Hantavirus infections in The Netherlands: epidemiology and disease

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(Accepted 18 November 1994)

SUMMARY

A serological survey for the prevalence of hantavirus infections in The Netherlands was carried out on > 10000 sera, from selected human populations, and different feral and domestic animal species. Hantavirus-specific antibodies were found in about 1% of patients suspected of acute leptospirosis, 10% of patients with acute nephropathia, and in less than 0·1% haemodialysis and renal transplant patients. Among individuals with a suspected occupational risk, 6% of animal trappers, 4% of forestry workers, 2% of laboratory workers and 0·4% of farmers were seropositive. The majority of the seropositive individuals lived in rural and forested areas. The main animal reservoir of the infection was shown to be the red bank vole (Clethrionomys glareolus). Epidemiological, clinical and laboratory findings seen in serologically confirmed human cases were similar to those associated with nephropathia epidemica.

INTRODUCTION

The severity of haemorrhagic fever with renal syndrome (HFRS), caused by members of the genus *Hantavirus* (HV) of the family Bunyaviridae, is largely dependent on the serotype of the virus involved. So far at least four different subtypes of HV have been characterized [1, 2]. These subtypes are all closely associated with the genus of the reservoir host involved: *Apodemus* – Hantaanlike viruses; *Rattus* – Seoul-like viruses; *Clethrionomys* – Puumala-like viruses and *Microtus* – Prospect-Hill-like viruses. Recently a HV has been identified in the USA as the cause of a severe respiratory disease in humans with high mortality. This infection is transmitted to humans by the deer mouse (*Peromycus*

^{*} Requests for reprints.

maniculatus) [3]. Infections with Hantaan-like, Seoul-like and Puumala-like viruses may cause renal failure in humans and are predominantly transmitted by the air-borne route. The clinical symptoms related to Hantaan- and Seoul-like virus infections are generally characteristic, whereas the symptoms associated with Puumala-like virus infections are more variable and often difficult to define as belonging to a disease entity [4]. As impairment of renal function of varying severity is a consistent feature of infection with Puumala-like viruses, the syndrome is referred to as nephropathia epidemica (NE). Seroepidemiological studies in Asia, Europe and the USA have demonstrated HV-specific antibodies in the sera of individuals, such as farmers and army personnel, at high risk of contact with feral rodents [5–10]. Recently, serological evidence of HV infection was also found in patients with chronic renal failure, haemodialysis patients, and in renal transplant candidates [11–13]. Furthermore, HV-specific serum antibodies have been found in individuals with acute respiratory disease and in patients suspected of acute leptospirosis [3, 6, 8]. The geographical distribution of HV infection in humans is usually focal probably due to the distribution of infected reservoir animals [9]. Although HV has been detected in a large variety of feral and domestic animals [14] the main reservoirs are rodent species. In 1984 we documented the first cases of HV infections in The Netherlands among laboratory workers of the National Institute of Public Health and Environmental Protection (RIVM), who had been in contact with infected laboratory Lou/M rats [5]. One of the laboratory workers suffered severe transient renal failure whereas the others showed milder clinical symptoms. Subsequently a number of serologically confirmed human cases of NE, not related to contacts with laboratory animals, was found in the eastern and southern parts of The Netherlands [6, 7]. In these areas we also identified HV-infected feral rodents [15]. We subsequently showed that the laboratory workers at the RIVM had been infected with Seoul-like viruses whereas the majority of the other seropositive human cases had been infected with Puumala-like viruses [15, 16].

In the present study we have investigated the geographical distribution of HV-specific serum antibodies in individuals in The Netherlands with or without a history of renal disease or suspected occupational risk. In addition, sera from a large collection of feral and domestic animals in The Netherlands were tested. Clinical and laboratory findings of the individuals with a history of serologically confirmed NE infection are presented.

MATERIALS AND METHODS

Human serum samples

Human serum samples were collected between 1972 and 1994 from 1783 individuals with renal diseases, including patients with suspected acute leptospirosis, established renal disease, haemodialysis and kidney transplant patients. Sera from 2172 individuals with suspected occupational risk of HV infection (laboratory workers, farmers, hunters, forestry workers, veterinary surgeons, zoologists, gardeners, trappers and military recruits) and 4474 sera from a control group consisting of office personnel and healthy blood donors in apparently enzootic areas (see below) were also collected. Samples were also collected from 463

military office personnel from all over the country. All sera were heat inactivated for 30 min at 56 °C directly after collection and stored at -20 °C until used.

Feral animal serum and organ samples

Feral animal samples were collected between 1984 and 1993 from 829 animals from 20 different species. Rodents were trapped between 1987 and 1993 at several places in The Netherlands, around houses surrounded by forests and fields, and on camp sites. The rodent species were identified, blood was collected and lung suspensions were prepared (for survey see Table 1). A separate group of rats was obtained from Rotterdam Harbour from an animal pest control service. Bats suspected of rabies were obtained from the Central Veterinary Institute in Lelystad. Serum samples from other feral mammals (Table 1; hare, roe deer, fox and wild boar) were collected between 1992 and 1993 during the hunting season.

Serum samples from domestic animals

Serum samples were collected from 2025 domestic animals, representing 14 species, at several locations in The Netherlands between 1984 and 1992. Sera from lagomorphs and rodents (Table 1) were obtained from a veterinary clinic, where the animals had been admitted with a variety of clinical symptoms. Serum samples from dogs with a history of acute gastrointestinal disease and from cats routinely examined for feline leukaemia virus infection were collected through veterinary practitioners throughout the country. Sera from cows, sheep and pigs were collected from different slaughter-houses.

Serology and antigen detection

Hantavirus-specific antibodies against Puumala-like virus (strain Hällnäs) were detected by a previously described indirect enzyme-linked immunosorbent assay (ELISA) [16]. For the detection of antibodies of species for which no species-specific conjugates were available (members of the families Talpidae, Sciuridae, Cricetidae, Castoridae, Chinchillidae, Caviidae, Capromyidae, Vespertilionidae and Dasyproctidae) a protein-A peroxidase conjugate (Amersham International, Amersham, UK) was used. The specificity and sensitivity of this conjugate for immunoglobulins of different animal species had been evaluated previously [17]. OD 450 values were obtained by subtracting the OD 450 values of control antigencoated wells from corresponding viral antigen-coated wells. Values > 0·2 were tested in a confirmatory indirect immunofluorescence test (IFA) at a 1/16 dilution, using drop slides fixed with Vero E6 cells infected with HV strain Hällnäs [16]. Results were considered positive when a characteristic dot-like immunofluorescence pattern was observed in the cytoplasm of infected cells.

HV antigen in lung tissues of feral animals was detected by a previously described antigen capturing ELISA [16]. For this purpose 10% lung suspensions were prepared in a glass grinder in phosphate buffered saline (PBS) containing 1% Triton X-100 and used as antigen.

Clinical and laboratory findings

The medical records of the 27 hospitalized and seropositive NE patients obtained from three hospitals in The Netherlands were reviewed for clinical chemistry, haematological, clinical findings and epidemiological data. These data

specific serum antibodies in feral and domestic ani Prevalence of

Peral Insectivora Tabpiake Tabpia curopora Common shows 89 0/38 1-5 Rodentin Cricetidae Ondina strediae Common shows 89 1/66* 1-5 Rodentin Cricetidae Ondina strediae Real bank vole 84-80 1/111* 10-8 Action of Maria Articlacus articlas Real bank vole 8-80 1/111* 10-8 Lagemorpha Leperidae Total proper of the more of the m	Group	Order	Family	Species	Common name	Year	numbers tested	%
Rodentin Sorieidae Cricetidae Condutar sussula Common shrew SB 1/66* Auridae Alfrediae arcalis Field vole 84-89 1/16* Auridae Apalemia supergiarea Nood mouse 84-89 1/111† Apalemia supergiae Nood mouse 84-89 1/111† Antidae Apalemia supergiae Nood mouse 89 0/12 Anticalaetyla Activaliae Arganiae 1/111† 89 0/12 Articalaetyla Beveldae Indiae supergiae Red fox 89 0/12 Articalaetyla Beveldae Capace capacasis Red fox 89 0/12 Articalaetyla Beveldae Capace capacasis Red fox 89 0/13 Chiroptera Varidae Apalemia capacasis Red fox 89 0/14 Chiroptera Varidae Suridae Savidae Capacas capacasis 80 0/14 Chiroptera Varidae suridae Capacas capacasis Brangento and to an acadas capacas capacas capacas capa		Insectivora	Talpidae	6	Common mole	68	0/33	0
Rodentia Cricetidae Muskvat 89 0/192 Appelmus Splenders Mircelae analyse Red bank vole 84–89 1/68 Appelmus splenders Nood mouse 89 0/12 Appelmus splenders Norway rat 89 0/42 Antidae Challes routes Norway rat 89 0/42 Carnivora Caurive contract Chance routes 89 0/42 Artiodactyla Mustelidae Lopus copensis Have 93 0/42 Artiodactyla Mustelidae Lopus copensis Badger 93 0/42 Artiodactyla Brovidae Common brown 93 0/17 Brovidae Properlelus cupriodus Common brown 93 0/17 Pleodis curriculae Properlelus curriculus Bropsen rabbit 93 0/1 Mychisculus curriculus Common brown 93 0/1 Agontidae Sciurus curriculus Bropsen rabbit 92 0/1 Agoridae Costoridae Craincelae			Soricidae	n.	Common shrew	68	1/66*	1.5
Mirridae Alicinomany applications Red bank vole 84, 89 12/111† Cathriconomy applications Red bank vole 84, 89 12/111† Rains noregious Wood mouse 89 0/12 Rains noregious Root mouse 89 0/12 Alts norecolus Rains noregious Root mouse 89 0/12 Auto mouse Carnivora Candidae Lepovidae Lepovidae Lepovidae Capred se opposition Capre		Rodentia	Cricetidae	zil	Muskrat	88	0/192	0
Murique Apodemous glaverlass Red bank vole 84-89 12/111† Ratius norregiens Ratius norregie				Microtus arvalis	Field vole	88	1/68	1.5
Muridae Apademus sploutieus Word mouse 89 0/56 Radiae radiae Radiae radiae Black rat 89 0/12 Radiae radiae Ratiae radiae Black rat 89 0/12 Artiodacyla Leporidae Leporidae 1 Alex mouse 89 0/12 Artiodacyla Mustelidae Leporidae 1 Alex mouse 89 0/12 Suidae Artiodacyla Revidae 1 Alex mouse 93 0/13 Chiroptera Vespertilionidae Na sendinas Common brown bat 93 0/11 Artiodacyla Perceicus sendinas Common brown bat 93 0/11 Phecola surdiae Common brown bat 93 0/11 Phecola surdiae Common brown bat 93 0/11 Apperentiae Phistolicalus surdiae Common brown bat 93 0/11 Apperentiae Pripatellae surdiae Physicalus surdiae 1 Alex bat 93 0/11 Apperentiae Leporidae Pripatellae surdiae Pripa				Clethrionomys glareolus	Red bank vole	84 - 89	12/111†	10.8
Retties norregions			Muridae	Apodemus sylvaticus	Wood mouse	88	0/26	0
Lagomorpha Leporidae Ratuss captus Back rat 89 0/4 Carmivora Cancidae Lepus expensis Hare 89 0/12 Carmivora Cancidae Lepus expensis Rect fox 93 0/62 Artiodactyla Mustellae Mesperalion and process an				Rattus norvegicus	Norway rat	68	0.12	0
Lagomorpha Leporidae Pupus capeanish Honse mouse 89 0/12 Garnivora Gainidae Fupus capeanish Hear 93 0/63 Artiodactyla Garnidae Tulpes nucles Badger 93 0/63 Artiodactyla Bovidae Capeanica Ninstelidae Ninstelidae 0/3 Artiodactyla Sundae San scroft Wild boar 92 0/10 Physicalica Ninstelidae Ninstelidae Ninstelidae 0/3 0/11 Physicalica Pripstelle bat 93 0/1 0/1 0/1 Placedus auritus Common long cared bat 93 0/1 0/1 Placedus auritus Phistorelle bat 93 0/1 Mygich desymene Pond bat 93 0/1 Mygich desymene Brond bat 93 0/1 Mygich desymene Brond bat 92 0/1 Chieveridae Cricertae Cricertae 0/1 Chieveridae Cricertae				Rattus rattus	Black rat	68	0/4	0
Lagomorpha Leporidae Lepos caponsis Hare 93 0/63 Carnivora Canidae Lepos caponsis Red fox 93 0/62 Artiodaetyla Bovidae Capreolus capreolus Roe 92 0/80 Suidae Susceptilionidae Pipsterfulus pipistrellus Pipistrelle bat 93 0/10 Chiroptera Vespertilionidae Pipistrellus pipistrellus Pipistrelle bat 93 0/1 Rodenta Pripaterlus suntuas Common brown bat 93 0/1 Myclates nortula Pripaterlus vallussi Prod bat 93 0/1 Myclates nortulas Brod bat 93 0/1 Rodentia Sciuridae Sciuridae Sciuridae Sciuridae Castoridae Cricetias cricetus Brond bat 92 0/1 Chirchildae Cricetias cricetus Brond bat 92 0/1 Chastoridae Cricetias cricetus Brond bat 92 0/1 Chastoridae Cricetias cricetus Castorid				$Mus\ musculus$	House mouse	88	0/12	0
Carnivora Guidae Valpos culpes Red fox 93 0/62 Artiodactyla Bovidae Guprelus emptedus Red fox 92 0/70 Chiroptera Vespertilionidae Pipistellus pipistellus Wild boar 92 0/10 Chiroptera Vespertilionidae Pipistellus pipistellus Common brown bat 93 0/11 Pipistellus auritus Common brown bat 93 0/17 0/17 Pipistellus auritus Common brown bat 93 0/1 Pipistellus auritus Common brown bat 93 0/1 Mypteduls auritus Cricelus auritus Cricelus auritus 0/1 Cricelus auritus Cricelus auritus Cricelus auritus 0/1 Caprideus auritus Caprideus auritus Cau		Lagomorpha	Leporidae	Lepus capensis	Hare	93	0/63	0
Mustelidae Mites meles Badger 93 0/3			Canidae		Red fox	93	0/62	0
Artiodactyla Bovidae Capreolus capreolus Roe 92 0/80			Mustelidae	Meles meles	Badger	93	0/3	0
Chiroptera Nies scroff Wild boar 92 0/10 Recours Vespertilionidae Pripsixtellas pipistellus Pripsixtellas 93 0/17 Plecotus aurituas Common brown bat 93 0/17 Plecotus aurituas Common long-eared bat 93 0/1 Plecotus aurituas Pripsixtelle bat 93 0/1 Rode Pripsixtellus nathusii Pond bat 93 0/1 Mydis dasquement Pond bat 93 0/1 Rodentia Sciurus cordine Carior bat 92 0/1 Rodentia Sciurus cordines Carior bat 92 0/1 Rodentia Sciurus cordines Carior grandines Mygocastor cogpus Nutria 92 0/1 Carnivora Carnivora Carnivora Princibilitais Carior grandines Carior grandines Carior grandines Carior grandines Carior grandines Carior grandines		Artiodactyla	Bovidae	Capreolus capreolus	Roe	92	08/0	0
Chiroptera Vespertilionidae Pipistrellus pipistrellus Pipistrellus serotinus Pipistrellus of Mananan long-eared bat paga dolf pleotas auritus 93 0/17 mestie Lagomorpha Pipistrellus nathusii Pipistrellus auritus Pipistrellus auritus 0/1 mestie Lagomorpha Leporidae Orgedugus cuniculus European rabbit paga dolf paga dolf paga dolf paga dolf paga dolf paga dolf paga delf paga d			Suidae	Sus $scrofa$	Wild boar	92	0/10	0
Price Price Price Common brown bat 93 0/17 Price Price Common brown bat 93 0/17 Price Price Common Price 93 0/1 Nyclaus noctula Red bat 93 0/1 Nyclaus noctula Red bat 93 0/1 Nyclaus noctula Red bat 93 0/1 Nyclaus noctula Price Price 93 0/1 Rodentia Cricetidae Cricetiae Chinchilla lamiger Chinchilla Dolichotis patagona Mara 92 0/14 Caviidae C		Chiroptera	Vespertilionidae	Pipistrellus pipistrellus	Pipistrelle bat	93	0/31	0
Plecotus auritus Plecotus auritus Propistrelle bat Propistrell				Epitesicus serotinus	Common brown bat	93	0/17	0
mestic Lagomorpha Pipistrellus nathassi Pipistrelle bat 93 0/1 Myotis dasyneme Red bat 93 0/1 Rodentia Sciuridae Sciuridae Sciuridae O/113 Cricetidae Cricetus cricetus Grave squirrel 92 0/1 Cricetidae Cricetus cricetus Hamster 92 0/1 Castoridae Cricetus Castoridae Castoridae Chinchilla Caviidae Caviidae Caviidae O/14 Carnidae Caviidae Opiichotis patagona Nutria 92 0/1 Carnidae Caviidae Myorocta acouchy Agouti 92 0/1 Carnidae Canis fumiliarts Dog 92 0/1 Felicae Felis catus Cow 91 0/250 Relidae Bos tarrus Cow 91 0/200 Suidae Sus scrofa Pig 84 0/208 Red Pig 84 0/200				Plecotus auritus	Common long-eared bat	93	9/0	0
Mycles desgreenee				Pipistrellus nathusii	Pipistrelle bat	93	0/1	0
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Capromyidae Mycocastor coypus Nutria 92 0/2 Dasyproctidae Myoprocta acouchy Agouti 92 0/1 Carnidae Canis familiaris Dog 91 0/585 Felidae Felis catus Cat 84 0/200 Artiodactyla Bovidae Bos taurus Sheep 91 0/254 Suidae Sus scrofa Pig 84 0/208 tal 34 species 84-93 14/2854			Caviidae	por	Guinea pig	92	0/59	0
Carnivora Dasyproctidae Myoprocta acouchy Agouti 92 0/1 Carnivora Canidae Canis familiaris Dog 91 0/585 Felidae Felis catus Cat 84 0/200 Bovidae Bos taurus Sheep 91 0/579 Ovis aries Sheep 91 0/254 Suidae Sus scrofa Pig 84 0/208 tal 34 species 14/2854 14/2854			Capromyidae	Mycocastor coypus	Nutria	92	0/2	0
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Felidae Felis catus Cat 84 0/200 Bovidae Bos taurus Cow 91 0/579 Ovis aries Sheep 91 0/254 Suidae Sus scrofa Pig 84 0/208 tal 34 species 14/2854 14/2854		Carnivora	Canidae		Dog	91	0/585	0
Artiodactyla Bovidae Bos taurus Cow 91 0/579 Ovis aries Ovis aries Sheep 91 0/254 Suidae Sus scrofa Pig 84 0/208 tal 34 species 84-93 14/2854			Felidae	Felis catus	Cat	84	0/200	0
Suidae Ovis aries Sheep 91 $0/254$ Suidae Sus scrofa Pig 84 $0/208$ tal 84-93 $14/2854$		Artiodactyla	Bovidae	Bos taurus	Cow	91	0/579	0
Suidae Sus scrofa Pig 84 0/208 tal 34 species 14/2854				Ovis aries	Sheep	91	0/254	0
34 spec			Suidae	Sus scrofa	Pig	84	0/208	0
				S		84 - 93	14/2854	0.4

Animal

antigen positive and HV antibody negative.
positive animals: no serum available and tested for HV

were compared with the published findings of NE cases in neighbouring countries [18, 19].

RESULTS

Prevalence of HV-specific antibodies in human sera

Between 1972 and 1994, 8892 serum samples from 1783 patients with renal disease, 2172 individuals with suspected occupational risk of HV infection and from 4937 individuals without apparent symptoms of renal disease or risk of HV infection were tested for the presence of HV-specific serum antibodies. In the first group 33/1783 patients (2%) were HV seropositive (Table 2). No HV-specific serum antibodies were found in 284 renal transplant patients and only 1/358 haemodialysis patient (0·3%) was seropositive. Five of the 865 leptospirosis suspected individuals (0·7%) were seropositive. All the seven serum samples collected retrospectively from patients who had been diagnosed with NE on clinical grounds between 1974 and 1988 were seropositive. In the years between 1989 and 1992 HV seropositivity in clinically suspected NE cases ranged from 4 to 11% (Table 2). In the period January–June 1993 7/33 suspected NE cases (21%) were HV seropositive. The geographical distribution of all the clinically documented and serologically confirmed cases is shown in Fig. 1.

Among the 2172 individuals with suspected occupational risk of HV infection, HV-specific serum antibodies were found in 4/180 laboratory workers (2·2 %) who had worked with contaminated laboratory rodents, in 3/679 farmers (0·4 %), 4/68 trappers (6 %) and in 6/151 sera from forestry workers (4 %). No HV-specific antibodies were found in hunters, zoologists, military personnel, gardeners or veterinarians. The geographical distribution of HV seropositive laboratory workers, farmers, animal trappers and forestry workers is indicated in Fig. 1. Of the 4474 serum samples from individuals in the control groups collected in apparently endemic areas between 1989 and 1992, 33 (0·7 %) had HV-specific serum antibodies. No antibodies were found in 151 sera from army office personnel collected at several locations in The Netherlands.

$Prevalence\ of\ HV$ -specific antibodies or HV antigen in animal samples

Sera from 2855 feral and domestic animals collected between 1984 and 1994 were tested for the presence of HV antibodies. Among 829 sera from feral animals (Table 1) representing 20 different species, HV-specific antibodies were detected in 10/111 (9%) red bank voles (Clethrionomys glareolus) and in 1/68 (1%) field voles (Microtus arvalis). HV antigen was detected in the lungs of one common shrew (Crocidura russula) and two red bank voles by ELISA from which no serum was available. No evidence of HV serum antibodies was found in the members of the Muridae, Leporidae, Canidae, Mustelidae, Bovidae, Suidae and Vespertilionidae families (Table 1). No HV antibodies could be demonstrated in the 2025 sera of domestic animals from 14 different species (Table 1). The geographical distribution of all the HV positive animals is shown in Fig. 1.

The distribution of the locations where the red bank voles, apparently the main HV reservoir in The Netherlands, were trapped is shown in Table 3. In five of the nine locations where HV seropositive or HV antigen positive animals were

Table 2. Prevalence of han	tavirus-specific se	$erum\ antibodies$	$in\ selected$	human
pop	ulations in The N	Netherlands		

Selected groups	Individuals	Year	Number	Positive	%
Renal disease	Kidney transplants	72 - 84	284	0	0
	Suspected acute leptospirosis	84-9	865	5	0.7
	Suspected NE	74-88*	7	7	100
		89	27	3	11.1
		90	60	3	5.0
		91	81	4	4.9
		92	68	3	4.4
		93	33	7	21.2
	Haemodialysis	93	358	1	0.3
Suspected occupational	Laboratory workers	81-4	180	4	$2 \cdot 2$
risk	Farmers	93	679	3	0.4
	Hunters	89	455	0	0
	Foresters	89 - 90	151	6	4.0
	Zoologists	91	17	0	0
	Trappers	91 - 3	68	4	5.8
	Military	92	460	0	0
	Gardeners	92	60	0	0
	Veterinary surgeons	93	102	0	O
Control	Office personnel†	89-90	151	1	0.7
	Healthy blood donors†	92	4323	32	0.7
	Army office personnel‡	92	463	O	O
Total		74 - 93	8892	83	0.9

^{*} Retrospective.

detected, the incidence in red bank voles ranged from 25 to $60\,\%$ per positive area. In four of the five areas where HV infected animals had been identified, clinically and serologically confirmed human cases of NE had been documented.

Epidemiological, clinical and laboratory findings in NE patients

Epidemiological and clinical findings of 27 HV seropositive individuals who had suffered from NE between January 1974 and June 1993 are shown in Fig. 2. Symptoms were observed more frequently in males (74%), with a peak between 30 and 40 years in 12/27 (44%) and no cases in children under 9 years. HV infections were observed in all seasons with a slight peak in the summer 11/27 (41%). Mapping of the residences of the clinical NE cases showed that 26/27 (96%) of the confirmed cases lived in rural and forested areas located in the eastern and southern regions of The Netherlands (Fig. 1).

The most pronounced symptoms found in hospitalized patients were: abdominal and/or flank pain in 24/25 patients, followed by fever (body temperature > $38.0~^{\circ}$ C) in 25/27 patients, vomiting in 14/25, nausea in 17/25, headache in 12/25 and myopia in 4/27 patients. Myopia may have been overlooked in some patients, because it is easily missed during clinical examination. Duration of illness, defined as from the first day of illness till discharge from hospital ranged from 11 to 41 days (mean 17 days).

[†] Sera collected in endemic areas.

[‡] Sera collected at different barracks all over the country.

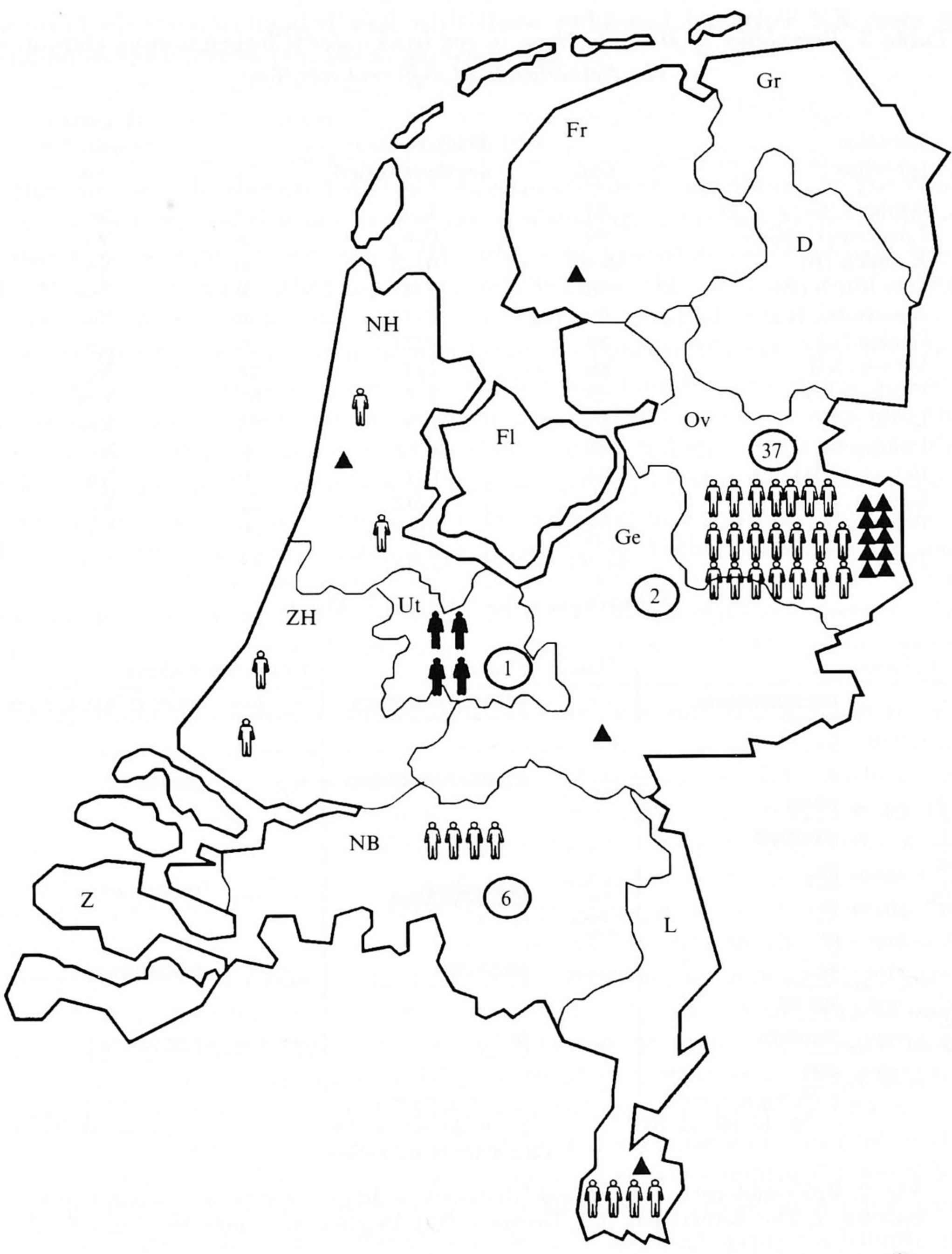


Fig. 1. Geographic distribution of hantavirus infections in The Netherlands; ↑, seropositive laboratory workers; ↑, serologically confirmed human cases of NE; ⊙, numbers of seropositive individuals in the province indicated; ▲, HV sero- or antigenpositive rodents. (Letters indicate the different provinces; (Fr), Friesland; (Gr), Groningen; (D), Drenthe; (Ov), Overijssel; (Ge), Gelderland; (Ut), Utrecht; (Fl), Flevoland; (NH), Noord-Holland; (ZH), Zuid-Holland; (Z), Zeeland; (NB), Noord-Brabant; (L), Limburg.

Table 3. Prevalence of HV infections in red bank voles (Clethrionomys glareolus) in The Netherlands at different locations

Location (province)*	Year	Positive mice/ numbers tested	%	Reported human NE case
Arnhem (Ge)	84	1/4	25	No
Loenermark (Ge)	86	0/5	20	No
Kempen (B)	86–87	0/22	0	No
Den Bosch (B)	88	0/1	0	Yes
Linschoten (Ut)	89	0/11	Õ	No
Geulen (L)	89	0/21	0	No
Velsen (NH)	89	1/4	25	No
Volthe (Ov)	89	3/5	60	Yes
Lutte (Ov)	89	1/4	25	Yes
Doldersum (D)	93	0/12	0	No
Bergen (NH)	93	0/4	0	No
Rossum (Ov)	93	3/12	25	Yes
Boekelo (Ov)	93	3/6	50	Yes
Total	84-93	12/111	11	5/9

^{*} Provinces abbreviated as in Fig. 1.

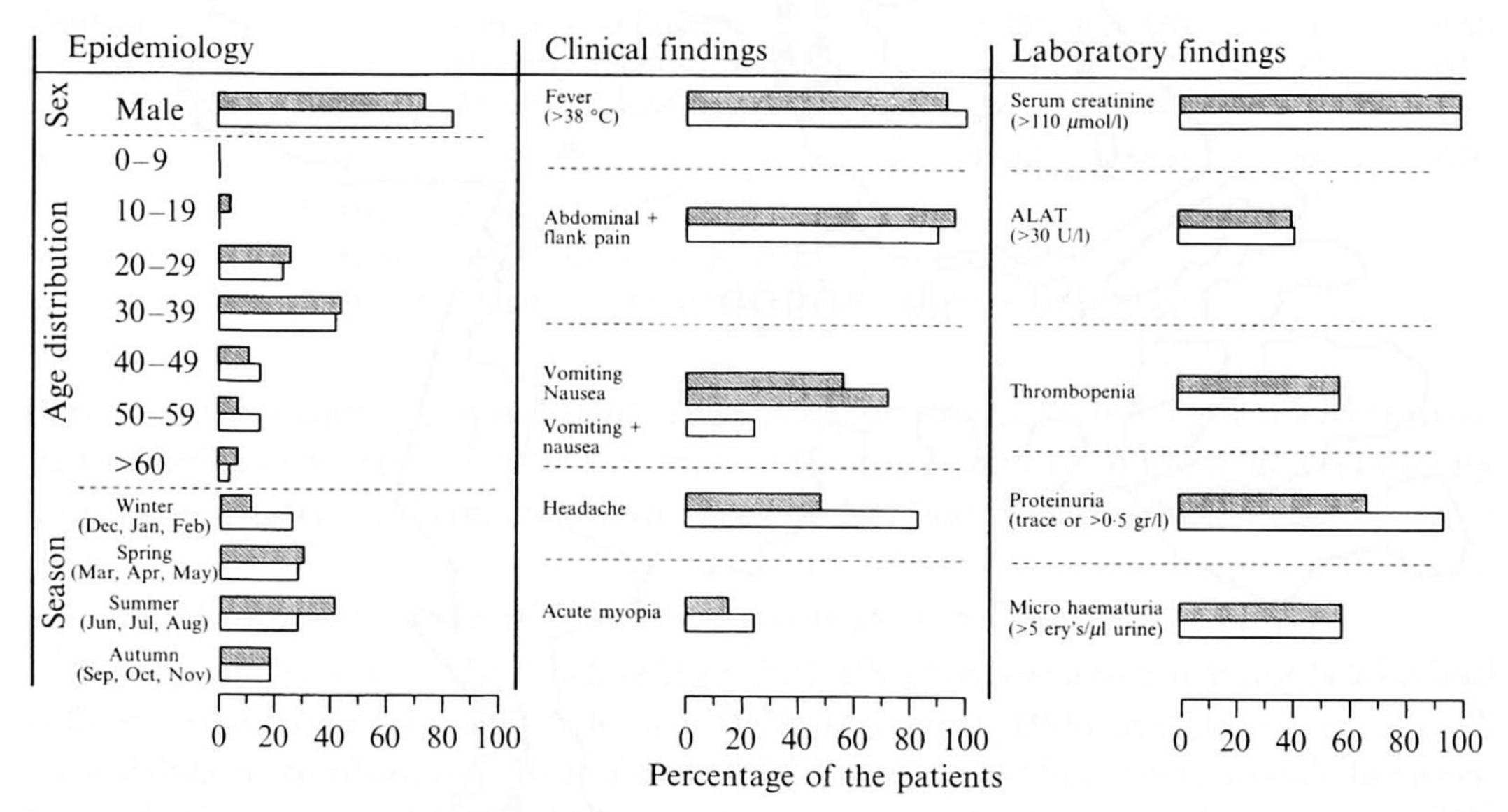


Fig. 2. Epidemiological, clinical and laboratory findings of hantavirus nephropathy patients in The Netherlands (♥), Germany [18], Belgium and northern France [19] (□).

Laboratory findings showed that the serum creatinine was increased (> 110 μ mol/l) in all 27 patients, ranging from 140 to 1300 μ mol/l with a mean value of 537 μ mol/l. Four of the 27 patients had to be given haemodialysis. Thrombocytopenia was found in 10/24 patients. Alanine aminotransferase (ALAT) was increased in 11/27 patients, ranging from 31 to 168 U/l. Protein was detected in the urine of 18/27 patients; in 14/27 the amount of protein was more than 0·5 g/l and in 4 of the 27 only a trace protein was detected. Microscopic haematuria, defined as > 5 erythrocytes/ μ l urine, occurred in 14/27 patients. All

the data observed coincided well with those published for other NE cases in neighbouring countries [18, 19] as shown in Fig. 2.

DISCUSSION

Here we have documented that HV infections caused by a Puumula-like virus occur in eastern and southern rural and forested areas in The Netherlands, in addition to laboratory-associated HV infections caused by a Seoul-like virus [15, 16]. As in neighbouring Germany and Belgium, the main reservoir of this infection is the red bank vole (Clethrionomys glareolus), which in a limited survey in the apparently enzootic areas showed a seroprevalence of HV-specific antibodies of 25-60%. Evidence of sporadic infection was also found in two animal species, the common shrew (Crocidura russula) in an apparently enzootic area and the common vole (Microtus arvalis). No evidence of HV infection was found in the other feral mammalian species investigated. These included bats, which have recently been shown to be a reservoir for HV infection in Korea [20]. Screening of the most common domestic mammalian species in The Netherlands indicated that domestic animals including the cat do not play an important role in the transmission of HV disease to humans. In the United Kingdom, however, cats have been shown to have a relatively high prevalence of HV-specific serum antibodies [21].

Evidence for HV infection in humans was predominantly found in apparently enzootic areas in individuals with typical signs of NE. In contrast to reports from other countries which demonstrated serological evidence of HV infections in individuals with chronic renal disease [9-13], we only found evidence for HV infection in 1 out of 642 haemodialysis and renal transplant patients. Furthermore, the seroprevalence of HV infections in patients suspected of leptospirosis (1%) was slightly lower than the incidence of acute leptospirosis in this group (3%), which indicates the relative importance of the HV infection in The Netherlands. HV seropositive individuals were more frequently identified amongst laboratory workers (2%), foresters (4%) and animal trappers (6%) than in the population at large (<0.5%), which indicates that the majority of these individuals run an occupational risk for acquisition of HV infections. It is of interest to note that the majority of the seropositive individuals identified in these studies had no history of clinically apparent renal disease, indicating that subclinical or non-typical HV infection may occur. The clinical signs of the serologically-confirmed human NE cases in The Netherlands are quite similar to those observed in neighbouring countries, with abdominal or flank pain, elevated levels of serum creatinine and proteinuria as most prominent features. Although most of the non-laboratory associated HV infections run a relatively mild course, more severe cases have been diagnosed. Recently we also investigated a patient with Guillain-Barré syndrome in association with HV infection [22]. The predominance of cases in males between 30 and 40 years of age, living in rural or forested areas, is also quite characteristic for NE cases observed in western Europe [6, 7, 10, 18, 23]. As in Germany, Belgium and France, NE cases occur throughout the year with a slight increase in the summer months. This is probably due to the mild climate, which largely allows rodents to survive outside the direct human environment. This is in contrast to the situation in Scandinavian countries, where rodents tend to seek human shelters in the autumn and winter [24]. Consequently the disease incidence peaks in these seasons. It is difficult to determine whether the recent increase in diagnosed cases of NE in The Netherlands is due to an increased awareness of the disease amongst the medical profession, or to an increased exposure of humans to infected rodents [3, 23, 25]. The recent outbreak of a previously unrecognized HV disease in the USA with a case fatality rate exceeding 60%, probably due to an increased exposure of humans to infected deer mice [3], stresses the need for a monitoring system for HV infections in humans and potential host animals.

ACKNOWLEDGEMENTS

We kindly thank Dr J. Vos, Dr J. Blankenstein, Dr A. Moll van Charante, Dr R. Diepersloot and Dr W. Wertheim for providing human serum samples and medical data, Dr R. Herbes, Dr A. E. J. M. van den Boogaard, Dr J. T. Lumey, Dr J. van Oirschot, Mr T. Brink, Mr A. Lefevré, and Mr R. van Apeldoorn for providing animal specimens, Mr H. Broeders, Mr B. van Ordel, Ms L. van Raay, Drs M. Schokker and H. Yuping for technical assistance. This work was supported by a grant from the Veterinary Public Health Inspectorate.

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