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OCCURRENCE AND DISTRIBUTION OF HANTAVIRUS IN WILD LIVING MAMMALS IN BELGIUM

R. VERHAGEN, *G. VAN DER GROEN, **A. IVANOV, J. VAN ROMPAEY, H. LEIRS, W.VERHEYEN

Laboratory of General Zoology, State University Centre Antwerp, Groenenborgerlaan 171, 2020 Antwerpen; *Institute of Tropical Medicine, 2000 Antwerpen, Belgium; **Institute of Poliomyelitis and Viral Encephalitides, Academy of Medical Sciences of the U.S.S.R., 142782 Moscow, U.S.S.R.

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Summary. — Small mammals were screened for the presence of antibodies to Hantaan virus (HTN) and Hantavirus (HV) antigen in Belgium. Antibody and antigen-positive animals were found in different parts of the country. One insectivore and five rodent species were found positive. The highest prevalence of infection was found in the bank vole (*Clethrionomys glareolus*). A relation between infected animals and wet habitats was observed. It was obvious that in bank vole the likelyhood of infection increased with age.

Key words: Hantavirus; rodents; epidemiology; Belgium

Introduction

Hantavirus disease is a collective name adopted for a viral disease in humans with a variety of clinical symptoms (Ladhevirta, 1971; Lee, 1982; Lädhevirta *et al.*, 1984). It is caused by viruses belonging to the Hantavirus group, a new genus in the *Bunyaviridae* family (Desmyter *et al.*, 1984). Hantaan virus, being the prototype of the Hantavirus group, was first isolated from the lungs of the Korean striped field mouse (*Apodemus agrarius*) in 1976 by Lee (Lee *et al.*, 1978). Evidence for infection of wild animals and humans by HV has now been reported worldwide (van der Groen, 1975). Hantaviruses are maintained primarily in rodents, especially members of the family *Cricetidae* and *Muridae* (van der Groen *et al.*, 1985).

In this study we report the preliminary results on the prevalence and distribution of HV in populations of wild living mammals from different geographic areas in Belgium and more specific on the results found in a heavily infested population of the bank vole (*Clethrionomys glareolus*) in a study area near the city Turnhout, province Antwerp (van der Groen *et al.*, 1983*b*; Verhagen *et al.*, 1986). In addition, the age and sex specific antibody and antigen prevalence of HV infection in these animals was investigated.

Materials and Methods

Animals. Musk rats (*Ondatra zibethicus*), water voles (*Arvicola terrestris*) and most of the brown rats (*Rattus norvegicus*) were collected by plant protection officers of the Ministry of Agriculture. All other species were collected by the authors using Sherman live traps or snap traps. All animals were transported to the laboratory in Antwerp where tissue and blood samples were taken. Musk rats, water voles, brown rats, in sectivores and carnivores were bled by cardiac puncture using a sterile 21G hypodermic needle. All other small mammals, if alive, were bled from the retro-orbital sinus using a sterile hematocrite tube.

After centrifugation, the sera were collected by aspiration and stored at −20 °C until the time of testing. After bleeding all animals were killed and examined at necropsy. Lung and several other tissue samples were preserved in 1.5 ml Eppendorf tubes and frozen until tested for the presence of HV-antigen. Dead animals were labelled and preserved in 7 % formaldehyde solution. All serologic as well as antigen tests were performed at the Institute of Tropical Medicine in Antwerp.

Serological and antigen tests. Antibodies (immunoglobulin G type) against HTN were detected using a modification of the indirect immunofluorescence antibody assay (IFA) as described by van der Groen (van der Groen *et al.*, 1983*a*). Serum antibody titres of 1 : 16 or greater were considered indicative of past infection with the virus. Evidence for HV-antigen in tissue samples was obtained using a modification of the enzyme-linked immunosorbent assay method described by Tkachenko (Tkachenko *et al.*, 1981). Differences in prevalence based upon sex and age were tested for significance by means of a Chi-square test, a Student t-test or an one way analysis of variance.

Results

National survey

Fig. 1 shows the location of collection sites for all species captured between February 1980 and December 1984. HV-positive mammals were found in five of the nine Belgian provinces. As the number of animals collected in some provinces are small, there is no reason to believe that HV are not present in these provinces too. Results of the serologic and antigen tests for all 17 species investigated (Table 1), revealed that HV-antibody or antigen was found in the sera or lungs of five species belonging to two different orders. Because most of the insectivores were found dead in the traps or died during transportation, only antigen-test could be made. The only species found positive for HV was the shrew *Sorex araneus*. Both positive animals were caught in the study area near the town Turnhout.

Fig. 1.
Number of rodents investigated for HV-infection (= N) and the number found HV-positive (N +) in the nine Belgian provinces

Table 1. Prevalence of Hantaan-antibody and Hantavirus-antigen in different species of wild mammals in Belgium

ORDER/Species	Total tested	Antibody AB-tot.	Antibody AB-pos.	Antibody %	Antigen AG-tot.	Antigen AG-pos.	Antigen %
INSECTIVORA							
Sorex araneus	47	—	—	—	47	2	4.3
Sorex minutus	5	—	—	—	5	0	0.0
Crocidura russula	13	—	—	—	13	0	0.0
Crocidura leucodon	3	—	—	—	3	0	0.0
CARNIVORA							
Mustela nivalis	5	—	—	—	5	0	0.0
Mustela erminea	1	—	—	—	1	0	0.0
RODENTIA							
Clethrionomys glareolus	789	458	37	8.1	507	80	15.8
Microtus agrestis	23	5	1	20.0	21	2	9.5
Arvicola terrestris	30	30	0	0.0	30	0	0.0
Ondatra zibethicus	135	130	0	0.0	132	1	0.8
Apodemus sylvaticus	1266	1260	3	0.2	65	0	0.0
Apodemus flavicollis	37	37	0	0.0	1	0	0.0
Micromys minutus	3	—	—	—	3	0	0.0
Mus musculus	7	7	0	0.0	3	0	0.0
Rattus rattus	21	8	0	0.0	17	0	0.0
Rattus norvegicus	68	54	2	3.7	51	1	2.0
Eliomys quercinus	10	7	0	0.0	3	0	0.0

The number of carnivores investigated up to now is too small to make any conclusions. Eleven species of rodents were collected and five of them showed evidence of HV-infection. Again, for several species the number investigated is too small to make any conclusions but it is very probable that, as numbers increase, more species will be found positive. The highest prevalence of antibody positive and antigen positive animals was found in the bank vole (*Clethrionomys glareolus*). Out of the 458 sera for HTN, 37 (8.1 %)

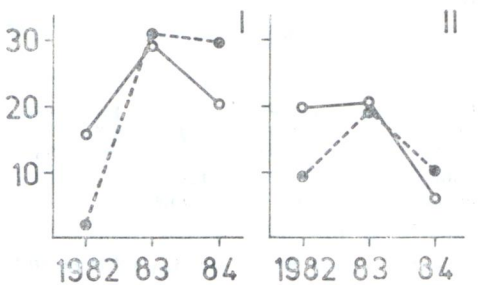

Fig. 2.
Prevalence of HTN-antibody positive (dashed line) and HTN-antigen positive (solid lane) males and females I — males; II — females
Abscissae: years; ordinates: % animals positive

Table 2. Results of the antibody against HTN strain 76—118 and antigen test for HV of bank voles

Year	Antibody (AB)			Antigen (AG)			AB + AG				Total tested		
	N	AB+	%	N	AG+	%	N	AB+	AG+	AB− AG+	N	N+	%
MALES													
1982	98	3	3.1	130	20	15.4	16	—	1	1	212	22	10.4
1983	39	12	30.8	57	17	29.8	36	3	2	9	60	20	33.4
1984	21	6	28.6	34	7	20.6	18	—	—	6	37	7	18.9
82—84	158	21	13.3	221	44	19.9	70	3	3	16	309	49	15.9
FEMALES													
1982	88	7	8.0	121	23	19.0	15	—	2	3	194	27	13.9
1983	37	7	18.9	50	10	20.0	33	1	1	6	53	11	20.8
1984	10	1	10.0	33	2	6.1	8	—	—	1	35	2	5.7
82—84	135	15	11.1	204	35	17.2	56	1	3	10	282	40	14.2
TOTAL													
1982	186	10	5.4	251	43	17.1	31	—	3	4	406	49	12.1
1983	76	19	24.7	107	27	25.5	69	4	3	15	113	31	27.4
1984	31	7	22.6	67	9	13.4	26	—	—	7	72	9	12.5
82—84	293	36	12.3	425	79	18.6	126	4	6	26	591	89	15.1

(N = number tested, AB+ = antibody positive, AG+ = antigen positive, AB+AG+ = both antibody and antigen positive, N+ = antibody and/or antigen positive).

were positive with titres ranging from 16 to 1024. Antigen tests revealed that 80 of the 507 (15.8 %) lungs tested possessed antigen. Most of the HV--infected bank voles were collected in the study area near Turnhout. Other rodent species found positive for HV were the field vole (*Microtus agrestis*), the muskrat (*Ondatra zibethicus*), the wood mouse (*Apodemus sylvaticus*) and the brown rat (*Rattus norvegicus*).

Table 3. Distribution of HV-infected bank voles in marsh (habitat 1), humid forests surrounding marsh (habitat 2) and other vegetation types (habitat 3)

Habitat	Males			Females			Total		
	N	N+	%	N	N+	N	N+	N+	%
Habitat 1	102	15	14.7	79	14	17.7	181	29	16.0
Habitat 2	92	25	27.2	92	17	18.5	184	42	22.8
Habitat 3	114	9	7.9	98	8	8.2	212	17	8.0
Chi-square		14.3			5.01			16.8	
Significance		$p < 0.001$			$0.05 < p < 0.1$			$p < 0.001$	

N = number tested, N+ = number antibody and/or antigen positive.

Table 4. Weight distribution of HV-positive and HV-negative bank voles

Weight class (g.)	< 9	9–11	12–14	15–17	18–20	21–23	> 23	Total
AB negative	4	18	94	81	35	18	7	257
AB positive	0	0	2	8	16	5	5	36
%	0	0	2.1	9.0	31.4	21.7	41.7	
AG negative	2	13	123	120	54	26	8	346
AG positive	0	0	9	23	31	10	6	79
%	0	0	6.8	16.1	36.5	27.8	42.6	

(AB = antibody, AG = antigen).

As most of the data on HV-positive animals came from the study area near Turnhout (province Antwerp) we will discuss these results in more detail.

Results of the study area near Turnhout

The results of HTN-antibody and/or antigen tests of 591 bank voles are presented in Table 2. The animals were collected between March 1982 and December 1984. As it was not always possible to test the animals for both antigen and antibody, the test results are presented separately. The total prevalence of HTN-antibody positive male bank voles was 13.3 % (21/158) and was very close to the value of 11.1 % found in females (15/135). The same resemblance between the sexes was found for the prevalence of antigen positive animals (males: 44/221 = 19.9 %; females: 35/204 = 17.2 %).

In Fig. 2 the course of prevalences over the three years was plotted. In 1982, the number of antibody-positive bank voles was very low when compared with the number of antigen-positive voles. By 1983 the prevalence of antibody- and antigen positive males and females was nearly equal, while in 1984 the situation was just the reverse of that in 1982. The difference in prevalences between the three years was found significant for males (antibody, $\chi^2 = 23.5$, $df = 2$, $p = 0.0001$; antigen, $\chi^2 = 5.19$, $df = 2$, $p = 0.07$), but not for females (antibody, $\chi^2 = 3.18$, $df = 2$, $p = 0.20$; antigen, $\chi^2 = 3.42$, $df = 2$, $p = 0.18$). The observed differences in prevalence of antibody- and antigen-positive animals as well as the course of the prevalences might indicate that infection of the bank vole population with HV is of recent date.

Out of the 126 bank voles who were tested for the presence of both antibody in their sera and antigen in their lungs, 26 (20.6 %) were found to have both antibody and antigen. Four animals (3.2 %) had only antibody in their sera and six (4.8 %) had only antigen. The observation that some animals showed only evidence for the presence of antibodies or antigen means that serological screening or screening for antigen alone does not give the real prevalence of infection in rodent populations.

Titre values

The antibody titres ranged from 16 to 256 and 16 to 512 in males and females respectively, while antigen titres ranged from 4 to 1024 in both sexes. No statistical significant differences in antibody and antigen titres between the sexes could be found (mean coded antibody titre (m.c.ab.t.) males = = 6.29, n = 21, m.c.ab.t. females = 6.36, n = 15, t-test = −0.09; mean coded antigen titre (m.c.an.t.) males = 5.39, n = 31, m.c.an.t. females = = 5.80, n = 15, t-test = −0.63).

Differences in titre values between years was tested using an one way analysis of variance. As no difference between the sexes was found, the data were pooled. Even after pooling both sexes there is no indication that there are differences in titre values between the three years (antibody titre, F--value = 1.62, not sign., antigen titre, F-value = 0.67, not sign.).

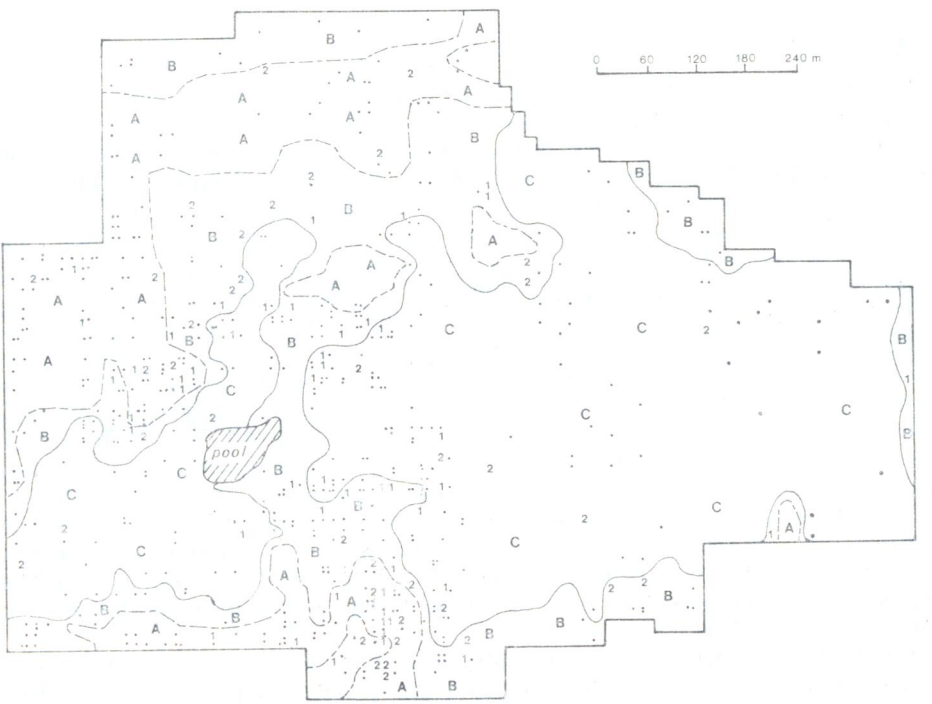

Fig. 3.

Distribution of captured animals in marsh (A), humid forests surrounding marsh (B) and dry vegetation types (C).

Points represents capture points of non-infected bank voles, 1 and 2 are antibody and/or antigen positive males and females

Table 5. Incidence of HV-infected bank voles over two month periods

Time interval	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
Number tested	1	30	27	11	120	104
Antibody positive	0	7	10	1	6	12
%	0	23.3	37.0	9.1	4.6	11.5
Number tested	23	46	33	14	166	141
Antigen positive	1	9	13	2	34	20
%	4.3	19.6	39.4	14.3	20.5	14.2

Habitat distribution of HV-infected bank voles

For 577 out of the 591 bank voles collected in the study area near Turnhout, the exact position of capture was known so that the habitat distribution of HV-infected animals could be studied. The study area measured 61.2 ha and was divided in 2720 trap stations set in a grid pattern. Spacing between successive trap stations was 15 m. Eleven different habitat types were recognized in the field and for each trap station the corresponding habitat type was noted. As bank voles showed a very strong preference for particular habitat types, there were several habitats without or with only very few captures. Therefore, we will use in the following analyses only three habitats with the following characteristics:

— marsh habitat: very wet forest with almost permanently water above the ground surface throughout the year. The tree and shrub layer are dominated by *Alnus glutinosa*, *Fraxinus excelsior* and different *Salix* species. The ground layer is characterized by *Lycopus europaeus*, *Solanum dulcamara*, *Iris pseudacorus*, *Hottonia palustris* and a variety of ferns, grasses, sedges and mosses especially *Spaghnum* species,
— humid forest surrounding the marsh habitat: pine and deciduous forest bordering the swampy forest. They are humid and have a rich and dense ground vegetation,
— dry vegetation types: these include pine and deciduous forests, abandoned fields, brushwood and heather. They have in common the dry ground in most times of the year and the relatively poor ground vegetation. In Fig. 3 the distribution of captures of bank voles which were tested for HV-infection and the border lines of the three distinguished habitats were represented. Most bank voles were captured in the swampy forest and the surrounding forests. In Table 3 the percentage of HV-infected animals (either antibody- or antigen-positive) were compared for the three habitats. The highest percentage of HV-infected bank voles was found in habitats surrounding the swampy forest and the lowest in the group which included all habitats on dry grounds. These differences in prevalence depending on habitat type were statistically significant.

Relation between HV-infection and age

Despite the numerous justified criticisms of the use of body weight as an approximate guide to age, it is one of the few methods which can be easily applied in field studies. When we do not need a high degree of precision, body weight can be used as a suitable indicator of age. We arranged the animals according their weight in 3 g intervals. Seven weight classes were distinguished and for each the number of HV-positive and negative animals was noted as shown in Table 4. The weight distributions of males and females were similar and therefore the results of the sexes were pooled. There was an obvious correlation between the number of antibody- and antigen--positive animals and weight (age) of the animals. When comparing the frequency distributions of HV-positive and negative animals, the difference was found highly significant (antibody: $\chi^2 = 41.93$, df = 4, p = 0.0; antigen: $\chi^2 = 41.19$, df = 4, p = 0.0).

From Table 4 it is also clear that very young animals (less than 15 g) were hardly affected by HV, while for old bank voles (weight more than 24 g) over 40 % of the animals were found HV-positive. The increase in prevalence with weight is very similar for antigen and antibody.

The mean coded titre values for the different weight classes decreased gradually with weight, although when tested for significance using one way analysis of variance, the decrease was not statistically significant (antibody F = 0.725, not sign.; antigen F = 0.272, not sign.).

Seasonal changes in prevalence

The number of animals caught in each two month interval and those found positive for HV are presented in Table 5. Unfortunately, the data are not equally distributed over the different time intervals and there are several intervals with only few data. Moreover, as we discussed before, there are significant differences in prevalence between the years. These shortcomings make it difficult to interpret the data. The incidence of HV-infected animals showed considerable seasonal variation (antibody Chi-square = = 24.74, df = 4, $p < 0.0001$; antigen Chi-square = 14.87, df = 5, $p < 0.05$). The number of antibody-positive as well as antigen-positive animals reached a peak in May-June.

Discussion

The preliminary results of the mammal survey presented here provide substantial evidence that the bank vole is the primary host for rural type HV in Belgium and therefore corresponds with the findings in Finland (Brummer-Korvenkontio *et al.*, 1980, 1982; Yanagihara *et al.*, 1984) and in the Western part of the U.S.S.R. (Gavrilovskaya *et al.*, 1983; Tkachenko *et al.*, 1983). Beside the bank vole, 5 other small mammal species were found to be either antibody or antigen positive for HV. Despite the large number of investigated wood mice, the prevalence of infected animals was extremely

low when compared with the bank vole. This fact might indicate that there is a different susceptibility to HV-infection depending on the species involved. In our study this is very likely as both species were collected at the same time, in the same habitats, the same locality (Turnhout) and are known to live in close vicinity (Verhagen, 1980). So it is evident that HV in Belgium, as well as in other geographical regions (Schmaljohn *et al.*, 1985; Van der Groen *et al.*, 1985) are polyhostal in nature with a main reservoir species and several other species that can carry the antigen but of which it is not certain that they play a role in the circulation of the virus.

In 1983, a significant higher proportion of HV-positive bank voles was reported in comparison with 1982 and 1984. We do not know to what degree this reflects the situation among bank voles living in other parts of Belgium. But in that particular year 50 % of the clinically documented human cases we are aware of so far have been reported. According to one of the patients, a farmer, a remarkable high density of rodents was observed around his farm during 1983 as compared to the previous years (van Ypersele de Strihou *et al.*, 1983). Several authors mentioned a pronounced regional occurrence of HV-infected populations (Brummer-Korvenkontio *et al.*, 1982; Yanagihara *et al.*, 1984) and a similar situation was observed in our study. A possible explanation for this regional variation in prevalence might be related to the presence of particular habitat types. We found a close association between high prevalence of HV-infection and wet habitat types and a similar relation was observed by Xu (Xu *et al.*, 1985).

It seems that the likelyhood of infection increases with the age of the bank voles, starting from the weight class of 12 — 14 g. Before this weight class the animals are less susceptible, perhaps due to the presence of maternal passive antibodies (Verhagen *et al.*, 1986).

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