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Seroprevalence of Tick-borne Diseases in the Population of the European North of Russia

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Abstract

Objective: The uptrend in the incidence of Tick-borne Diseases (TBD) is a new challenge for public health in many countries, especially those in the Arctic zone. The objective of our study was to assess the TBD seroprevalence in the population of Komi Republic (KR) located in the northeast of European Russia.

Materials and methods: Blood serum was sampled from 343 (183 men, 160 women) healthy donors aged 20-70 and tested with Enzyme-Linked Immunosorbent Assay (ELISA).

Results: IgG antibodies to TBD pathogens were detected in 66 (19.2%) samples: 47 (13.7%) samples contained antibodies to TBE virus, 18 (5.3%) those to *Borrelia*, and 5 (1.5%) those to *Anaplasma phagocytophilum*, 4 samples contained antibodies to TBE mixed with those to another TBD. The results were compared with those of an earlier serological survey and showed a significant increase in the seroprevalence IgG antibodies to TBE (13.7 ± 1.9% in 2013, and 3.5 ± 0.75% in 2001, respectively). In the sera samples of occupationally-risk professionals the IgG antibodies to TBD were more common than in the rest of donors (36.2 ± 7.0% and 16.6 ± 2.2%; p<0.05), in men more common than in women (25.1 ± 3.2% and 12.5 ± 2.6%; p<0.01), and young men (20-34 years) were the most affected.

Discussion: The situation in KR justifies the need for professional advancement of medical practitioners in TBD treatment, and revision of regional plans for anti-epidemic measures. Attention should be given to the effectiveness of health education, particularly among indigenous people who inhabit territory where tick bites are recorded.

Conclusion: Significant growth of TBE seroprevalence evidences the increased risk of acquiring TBE by KR population, including the inhabitants of settlements where this infection was never reported previously.

Keywords: Seroprevalence; Tick-borne diseases; Tick-borne encephalitis; Human Granulocytic Anaplasmosis (HGA); Enzyme immunoassay method; The Komi Republic

Abbreviations:

HGA: Human Granulocytic Anaplasmosis; HME: Human Monocytic Ehrlichiosis; KR: Komi Republic of Russia; TBD: Tick-Borne Diseases; TBE: Tick-Borne Encephalitis; TBEV: Tick-Borne Encephalitis Virus; TBI: Tick Bite Incidence

Introduction

Ticks being obligatory hematophagous ectoparasites are responsible as vectors or reservoirs at the transmission of pathogenic fungi, protozoa, viruses, rickettsia and others bacteria during their feeding process on the hosts [1]. Tick-Borne Diseases (TBD) are becoming an increasing and serious problem all over the world.

Among all the TBD Tick-Borne Encephalitis (TBE) is now of the most concern due to its wide distribution, polymorphism of clinical manifestations, affliction of nervous system with consequent therapeutic failure, lethal cases, and almost lack of specific therapies.

Thus in 2015 in Russia 2116 TBE cases are reported, and 24 (1.13%) patients died, *inter alias*, one child under 17 [2]. In some regions of northern Europe there is uptrend in TBE incidence [3-5]. In Russia, after 2000, TBE incidence dropped dramatically nationwide but increased notably in the north of the country [6-8].

The Komi Republic (KR) is one of Russian administrative territories where uptrend in TBE incidence rate is reported [9].

KR is located in the northeast of European Russia, between 59°12'-68°25' north latitude, and 45°25'-66°10' east longitude. KR territory is 416.8 km^2 and lies 840 km north and south, thus covering a few climatic zones: Arctic deserts, tundra, forest tundra, conifer forest, mixed forest.

Natural and climatic conditions, presence of ixodic ticks (*Ixodes persulcatus* Schulze, 1930 dominates) [10], and diversity of hosts provide the best conditions for TBD distribution over KR.

The KR population is about 900 thousand, with small downtrend within the studied period (2001-2013), and the share of Komi, the local indigenous people, is about 22.5% (2010) (for statistics [11]).

The statistical data on TBD incidence and TBI (number of humans seeking medical care in connection with tick bites) are being regularly reported by KR medical practitioners.

The incidence rate is among the most important indicators defining both scope and methods for the TBD prevention. However, it does not always adequately display the true TBD distribution as it depends largely on the awareness of the local population, their health-seeking behaviour, accessibility and quality of the medical care, skill of the medical practitioners, availability of laboratory diagnostics, etc.

Besides, for many TBD subclinical forms dominate, and that is why patients with clinically mild course of disease usually do not seek medical aid at all, and consequently the reported TBD incidence is underestimated. However, to develop efficient preventive measures we must know the true TBD distribution within the territory under study.

Seroprevalence surveillance with detection of antibodies to TBD pathogens in the sera of local inhabitants is a method for better assessment of the real rate of infection in humans.

The objective of this our study was to conduct a seroprevalence survey and assess the real presence of TBEV, *Borrelia burgdorferi sensu lato (s.l.), Anaplasma phagocytophilum, Ehrlichia chaffeensis,* and *Ehrlichia muris* in KR population.

Materials and Methods

In 2013, January-March, blood was sampled from 343 healthy donors in 5 KR districts (Figure 1).

The survey subjects were divided into 3 groups: Southern (S) group involved donors (n=132) from S1 and S2 districts below 61° n.l., where both in 2001 and 2012 tick bites and TBE cases were reported. Central (C) group involved donors (n=111) from C1 and C2 districts between 61° and 62.8° n.l., here in 2001 tick bites, but no TBE cases were reported, and in 2012 both tick bites and TBE cases were reported. Northern group (N group) involved donors (n=100) from N district. In 2001 neither tick bites nor TBE cases were reported, and in 2012 tick bites, but no TBE cases were reported.

The line-up distribution of the donors by age and gender is shown in Table 1. Among the survey subjects 47 had occupational risk of exposure to tick bites and TBD contracting.

Prior to blood sampling the survey subjects were informed about the study objectives, and consented to provide their personal data.

The entry criteria were:

- No health-related complaints;
- No TBE vaccination history;
- No manifested TBD forms, or diseases that could affect the results of serological tests;
- Permanent stay in the district. The donors never left the territory of their district at least during several years prior to the survey.

The blood was sampled from the cubital vein into EDTA tubes, centrifuged (3000 rev/min for 10 min), frozen, and the sera samples were stored at -70°C.

All subsequent transportation was carried out in compliance with the "cold chain" rules.

The sera samples were examined with Enzyme-Linked Immunoassay (ELISA) using the test systems as follows for the detection of IgG to:

- TBEV: "VektoVKE-of IgG" (JSC "Vector-Best", Novosibirsk);
- *Borrelia burgdorferi sensu lato*: "LymeBest" (JSC "Vector-Best", Novosibirsk);
- *Anaplasma phagocytophilum* EV: GACH-IFA- IgG (JSC Omniks, St. Petersburg);
- *Ehrlichia chaffeensis u E. muris*: MACH-IFA- IgG (JSC Omniks, St. Petersburg).

To understand the relation of recent TBE incidence growth with TBI we compared annual figures for 2001 (from archive of "Hygiene and Epidemiology Center in the Republic of Komi") and for 2013 (our results). The corresponding primary information on TBE cases and TBI, including date and place (KR district), was provided by medical - prophylactic institutions to the corresponding district Rospotrebnadzor offices.

We also analyzed the results of testing for antibodies to TBEV of 597 sera samples within the same RK districts in 2001. ELISA test-systems used in both surveys (2001 and 2013) were provided by the same manufacturer.

Statistical processing of the data was carried out with the help of Statistica 6.0 software.

The normality of the distribution in the data retrieval was tested with the Shapiro-Wilk W test.

We calculated relative performance (P), non-sampling error of the relative magnitude (m), and Student (t) test was used to compare the two groups. The differences were considered as significant if $p<0.05$.

Results

66 of 343 (19.2%) sera samples contained IgG antibodies to TBD pathogens, and 4 samples contained IgG antibodies to 2 pathogens simultaneously.

The most common were IgG antibodies to TBEV: 47 (13.7%), and to Borrelia: 18 (5.2%). In 5 samples (1.5%) IgG antibodies to *Anaplasma phagocytophilum* were detected, for the first time in KR (Figure 1).

None of our samples contained antibodies to the HME causative agent. 4 samples contained IgG antibodies to TBEV mixed with those to Borrelia (3 samples), or to the HGA pathogen (1 sample).

The geographical dependence of the seropositivity rate varied considerably for different pathogens. Thus, the percentage of samples with IgG to TBEV decreased northward: (S: 19.7 ± 3.5%; C: 12.6 ± 3.1%; N: 7.0 ± 2.6%), while that with IgG to *Borrelia burgdorferi sensu lato* decreased southward (S: 2.3 ± 1.3%; C: 4.5 ± 2.0%; N: 10.0 ± 3.0%).

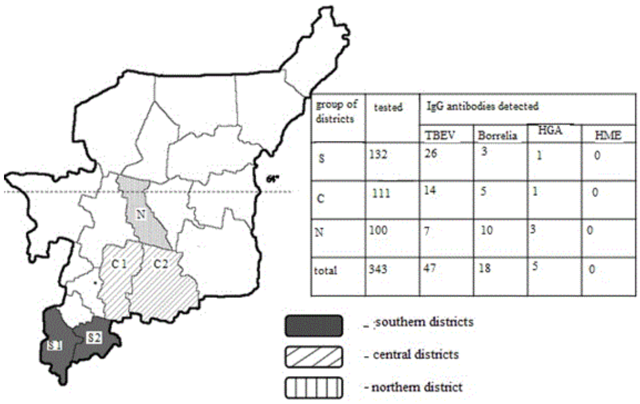

Figure 1: The map of Komi Republic with administration division. Occurrence (number of positive samples) and geographic distribution of IgG antibodies to TBD in sera samples of KR population in 2013.

Regardless of the district the seroprevalence of IgG antibodies to TBD pathogens was larger in men than in women, and reached its maximum in young men, aged 20-34 years (Table 1).

In the sera samples of occupationally-risked cohorts (agriculture, construction, logging, fishing, geological works) IgG antibodies to TBD pathogens were more common than in the rest of local population (36.2 ± 7.0% and 16.6 ± 2.2 %; $p<0.05$).

The analysis of our results has shown the growth both of TBI and TBE incidence in 2013 as to compare with 2001. The results of the serological monitoring bear witness as well of reliable increase in the frequency of detection of IgG antibodies to TBEV in sera of KR inhabitants belonging to S and C groups (Table 2).

Discussion

Our results testify to the presence of active natural foci of TBE, Lyme, and HGA in KR.

The variation in TBEV and *Borrelia burgdorferi sensu lato* seroprevalence in different KR districts may be due to different pathogen content in local ticks, thus giving ground for further studies on TBD-pathogen prevalence in those vectors.

This was the first study on a representative sample of KR population targeted to detect the antibodies to *Ehrlichia* and *Anaplasma*. The presence of antibodies to *Anaplasma phagocytophilum* in several sera samples proved that the regional population had been infected with this pathogen, and manifest forms of Human Granulocytic Anaplasmosis (HGA) possibly may develop in KR. This is consistent with some earlier published data: *Anaplasma phagocytophilum* DNA is detected in 1.0% of ticks collected in KR [12]. To date, HGA cases are reported in many regions of Russia, though its detectability is still insufficient [13]. By now HGA is not on record in KR.

Many researchers report the recent uptrend in the incidence of mixed TBD forms [14-17]. Our results also testified of co-infection with a few TBD pathogens thus threatening KR population with the development of mixed infections.

The antibodies to TBD pathogens were shown to be much more often detected in sera samples of occupationally-risked contingents, as to compare with other professionals. It is consistent with some results by Polish and Italian researchers [15,18].

Our data supported the occupation-related nature of TBD, and argued the need to improve organizational measures to protect those professionals.

The gender-related variation in TBD antibodies seroprevalence, in our opinion, was due to the way of life typical for KR population: men's professional occupation and hobbies (fishing, hunting) suggest more frequent and close contacts with TBD foci.

The dominance of men among TBE patients is typical for many countries, e.g. Switzerland [19] and Italy [20], and more frequent occurrence of TBD antibodies in young men is probably due to their greater socio-economic activity.

The obvious TBI growth (in 2013 compared to 2001) in all KR districts under study, and significant increase in the frequency of detection of IgG antibodies to TBEV (2013 as compared to 2001) in the blood serum of S ($p<0.001$) and C ($p<0.05$) group residents, proved, in our opinion, that the recorded uptrend in TBE incidence rate was due to more frequent bites by TBEV-infected ticks, rather than thanks to improved diagnostics. Among the N-group residents there was also an uptrend in seroprevalence. It should be underlined that a certain disproportion in number of sera samples investigated in those two periods does not affect the validity of our conclusions because the groups of sera donors were comparable in terms of their distribution by age, gender, and professional status of patients.

Age	Women		Men	
	Positive/tested	% ± m	Positive/tested	% ± m
20-34	10/71	14.1 ± 4.1	23/67	34.3 ± 5.8*
35-50	3/35	8.6 ± 4.7	15/70	21.4 ± 4.9
>51	7/54	13.0 ± 4.6	8/46	17.4 ± 5.6**
total	20/160	12.5 ± 2.6	46/183	25.1 ± 3.2***
*$p<0.05$ as to compare with women of the same age; **$p<0.05$ as to compare with men aged 20-34; ***$p<0.01$ as to compare with women				

Table 1: Occurrence of IgG antibodies to TBD in sera samples of KR population in 2013, the dependence on age and gender.

Districts	2001		2013		Criteria
	positive/tested	% ± m	Positive/tested	% ± m	t and p
S	10/264	3.8 ± 1.2	23/132	19.7 ± 3.5	4.3; p<0.001
S1	5/145	3.4 ± 1.5	20/102	19.6 ± 3.9	3.9; p<0.001
S2	5/119	4.2 ± 1.8	6/30	20.0 ± 4.0	3.6; p<0.001
C	8/233	3.4 ± 1.2	14/111	12.6 ± 3.1	2.8; p<0.05
C1	4/108	3.7 ± 1.8	10/81	12.3 ± 3.6	2.1; p<0.05
C2	4/125	3.2 ± 1.6	4/30	13.3 ± 3.4	2.7; p<0.05
N	3/100	3.0 ± 1.7	7/100	7.0 ± 2.6	1.3; p>0.05
Total	21/597	3.5 ± 0.75	47/343	13.7 ± 1.9	5.1; p<0.001

S-Southern districts: S1-Priluzskiy; S2-Koigorodskiy

C-Central districts: C1-Ust-Kulomskiy; C2-Kortkerosskiy

N-Northern district: Intinskiy

Table 2: IgG antibodies to TBEV in sera of KR population in 2001 and 2013.

Some KR districts (C1 and N), where the donors' sera contained IgG antibodies to TBEV never were officially classified with TBE endemic zones. Probably, the growth of TBEV seroprevalence in KR residents does not testify to their better protection against the pathogen, since the presence of antibodies to TBEV, strictly speaking, is not a sign of immunity, and proves only the increased frequency of contacts with the pathogen.

It is known that people with rather high titers of antibodies to TBEV may contract TBE. A group of researchers [21] marks considerable increase in TBEV seroprevalence in some regions of Czechia, and conclude that those data do not prove better protection of the local population against TBEV, since clinical manifestations of asymptomatic TBE cases do not induce lifelong immunity, but they are likely to reflect the previous epidemiological situation.

The seroprevalence growth detected by us showed an increase in the risk of infection with the pathogen, which may be due to higher activity of TBE foci loymopotential. Testing of this hypothesis proves the usefulness of a special investigation. Identification of TBD in new territories is already mentioned by numerous authors [22-27].

Perhaps, in the northern regions of Europe, it is due to the expansion of the tick habitats, the main natural reservoirs of TBD pathogens. One of the reasons for tick expansion into new territories is likely to be a change in climatic factors [28-30].

The TBD invasion into new territories is a formidable new challenge to the public health. The dramatics of the situation is exacerbated by a number of circumstances.

Firstly, in KR most *Ixodes persulcatus* ticks are infected with TBEV of the Far East genotype known for its high mortality rate [31]. The expansion of this virus genotype to other Russian regions and further to Scandinavian countries is possible.

Secondly, historically, as most of the northern indigenous nations never contacted ticks, and they may turn out more susceptible to TBD than the southern population [32,33].

The new situation justifies the need for professional advancement of local medical practitioners in TBD treatment, and revision of regional plans for anti-epidemic measures. Particular attention should be given to the effectiveness of health education, including that among indigenous peoples who live in areas where tick bites are recorded.

Conclusions

1. Wide distribution of tick-borne diseases is recognized in Komi Republic. Significant growth of TBE seroprevalence in the local population reflects the increasing risk of infection, including the areas where this infection was never recorded previously, and those not considered endemic to these infections.
2. For the first time infection with HGA pathogen is found in KR population. This indicates the potential for development of mono- and mixed forms of the disease.
3. In KR the increase in TBE incidence in XXI century is due to the growth of tick bite incidence in KR southern and comparatively northern districts, and, as a result, more frequent infection of people with TBEV.
4. The results justify the need to improve the diagnostic methods for detection of tick-borne infections, sanitary-epidemiological and preventive measures against those infections, and educational activities, including the indigenous peoples of the North.

References

1. Brites-Neto J, Duarte KMR, Martins TF (2015) Tick-borne infections in human and animal population worldwide. Vet World 8: 301-315.
2. Nikitin A, Noskov A, Andaev E, Pakskina N, Yatsmenko E, et al. (2016) Epidemiological situation on tick-borne viral encephalitis in the Russian Federation in 2015 and Prognosis for 2016. Problems of Particularly Dangerous Infections 1: 40-43.
3. Jaenson TG, Hjertqvist M, Bergstrom T, Lundkvist A (2012) Why is tick-borne encephalitis increasing? A review of the key factors causing the increasing incidence of human TBE in Sweden. Parasit Vectors 5: 184.

4. Skarpaas T, Golovljova I, Vene S, Ljostad U, Sjursen H, et al. (2006) Tickborne encephalitis virus, Norway and Denmark. Emerg Infect Dis 12: 1136-1138.
5. Süss J (2012) TBE – A short overview on epidemiological status in Europe. ISW-TBE 2012. Vienna, pp: 2-3.
6. http://www.tbe-info.com/Kontext/WebService/SecureFileAccess.aspx
7. Burmagina IA, Agafonov VM, Burmagin DV (2014) Characteristics of extreme increase of vector-borne infections in the European [Russ] North. Kazan Med J 5: 731-735.
8. Noskov AK, Ilin VP, Andaev EI, Pakskina ND, Verigina EV, et al. (2015) Morbidity rates as regards tick-borne viral encephalitis in the russian federation and across federal districts in 2009-2013. Epidemiological situation in 2014 and prognosis for 2015. [Russ] Problems of Particularly Dangerous Infections 1: 46-50
9. Revich B, Tokarevich N, Parkinson AJ (2012) Climate Change and zoonotic infections in the russian arctic. Int J Circumpolar Health 71: 187-192
10. Glushkova L, Galimov R (2011) Ecological and epidemiological aspects of tick-borne encephalitis in the republic of komi and disease prevention. EpiNorth 12: 44-50.
11. Glushkova L, Korabelnikov I, Egorov YU (2011) Distribution of Ixodes persulcatus P. Sch. in southern and central districts of Komi Republic. [Russ] Meditsinskaya Parazitologiya i Parazitarnye Bolezni 3: 48-50.
12. http://komi.gks.ru/wps/wcm/connect/rosstat_ts/komi/ru/census_and_researching/census/national_census_2010/score_2010/
13. Glushkova L, Korabelnikov I, Ternovoy V, Protopopova E, Mikrukova T, et al. (2012) Detection of causative agents in Ixodes persulcatus in the Komi Republic [Russ]. Sibirskiy Medicinskiy Jurnal 4: 88-91.
14. Teterin V, Korenberg E, Nefedova V, Vorob'ova N, Frizen V (2012) Enzyme-linked immunosorbent assay and polymerase chain reaction in the laboratory diagnosis of human granulocytic anaplasmosis [Russ]. J Infectol 4: 33-39.
15. Ye A, Blagov N, Druzhinina T, Ye S (2008) Tick-borne mixed infections (human Ixodes tick-borne borreliosis and human granulocytic erhlichiosis) in the Yaroslavl Region [Russ]. Epidemiologiya i Infektsionnye Bolezni 2: 6-8.
16. Chmielewska-Badora J, Moniuszko A, Żukiewicz-Sobczak W, Zwoliński J, Piątek J, et al. (2012) Serological survey in persons occupationally exposed to tick-borne pathogens in cases of co-infections with Borrelia burgdorferi, Anaplasma phagocytophilum, Bartonella spp. and Babesia microti. Ann Agric Environ Med 19: 271-274.
17. Dvořáková Heroldová M, Dvořáčková M (2014) Seroprevalence of anaplasma phagocytophilum in patients with suspected lyme borreliosis. Epidemiol Mikrobiol Imunol 63: 297-302.
18. Kocianová E, Kost'anová Z, Stefanidesová K, Spitalská E, Boldis V, et al. (2008) Serologic evidence of Anaplasma phagocytophilum infections in patients with a history of tick bite in central slovakia. Wien Klin Wochenschr 120: 427-431.
19. Cinco M, Barbone F, Grazia Ciufolini M, Mascioli M, et al. (2004) Seroprevalence of tick-borne infections in forestry rangers from northeastern Italy. Clin Microbiol Infect 10: 1056-1061.
20. Schuler M, Zimmermann H, Altpeter E, Heininger U (2014) Epidemiology of tick-borne encephalitis in Switzerland, 2005 to 2011. Euro Surveill 19: 20756.
21. Rezza G, Farchi F, Pezzotti P, Ruscio M, Lo Presti A (2015) Tick-borne encephalitis in north-east Italy: a 14-year retrospective study, January 2000 to December 2013. Euro Surveill 20: 30034.
22. Kříž B, Kott I, Daniel M, Vráblík T, Beneš Č (2015) Impact of climate changes on the incidence of tick-borne encephalitis in the Czech Republic in 1982-2011.[Czech.] Epidemiol Mikrobiol Imunol 64: 24-32.
23. Shcherbakova S, Naidenova E, Bil`ko E, Vodina E, Logunova T, et al. (2011) Detection of specific antibodies to arboviruses in blood sera of people living in the territory of the saratov region [Russ]. Problems of Particularly Dangerous Infections 108: 72-74.
24. Aslan Başbulut E, Gözalan A, Sönmez C, Cöplü N, Körhasan B, et al. (2012) Seroprevalence of Borrelia burgdorferi and tick-borne encephalitis virus in a rural area of samsun, Turkey. [Turkish] Mikrobiyol Bul 46: 247-256.
25. Ergünay K, Saygan M, Aydoğan S, Litzba N, Sener B, et al. (2011) Confirmed exposure to tick-borne encephalitis virus and probable human cases of tick-borne encephalitis in Central/Northern Anatolia, Turkey. Zoonoses Public Health 58: 220-227.
26. Güneş T, Poyraz O, Ataş M, Alim A (2010) Seroprevalence of tick-borne encephalitis virus (TBEV) among the residents of rural areas in Sinop, central Black-Sea region, Turkey. [Turkish] Mikrobiyol Bul 44: 585-589.
27. Larsen A, Kanestrøm A, Bjørland M, Andreassen A, Soleng A (2014) Detection of specific IgG antibodies in blood donors and tick-borne encephalitis virus in ticks within a non-endemic area in southeast Norway. Scand J Infect Dis 46: 181-184.
28. Lommano E, Burri C, Maeder G, Guerne M, Bastic V, et al. (2012) Prevalence and genotyping of tick-borne encephalitis virus in questing Ixodes ricinus ticks in a new endemic area in western Switzerland. J Med Entomol 49: 156-164.
29. Lyubeznova O, Bondarenko A (2012) Influence of climatic factors on tick-borne infection extension in the field of kirov [Russ]. Epidemiol Vaccinal Prev 2: 48-51.
30. Andreassen A, Jore S, Cuber P, Dudman S, Tengs T, et al. (2012) Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. Parasit Vectors 5: 177.
31. Tokarevich NK, Tronin AA, Blinova OV, Buzinov RV, Boltenkov VP (2011) The impact of climate change on the expansion of Ixodes persulcatus habitat and the incidence of tick-borne encephalitis in the north of European Russia. Global Health Action 4: 844-888.
32. Mikryukova TP, Chausov EV, Konovalova SN, Yu K, Protopopova E, et al. (2014) Genetic diversity of the tick-borne encephalitis virus in Ixodes persulcatus in northeastern European Russia. Parazitologiya 48: 131-149.
33. Hedlund C, Blomstedt Y, Schumann B (2014) Association of climatic factors with infectious diseases in the Arctic and subarctic region – a systematic review. Glob Health Action 7: 24161.