Scandinavian Journal of Infectious Diseases
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713690438

Serological and genetic evidence for the presence of Seoul hantavirus in Rattus norvegicus in Flanders, Belgium

Paul Heyman [a]; K. Baert [b]; A. Plyusnina [c]; C. Cochez [d]; Å. Lundkvist [e]; M.Van Esbroeck [f]; E. Goossens [g]; C. Vandenvelde [d]; A. Plyusnin [c]; J. Stuyck [b]
[a] From the Research Laboratory for Vector-borne Diseases, Queen Astrid Military Hospital, Bruynstraat, Brussels [b] Research Institute for Nature and Forest, Kliniekstraat, Brussels [c] Haartman Institute, Department of Virology, University of Helsinki, Finland [d] National Reference Centre for Hantavirus Infections, Queen Astrid Military Hospital, Bruynstraat, Brussels, Belgium [e] Swedish Institute for Infectious Disease Control, Solna, Sweden [f] Institute of Tropical Medicine, Antwerp [g] Veterinary and Agrochemical Research Centre, Uccle, Belgium

First Published:2009

To cite this Article Heyman, Paul, Baert, K., Plyusnina, A., Cochez, C., Lundkvist, Å., Esbroeck, M.Van, Goossens, E., Vandenvelde, C., Plyusnin, A. and Stuyck, J.(2009)'Serological and genetic evidence for the presence of Seoul hantavirus in Rattus norvegicus in Flanders, Belgium',Scandinavian Journal of Infectious Diseases,41:1,51 — 56

To link to this Article: DOI: 10.1080/00365540802459994

URL: http://dx.doi.org/10.1080/00365540802459994

Scandinavian Journal of Infectious Diseases, 2009; 41: 51–56

informa
healthcare

ORIGINAL ARTICLE

Serological and genetic evidence for the presence of Seoul hantavirus in Rattus norvegicus in Flanders, Belgium

PAUL HEYMAN[1], K. BAERT[2], A. PLYUSNINA[7], C. COCHEZ[3], Å. LUNDKVIST[4], M. VAN ESBROECK[5], E. GOOSSENS[6], C. VANDENVELDE[3], A. PLYUSNIN[7] & J. STUYCK[2]

From the [1]Research Laboratory for Vector-borne Diseases, Queen Astrid Military Hospital, Bruynstraat, Brussels, [2]Research Institute for Nature and Forest, Kliniekstraat, Brussels, [3]National Reference Centre for Hantavirus Infections, Queen Astrid Military Hospital, Bruynstraat, Brussels, Belgium, [4]Swedish Institute for Infectious Disease Control, Solna, Sweden, [5]Institute of Tropical Medicine, Antwerp, [6]Veterinary and Agrochemical Research Centre, Uccle, Belgium, and [7]Haartman Institute, Department of Virology, University of Helsinki, Finland

Abstract

Seoul hantavirus (SEOV), carried by Rattus rattus (black rat) and R. norvegicus (Norway, brown rat), was reported to circulate as well as cause HFRS cases in Asia. As Rattus sp. are present worldwide, SEOV has the potential to cause human disease worldwide. In Europe however, only SEOV prevalence in rats from France was reported and no confirmed cases of SEOV infection were published. We here report genetic and serological evidence for the presence of SEOV virus in brown rat populations in Belgium. We also serologically screened an at-risk group that was in contact with R. norvegicus on a daily basis and found no evidence for SEOV infection.

Introduction

Hantaviruses are the aetiological agents of haemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus pulmonary syndrome (HPS) in the Americas [1–3]. In the natural rodent reservoir, hantaviruses establish a life-long, chronic infection and the rodents develop a strong neutralizing antibody response against the virus. At least 2 hantaviruses are known to be present in Belgium – Puumala hantavirus (PUUV), carried by Myodes (Clethrionomys) glareolus (bank vole), is found in most of Europe and causes the mildest form of HFRS, nephropathia epidemica (NE) [4,5]; Tula hantavirus (TULV) carried by Microtus arvalis (common vole) was recently also described [6]. Although long considered non-pathogenic to man, TULV was recently reported in connection with cases of HFRS and/or hantavirus disease [7,8]. PUUV is responsible for epidemics in Belgium [5,9]; TULV has, to date in Belgium, not been linked to human disease.

Seoul hantavirus (SEOV), carried by Rattus rattus (black rat) and R. norvegicus (Norway, brown rat), was also reported to cause HFRS and is responsible for approximately 25% of the HFRS cases in Asia [10]. As Rattus sp. are present worldwide, SEOV has the potential to cause human disease worldwide. SEOV in brown rats was found in Japan [11–13], South Korea [14], USA [15,16] and Brazil [17] and, most recently in Cambodia [18], Indonesia [19] and in France [20]. 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 [15–17]. 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.

Correspondence: P. Heyman, Research Laboratory for Vector-borne Diseases, Queen Astrid Military Hospital, Bruynstraat 1, B-1120 Brussels, Belgium. Tel: +32 2 2644044. Fax: +32 2 2644608. E-mail: paul.heyman@smd.be

(Received 4 May 2008; accepted 6 September 2008)

ISSN 0036-5548 print/ISSN 1651-1980 online
DOI: 10.1080/00365540802459994

Materials and methods

During this study, 195, 143 and 164 rats were trapped and sampled in, respectively, 2004, 2005 and 2006. The examined rats all 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), rodent management department (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 captured animals trapped in 2004 and 2005, but was not available from those trapped in 2006.

Initial screening of the rodent serum samples was performed using enzyme immuno- assay (EIA) tests, immunofluorescence assay (IFA) was applied to confirm positive EIA findings, and RT-PCR was applied on tissue (lung) samples of all seropositive rats for demonstration of the hantavirus genome.

IFA was performed as described earlier [20]. Screening for SEOV IgG antibodies was performed by applying a standard IFA technique (PROGEN Biotechnik GmbH, Heidelberg, Germany). In brief, 10-well slides coated with SEOV infected cells (strain R22) were re-hydrated and to each well 25 µl of a 1/64 dilution of rat serum was added. After appropriate incubation, slides were washed and 25 µl of anti-rat-IgG FITC labelled conjugate was added to each well. After incubation and washing, the slides were examined under a fluorescence microscope. Samples showing a positive reaction at a 1/64 dilution were further diluted and tested until the endpoint titre was obtained.

EIA screening was performed by applying a standard EIA technique (de Carvalho-Nicacio and Lundkvist, unpublished), using native irradiation-inactivated viral antigen (SEOV, strain R22VP30, kindly provided by Dr. Avsic-Zupanc) and recombinant N-antigens (PUUV, strain Kazan, and DOBV, strain Slovenia) for the detection of IgG antibodies. In brief, 96-well microtitre plates were coated with antigen and incubated overnight at 4°C. Unsaturated binding sites were blocked, followed by incubation of rodent sera diluted at 1:200 at 37°C for 1 h. Where appropriate, peroxidase (Sigma, St. Louis, MO) or alkaline-phosphatase-labelled (Jackson, West Grove, PE) anti-rat IgG antisera was incubated for 1 h. Subsequently, TMB or p-nitrophenyl substrates (Sigma, St. Louis, MO) were added and, after colour development, the reaction was stopped with 2*M* H_2SO_4 (for TMB substrate). The optical density (OD) was determined at 450 nm or 405 nm against a reference wavelength of 620 nm [20].

A group that was professionally – and on a daily basis – in close contact with R. norvegicus was asked to participate in a screening. This group, consisting of 98 individuals, gave a blood sample for screening for the presence of antibodies to hantaviruses (PROGEN Biotechnik GmbH, Heidelberg, Germany), Leptospira serovars (in-house MAT IgG test), Echinococcus multilocularis (Bordier Affinity Products, Crissier, Switzerland), Borrelia spp. (ELISA: Enzygnost, Dade Behring, Marburg, Germany; Western Blot: Mikrogen, Martinsried, Germany) and Anaplasma phagocytophilum (Focus Diagnostics, Cypress, CA, USA).

RT-PCR and sequencing

Approximately 50 mg of lung tissue was ground using MagNA Lyser Green Beads in the Roche MagNA Lyser Instrument (Roche Diagnostics, Belgium, Vilvoorde, Belgium), according to the manufacturer's protocol. RNA was extracted from lung tissue samples with the High Pure RNA Tissue Kit (Roche Diagnostics Belgium, Vilvoorde, Belgium) according to the manufacturer's instructions. RNA was then subjected to a nested RT-PCR yielding a 324-bp PCR amplicon from the M segment (nt 1979 to 2302, primers excluded) or a 320-bp amplicon from the S segment (nt 509 to 782, primers excluded) [7]. PCR amplicons were gel-purified using QIAquick Gel Extraction-kit (QIAGEN GmbH) and sequenced automatically using ABI PRISM (Dye Terminator sequencing kit (Perkin Elmer/ABI, NJ). Multiple nucleotide and amino acid sequence alignments were prepared manually using SeqApp 1.9a169 sequence editing program.

Results

Rattus norvegicus

Screening of rat serum samples for the presence of Seoul hantavirus IgG antibodies showed that in 2004, 30.8% of the rats tested positive; in 2005, 32.9%; and in 2006, 18.3%. Over the study period the average seroprevalence was 27.1% (Table I). In 2004 and 2005 no gender differences in hantavirus prevalence were found, but in 2006 twice as many female rats as male rats were seropositive (Table I). Seasonal changes in prevalence were found during the 3 y. In the second half of the y, fewer seropositive rats were captured (Table II). Distribution of SEOV-positive brown rats in the Flanders region seems to be evenly spread over the total area, and no hotspots of hantavirus prevalence were found (Figure 1).

Table I. Seropositive and seronegative R. norvegicus by y and gender.

Gender	−/+	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	

In total, 6 WB-positive samples have been selected for genetic analysis: ##295, 297, 415, 430, 500 and 895. All these were found also to be RT-PCR-positive when tested with the hantavirus S and M segment-specific primers. Partial sequences of the hantaviral M segment (nt 1990–2272, primers' sequences excluded) and S segment (nt 508–782, primers' sequences excluded) were recovered from 1 of the samples (#895). Both newly recovered M and S segment sequences showed the highest level of identity to the corresponding sequences of previously described SEOV strains, thus confirming that they belonged to the SEOV genotype. The wild-type hantavirus strain was designated SEO/Belgium/Rn895/2005, or Belgium895 for short. The partial S segment sequence of Belgian strain showed 97–98% sequence identity to the S sequences of SEOV strains from France and Indonesia and also Rattus norvegicus-associated strains from Cambodia (e. g. Cambodia032), all belonging to the SEOV genetic lineage #7 [20]. The level of sequence identity to other SEOV strains was 95–96%. The S segment sequences of other hantaviruses associated with Murinae rodents (mice and rats of the Old World) were much less related to the Belgium895 S sequence: identity was 70–76%.

Analysis of the partial M segment sequence of Belgium895 strain showed a similar pattern. The highest level of sequence identity (97–98%) was observed toward French and Indonesian strains. Other SEOV strains showed lower sequence identity (92–95%) and other Murinae-associated hantaviruses were less related (66–76%).

Table II. Seropositive and seronegative R. norvegicus, seasonal distribution.

	2004–2006				
	Positive		Negative		Total captured
Jan-Mar	22	33%	44	67%	66
Apr-Jun	45	33%	90	67%	135
Jul-Sep	44	28%	113	72%	157
Oct-Dec	18	15%	100	85%	118
Total	129	27%	347	73%	476

Human at-risk group

The results of the serological screening are summarized in Table III.

Six out of 98 (6.1%) individuals were positive in PUUV IgG but had no clinical signs of NE. Two out of 98 were positive for L. grippotyphosa, 1 out of 98 for L. icterohaemorrhagica, and 1 out of 98 for L. canicola. The first 2 serovars were found present in local rodent populations while the last was probably vaccine-related. Titres were no higher than 1/30 (cut-off 1/10); only titres higher than 1/100 are associated with acute illness [21]. Serum samples were also tested against serovars L. Bratislava, L. autumnalis; L. castellonis, L.bataviae, L. celledoni; L. cynopteri, L. djasiman, L. poi, L. Louisiana, L. lichuan, L. tabaquite, L. panama, L. Pomona, L. pyrogenes, L. ranarum; L. hardjo, L. shermani and L. tarassovi, but all were found negative.

Two persons showed positivity against E. multilocularis IgG antibodies and 2 others were found Borrelia sp. and Anaplasma phagocytophilum IgG antibody-positive and were confirmed by Western blot.

Discussion

Rattus norvegicus can be considered as 1 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 UK, R. norvegicus was found to harbour no less than 13 zoonotic agents and 10 non-zoonotic parasite species [22]. Archaeological data regarding the introduction

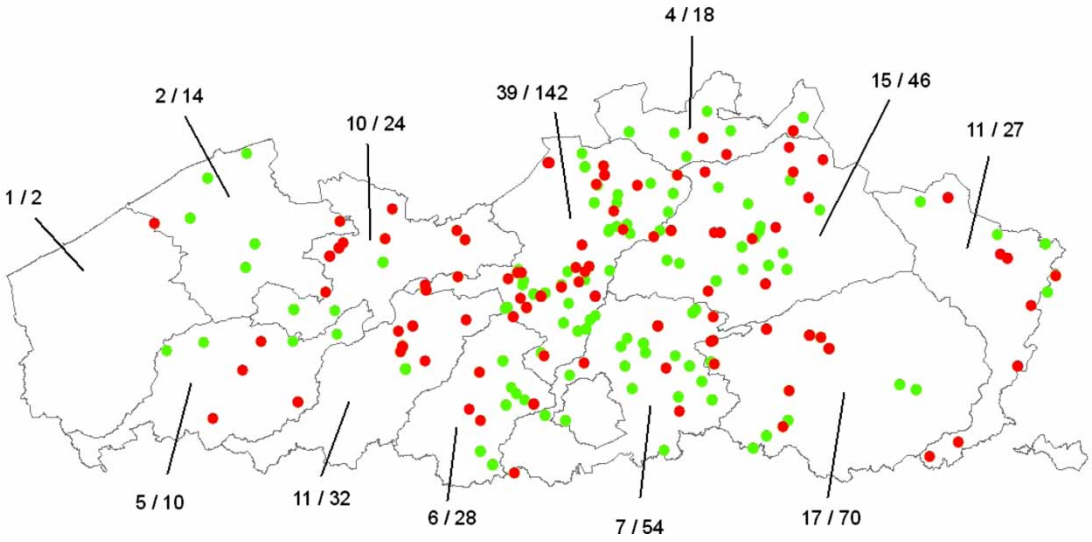

Figure 1. Flanders, distribution of seropositive R. norvegicus. Red: seropositive rats; Green: seronegative rats. Positive/negative ratio by trapping region indicated by lines.

of either black or brown rat are scarce, but evidence for the presence of the black rat (R. rattus) in Roman times on Belgian territory (Ervynck, unpublished data) and its presence in late mediaeval and post-mediaeval Belgium was reported [23,24]. R. norvegicus probably appeared in Belgium during the 18th century [23,24]. Reports from that era describe the displacement of black rats by the more aggressive and larger Norwegian rats throughout Europe [25]. Today, R. norvegicus has almost completely replaced R. rattus in Europe, and black rats are now rare or absent in much of their former range. In tropical zones, however, R. rattus is well established and is more common than R. norvegicus [26]. Recently, however, more and more observations of black rat

Table III. Analysis of the human at-risk group (n = 98) was performed by ELISA for leptospirosis, hantaviruses, Borrelia sp. IFA was used for A. phagocytophilum.

Pathogen	Serovars/serotype	Npos at-risk group
Leptospirosis §	Canicola	1
	Grippotyphosa	2
	Hebdomadis	1
	Icterohaemorrhagiae	1
Hantavirus	PUUV IgG	6
	HTNV IgG	4*
	SEOV IgG	4*
Borrelia	Burgdorferi, IgG	2#
Echinococcus	Multilocularis, IgG	3
Anaplasma	Phagocytophilum, IgG	2#

§ All serum samples were negative against serovars L. Bratislava, L. autumnalis; L. castellonis, L.bataviae, L. celledoni; L. cynopteri, L. djasiman, L. poi, L. Louisiana, L. lichuan, L. tabaquite, L. panama, L. Pomona, L. pyrogenes, L. ranarum; L. hardjo, L. shermani and L. tarassovi.
*Cross-reaction with PUUV.
confirmed by Western blot.

populations in pig and poultry houses were recorded in Belgium (K. Baert, personal communnication). SEOV-caused laboratory outbreaks in humans were reported in Belgium, France, The Netherlands, and England [27], and the strain of SEOV virus in R. norvegicus from France has been genetically characterized [20], but SEOV has to date not been 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 populations in the Flanders region of Belgium. The seroprevalence rate among brown rats of approximately 27% over the 3-y period is in agreement with similar studies in other parts of the world; an approximately 50% seroprevalence in Baltimore (Maryland, USA) rats [28], a 31.6% seroprevalence in Buenos Aires, Argentina [1], a 10% seroprevalence in R. norvegicus in Indonesia [29], a 13.5% seroprevalence in China [10] and, closer to home, McCaughey et al. noted a 21.6% seroprevalence in Northern Ireland [30].

Klein et al. [31] discovered no seasonal changes in Seoul hantavirus infection in Norwegian rats, despite other field observations of several rodent species suggesting that intensity and prevalence of hantavirus infection varies seasonally. This variation may be related to changes in environmental factors such as food availability, precipitation and temperature. Environmental factors may directly affect host immune responses against infection or act indirectly through changes in population densities and subsequent exposure to and transmission of hantavirus. The seasonal changes in the seroprevalence, with fewer seropositive rats in the second half of the y, which we have found in each of the 3 study y,

correspond with these previous studies. An explanation of these findings could be that relatively more young animals were caught in autumn as a result of higher breeding activity in spring and summer. It is clear that young animals had less chance to come into contact with infectious virus especially if the virus is transmitted via wounds [31,32], which occur more frequently when rats become sexually active and fight. Most of the rats caught in 2006 were trapped between September and December; these seasonal changes are probably also responsible for the lower overall prevalence of 18.3% found in 2006 compared with 30.8% and 32.9% seropositive rats found in 2004 and 2005, respectively. A more detailed genetic analysis of strain Belgium895 will be published in the near future.

In contrast to previous studies [31,32], which suggest that more male than female rats are infected with Seoul hantavirus, no gender differences were noted in our data for 2004 and 2005. In 2006, however, there were twice as many female as male rats infected.

Although brown rats thus seem to be worldwide massively infected with the human pathogenic Seoul hantavirus, reports of confirmed human cases of SEOV infection outside Asia are scarce [31,33,34].

We noted an elevated (6.1%) seroprevalence for hantavirus antibodies in the examined human at-risk group, where the overall PUUV seroprevalence in Belgium is around 1.5%. All 6 individuals showed higher titres for PUUV than for other hantaviruses in IFA. Importantly, however, none was confirmed in FRNT as being SEOV infection. SEOV thus does not seem to be a competent pathogen for humans even after prolonged contact with rats.

Acknowledgements

The study was made possible by grants from the Belgian Ministry of Defence and from The Academy of Finland.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] McCaughey C, Hart CA. Hantaviruses. J Med Microbiol 2000;49:587–99.

[2] Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis 1997;3:95–104.

[3] Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. Lancet Infect Dis 2003;3:653–61.

[4] Heyman P, van Mele R, de Jaegere F, Klingstrom J, Vandenvelde C, Lundkvist A, et al. Distribution of hantavirus foci in Belgium. Acta Trop 2002;84:183–8.

[5] Heyman P, Vervoort T, Colson P, Chu YK, Avsic-Zupanc T, Lundkvist A. A major outbreak of hantavirus infection in Belgium in 1995 and 1996. Epidemiol Infect 1999;122:447–53.

[6] Heyman P, Klingstrom J, de Jaegere F, Leclercq G, Rozenfeld F, Escutenaire S, et al. Tula hantavirus in Belgium. Epidemiol Infect 2002;128:251–6.

[7] Klempa B, Meisel H, Rath S, Bartel J, Ulrich R, Kruger DH. Occurrence of renal and pulmonary syndrome in a region of north-east Germany where Tula hantavirus circulates. J Clin Microbiol 2003;41:4894–7.

[8] Schultze D, Lundkvist A, Blauenstein U, Heyman P. Tula virus infection associated with fever and exanthema after a wild rodent bite. Eur J Clin Microbiol Infect Dis 2002;21: 304–6.

[9] Heyman P, Vervoort T, Colson P, Chu YK, Avsic-Zupanc T, Lundkvist A. A major outbreak of hantavirus infection in Belgium in 1995 and 1996. Epidemiol Infect 1999;122:447–53.

[10] Li YL, Ruo SL, Tong Z, Ma QR, Liu ZL, Ye KL, et al. A serotypic study of haemorrhagic fever with renal syndrome in rural China. Am J Trop Med Hyg 1995;52:247–51.

[11] Kariwa H, Yoshimatsu K, Araki K, Chayama K, Kumada H, Ogino M, et al. Detection of hantaviral antibodies among patients with hepatitis of unknown aetiology in Japan. Microbiol Immunol 2000;44:357–62.

[12] Lokugamage N, Kariwa H, Lokugamage K, Iwasa MA, Hagiya T, Yoshii K, et al. Epizootiological and epidemiological study of hantavirus infection in Japan. Microbiol Immunol 2004;48:843–51.

[13] Sugiyama K, Takagi K, Kinjo T, Nakata Y, Komatsu T, Kitamura T. Isolation and characterization of a hantavirus from Rattus norvegicus in a residential area of Nagoya City, Japan. J Vet Med Sci 1995;57:51–4.

[14] Kim YS, Ahn C, Han JS, Kim S, Lee JS, Lee PW. Haemorrhagic fever with renal syndrome caused by the Seoul virus. Nephron 1995;71:419–27.

[15] Glass GE, Watson AJ, Le Duc JW, Childs JE. Domestic cases of haemorrhagic fever with renal syndrome in the United States. Nephron 1994;68:48–51.

[16] Hjelle B, Jenison SA, Goade DE, Green WB, Feddersen RM, Scott AA. Hantaviruses: clinical, microbiological, and epidemiological aspects. Crit Rev Clin Lab Sci 1995;32:469–508.

[17] Iversson LB, da Rosa AP, Rosa MD, Lomar AV, Sasaki MG, Le Duc JW. Human infection by hantavirus in southern and south-eastern Brazil. Rev Assoc Med Bras 1994;40:85–92.

[18] Plyusnina A, Ibrahim IN, Winoto I, Porter KR, Gotama IB, Lundkvist A, et al. Identification of Seoul hantavirus in Rattus norvegicus in Indonesia. Scand J Infect Dis 2004;36: 356–9.

[19] Reynes JM, Soares JL, Hue T, Bouloy M, Sun S, Kruy SL, et al. Evidence of the presence of Seoul virus in Cambodia. Microbes Infect 2003;5:769–73.

[20] Heyman P, Plyusnina A, Berny P, Cochez C, Artois M, Zizi M, et al. Seoul hantavirus in Europe: first demonstration of the virus genome in wild Rattus norvegicus captured in France. Eur J Clin Microbiol Infect Dis 2004;23:711–7.

[21] Desmecht M, Korver H, Terpstra W. Isolation of Leptospira interrogans sezrotypen saxkoebing, grippotyphosa and copenhageni from muskrats in Belgium. Vlaams Diergeneesk Tijdschr 1991;60:59–63.

[22] Webster JP, Macdonald DW. Parasites of wild brown rats (Rattus norvegicus) on UK farms. Parasitology 1995;111: 247–55.

[23] Ervynck A. University of Amsterdam. Archeozoölogisch onderzoek van de zwarte rat (Rattus rattus) en de bruine rat (Rattus norvegicus). 1989.
[24] Ervynck A, van Neer W, Lentacker A. Introduction and extinction of wild animal species in historical times: the evidence from Belgium. Benecke N, editor. Archäologie in Eurasien 6. (The Holocene History of the European Vertebrate Fauna – Modern Aspects of Research 1999;399–407. Berlin, Germany, Deutsches Archäologisches Institut, Eurasien-abteilung.
[25] Krinke GJ. The laboratory rat. Academic Press; 2000.
[26] Nowak, Ronald M. Walker's Mammals of the World, 5th ed. Baltimore and London: The Johns Hopkins University Press; 1991. pp. 1345–71.
[27] Lee HW. Epidemiology and pathogenesis of haemorrhagic fever with renal syndrome. In: Elliott RM, editor. The Bunyaviridae. New York: Plenum Press; 1996. p. 253–67.
[28] Easterbrook JD, Shields T, Klein SL, Glass GE. Norway rat population in Baltimore, Maryland, 2004. Vector Borne Zoonotic Dis 2005;5:296–9.
[29] Ibrahim IN, Sudomo M, Morita C, Uemura S, Muramatsu Y, Ueno H, Kitamura T. Seroepidemiological survey of wild rats for Seoul virus in Indonesia. Jpn J Med Sci Biol 1996;49: 69–74.
[30] McCaughey C, Montgomery WI, Twomey N, Addley M, O'Neill HJ, Coyle PV. Evidence of hantavirus in wild rodents in Northern Ireland. Epidemiol Infect 1996;117:361–5.
[31] Klein SL, Marson AL, Scott AL, Ketner G, Glass GE. Neonatal sex steroids affect responses to Seoul virus infection in male but not female Norway rats. Brain Behav Immun 2002;16:736–46.
[32] Klein SL, Bird BH, Glass GE. Sex differences in Seoul virus infection are not related to adult sex steroid concentrations in Norway rats. J Virol 2000;74:8213–7.
[33] Lledo L, Gegundez MI, Saz JV, Beltran M. Seroprevalence of antibodies to Seoul virus in occupational risk groups. Enferm Infec Microbiol Clin 2004;22:217–9.
[34] Smith HM, Reporter R, Rood MP, Linscott AJ, Mascola LM, Hogrefe W, Purcell RH. Prevalence study of antibody to rat-borne pathogens and other agents among patients using a free clinic in downtown Los Angeles. J Infect Dis 2002;186:1673–6.