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OCCURRENCE AND MAINTENANCE OF HANTAVIRUS INFECTIONS AMONG RODENT POPULATIONS IN THEIR NATURAL HABITAT - RESULTS OF A FIELD STUDY FROM PODKARPACKIE PROVINCE, POLAND 2010-2012*

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ABSTRACT

Human cases of hantavirus infection have been reported annually in Poland's Podkarpackie province, since 2007. In 2014 the number of cases reported significantly increased prompting a rise in studies focusing on the infection.
OBJECTIVE. The purpose of this study was to evaluate the prevalence, maintenance and the dynamics of hantavirus infection among rodent species, including the bank vole *(Myodes glareolus)* and yellow-necked mouse *(Apodemus flavicollis)* which act as reservoirs of hantavirus in the environment.
MATERIAL AND METHODS: Rodent capture was carried out on seven research grids in the Podkarpackie province, from summer 2010 to spring 2012. They were caught in live-capture traps used in accordance with the protocol CMR (Catch-Mark-Release). The population was estimated as MNA (Minimum Number Alive). Blood samples were collected for serological testing on live animals by puncture of saphenous vein. In order to determine the hantavirus infection we used ReaScan Ab-Dect Puumala IgG - Reagena® for bank voles, and for mice ELISA - Mouse Hanta Virus Test ELISA Kit (Version with Control Antigen Wells) - BioCat GmbH®. The relationship between age, gender, seasons, population size and prevalence of hantavirus infection was tested by Pearson's chi-squared test or Fisher's exact test and by calculating the Pearson's correlation coefficient.
RESULTS. A total of 854 rodents were captured: 222 bank voles, 592 yellow-necked mice, 4 common voles and 36 striped field mice. Of these, 564 were tested. The presence of specific anti-hantavirus was found in 9.7% of bank voles and 9.5% of yellow-necked mice. There was a statistically significant difference in the frequency of infection between the groups of male and female yellow-necked mice as well as in the groups of adult group in both species. The dynamics of spread of hantavirus infection was clearly evident in the studied area of Sanok. The correlation coefficient between the number of individuals caught and the prevalence of hantavirus infection in yellow-necked mice was -0,87 but for the bank vole it was 0,76.
SUMMARY AND CONCLUSIONS. The results of the study indicate that hantavirus infection among yellow-necked mice and bank voles are unevenly distributed in certain hot spots, vary over time, and are most in the spring season. In addition, differences observed in the dynamics of infection depended on the species of animal hosts. It would be advisable to conduct long-term study, which would allow for a risk assessment of the possibility of turning the spot located outbreaks into endemic area of hantavirus occurrence.

Key words*: hantavirus, Poland, Myodes glareolus, Apodemus flavicollis, Dobrava, Puumala*

INTRODUCTION

Hantavirus is one of five genus belonging to the large *Bunyaviridae virus* family. Within this genus there are at least 29 genotypes and 23 serotypes, of which 17 are considered pathogenic to humans (1).

In their natural habitat rodents, insectivores such as shrews and moles, and as recently discovered recently -

* This study was financed by National Science Centre within the funds allocated to finance research projects, project No. NN303 590138, (Contract No. 5901/B/P01/2010/38).

bats act as a reservoir for Hantaviruses (2, 3). Hantaviruses, in contrast to the other four genus of *Bunyaviridae* are not transmitted by arthropods. Rodent which are natural hosts of Hantaviruses, can remain persistently infected for life, which results in lifelong viral excretion. Infectious material for people are rodent excretions and secretions.

The infection is spread by inhalation of aerosol or dust particles containing rodent excretions and secretions or by direct contact with them. The fact that rodents are widespread throughout the world is the cause that the hantavirus infection may be one of the most widespread zoonosis in the world (2,3).

Within the territory of Poland there are at least 6 species of rodents, which could act as hosts for Hantaviruses. One of the most common is the bank vole *(Myodes glareolus)*, natural reservoir of the Puumala serotype (PUUV) as well as a house mouse *(Mus musculus)*. A reservoir of the Dobrava serotype (DOBV) lives in forests yellow-necked mouse *(Apodemus flavicollis)*, Saarema (SAAV) striped field mouse *(Apodemus agrarius)* and serotype Tula (TUUV) - a common vole *(Microtus arvalis)*.

In Poland, the first outbreak of hantavirus disease involving 9 human cases was registered in 2007 in Podkarpackie province (4). Since then, every year human infections have been diagnosed in this region, suggesting the existence of areas with an increased risk of human infection. In 2014, as many as 55 cases were reported in Podkarpackie in routine surveillance (5). Moreover, the results of previous studies on the prevalence of antibodies against hantaviruses among mammologists, who are occupationally exposed to rodents, carried out by National Institute of Public Health- National Institute of Hygiene (NIPH-NIH), showed the presence of antibodies in 26% of studied population (6).

The mechanisms by which the virus transmits to human is based on both the prevalence of infection among the animal hosts and the size of host population. In Poland, until now, no long-term field research of environmental epidemiology has been conducted. Such research is crucial for understanding the emergence of foci of occurrence, spread and persistence of hantaviruses, including human pathogenic serotypes, in populations of rodents.

The aim of this study was to assess the prevalence of hantavirus infections in the rodent population in selected areas of Podkarpackie province and estimate the intensity of the virus circulation in their natural hosts. In contrast to the earlier studies methodology used here allowed for tracking the same animal populations in its natural conditions, in real time.

MATERIALS AND METHODS

The area of research. Capture of rodents was conducted in the Podkarpackie province, in the forest inspectorate units Brzozow and Lesko. Both settings are located in the forest region of the 8-th Carpathian Natural Forest Land, in the sub montane Beskidy and Bieszczady Mountains. Significant differences in relative elevation and terrain variation affect the climate diversity, which was classified as the mountain and foothill climate. Brzozow is dominated by mixed forests with a predominance of beech and fir (50% and 25% of the forest province). In the further southernmost forest inspectorate Lesko the species composition is dominated by pine (33%), followed by beech and fir (28% and 25% respectively).

To conduct research on the environments most comparable in terms of the type of vegetation, in each of the inspectorates, we selected a similar type of forest habitat as indicated by foresters. This most frequently was small patches of oak - lime - hornbeam forest (Tilio-Carpinetum), occurring in these areas often with a mixture of maple, fir and beech. All captures were carried out on 7 research grids, 1 hectare in size and one with surface of 0.3 ha, and on 5 transects, at which traps aligned. Transects means traps are placed every 10 m in a line drawn to cover the most different habitats. Transects allow monitoring of larger area, capturing more individuals and collection of more specimens in comparison to the grid method, however it does not allow the researcher to track the activity of the animals. The purpose of introducing transects to the methodology was to identify foci of the infected rodent populations.

Capture of rodents. Rodents were captured in 6 sessions: 2010. (summer, fall), 2011. (spring, summer, autumn), 2012 (spring). The capture area mainly covered the inspectorate of Brzozow - 8 grids and the inspectorate Lesko - 4 grids.

CMR method (Catch-Mark-Release) was used. After determination of the species, all captured animals were weighed, marked and their sex determined. Blood was drawn and the individual was released at the point of capture. Marking each newly caught individual allowed the estimation of the population and the traceability of animals in successive captures.

On the basis of preliminary studies in 2010, two grids were selected where we continued to monitor the dynamics of infection prevalence in spring, summer and autumn seasons in 2011, and in spring of 2012. This allowed us to trace the history of infected individuals caught again. In 2011 an additional third grid was selected (Inspectorate Weremień) and the study was conducted here in autumn 2011 and the spring of 2012.

The population was estimated as MNA (Minimum Number Alive), the number of individuals caught during one of a series of catches.

Collection of blood samples. Individuals for blood collection were not premedicated. Blood was taken from the saphenous vein, after preparing the needle insertion site. After puncturing the blood vessel with a disposable

needle - a drop of blood, appearing on the surface was collected with a pipette and / or special Microvette type of capillary, as appropriate.

Laboratory tests. In order to determine the infection of hantavirus in each animal, serological tests were used to detect the presence of specific antibodies against these viruses. The test used to study voles was ReaScan Ab-Dect Puumala IgG produced by Reagena. The test was performed in accordance with the procedure specified by the manufacturer. To read the result an electronic reader was used, which allowed the intensity of the immune response to be assessed. To study blood samples collected from yellow-necked mice in the first year of the study we used the same test, as was used for testing bank voles, as it was officially allowed by the manufacturer specification of the test. As we did not obtained any positive or inconclusive test results from mouse blood during the first two session, following contact with the manufacturer it was decided to change the test and subsequent testing was performed using the ELISA - Mouse Hanta Virus Test, (ELISA Kit Version with Control Antigen Wells) – produced by BioCat GmbH.

Prevalence study of the infection in populations of rodents. The prevalence ratio of infection was calculated. The relationship between age, gender and population size and prevalence of hantavirus infections was assessed by Pearson's chi-squared test or Fisher's exact test and by calculating the correlation coefficient.

RESULTS

Hantavirus rodent infection. A total of 854 animals were captured: 222 bank voles, 592 yellow-necked mice, 4 common voles and 36 striped field mice. Of these, 564 were tested for the presence of anti -hantavirus antibodies. In the captures made in 2010 none of the animals tested were seropositive for hantavirus infection. Because the test used in 2010, ReaScan Ab-Dect Puumala IgG was not suitable for mice, results obtained with this test were not included in any further analysis.

In the vast majority of studied groups of rodents, the dominant species was yellow-necked mouse, then the bank vole, and least numerous was field mouse that comes to the edge of forested area mostly in autumn.

Among the 564 tested animals, specific antibodies for hantaviruses were found in 55 subjects. During the period from the summer of 2010 until the spring of 2012 the number of tested bank voles was 222, out of which

Table I. Rodent species captured and the prevalence of antibodies against hantavirus in Podkarpackie province, Poland, 2010-2012[1].

Capture method	Name of sampling area	Rodent species	Number of positive / tested animals						TOTAL
			2010		2011			2012	
			summer	autumn	spring	summer	autumn	spring	
			positive / tested	positive / tested	positive / tested	positive / tested	positive / tested	positive / tested	
line-transect	Zahutyń	*M. glareolus*	0/6	-	-	-	-	-	0/6
	Górki	*M. glareolus*	-	0/1	-	-	-	-	0/1
	Graby	*M. glareolus*	-	0/3	-	-	-	-	0/3
	Weremień_1	*M. glareolus*	-	0/16	-	-	-	-	0/16
	Weremień_2	*M. glareolus*	-	-	0/3	-	-	-	0/3
		A. flavicollis	-	-	4/27	-	-	-	4/27
	Sanok	*M. glareolus*	-	-	7/15	-	-	-	7/15
		A. flavicollis	-	-	7/48	-	-	-	7/48
1 ha grid	Turze Pole	*M. glareolus*	0/21	0/6	0/8	0/6	1/13	0/3	1/57
		A. flavicollis	*	*	0/4	3/47	1/77	3/3	7/131
		A. agrarius	*	*	-	-	1/3	-	1/3
		Microtus sp.	0/2	0/0	-	0/1	0/1	-	0/4
	Biała Góra	*M. glareolus*	0/10	-	-	-	-	-	0/10
	Trepcza	*M. glareolus*	0/3	-	-	-	-	-	0/3
	Gruszki	*M. glareolus*	-	0/4	-	-	-	-	0/4
	Sanok	*M. glareolus*	-	-	7/21	6/26	4/21	0/4	17/72
		A. flavicollis	-	-	8/46	3/51	3/26	-	14/123
		A. agrarius	-	-	-	1/1	0/1	-	1/2
	Sanok leśniczy	*M. glareolus*	-	-	-	1/15	-	-	1/15
		A. flavicollis	-	-	-	3/44	-	-	3/44
	Weremień	*M. glareolus*	-	-	-	-	1/25	0/8	1/33
		A. flavicollis	-	-	-	-	0/28	-	0/28
		A. agrarius	-	-	-	-	0/1	-	0/1
TOTAL			0/36	0/30	33/172	17/191	11/196	3/18	

[1] Animals recaptured in different seasons are included in the table.
" -" - area not investigated
"*" - results not included

in 24 (9.7%) specific antibodies were detected (Tab. I). Among four tested common voles (*Microtus arvalis*), none had antibodies indicative of infection. In the case of yellow-necked mouse, studies were performed on material taken from the 348 individuals caught from spring 2011 to the spring of 2012. In this group, the antibodies were found in 33(9.5%) animals (Tab. I). In addition, 36 striped field mice were tested, demonstrating the presence of antibodies in two of them. Analysis showed a significantly higher percentage (12.9%) of infected males than females (5.6%) of the mice (p = 0.00). Significantly higher proportion of infection were found among adult yellow-necked mice (17%, p=0,000) and adult bank voles (18.1%, p=0.000) in comparison to the groups of subadults and juveniles. The percentage of infected animals also differed significantly among seasons and was the highest in the spring for both bank voles (23%; p = 0.003), as well as for yellow-necked mice (16%; p = 0.008).

During the study infected individuals were detected in four out of seven research areas. Since the individual serotypes of hantaviruses are specific for the host animals, and the prevalence of infection and its changes can best be observed in the population from the same area over prolonged period of time, further analysis was conducted separately for each species of animal hosts. The analysis of the results was performed in the research areas where antibodies were found in at least one individual during at least one previous series of catches.

Hantavirus infections among bank vole *(Myodes glareolus).* When analyzing the prevalence of infection among bank voles (Tab. II) in all 4 grids during all periods of research, it was found that the prevalence of infection in the tested animals was 12.5%. The antibodies were detected in both juveniles, as well as in individuals in the groups of subadults and adults. The percentage of prevalence of antibodies in adults was 22.7% and was higher than among juveniles - 3.5% and subadults - 3.6 (p=0,002) (Tab. II). There were no statistically significant differences in infection between males and females. In the following season 23 individuals were recaptured and re-examined, of which two were also caught for the third time. The status of infection remained unchanged.

Prevalence ratio differed between captures in particular grids in the same season and between captures in the same grid and season in different years. While in autumn of 2011 infected individuals were found on all studied grids, in the spring of 2012 there were not any (tab. II). The dynamics of the prevalence of hantavirus infection was the most evident on the research grid of Sanok. The correlation coefficient between the number of individuals caught during one series of catches and prevalence of infection was 0.5. It indicates a significant positive relationship between changes in the vole population and the prevalence of infection.

Hantavirus infections among yellow-necked mouse *(Apodemus flavicollis).* The prevalence ratio of infection in total for all four grids and for all studied periods was 9%. The antibodies were detected only in groups of subadults and adults. There was no detection of antibodies among juveniles (tab. III). The proportion of infections among males was higher than among females and amounted to 12.9 and 4.7 respectively, and it was statistically significant (p = 0.012).

Table II. Prevalence of antibodies against hantavirus among bank vole *Myodes glareolus* captured on 4 grids in Podkarpackie province, Poland, 2010-2012.

Name of sampling grid	Year	Season	Bank vole												% positive
			Females						Males						
			adults		subadultus		juvenile		adults		subadultus		juvenile		
			tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	positive	positive	
Turze Pole	2010	summer	6	0	1	0	1	0	8	0	2	0	3	0	0
		autumn	1	0	1	0	1	0	3	0	0	0	0	0	0
	2011	spring	2	0	0	0	0	0	4	0	2	0	0	0	0
		summer	1	0	0	0	0	0	4	0	0	0	0	0	0
		autumn	3	1	1	1	1	0	1	0	5	0	0	0	18.18
	2012	summer	1	0	0	0	0	0	2	0	0	0	0	0	0
Sanok	2011	spring	11	8	1	0	4	0	6	4	5	0	3	0	40
		summer	3	2	6	0	2	0	0	0	5	0	3	0	10.53
		autumn	2	0	3	0	1	0	2	1	1	0	3	1	16.67
	2012	spring	1	0	0	0	0	0	3	0	0	0	0	0	0
Sanok Leśniczy	2011	summer	5	0	2	0	1	0	4	1	1	0	2	0	6.67
Weremień	2011	autumn	3	0	9	0	3	0	1	1	8	0	1	0	4
	2012	spring	5	0	0	0	0	0	4	0	0	0	0	0	0
TOTAL			44	11	24	1	14	0	42	7	29	0	15	1	

Table III. Prevalence of antibodies against hantavirus among yellow-necked mouse *Apodemus flavicollis* captured on 4 grids in Podkarpackie province, Poland, 2010-2012.

Name of sampling grid	Year	Season	Yellow-necked mouse												
			Females						Males						% positive
			adults		subadultus		juvenile		adults		subadultus		juvenile		
			tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	
Turze Pole	2011	spring	2	0	0	0	0	0	0	0	1	0	1	0	0
		summer	12	1	2	0	1	0	18	2	13	0	1	0	6.38
		autumn	16	0	10	1	4	0	7	1	15	0	3	0	3.64
	2012	spring	3	0	0	0	0	0	4	3	0	0	0	0	50
Sanok	2011	spring	19	1	14	1	3	0	19	6	27	0	0	0	9.76
		summer	9	2	10	0	3	0	6	1	9	0	0	0	8.11
		autumn	6	0	4	0	1	0	7	3	2	0	0	0	15
	2012	spring	0	0	0	0	0	0	0	0	0	0	0	0	0
Sanok Leśniczy	2011	summer	8	0	9	0	1	0	14	3	9	0	2	0	6.98
Weremień	2011	autumn	7	0	4	0	2	0	4	0	8	0	2	0	0
	2012	spring	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL			82	4	53	2	15	0	79	19	84	0	9	0	

Thirty-six individuals were recaptured and tested again in successive seasons, of which three were also caught for the third time. In three individuals the infection status changed from negative to positive between successive captures.

Correlation coefficients between the number of individuals caught within all series of captures and the prevalence of hantavirus infection was for the grid Sanok-0.87 and for Turze Pole it was -0.5. This indicates that the correlation between the prevalence of infection and the number of individuals in yellow-necked mice is reversed than for bank voles. It means that along with an increase in the population size, prevalence of hantavirus infections decreases among yellow-necked mice.

DISCUSSION

Previously conducted research in Poland on hantavirus infections in animal hosts, focused only on detection and assessment of their occurrence. The current study indicates that hantavirus infection of yellow-necked mice and bank voles are spot-located, variable over time, and these changes are most visible in the spring season. The nature of infection indicates that a major challenge in finding rodent populations where the prevalence of infected individuals is evident.

To support the results and conclusions , studies should be planned for a longer period of time: namely for three seasons and minimum of three years, optimally 5 to 10 years. This is based on observations carried out on Turze Pole, where captures of bank vole through 6 sessions led to finding infected animals only in one, the fifth, session. We are unable to draw firm conclusions from the results of this study, rather findings are more hypothesis generating. However, some of the results, like the correlation between the number of individuals caught during one of a series of catches and prevalence of hantavirus infection among tested ones or differences in prevalence of infection related to the age and gender, clearly show that the spread of hantaviruses among bank vole and yellow-necked mouse populations have a different course.

This indicates that different factors are more dominant in the spread of infection among mice - presumably gender and age of individuals, but in bank vole perhaps more meaningful is the number of individuals in a population or its density. These differences can be related to different social systems of these species, and thus with a different mechanism of increasing the frequency of contact between individuals in the population, which is crucial for spread of the infection.

CONCLUSIONS

Understanding the dynamics of the spread of hantavirus infection in the population of animal hosts, may have important implications for public health. Such long-term study would lead to risk assessment on the possibility of turning the spot located outbreaks into endemic area of hantavirus occurrence in animal habitat. The significant increase of human hantavirus infections observed in 2014 indicates an important cognitive aspect of such research. Knowledge of the mechanisms and factors favoring the consolidation of the virus in the environment would allow for the development of evidence-based information to the public about the risk factors posing a threat to hantavirus infections and ways to prevent them.

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Acknowledgements

We would like to thank forestry managements in Brzozów and Lesko, and Jerzy Ginalski Director of the of Folk Architecture Museum in Sanok for their approval for conduction the study. Additionally, we thank Wiktor Gregorowicz, Monika Leszkiewicz, Jean-Francois Godeau, Piotr Ceryngierow, Agnieszka Napiórkowska, Agnieszka Kloch, Maciej Sierakowski, Joanna Ostrek, students and teachers from Zespół Szkół Leśnych in Lesko for a great help in field work activities. We also thank Anna Zielicka-Hardy for her support with the English version of the article.

Received: 18.05.2015
Accepted for publication: 3.06.2015

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