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HANTAVIRAL ANTIGENS AND ANTIBODIES IN WILD RODENTS IN PORTUGAL

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Summary. - Small rodents of the species *Rattus norvegicus* and *Rattus rattus* have been captured between 1986 and 1988 in several areas of Southern Portugal. A total of 135 animal specimens were examined for hantaviral antigens in lung sections and 5 have been found positive. Some of the rodents were shown to have serum antibodies as detected by immunofluorescence in titres up to 1 : 256. This investigation proves for the first time the presence of Hantavirus in wild rodent populations of Portugal.

Key words: Hantaan virus; Iberian Peninsula; wild rodents; Portugal

Introduction

Serological studies utilizing different techniques have demonstrated that hantavirus infections are widely distributed throughout the world, even when the cases of human disease are usually sporadic. Haemorrhagic fever with renal syndrome (HFRS) has been reported mostly from Far East Asia and Eastern Europe (Yanagihara *et al.*, 1988). But the clinical disease was recognized also in the Western European countries , since 1933 in Sweden (Zetterholm, 1934), 1942 in Finland (Lähdevirta, 1971), 1948 in Norway (Knutrud, 1949), 1957 in Denmark (Hansen, 1958), 1979 in Belgium (Desmyter *et al.*, 1983), 1983 in France (Chanard *et al.*, 1984), and 1984 in United Kingdom (Lloyd *et al.*, 1984). It was also described in Yugoslavia (Mandic, 1969), and Greece (Lee and Antoniadis, 1981). However, till now there was no reliable information concerning the presence of hantavirus in Southwestern Europe.

Clethrionomys glareolus and *Apodemus* species are the most important reservoirs for hantavirus in other areas of Europe and Asia. But this group of rodents is absent in the Iberian Peninsula, except in some areas of Northwestern Spain. However, other important reservoir rodents as *Rattus norvegicus* and *R. rattus*

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are very widely distributed all over the Iberian Peninsula, while in the south of Portugal the *Apodemus* species survive in very localized and restricted areas (Madureira and Ramalhinho, 1981).

The aetiologic agent of HFRS is able to establish persistent infections in wild rodents (Lee *et al.*, 1981). Capture and analysis of such animals is probably the most effective way to demonstrate the existence of hantavirus in a country where cases of human disease are not easily diagnosed. The present paper present evidence of antigens in the lungs of wild rodents and hantaviral antibodies in the sera of rats trapped on some farms around a small village of Southern Portugal.

Materials and Methods

Trapping area. Small rodents were captured between 1986 and 1988 in the farms around Águas de Moura, a small village of Palmela county (38º 35N; 8º 40W) 60 km south of Lisbon. Traps for live capture, similar to the tomahawk model vere mostly used. The traps were provided with different kinds of bait, including fresh apples.

Handling of animals. The animals were bled by cardiac puncture. The blood was allowed to clot and the sera were separated and stored at -25 ^{0}C until used. Organs (lungs, liver, kidneys, brains, and spleen) were stored individually frozen at -80 ^{0}C in small tubes until examined. All the captured animals were identified by species and skulls were saved for later studies (Gama, 1975).

Detection of hantavirus antigens in lungs. Sections, 4 μ m thick, were cut from lungs in a cryostat, dried and acetone fixed; 2 to 4 sections were positioned on each slide. The sections were kept at -80 ^{0}C until used. One or two sections were covered with a nephropatia epidemica (NE)-positive human convalescent serum, while the other section (or sections) were covered with a NE-negative human control serum. FITC - conjugated anti-human IgG diluted to give optimal specific fluorescence was then applied to the sections. The technique was carried out as described before (Traavik *et al.*, 1983).

Reference slides. Slides containing Vero E-6 cells infected with the following hantavirus: Hantaan, CG 18-20 (USSR strain), Nephropatia epidemica (NE), Prospect Hill, Tchoupitoulas and CG 13891 (Belgian), were kindly supplied by Dr. van der Groen, Institute of Tropical Medicine, Antwerpen, Belgium.

Antibody detection in rodent sera. The rodent sera were screened by IFA in dilutions 1:20. The following fluorescein isothiocyanate conjugates were used: FITC conjugate specific antibody (goat) to human IgG (fluoroabody) from Bionetics; FITC - conjugate rabbit immunoglobulins to mouse immunoglobulins, from Dako.

Sera giving the characteristic pin-point fluorescence pattern in the cytoplasma of the cells were considered suspect, and tested again after dilution to 1:32. Sera presenting the fluorescence pattern in this dilution were considered antibody positive. The positive sera were retested in twofold serial dilutions up to 1:256. Reference monoclonal antibodies in mouse ascitic fluids (MAF) were used as positive controls. The antibodies were directed against: Hantaan (HTN), Tchoupitoulas (TCH), SR-11, and Prospect Hill (PH) viruses and were kindly supplied by Dr. J. McCormick from CDC, Atlanta, Geo. U. S. A.

Results

Lung sections from 135 small rodents were examined for hantaviral antigens

in the lungs using a positive reference serum (Table 1). The antigen was detected in the cytoplasma of alveolar cells either in a form of pin-point fluorescence or as a grossly granular pattern. In some lung sections there was specific immunofluorescence (IF) all over the tissues, while in other sections only isolated cells or group of positive cells could be seen. The extent of the detected antigens area varied from section to section in the different animals. A total of 5 antigen-positive lungs were found in 3 *Rattus norvegicus* species, in 1 *Rattus rattus*, and in 1 *Mus spretus*.

The sera from all the animals with positive or probably positive immunofluorescence pattern in their lung sections (a total of 30) were selected for antibody determination (Table 1). Thus, sera from the 5 antigen-positive animals, and additional 25 sera from animals with suspect IF patterns in the lungs, were examined for anti-hantavirus specific antibodies by IF. From these, sera from 14 animals gave positive IF reactions at a dilution of 1:32. Two of the sera in question were tested in further two-fold dilutions up to 1:256. Both sera gave bright IF reaction in all dilutions. Due to scarcity of hantavirus-infected cell culture slides, no further serological examinations were possible.

Discussion

Some years ago we have tried to identify human cases of HFRS in Portugal. Several patients with nephropatia living in rural areas of Southern Portugal and also haemodialysis patients have been examined to determine whether a hantavirus might have caused their disease. In some cases the epidemiologic features were compatible with HFRS. These cases mainly concerned workers involved in agricultural activities.

Using the indirect IF we found several human cases with low hantavirus antibody titre, usually 1:32. In some cases we noted that these sera reacted better

Table 1. Virus antigens in the lungs and serum antibodies to hantavirus antigens in small rodents from Southern Portugal

Species	Number of positive animals		
	Lungs	Antibodies	Both
Rattus norvegicus	3/60	8	1
Rattus rattus	1/71	4	-
Mus spretus	1/4	-	-

* No. of positive out total lung specimens examined by IF
** Out of 30 selected animals including the 5 hantavirus antigen carriers

with the NE antigen than with other hantavirus antigens. However, western blot confirmation test was inconclusive. Some investigators have reported that even in some areas where HFRS was endemic, antibodies against hantavirus are usually of low prevalence in the population.

It seemed, therefore, reasonable to identify the putative local hantavirus strain(s) in reservoir animals. No *Clethrionomys* rodents are present in Portugal, neither are *Apodemus* species distributed regularly all over the country. Our effort became concentrated to one area of Southern Portugal located around a village called Águas de Moura in the Palmela county, where the wild rodent populations are mainly composed of *Rattus norvegicus*, *Rattus rattus* and of *Mus* species. We have shown that some captured *Rattus norvegicus* had hantavirus antigens in their lungs and that they had high titres of hantavirus antibodies in their sera. Curiously, the sera reacted intensively with the antigen of the Tchoupitoulas virus isolated from *R. norvegicus* in North America. Probably the Águas de Moura strain is antigenically more closely related to the Tchoupitoulas virus than to any other of the hantavirus strains used as antigens.

Hantavirus strains from *Rattus norvegicus* and *Rattus rattus* are less pathogenic for humans than other strains (Tsai *et al.*, 1985). It has also been indicated that hanta-related viruses isolated from commensal rats probably do not cause clinical disease, except under unusual circumstances (Yanagihara *et al.*, 1988). It is, however, hard to define what unusual circumstances are in the context of a viral zoonoses. Attempts to isolate the hantavirus strain(s) circulating in Portugal are now under way.

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