

Seroepidemiologic Evaluation of Exposure to Infection with Hantavirus (serotype *Puumala*) Among Forestry Workers in Poland

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Abstract

The genus *Hantavirus* belongs to a large family of viruses *Bunyaviridae*. In humans, infection happens through the respiratory system (inhalation of secretions of infected rodents with dust) or through the direct contact with a rodent. The bank vole (*Myodes glareolus*), which inhabits areas of almost the whole of Europe, is especially responsible for the transmission of hantavirus serotype *Puumala*. The primary goal of the study was evaluation of the state of health of forestry workers by laboratory diagnosis of diseases caused by hantaviruses serotype *Puumala*. The study covered a group of 820 randomly selected workers of the State Forests from 32 Forest Districts located in the area of the whole Poland. The results show that the largest number of seropositive results among forestry workers was obtained in the Regions of Łódź, Rzeszów and Bydgoszcz. The largest number of positive results, 3 (11.5%), were noted in the Piotrków Trybunalski Forestry District in the Łódź Region. In the whole of Poland, 12 seropositive results were noted (1.5%) and 26 doubtful (3.2%). The distribution of the positive and doubtful results obtained may suggest that in given regions large populations of bank vole may occur. Bank voles is the vector of hantavirus serotype *Puumala*. The conducted studies suggest the occurrence of hantaviruses *Puumala* in large forest complexes in the area of eastern and central Poland, and in the Kłodawa area of the Zielona Góra Region. People residing and working in these areas are exposed to infection. This infection seems to be comparable with the data from other countries. The majority of the cases of infection probably have an asymptomatic course.

Keywords: Hantavirus *Puumala*, zoonoses, forestry workers, rodents, *Myodes*

Introduction

The genus *Hantavirus* belongs to a large family of viruses *Bunyaviridae* (more than 300 viruses capable of infecting humans, animals, plant and arthropods) (Bishop et al. 1980, Fenner 1975, Schmaljohn and Hooper 2001). At present, more than 21 hantaviruses are known, which cause diseases in humans with various symptoms, starting from proteinuria, pulmonary oedema, and ending with haemorrhagic diseases. Hantaviruses are commonly divided into two groups: Old World and New World viruses, according to the occurrence of rodents transmitting these germs and diseases caused in humans: haemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS), or hantavirus cardiopulmonary syndrome (HCPS)

(Schmaljohn 1996). The infectious materials are excrements and secretions of infected rodents. Infection in humans takes place through the respiratory system (inhalation of secretions with dust) or by direct contact with a rodent. The bank vole (*Myodes glareolus*), which inhabits areas of almost the whole of Europe, except the Mediterranean region, is especially responsible for the transmission of hantavirus serotype *Puumala* (Avsic-Zupanc et al. 2007, Bernshtein et al. 1999, Cvetko et al. 2005, Olsson et al. 2003). The number of bank voles increases after mast year. Easy acquisition of food may contribute to massive reproduction even in winter, leading to very high densities in spring and summer. The increase in the number of bank voles may also affect the increase in the number of *Puumala* infections (Clement et al. 2009, Zwolak et al. 2016).

The climatic data and areas inhabited by rodents are important in foreseeing infections with hantaviruses (Olsson et al. 2009). In humans, hantavirus serotype *Puumala* causes HFRS, usually with a mild to moderate course, often called *nephropathia epidemica* (NE), and with a low death rate (Pilaski et al. 1994, Valtonen et al. 1995, Lahdevirta 1989). The risk of contracting HFRS is most often associated with agriculture or forestry (Olsson et al. 2003, Abu Sin et al. 2007, Vapalahti et al. 2010, Dutkiewicz et al. 2008). At present, cases of HFRS caused by the serotype *Puumala* have been noted in Europe, Asia and in North America. To date in America, no cases of human-to-human transmission of hantaviruses have been noted (Wells et al. 1997a), although such cases were observed in Argentina and Chile (Wells et al. 1997b, Padula et al. 1998). Other data show that in the saliva of patients infected with hantavirus *Puumala*, inhibitory substances are present which prevent the transmission of the virus from human-to-human (Pettersson et al. 2008).

The disease caused by the serotype *Puumala* usually begins suddenly with a fever, headache and abdominal pain, sometimes with vomiting or diarrhea (Mustonen et al. 1994, Settergren et al. 1989). There may also occur symptoms on the part of the central nervous system, such as sleepiness or dizziness. In addition, the RNA of the *Puumala* virus has also been detected in the cerebral fluid (Mahonen et al. 2007). In one-third of patients suffering from NE (*nephropathia epidemica*) there occurs vision disorders, often in the form of suddenly occurring short-sightedness (Kontkanen et al. 1994). There also occur symptoms on the part of the urinary system: oliguria, anuria, proteinuria, while in the second week of the disease, polyuria, accompanied by haematuria develops. Approximately 5% of patients require dialyses (Mustonen et al. 1994, Settergren et al. 1989). In severe cases, there may occur symptoms on the part of the heart, pulmonary oedema, or shock. According to the latest data, mortality associated with contracting serotype *Puumala* is about 0.1% (Mustonen et al. 1996, Makela et al. 2009). The disease is rarely observed in children and has a considerably milder course than in adults; however, abdominal symptoms occur more frequently (Mustonen et al. 1994).

The primary goal of the study was evaluation of health state of health of forestry workers by laboratory diagnosis of diseases caused by hantavirus serotype *Puumala*.

Materials and Methods

The study covered a group of 820 randomly selected workers of the State Forests from 32 Forest Districts located in the area of the whole Poland. The research group included 678 males and 142 females; mean age 47±8.

Prior to the study, each forestry worker was precisely informed concerning its goal and course, and the possibility to withdraw from participation in the study at each stage, without incurring any consequences. From all study partici-

pants, blood was collected, centrifuged on the spot, and sera preserved for further studies. In addition, medical history was taken concerning the exposure to contact with rodents, number of hours of work in the forest, and presently reported complaints.

In all 820 study participants, serologic tests were performed for Hantavirus *Puumala* IgM and IgG, immunoenzymatic test (ELISA) by PROGEN Biotechnik GmbH, Germany. Antibodies (class IgM and IgG) against hantaviruses were examined using *Puumala* strain antigens. The tests were performed in accordance with the research procedure provided by the manufacturer. Statistical analysis of the results was obtained via using Chi-square test.

Results

Table 1 presents the results obtained in individual Forest Districts.

The above-presented results show that the largest number of seropositive results among forestry workers were obtained in the following Regions: Łódź, Rzeszów and Bydgoszcz. The largest number of positive results, 3 (11.5%), were noted in the Piotrków Trybunalski Forestry District, the Łódź Region. In the whole of Poland, 12 seropositive results were noted (1.5%) and 26 doubtful (3.2%). Figure 1 demonstrates the distribution of positive results in the area of the whole Poland.

Figure 1 demonstrates that positive results were most frequently observed in south-eastern and central Poland, whereas doubtful results were noted in the whole of eastern Poland. In western Poland, the only Forestry District where both positive and doubtful results were found was Kłodawa Forestry District in the Zielona Góra Region. The distribution of the positive and doubtful results obtained may suggest that in given regions may occur large populations of bank vole, which is the vector of hantavirus *Puumala*. In addition, this may be associated with the type of forests in these areas, for example, abundant with beech trees, the nuts of which are favourable food for this rodent.

Table 2 presents the distribution of seropositive results according to gender and type of work performed

Positive results were found in 11 (1.6%) males and 1 (0.7%) female (Table 2). It was confirmed that gender had no effect on the frequency of occurrence of infection with hantavirus *Puumala*, the results being statistically insignificant ($\chi^2 = 0.6864$; $p = 0.407375$). The lack of statistical significance ($\chi^2 = 0.6932$; $p = 0.405062$) of infection with hantavirus *Puumala* was also confirmed with respect to the working time outdoors. In employees working outdoors there were 10 (1.7%) positive results, while in office workers only 2 (0.9%) positive results were found. Both cases of positive results in office workers were observed in Piotrków Trybunalski. The ratio between office work to work outdoors was for the 2 cases 6:2 and 7:1, respectively. The ob-

Table 1. Examination results for presence of antibodies class IgM and IgG against Hantaviruses *Puumala*, with division into administrative regions of Poland and the Forest Districts in the study

Region	No.	Forest District	No. of forestry workers examined	No. of doubtful results		No. of positive results		% of positive results for individual regions
				IgM	IgG	IgM	IgG	
Szczecin	1	Międzyzdroje	25	0	0	0	0	0
	2	Drawsko Pomorskie	25	0	0	0	0	
Gdańsk	3	Łupawa	25	0	0	0	0	0
	4	Starogard Gdański	25	0	0	0	0	
Olsztyn	5	Srokowo	26	0	1	0	0	1,9 %
	6	Szczytno	26	0	1	0	1	
Białystok	7	Augustów	30	0	1	0	0	0
	8	Bielsk Podlaski	25	0	0	0	0	
Warsaw	9	Samaki	22	0	2	0	0	0
	10	Dwukopy	25	0	2	0	0	
Bydgoszcz	11	Woziwoda	25	0	0	0	0	3,9 %
	12	Włocławek	26	0	0	2	0	
Poznań	13	Oborniki Wielkopolskie	25	0	0	0	0	0
	14	Kalisz	25	1	0	0	0	
Zielona Góra	15	Kłodowa k. Gorzowa	25	1	4	1	0	2 %
	16	Wymiarki	25	0	0	0	0	
Wrocław	17	Szczytna Góra Śląska	25	0	0	0	0	0
	18	(Wrocław District, Poznań Region)	26	0	0	0	0	
Opole	19	Tułowice	25	0	3	0	0	0
	20	Namysłów	25	0	0	0	0	
Katowice	21	Wisła	25	1	0	0	0	0
	22	Herby	25	0	1	0	0	
Łódź	23	Grotniki	25	0	1	0	0	5,9 %
	24	Piotrków Trybunalski	26	1	0	1	2	
Kielce	25	Stąporków	24	1	0	0	1	2 %
	26	Staszów	25	0	0	0	0	
Kraków	27	Olkusz	32	1	1	0	1	1,8 %
	28	Stary Sącz	25	0	0	0	0	
Rzeszów	29	Nowa Dęba	25	0	1	0	1	4 %
	30	Lesko	25	0	0	0	1	
Lublin	31	Janów Lubelski	31		0	0	0	1,8 %
	32	Radzyń Podlaski	26	1	0	1	0	
TOTAL			820	8	18	5	7	12 (1.5 %)

served positive results in office workers may suggest the location of the Forest District in an area which is the habitat of bank vole, or the presence of excrements of these rodents in office rooms (due to insufficient protection against rodents).

Discussion and Conclusion

Both in central and northern Europe an upward tendency is observed in the number of HFRS cases. This is partially due to better surveillance and diagnostics, and partially related with climatic changes, especially warmer conditions in summer (Heyman et al. 2008, Schwarz et al. 2009).

Also, in northern Europe, climatic factors exert an effect on the occurrence of HFRS: therefore, in winter at the turn of 2006-2007 in Sweden, a rapid increase was observed in the number of *Puumala* infections (313 cases) (Pettersson et al. 2009). It is considered that worldwide there are as many as 150,000 cases of HFRS annually, a half of them observed in China (Lee 1996, Song 1999).

In Europe, a large number of HFRS infections are caused by serotype *Puumala* (Heyman et al. 2009). 95% of cases were observed as late as after 1990 in association with improvement of diagnostics. Diagnostic tests for the detection of *Puumala* hantavirus were introduced in some countries in 1979, although in many countries the reported cases

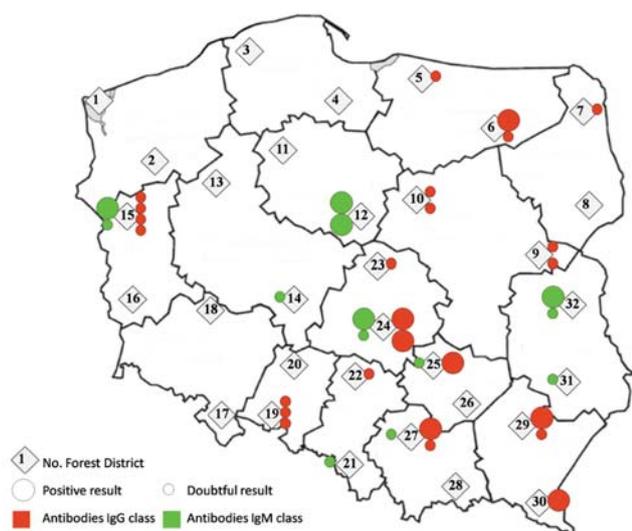


Figure 1. Distribution of seropositive and doubtful results in the area of Poland, according to division into administrative regions and Forestry Districts examined

Table 2. Distribution of seropositive results according to gender and type of work performed in the Forestry District

Forestry District	No. of positive results	For positive results			
		Males n=678	Females n=142	Outdoors ¹ n=594	Office ² n=223
Szczytno	1	1	0	1	0
Włocławek	2	2	0	2	0
Kłodawa k. Gorzowa	1	1	0	1	0
Piotrków Trybunalski	3	3	0	1	2
Stąporków	1	1	0	1	0
Olkusz	1	1	0	1	0
Nowa Dęba	1	1	0	1	0
Lesko	1	1	0	1	0
Radzyń Podlaski	1	0	1	1	0
Total	12	11	1	10	2

¹Outdoors: 50% and more working hours outdoors

²Office: less than 50% of working hours indoors

Lack of data concerning working hours of employees.

of infection appeared as late as in the last decade (Antoniadis et al. 1996, Lundkvist et al. 1997, Papa et al. 1998). Fatal cases are usually due to complications related with renal failure or haemorrhage, although the cases caused by *Puumala* are most often mild, and mortality is approximately 0.1% (Papa et al. 1998, Avsic-Zupanc et al. 1999).

Many studies and observations undoubtedly indicate the endemic occurrence of some zoonoses in Poland, often associated with exposure to contact with wild animals. These zoonoses constitute a serious epidemiological problem, especially in the environment of forest exploitation, where they are frequently of a character of occupational diseases in forestry workers (Dutkiewicz et al. 2011). The first case of HFRS in Poland was reported in the area of Rzeszów in 2007 (Nowakowska et al. 2009). Cases of diseases caused by hantaviruses have been reported to the National Institute of

Public Health since 2007. These reports come mainly from the Rzeszów Region. Epidemiological reports from 2007-2015 issued by the National Institute of Public Health show about 6-8 cases, on average, annually, which constitutes approximately 0.02 of the incidence of this disease per 100,000 population. An exception is the year 2014, when as many as 54 cases of diseases caused by hantaviruses were reported, including 53 cases from the Rzeszów Region, which indicates that this is an endemic region of occurrence of hantaviruses. Nearly 100% of the reported cases were hospitalized (Report – National Institute of Public Health – National Institute of Hygiene).

German researchers investigated for hantaviruses (serotype *Dobrava/Tula*) a population of 563 forestry workers from the southern regions of Germany. Serologic tests showed 9.1% positive results (Mertens et al. 2011). Polish researchers carried out studies concerning the occurrence of infections with *Puumala* hantavirus in the area of Poland. In the group of patients diagnosed with symptoms on the part of the urinary system no antibodies against serotype *Puumala* were found. Also, no seropositive results were observed in the group of 86 forestry workers from the area of the Upper Silesian Region. The only seropositive results were found in 19% of zoologists examined (from the group of 76 zoologists from the whole Poland) (Sadkowska-Todys et al. 2007). Another study investigated antibodies against *Puumala* hantaviruses among patients with the symptoms of *nephropathia epidemica* (n=9) and renal insufficiency (n=21), as well as among healthy zoologists (n=78). In the examined groups, no antibodies were found against *Puumala* in IgM class. However, seropositive results IgG were noted in the group of 11 zoologists (14%) (Gut et al. 2007b). Another study conducted in Poland also did not show any positive results among forestry workers (from the Upper Silesian Region, n=86). Nevertheless, positive (14.9%) and doubtful (10.6%) results were found in the group of zoologists (Gut et al. 2007a). Studies conducted among forestry workers (n=216) from the area of south-eastern Poland showed 4 positive results (1.9%) and 8 which were doubtful (3.7%) for antibodies against *Puumala* hantaviruses. Forestry workers who had positive results stayed in the forest for more than 50% of their working time (Żukiewicz-Sobczak et al. 2014). Also, another study concerning the exposure of forestry workers to infections with hantaviruses, included 161 employees of Forest Districts in the area of south-eastern Poland (Puławy, Biała Podlaska, Zwierzyniec, Roztocze National Park). The results showed that the presence of anti-hantavirus antibodies IgG was found in only 4 people examined (2.5%), and 2 infections were caused by the serotype *Puumala*. No specific antibodies of the IgM class were observed (Knap et al. 2010). The results of the presented study also confirm a low percentage (1.5%) of seropositive results among forestry workers in the whole Poland. Grygorczuk S. et al. found 4 positive results (5.7%) confirming the pres-

ence of antibodies against hantaviruses *Puumala* IgG class observed in the group of 69 employees of Forest Districts in north-eastern Poland (Regions of Białystok and Olsztyn) (Grygorczuk et al. 2008). The presented study also indicates the presence of antibodies against hantaviruses *Puumala* in the regions of eastern Poland. Serologic data suggest the occurrence of probably asymptomatic or poorly symptomatic, and in consequence, undiagnosed hantavirus infections among the population in the area of the whole Poland (Sadkowska-Todys et al. 2007, Grygorczuk et al. 2008).

Every several years in Central Europe, an increase in morbidity is observed related with the periodical growth of an abundance of the population of the main reservoir and carrier of hantavirus *Puumala*, the bank vole. An increase in the population of this rodent also depends on the amount of food available, which are frequently beech nuts; hence, HFERS more often occurs in the area of beech forests, e.g. in Austria or the Czech Republic (Olsson et al. 2002, Mailles et al. 2005). In Europe, the majority of HFERS cases are caused by the serotype *Puumala*, until 2006, 35,424 cases were reported (Heyman et al. 2008, Heyman et al. 2009). In Germany, many cases of HFERS are reported annually, although the highest peak was noted in 2007 (1,687 cases). The majority of these cases were caused by *Puumala* infection (Winter et al. 2009). Lower Bavaria is an endemic region for the occurrence of hantavirus infections, especially *Puumala* (Mertens et al. 2009). Other regions of endemic occurrence of hantaviruses is north-eastern France and Belgium. In 2005, in these countries many cases of HFERS were registered: 253 in France and 372 in Belgium (Heyman et al. 2008, Tersago et al. 2009). Approximately 40% of Finnish researchers engaged in studies of small mammals possess antibodies against *Puumala* virus. Annually in Finland, about 1,000 laboratory confirmed symptomatic cases of infections with hantaviruses are confirmed, from 100 - 300 in Sweden and about 50 in Norway. The frequency of occurrence of antibodies against *Puumala* has been estimated at 5% among the Finnish population; therefore, it is considered that in this country there occur approximately 4,000 infections with this virus in humans. It follows that about 75% of infections have an asymptomatic, poorly symptomatic or atypical course. Similar calculations were performed in Sweden where it was estimated that only approximately 10-12.5% of cases of infections are clinically and serologically confirmed (Vapalahti et al. 2003, Heyman et al. 2011).

In Poland, studies were carried out concerning the degree of infection of bank voles with hantavirus (Wójcik-Fatla et al. 2013a, Sheikh Ali et al. 2014). In 2009, 2 cases of the bank vole which was the carrier of *Puumala* were found (out of 45 examined voles from the areas of north-eastern Poland), which constitutes about 4% (Sheikh Ali et al. 2014).

Hypotheses were posed concerning the transmission of hantaviruses by ticks; however, they have not been confirmed. Some types of viruses belonging to the family *Bunyaviridae* are transmitted by ticks; nevertheless,

hantaviruses are transmitted to humans only via rodents: by the respiratory route (Elliott 2009, Horne and Vanlandingham 2014). The presence of the genetic RNA material of hantaviruses has not been confirmed in *Ixodes ricinus* or *Dermacentor reticulatus* ticks (Wójcik-Fatla et al. 2013b, 2011).

The conducted studies suggest the occurrence of hantavirus serotype *Puumala* in large forest complexes in the areas of eastern and central Poland, and in the Kłodawa area of the Zielona Góra Region. People residing and working in these areas are exposed to infection. This infection seems to be comparable with the data from other countries. The majority of the cases of infection probably have an asymptomatic course.

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