Serological and genetic evidence for the presence of Seoul hantavirus in *R.norvegicus* in the Flanders, Belgium.

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Introduction

Hantaviruses are the etiological agents of hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus pulmonary syndrome (HPS) in the Americas. In the natural rodent reservoir hantaviruses establish a life-long, chronic infection and the rodent develops a strong neutralizing antibody response against the virus. At least two hantaviruses are known to be present in Belgium, i.e. Puumala hantavirus (PUUV), carried by *Myodes* (*Clethrionomys) glareolus* (bank vole), which is found in most of Europe and causes the mildest form of HFRS, Nephropathia epidemica (NE). Tula hantavirus (TULV) carried by *Microtus arvalis* (common vole) was recently also described. Although long considered non-pathogenic to man, TULV was recently reported in connection to cases of HFRS and/or hantavirus disease. PUUV in responsible for epidemics in Belgium, TULV was so far, in Belgium, not linked to human disease. Seoul hantavirus (SEOV), carried by *Rattus rattus* (black rat) and *R. norvegicus* (Norway, or brown, rat), was also reported to cause HFRS and is responsible for approximately 25% of the HFRS cases in Asia. As *Rattus sp.* are present worldwide, SEOV has the potential to cause human disease worldwide. SEOV in brown rats was found in Japan, South Korea, USA and Brazil and, most recently in Cambodia, Indonesia and in France. Although, SEOV seems to be responsible for human infections in Asia, there are at present only a few reports concerning confirmed human SEOV infections outside Asia, i.e. from the USA and Brazil. This study aimed to evaluate the presence of SEOV in *R. norvegicus* in the Flanders region in Belgium and the impact of its presence on an at-risk group, rodent-control teams.

Materials & Methods

During this study 195, 143 and 164 rats were trapped and sampled in, respectively, 2004, 2005 and 2006. The examined rats all except one *Rattus rattus* belong to the species *Rattus norvegicus* and were obtained by live trapping in the Flanders region in Belgium. The Research Institute for Nature and Forest (INBO, Brussels, Belgium) department rodent management (Merelbeke, Belgium), provided the samples. In 2005 and 2006, serum samples were obtained from all animals, in 2004 only 169 serum samples were obtained from 195 rats. Lung tissue was available from all animals trapped in 2004 and 2005, not from those trapped in 2006.
The employees of the Water Division of the Flemish Government, a group that is professionally in close contact with *R. norvegicus*, were asked to participate in a screening for hantaviruses. Of this group, 116 individuals gave a blood sample which was also screened for the presence of antibodies to hantaviruses. A control group (N = 121) was tested also.
Initial screening for both human and rodent serum samples was performed using enzyme immuno assay (EIA) tests, immunofluorescence assay (IFA) was applied to confirm EIA findings, after which serum samples that showed to be positive in these techniques were subsequently submitted to focus reduction neutralization tests (FRNT) for determination of the causal hantavirus and, in the case of the rodent tissue samples, RT-PCR was used for demonstration of the hantavirus genome.

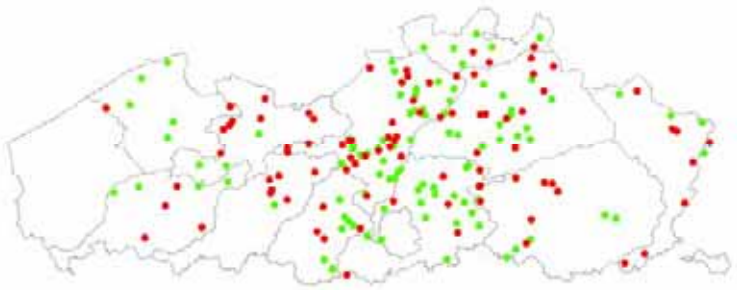
Table 1 : Rattus norvegicus serology results

sex	-/+	2004		2005		2006		total	
male	positive	26	30.6%	24	34.8%	5	7.6%	55	25.0%
	negative	59		45		61		165	
	Subtot	85		69		66		220	
female	positive	25	30.5%	23	31.5%	6	15.8%	54	28.0%
	negative	57		50		32		139	
	Subtot	82		73		38		193	
unknown	positive	1	50.0%	0	0.0%	19	31.7%	20	31.7%
	negative	1		1		41		43	
	Subtot	2		1		60		63	
total	positive	52	30.8%	47	32.9%	30	18.3%	129	27.1%
	negative	117		96		134		347	
	Subtot	169		143		164		476	

Results

Screening of rat serum samples for the presence of Seoul hantavirus IgG antibodies showed that in 2004, 30,77% of the rats tested positive, in 2005 32,87% and in 2006 18.29%. Over the three-year period the average seroprevalence was 27,10% (table 1). In 2004 and 2005 no sex differences in hanta virus prevalence were found, in 2006 twice as much female rats than male rats were infected. Seasonal changes in prevalence were found during the observing years. In the second half of the year less seropositive rats were captured.

Fig 1: Distribution of SEOV-seronegative (green) and SEOV-seropositive (red) brown rats in the Flanders



Distribution of SEOV-positive brown rats in the Flanders region seems to be proportionally spread over the total area, and no hot spots of hanta virus prevalence were found (fig.1).
The human at-risk group presented a significantly higher seroprevalence rate for SEOV and PUUV-IgG, i.e. 6.12% (6/98), than the PUUV seroprevalence rate in the normal population in the Flanders region which is 1.4%. FRNT tests (no results at the moment). RT-PCR revealed the presence of SEOV in lung tissue of several rats.

Discussion

Rattus norvegicus can be considered as one of the most harmful mammal species to mankind and rat-borne diseases have probably taken more human lives than all the wars in human history combined. In the United Kingdom *R. norvegicus* was found to harbour 13 zoonotic agents and ten non-zoonotic parasite species.
SEOV-caused laboratory outbreaks, related to working with *Rattus sp.*, in humans were reported in Belgium, France, The Netherlands, and England, and the strain of SEOV virus from France has been genetically characterized, but SEOV was so far not described in the wild brown rat populations in Belgium. Our study provides evidence for a significant seroprevalence, and for the presence of the SEOV genome, in wild rat population in the Flanders region in Belgium.
The seroprevalence rate amongst brown rats of approximately 27% over the three-year period is in agreement with similar studies in other parts of the world.
We noted a 6.12% seroprevalence in the examined at-risk group, FRNT will tell us if this seroprevalence was caused by SEOV infections or not.

Table 2 : seasonal changes

2004-2006	positive		negative	
jan-mar	22	33%	44	67%
apr-jun	45	33%	90	67%
jul-sep	44	28%	113	72%
okt-dec	18	15%	100	85%
total	129	27%	347	73%

The seasonal changes of the seroprevalence (table 2), with less seropositive rats in the second half of the year, which we have found in each of the three observing years, corresponds with previous field observations of several rodent species which suggest that intensity and prevalence of hantavirus infection varies seasonally. A simple explanation of these findings could be that relative more young animals were caught in autumn as a result of higher breeding activities in spring and summer. It is obvious that young animals had less chance to get in contact with infectious virus especially when one knows that the virus is transmitted via wounding and fights, which occur more when rats reach the age of puberty.
In contradiction with previous studies which suggest that more male than female rats are infected with Seoul hantavirus, no sex differences were found from our data for 2004 and 2005. In 2006 there were even twice as much female then male rats infected.

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