

EVIDENCE OF PUUMALA *HANTAVIRUS* INFECTION IN RED FOXES (*VULPES VULPES*) IN BELGIUM

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MEMBERS of the genus *Hantavirus* are rodent-borne negative-stranded RNA viruses within the family Bunyaviridae (Schmaljohn and Hjelle 1997). Hantaviruses are causative agents of human diseases called haemorrhagic fever with renal syndrome (HFRS) and *hantavirus* pulmonary syndrome (HPS) (Mertz and others 1998). Prototype viruses associated with HFRS are Hantaan (HTN) and Seoul (SEO) in Asia, and Puumala (PUU) and Dobrava (DOB) in Europe (Lee 1996, Kanerva and others 1998). Sin Nombre (SN) and related viruses cause HPS in the Americas (Nichol and others 1993, Peters and others 1999). Some hantaviruses, such as Tula (TUL), Khabarovsk (KBR) or Prospect Hill (PH), are not known to be pathogenic for human beings.

Each *Hantavirus* is carried predominantly by its own rodent or insectivore host. Transmission of the virus occurs through aerosolised excreta from infected animals. The geographical distribution of HFRS and HPS cases generally overlaps the biotope of the *Hantavirus*-related reservoir. In Europe, the red bank vole (*Clethrionomys glareolus*) is the main rodent host of PUU and preferentially occupies forested areas. Two major epidemics of HFRS recorded in Belgium in 1992 to 1993 and in 1995 to 1996 were focused on the same wooded south-west region between the River Sambre and the River Meuse (Clement and others 1994, Heyman and others 1999); both outbreaks were concomitant with irruptions in bank vole populations.

Apart from human beings, there is limited knowledge of *Hantavirus* infections in non-reservoir species. In Belgium, sporadic detection of seropositive and/or antigen-positive animals has been reported in five different rodent species and in two insectivores (Verhagen and others 1987). In

Europe, China and the USA, the detection of antibodies *Hantavirus* has been recorded occasionally in predators such as cats (*Pelis catus*) and weasels (*Mustela frenata*) (Childs and others 1988, Bennett and others 1990, Yanagihara 1990, Nowotny 1994), although the actual *Hantavirus* serotype was not determined. In Sweden, serological evidence of PUU virus infection has recently been shown in moose (*Alces alces*) originating from an endemic region for HFRS (Ahlm ro and others 2000).

To investigate the susceptibility of a predatory species of the red bank vole to *Hantavirus* infection, sera collected from foxes (*Vulpes vulpes*) inhabiting the southern part of Belgium (Fig 1) were submitted to a serological study. All samples were obtained between 1995 and 1997, which coincided with the peak period of the largest HFRS outbreak recorded in Belgium (Heyman and others 1999) so far.

The primary aim for collecting fox sera was to investigate the efficacy of an antirabies vaccination campaign in the province of Luxembourg; these sera were also used for the present study (Brochier and others 1997). The foxes were shot during the night and blood was collected from the thoracic cavity within five minutes after the death of the animals.

Sera samples were screened by ELISA for the presence of *Hantavirus*-specific immunoglobulin G (IgG) antibodies. PUU (strain CG 18-20)-, HTN (strain 76-118)-, DOB (strain Dobrava)- and SEO (strain SR-1 1)-infected Vero E6 cell lysates, and non-infected Vero E6 cells used as negative control antigens, were adsorbed to microtitre plates at 4°C overnight. Serum samples at a dilution of 1:100, were incubated for one hour at 37°C. Specific antibody binding was detected by peroxidase-conjugated rabbit anti-dog IgG (Sigma), at a dilution of 1:5000. Tetramethylbenzidine substrate (Sigma) was added after one hour at 37°C and the reaction was stopped after 10 minutes with IN sulphuric acid. The optical density was determined on both viral and negative control antigens at 450 nm. The cut-off value was estimated by a log-likelihood ratio method (Parker and others 1990, Vizard and others 1990). To confirm the antibody response, positive and doubtful sera were also tested by using an immunofluorescence assay (IFA) (Lundkvist and others 1991) and focus reduction neutralisation test (FRNT) using PUU, DOB, SEO and TUL *Hantavirus* strains (Lundkvist and others 1997).

One hundred and twenty-five fox sera were examined for antibodies against PUU, DOB, SEO and HTN antigens by ELISA. Twenty sera were positive, or borderline, to at least one of the *Hantavirus* antigens. Of these 20 sera, three (samples 9673, 96113, 9708) showed a significant neutralising activity against PUU with 100 per cent, above 95 per cent, and 80 per cent inhibition, respectively, at a dilution of 1:40 (Table 1). Sample 9673 showed more than 80 per cent inhibition at a dilution of 1:160. None of the samples had reactivity with other prototypic hantaviruses. Of the 20 ELISA-positive samples tested by IFA, sample 9673 showed a highly specific reactivity to PUU virus, while sample 96113 showed a borderline reaction (Table 1).

The 20 fox sera submitted to FRNT and 13 additional sera doubtful for DOB, HTN and/or SEO by ELISA, were assessed for the presence of viral RNA by reverse-transcription (RT) nested PCR (Pilaski and others 1994). All samples were negative for both genus and PUU-specific amplification.

In previous surveys on domestic cats, antibodies to *Hantavirus* were found in 9·6 per cent (15/157) of animals tested in Great Britain and in 5 per cent (10/200) of animals tested in Austria (Bennett and others 1990, Nowotny 1994). A 6·5 per cent (42/649) seroprevalence of *Hantavirus* infection was shown in domestic cats in Baltimore and an epidemiological survey in Arizona revealed a reactivity to SN *Hantavirus* in 2·8 per cent (4/145) and 4·7 per cent (4/85) of tested cats and dogs, respectively (Childs and others 1988, Malecki and others 1998). *Hantavirus* infection was also detected in two out of five laboratory cats, after a rat- associated episode of HFRS at a Belgian university (Desmyter and others 1983). Although these results were not confirmed by a neutralisation test, the only serological test at present available for the confirmation/serotyping of *Hantavirus* infections, these observations indicate that cats and dogs are susceptible to *Hantavirus* infection and that spill-over events to these species may not be uncommon.

The present study describes the first evidence of PUU *Hantavirus* infection in foxes. Sera from three of 125 (2·4 per cent) foxes caught in southern Belgium were positive by ELISA and confirmed by FRNT. The RT nested PCR did not reveal the presence of viral RNA in 33 sampled sera. The detection of antibodies in foxes suggests that this species is a potential carrier of hantaviruses. However, the low seroprevalence and virus titres found in the positive animals suggest that foxes are probably a dead-end host for *Hantavirus* infection. Further investigations may be necessary to determine whether these agents are pathogenic for this species.

The presence of seropositive foxes in the southernmost region of Belgium where human cases of HFRS were rare during the 1995 to 1996 epidemic, suggests that the distribution of PUU *Hantavirus* is not focal in the country. Further epidemiological studies are required to determine the actual localisation of hantaviruses in wild mammals in Belgium, to evaluate more accurately the seroprevalence among foxes, and to investigate the susceptibility of other rodent predatory species to *Hantavirus* infection.

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Table 1. Reactivity of the positive fox sera to Hantavirus strains by ELISA. focus reduction neutralisation test (FRNT) and immunofluorescence assay (IFA)

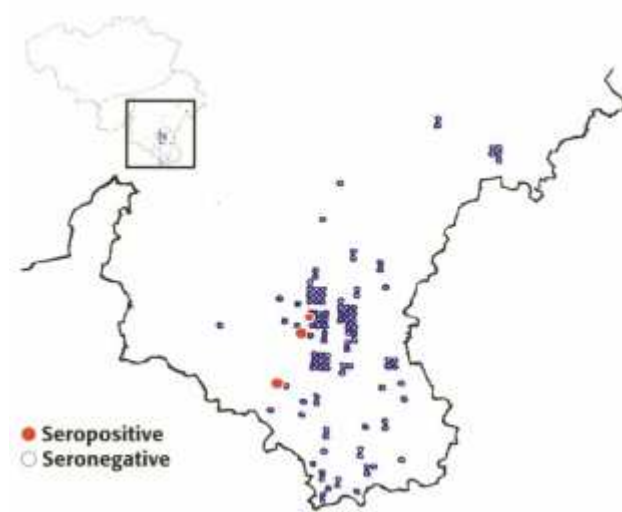
Serum sample	Elisa				FRNT				IFA
	PUU	DOB	HTN	SEO	PUU	DOB	SED	TULL	PUU
9673	+	+	-	-	160*	<80	<80	<80	+
96113	+	+	+	+	40	<80	<80	<80	+/-
9708	+	-	-	-	40	<80	<80	<80	-

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Reciprocal FRNT endpoint titre

PUU Puumala, DOB Dobrava, HTN Hantaan, SEO Seoul, TUL Tula, + Positive, - Negative, +/- Borderline

Figure 1. Geographical origin of the foxes tested for Hantavirus infection in southern Belgium between 1995 and 1997



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