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Microbiological monitoring of yersinioses in Yakutia

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Summary

Here, we report results of long-term complex epidemiological and microbiological monitoring of yersinioses in Yakutia. The circulating pathogenic yersinia strains were screened for presence of the major virulence genes. A persistence of human- and rodent- non-pathogenic *Y. enterocolitica* biotype 1A and *Y. kristensenii* in the internal organs of the most prevalent rodent species was detected.

Key words: *monitoring, pseudotuberculosis, enteric yersiniosis, small mammals, yersinia virulence genes*

Introduction

Yersinia pseudotuberculosis and *Yersinia enterocolitica* are widely prevalent in many countries of the world and cause sporadic and group morbidity. At the same time, pseudotuberculosis is more characteristic mainly in the areas with moderate and cold climate while enteric yersiniosis is omnipresent [4,6,10,11,13]. A total of 6500 cases of pseudotuberculosis are notified every year (the mean incidence rate is 4.5 per 100,000 population (‰) while most of them are registered in Siberia (67.2 %) [15]. According to the official statistics in 2010, the prevalence of enteric yersiniosis in some oblast of North-West, Siberian and Far East Federal Districts is more than double higher than mean national values.

A variability of clinical manifestations of yersiniosis due to multiplicity of the virulence factors of the pathogens [1,5], makes it difficult their diagnostics and differentiation from other infectious and somatic diseases. The standardized diagnostic methods of detection of yersinia and antibodies against them play a leading role in confirmation of the yersinia etiology of the disease and identification of the sources and factors of the pathogen's transmission.

It is known that psychrophilic properties of yersinia allow for their long-term persistence in water and soil thus leading to the permanent circulation of the pathogen in natural ecosystems in the soil-plant-animal chain [7,9]. Previous observations in the areas of focal reservoirs of

yersinia in Yakutia demonstrated that small animals, mainly wild mouse-like rodents, grey rats, were shown to be more sensitive to yersiniosis [2,3,8,10].

The focused monitoring of yersinia in Sakha Republic (Yakutia) that comprises a half of the Far Eastern Federal District is carried out since 2006 in the context of implementation of megaprojects in the northeastern Russia (railroad and oil pipeline construction).

Aim of this study was to determine the prevalence of different species of yersinia in Yakutia and to study their epidemiological and microbiological characteristics.

Materials and methods

The following methods were used: bacteriological, serological (reaction of indirect hemagglutination), molecular genetic (polymerase chain reaction [PCR]). PCR was used to detect chromosomal virulence genes coding for proteins of yersinia adhesion/invasion into intestinal epithelium (*ail*, *inv*), *Y. pseudotuberculosis* superantigen toxin (*ypma/c*), *Y. enterocolitica* thermostable toxin (*ystB*) and yersinia virulence plasmid (*yscQ*) [12,14].

Results and discussion

Yersinioses are registered throughout Yakutia although their distribution is non-uniforumous across the territory while large cities (Nerungri, Yakutsk) and rural areas are marked with highest prevalence of the disease. Rural and urban population have similar incidence rates.

Diseases caused by *Y. enterocolitica* and *Y. pseudotuberculosis* in Yakutia are notified as group outbreaks and sporadic cases. The periods of epidemic incidence (1974-1998) (Table 1) and sporadic incidence (since 1999 until present) (Table 2) have been described. A total of 13 outbreaks of pseudotuberculosis and enteric yersiniosis were registered in 1974 to 1998 in child and day-care institutions and workers settlements during spring-summer and autumn-winter periods. All outbreaks were food-born and were due to consuming salads of fresh vegetables. Yersinia etiology of the disease was suggested based on clinical and epidemiological data, positive culture and detection of specific antibodies (Table 1).

It has been demonstrated that liver disorders (hepatomegaly, hepatitis) and fever are the most frequent clinical manifestations of pseudotuberculosis in Yakutia. The etiological role of *Y. enterocolitica* was also shown in cases of acute surgical pathology of the abdominal cavity organs in infectious patients from Srednekolymsky district (Argakhtakh and Svatay villages, Srednekolymsk city). During the short time-span in 1996 (27.12.96 - 13.01.97) 3 out of 143 patients diagnosed with acute appendicitis were culture positive for *Y. enterocolitica* serotype O9

(biotype 2) isolated from appendix. The incidence of acute appendicitis in Srednekolymsky district in that period was high with mean values 14-15 ‰ while children from 3 to 16 years old constituted 60 %. *Y. kristensenii* were also isolated from the excrements of two patients with acute enteric infection. The food-born transmission of yersinia was demonstrated (dairy products) although the water-born mode of transmission cannot be excluded since water from surface ponds was used for drinking and economic purposes in some locations, however no yersinia were isolated from water specimens. Isolation of such strains from patients along with non-isolation of other pathogenic agents suggests a potential role of yersinia as etiological agents of enteric yersiniosis in humans. These results should be taken into consideration and a further diagnostic study should be performed for enteric yersiniosis of patients diagnosed with "acute enteric infection of unknown etiology".

Multiyear dynamics of the yersinia isolation is wave-like. Bacteria of the *Yersinia* genus were isolated from organs of small mammals, swabs of vegetables and fruits, swabs of packing materials and surfaces of equipment of large vegetable stores, soil, water from surface water ponds. This isolation is a peculiar kind of indicator of favorable conditions for yersinia circulation. A circulation of *Y. pseudotuberculosis* serotype O:1 and *Y. enterocolitica* serotypes O:3 (biotype 4); O:9 (biotype 2); O:5; O:6,30; O:6,31; O:7,8; O:19,8; O:22 (biotype 1A) has been demonstrated. At the same time, a use of the more sensitive PCR method permitted us to detect infected samples >5 times more frequently than bacteriological method (Tables 3, 4).

The serological screening of the rural population revealed that antibodies to *Y. pseudotuberculosis* and *Y. enterocolitica* are detected in agricultural workers in 2,7 % and 7,4 % studied individuals, respectively (Table 5). A retrospective serological investigation of the blood sera from farm animals (cattle, pigs, dogs) identified antibodies to *Y. pseudotuberculosis* and *Y. enterocolitica* in 2.7 % and 6.5 % samples, respectively (Table 6).

As a result of complex epidemiological and microbiological monitoring of yersinia in Yakutia the natural and antropurgic foci of yersinioses have been detected. The following rodent species as yersinia reservoirs captured in their natural habitats were identified: gray rat (36 %), red vole (20 %), house mouse (15 %), shrew (7 %), root-vole (5 %), red-gray vole (4 %). In the recent years, a trend of increasing rodent population has been noticed in human settlements due to the favorable conditions of wintering and the period of the reproduction. The level of rodent prevalence in antropurgic foci is 60 per 100 traps/day, in natural biotopes – up to 25. While infecting the environment and being subjected to hunting, the rodents in antropurgic foci infect the domestic and farm animals with *Yersinia* species via water- and food (forage)-born modes.

The correct delineation of the area of distribution of yersinia and infected animals is important for implementation of the anti-epidemic measures in the foci of yersinioses.

It may be that a severe climate of the Far North with drastic changes in temperature (from -67 ^{0}C in winter to 35 ^{0}C and more in summer), long cold period acting as a stress factor, create favorable conditions for preservation and accumulation of the pathogenic agents of pseudotuberculosis and enteric yersiniosis due to their psychrophilic properties. The global warming trend of the climate changes implying increasing temperature and humidity (from 20 to 70%), lead in particular to the permafrost warming that in its turn influences the changing ecology of microorganisms and possible emergence of new subspecies with modified biological and genetic properties [8].

This is a first focused study in Yakutia that analysed presence of the major virulence genes of yersinia circulating herein. The following species and genes were found: *Y. pseudotuberculosis* serotype O:1, carrying yersinia virulence plasmid pYV and gene coding for superantigen YPM a/c; pathogenic *Y. enterocolitica* serotypes O:3 (biotype 4) and O:9 (biotype 2), carrying plasmid pYV and adhesin/invasin coding gene *ail*, isolated also from rodents. A circulation of non-pathogenic *Y. enterocolitica* serotypes O:6,30; O:5, O:7,8 biotype 1A carrying thermostable enterotoxin YSTB encoding gene *ystB* has also been observed. At the same time, a fragment of the *ystB* gene was detected in 3 samples of intestinal homogenates and in 1 sample of spleen homogenate obtained from red voles captured in the areas of construction of oil pipeline stations NPS-5 Talakan-Vitim, NPS-14, oil pipeline Eastern Siberia – Pacific Ocean and on the location of Verhne-Munskiy diamond mines. Three cultures of *Y. enterocolitica* biotype 1A and cultures of *Y. kristensenii* were also isolated from organ homogenates. These results demonstrate a persistence of the *Yersinia* species considered to be non-pathogenic for humans and rodents, in the internal organs of rodents. Further investigations are needed to study virulence properties of this group of yersinia and their possible role as etiologic agents.

As a part of implementation of large national megaprojects in North-East Russia, a railway road is under construction that will connect central part and northeastern Russia (Sakha Republic (Yakutia). The Eastern Siberia – Pacific Ocean oil pipeline exports Russian crude oil to the Asia-Pacific countries (Japan, China and Korea) since 2010. Apparently, such an active influence of the technogenic factors in this region would favor increasing the spectrum of the circulating microorganisms pathogenic for humans and animals, forming various habitats and ecological niches, suitable for potentially pathogenic microorganisms. On the other hand, increasing human migration and cargo flows, load of goods, number of terminals, storage facilities etc, in Yakutia would require focused preventive measures in relation to different

infections, including yersinioses. The above perspectives of the economical exploration and development of the Yakutia territory would need strengthening measures of monitoring of yersinia as well.

Conclusions

1. Our results demonstrate a high reservoir role of rodents living in Yakutia. Gray rat and house mouse were found to be the major rodent species infected with yersinia in the antropurgic foci, red vole and root-vole – in the natural biotope.
2. Use of molecular genetic method demonstrated that 10 % of the studied rodents were infected with pathogenic yersinia.
3. *Y. pseudotuberculosis* serotype O:1 and pathogenic *Y. enterocolitica* serotypes O:3 (biotype 4) and O:9 (biotype 2), carrying virulence genes circulate in Yakutia.
4. An important epidemiological feature of yersinioses in Yakutia is isolation of non-pathogenic *Yersinia* from humans and rodents.

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Table 1

Characteristics of outbreak incidence of pseudotuberculosis and enteric yersiniosis in 1974-1998

Year	Location	Population subgroup	Incidence rate, ‰	Transmission factor	Aetiological agent/Method of confirmation
1974 1975 1987	Mirninsky district, Township Nadezhnyy, City Mirnyy	Children in children's pre-school institutions	>10.0	Salad of fresh cabbage	*Y. pseudotuberculosis*/ Bacteriology, RIHA
1977 1978 1983	City Nerungri	Adults, schoolchildren	>10.0	Russian Vinaigrette	*Y. pseudotuberculosis*/ Bacteriology, RIHA
1979	Oymyakon district, Mine “Yubileinyy”, location “Partizan”,	Adults, children	4.0 - 10.0	Salad of fresh vegetables	*Y. pseudotuberculosis*/ Bacteriology, RIHA
1981	City Yakutsk, children's tubercular hospital	Children	8.0-9.0	Salad, vegetable dishes	*Y. enterocolitica*/ Bacteriology, RIHA
1985, 1987	Lenskiy district, Township Razvedchik, City Lensk	Workers of plant “Lenskiy”, children	4.0 - 10.0	Salad of fresh cucumbers	*Y. pseudotuberculosis*/ RIHA
1993, 1997	Srednekolymsky district, Villages Argakhtakh and Svatay, City Srednekolymsk	Adults, children	3,8-6,7	Dairy products	*Y. enterocolitica*/ Bacteriology, RIHA
1998	City Viluysk, psychoneurological nursing house	Patients	>10.0	Foodstuffs	*Y. enterocolitica*/ RIHA

Table 2

Dynamics of incidence of enteric yersiniosis in Sakha (Yakutia) Republic in 2001-2010*

Year	Absolute values	Incidence rate, ‰000
2001	1	0.10
2002	0	0
2003	1	0.10
2004	0	0
2005	1	0.10
2006	0	0
2007	0	0
2008	3	0.31
2009	8	0.84
2010	7	0.74

* data of the official statistics Rospotrebnadzor in Republic Sakha (Yakutia)

Table 3

Detection of yersinia in small animals in 2001-2010

Year	Bacteriology		PCR	
	Number of tested samples	Isolated cultures number/%	Number of tested samples	Detected virulence genes number/%
2001	121	12/9.9	-	-
2002	151	0	-	-
2003	106	0	-	-
2004	261	0	-	-
2005	325	5/1.5	-	-
2006	221	0	12	4/8.3
2007	256	1/0.4	357	0
2008	320	4/1.3	26	7/26.9
2009	279	11/3.9	334	64/19.1
2010	359	1/0.3	366	35/9.5
TOTAL	2399	34/1.4	1095	110/10

Table 4

Detection of yersinia in environmental samples (swabs, vegetables, surface ponds water, soil) in 2001-2010

Year	Bacteriology		PCR	
	Number of tested samples	Isolated cultures number/%	Number of tested samples	Detected virulence genes number/%
2001	4847	26/0.5	-	-
2002	4289	7/0.2	-	-
2003	3636	4/0.1	-	-
2004	3692	5/0.1	-	-
2005	2431	5/0.2	-	-
2006	2831	1/0.03	-	-
2007	3750	7/0.2	-	-
2008	3070	5/0.2	48	5/10.4
2009	3610	17/0.5	35	2/5.7
2010	4012	22/0.5	116	4/3.4
TOTAL	36168	99/0.3	199	11/5.5

Table 5

Results of serological investigation of agricultural workers

Year	Number of tested individuals	Detected antibodies to the pathogenic agents of			Number of positive findings, %
		Pseudotuberculosis	Enteric yersiniosis		
			O:3	O:9	
2001	49	5	2	-	14
2002	163	4	2	-	3.6
2003	102	-	7	-	6.8
2004	159	3	1	1	3.1
2005	21	1	-	-	4.7
2006	86	4	11	-	17
2007	157	4	20	5	18
2008	24	3	10	-	54
2009	145	-	4	4	5.5
2010	223	6	16	-	9.8
TOTAL	1129	30	73	10	10.0

Table 6

Serological investigation of blood sera from farm animals (Sosnovka village, Viluyskiy district) in 1999

Animal	Number of samples	Number of samples positive for antibodies to pathogenic agents of			Number of positive findings, %
		Pseudotuberculosis	Enteric yersiniosis		
			O3	O9	
Cattle	77	-	5	-	6.4
Pigs	14	3	-	-	21.0
Dogs	17	-	2	-	11.7
TOTAL	108	3	7	-	9.2