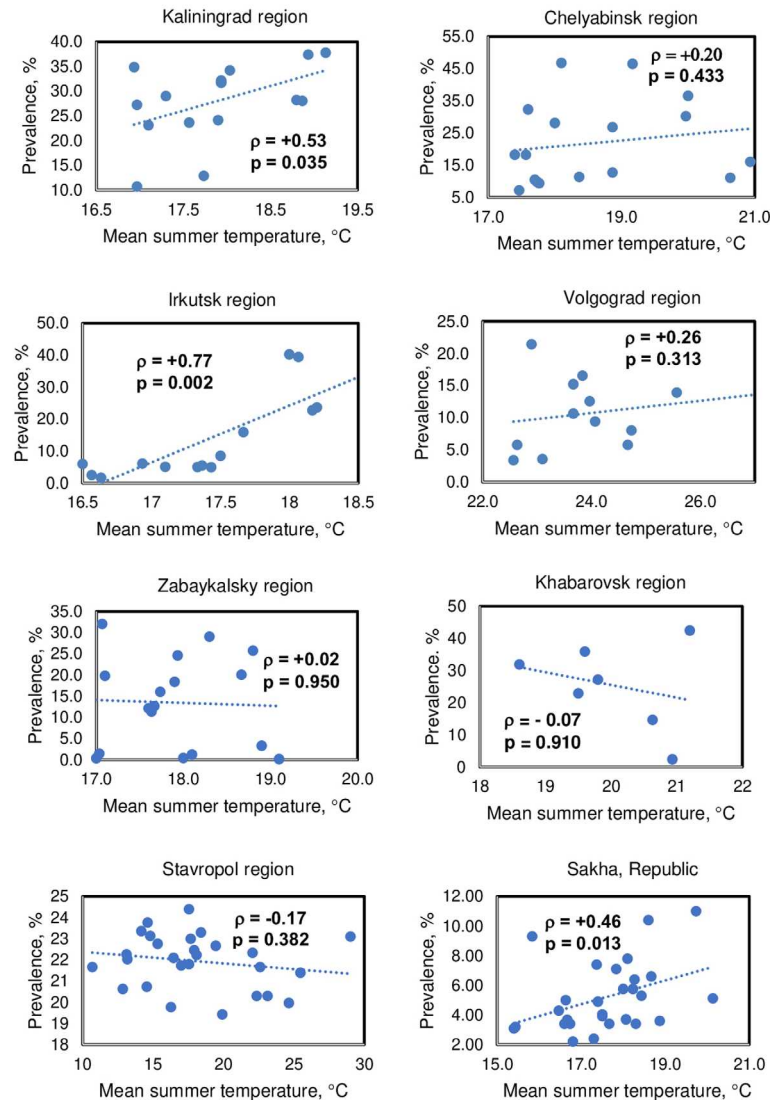
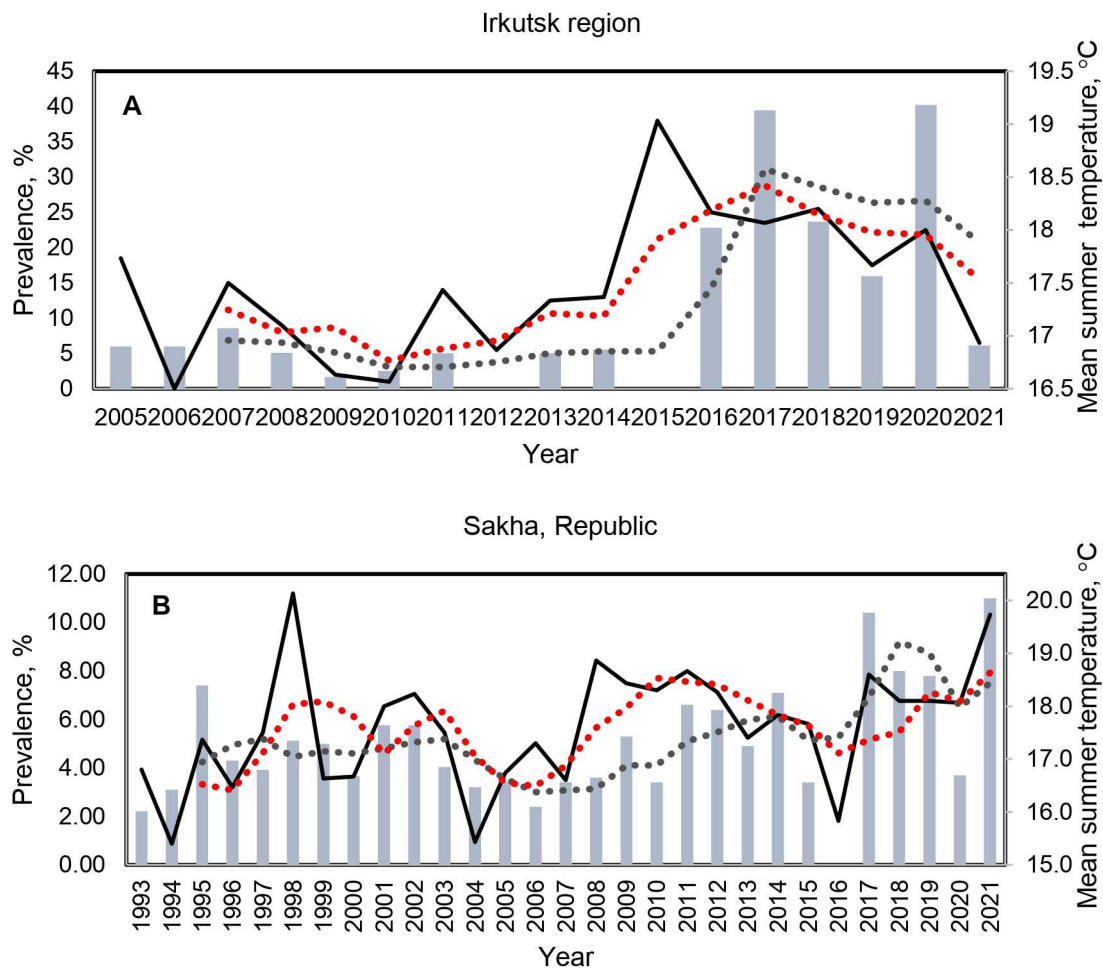


Figure 2. Scattergram showing Spearman's correlation (ρ) between the annual prevalence of *V. cholerae ctx* strains for the selected clusters with significant positive temporal trends over the study period 2005-21 and 1993-2021 (only available data set for the Stavropol region and Sakha, Republic)

The Irkutsk region is located in southeastern Siberia in the basins of the Angara, Lena, and Nizhnyaya Tunguska Rivers. There are seven main recreational water reservoir points in Angara River and its tributaries for monitoring non-toxicogenic *Vibrio* strains in Irkutsk and the Usolsky municipality that are located approximately 95 km from each other. The prevalence rate of isolating *V. cholerae* *ctx* changed from 1.7 to 8.6% for the period 2006-2015. Reports about 4 times an increased number of *V. cholerae* *ctx*- isolates started in 2016 (Fig. 3A). Noticeably, the pronounced increase in the temperature values took place during the years 2016–2020 corresponding to the rise in the *Vibrio* prevalence rate. The annual

official epidemiological reports do not contain any reasons for the noticeable rise in the *Vibrio* strains isolation. Most obtained strains of *Vibrio* belonged to non-O1/O139 serogroups. In 2013, three isolates of *V. fluvialis* and six isolates of *V. metschnikovii* were obtained.

The Republic of Sakha is the largest republic of Russia along the Arctic Ocean. Most points for water sampling are located in the capital of the Republic called Yakutsk and adjacent territories in recreational zones and zones with high anthropogenic load. Figure 3B shows that over the 28 years of observation, the prevalence of *Vibrio* isolates manually increased from 2.2 in 1993 to 11.0% in 2021. The prevalence and temperature curves explicitly have wavy



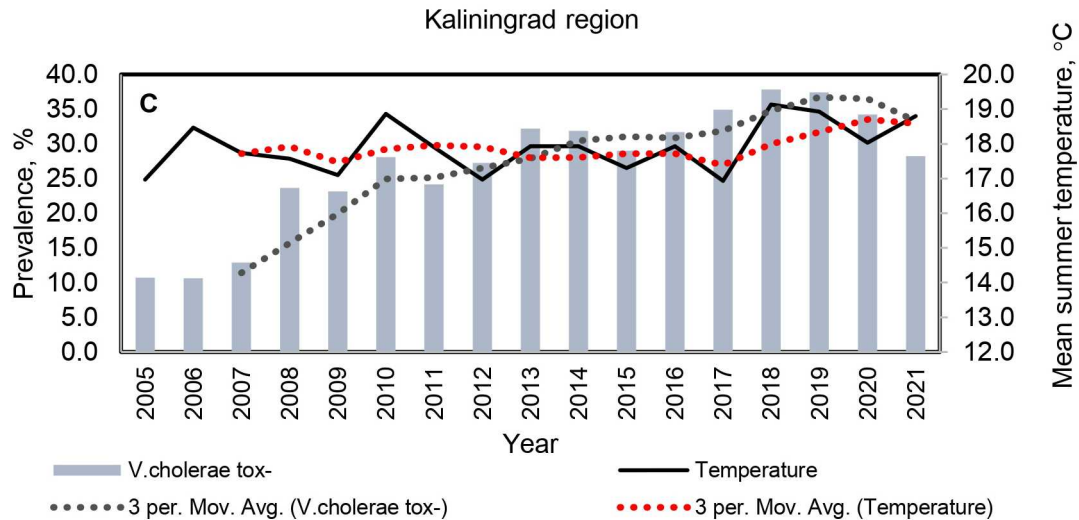


Figure 3. *V. cholerae* tox⁻ prevalence and yearly mean summer air temperature in three Russian regions. The chart inset A: Irkutsk region between 2005 and 2021. The chart inset B: Sakha Republic between 1993 and 2021. The chart inset C: Kaliningrad region between 2005 and 2021

patterns with 4-9 years periods.

The Kaliningrad region is the farthest western region of Russia. It is a semi-exclave that lies along the shores of the Baltic Sea. There are 130 stationary recreational and port points for monitoring *Vibrio* isolates in the Kaliningrad region that are sited near the capital city. The prevalence of *V. cholerae* ctx⁻ changed from 10.9-12.9 to 27.3-37.8% and increased in waves with the period from 3 to 5 years and at least three full waves can be observed in Figure 3C. Likewise, the temperature trend peaky changed, and 3-year moving average curves for the prevalence and temperature closely followed together since 2009.

Discussion

In the study, we first conducted a descriptive analysis of the spatial distribution of reported *V. cholerae* ctx⁻ isolates in Russia. A total of 16 persistent clusters were detected by using

space-time analysis with special adjustments for long-standing clusters (7-8 years). Overall, *V. cholerae* ctx⁻ strains are distributed across almost all regions. *V. cholerae* is a free-living in aquatic reservoirs with moderate salinity bacteria. Expectedly, most clusters with $RR \geq 2.00$ are located in territories that to some extent face the influence of marine climates such as all clusters in South, North Caucasian, and Far Eastern Federal Districts or the Kaliningrad region. The interesting phenomenon is that several significant persistent clusters (the Omsk, Kemerovo, Yaroslavl, Vologda regions, and Sakha, Republic) including the “most likely” cluster (the Chelyabinsk region) are located far from the pelagic territories. Previous studies have reported the presence of non-O1/O139 *V. cholerae* in some lakes and rivers in the USA, Germany, and Austria that are not considered to be endemic for cholera.^{20,21} It is known that only a few *Vibrio* species are able to survive under low temperature and low

salinity conditions: *V. cholerae* in brackish water reservoirs,^{22,23} and frozen under -40 °C food and beverages.²⁴ The finding for Russian regions requires precise analysis and is indirect evidence of a persistent reservoir of *V. cholerae ctx*. Vibrio-contaminated freshwater resources are usually the source of a natural outbreak of diarrheal diseases, wound infections, or even bacteriemia.^{4,25} However, these Russian territories have never reported any cases of vibrio-infectious. Our results support the hypothesis postulated in several comprehensive studies that *V. cholerae ctx* can survive not only a warm season but also a few years in water reservoirs of temperate and cold climatic areas.^{26,27} No studies to date have determined if *V. cholerae* strains from freshwater sources in areas with low temperatures have different biological features compared with strains isolated from water reservoirs in marine territories. If these strains have good adaptation abilities, they might quickly expand to other territories under changing climate conditions and increase the vibriosis infection rate in Russia.

In Russia, freshwater in recreational zones is a part of epidemiological monitoring and the detection of Vibrio isolates is one of the indirect markers of sanitary unfavourability of water reservoirs.¹¹ Based on our results, identified persistent clusters, specifically in non-pelagic regions, should become targeted as areas to investigate the source of Vibrio contamination and other accompanying factors that might contribute to microbial pollution. It can be concluded that the application of space-time statistics to explore Vibrio abundance provides useful information and should be a part of the public health surveillance system as an indicator of polluted areas and gut infection

risk.

Through correlation analysis of 16 identified space-time persistent clusters of *V. cholerae ctx*, a clear link between the prevalence of *Vibrio* and mean air summer temperature was discovered in three Russian regions the Kaliningrad region with temperate marine, and the Irkutsk region, and the Republic of Sakha with sharp continental climates. Significant temperature affection on the *V. cholerae ctx* abundance had not been shown for southern clusters including the Stavropol region with the 28-year data set. It might be the result of relatively warm climatic conditions in southern territories where the temperature effects are less pronounced due to low temperature variability. The relationship between Vibrio quantities and temperature is often insignificant in equatorial and tropical aquatic systems instead, salinity emerges as a crucial factor regulating the distribution and dynamics of Vibrio spp.²⁸

The positive thermo-climatic sensitivity of the Vibrio abundance in the Kaliningrad region is directly linked with the raised Baltic Sea water.^{3,29} Analysis of sea surface temperature trends in the Baltic Sea from 1982 to 2010 revealed a warming trend of 0.063-0.078 °C per year (6.3-7.8 °C per century), with the most recent peak temperatures being unprecedented in the recorded history of this region. The effects of global warming have led to a restructuring of geographical distribution, resulting in the expansion of Vibrio spp. into new territories.⁴ Since 2005, the Kaliningrad region has reported the isolation of various strains of *V. cholerae ctx*, and the prevalence rate continues to increase.

The Irkutsk region is identified as one of the clusters with pronounced positive temperature

effects on the *Vibrio* abundance. The rate of *Vibrio* isolates increased 4 times jointly with the mean ambient summer temperature over the observed period. There is no straightforward causality for the sharp rises that could be found. However, the region experiences a wide range of climatic peculiarities due to its unique location. Since 2000, the temperatures in the Irkutsk have been more like a humid continental climate, and the region was reclassified to Dwb according to the updated Köppen-Geiger climate zone classification.¹³ Lake Baikal has a moderating effect, resulting in Irkutsk having temperatures that are less extreme than other parts of Siberia. Even with being far away from any marine water source, Lake Baikal exhibits a multitude of similarities to oceans, such as depth, oxygen levels, oligotrophy, and microbiota. This makes it a distinctive model of pelagic microbiota that experiences conditions similar to those found in the ocean but without the presence of saltwater that *V. cholerae* is able to tolerate.³⁰

In Sakha, Republic, the prevalence of *V. cholerae ctx* slowly increased from 2.2 to 10% due to the annual cold weather conditions in the study period 1993-2021, and the significant temperature sensitivity of the prevalence rate could be revealed only for the 28-year data set. However, Yakutsk, despite its freezing winters, is far south of the tree line due to its warm summers, with July daytime temperatures in Yakutsk being even hotter than in some maritime subtropical areas that lead to the growth of *Vibrio* spp. Moreover, the shape of the curve for the large data set shows the existence of propagated sources of water contamination for local water basins, when the first wave of cases serves as a source of water contamination for subsequent cases, in turn, serves as a source

for later cases.

For other regions, specifically, from moderate climatic zones including high-risk clusters like the Chelyabinsk, and Omsk regions, the temperature affection on the *Vibrio* abundance was not found. Presumably, a relatively small data set and/or other factors like chemical pollution might contribute to high annual *V. cholerae ctx* isolation rates in local water basins. According to official reports, excess levels of chlorides, phosphates, sulfates, common coliform bacteria, and thermotolerant coliform bacteria in 15% of water samples containing cholera vibrios were reported over several years in the Chelyabinsk region. Annual official epidemiological reports do not contain explanations such high *Vibrio* isolation rate in the Omsk region.

Limitations

A primary limitation of the study is missing data on *vibrio* isolates for 14 Russian regions out of 83 (16.9%) and the 16-year regional data set of *V. cholerae ctx* isolates from water reservoirs might not be sufficient to reveal thermo-climatic patterns for southern regions with relatively high values of ambient summer temperature. In addition, we did not take into account that different serotypes of *V. cholerae* might have different temperature sensitivity patterns. The next limitation is the lack of detailed epidemiologic data on the source of *Vibrio* isolation and the employment of the air temperature of capital cities instead of water surface temperature for statistical analyses. The final limitation is that the results do not reveal the causality that underlies the correlations. The statistical correlations observed may be caused by other factors that were not considered in

the study such as mixed effects of different climatic variables and organic pollution of water reservoirs.

Conclusion

Our analysis showed that *V. cholerae ctx* persists annually throughout much of the country, particularly the Southern and North Caucasian Federal Districts. Remarkably, a relatively great number of clusters, including the “most likely” cluster in the Chelyabinsk region, are located far from marine coastlines that confirm the presence of territories with a persistent circulation of *V. cholerae ctx* in freshwater basins in Russia. Identified clustered areas should be used to target potential sources of diarrheal pathogens and generally improve surveillance.

Applying correlation analysis to all identified 16 space-time persistent clusters of *V. cholerae tox* we found a distinct association between mean air summer temperature and *V. cholerae ctx* prevalence for three Russian regions with temperate marine and sharp continental climatic conditions – the Kaliningrad, Irkutsk regions, and the Republic of Sakha.

The study offers valuable outcomes to support simplified empirical evaluations of the potential hazards of vibrio abundance that might be useful locally for public health authorities and globally as a part of the warning system of climate change effects in Russia.

Abbreviation

V. cholerae ctx, *Vibrio cholerae* non-toxigenic; WMO, World Meteorological Organization; LLR, Log-likelihood ratios; RR, Relative risk.

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Consent for publication

Not applicable.

Availability of data

The analysis in this study is based on publicly available data from the Rospotrebnadzor (www.rospotrebnadzor.ru) and Roshydromet (www.meteorf.gov.ru).

Conflict of interest

There is no conflict of interest.

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