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SEROLOGICAL EVIDENCE OF HUMAN HANTAVIRUS INFECTION IN ARGENTINA, BOLIVIA AND URUGUAY

MERCEDES C. WEISSENBACHER[1], ESTELA CURA[2], ELSA L. SEGURA[2], MARIA HORTAL[3], LUC JU BAEK[4], YONG KYU CHU[4], HO WANG LEE[4]

[1] Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires; [2] Instituto Fatala Chabén, Ministerio de Salud, Buenos Aires; [3] Laboratorio Central de Salud Pública, Montevideo, Uruguay; [4] Asan Institute for Life Sciences, Asan Foundation, Seoul, Korea

Summary A serological survey was conducted in 1985-1987 to determine the presence of infection for Hantavirus in the general population in Argentina, Uruguay, Paraguay and Bolivia, as well as among rodent-exposed laboratory workers in Argentina. Out of 748 individuals tested by immunofluorescence 20 proved positive for Hantaan virus 76/118 strain of whom 16 also reacted against Seoul virus 80/39 strain and 2 against Puumala virus Sotkamo strain. Ten out of 72 Argentine laboratory workers were positive for the first 2 viruses by ELISA, immunofluorescence and/or plaque reduction neutralization test, in 4 of whom recent infection was demonstrated by IgM antibody presence. Inapparent Hantavirus infection was thus demonstrated for the first time in 2.7% of regional inhabitants, together with 13.9% among rodent-exposed laboratory workers. Our data established the existence of human Hantavirus infection nearly 10 years before the recognition of clinical cases of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome in Argentina.

Key words: hantaan virus, hantaan hemorrhagic fever, seroprevalence of hantaan virus

Hemorrhagic fever with renal syndrome (HFRS) comprise a variety of clinically similar entities, known to be endemic in Euro-Asia continent[1-3]. After the etiologic agent of Korean hemorrhagic fever, the Hantaan virus, had been discovered in 1976 and isolated from Korean hemorrhagic fever patients in 1978[4, 5] its widespread distribution was demonstrated not only in areas where HFRS is endemic, but also in many parts of the world where the infection is mainly inapparent. Serological surveys in countries presumed to be free from HFRS showed anti-Hantaan antibodies in sera from humans without clinical sympto-matology[6-8]. In America human infection with Hantavirus was demonstrated serologically in USA[6], Bolivia[6] and Canada[7]. A disease similar to HFRS was not known to exist, but a few cases of mild HFRS have recently been identified retrospectively in USA[3].

In Argentina up to 1985 there had been no evidence of human infection with Hantaan virus. Subclinical infection in general population[9] and inapparent infection in laboratory workers[10] were communicated for the first time in 1986-1987.

Urban, wild and laboratory rodents are the reservoirs invariably associated with Hantavirus infection all over the world[3, 5, 11, 12]. The virus is transmitted to humans through rodent urine, feces or saliva.

In Argentina, anti-Hantaan antibodies were detected in 11% of Buenos Aires and Mar del Plata port rats in 1983-1984[13]. We demonstrated that 22% of experimental rats were infected with Hantavirus in some laboratories of Argentina and a 23.5% seroprevalence of Hantavirus infection was also reported for the first time in wild field rodents (Callomys musculinus) in 1985-1987[14].

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Postal adress: Dr. Mercedes C. Weissenbacher, PAHO/WHO, Avda. Brasil 2697, 11,300 Montevideo, Uruguay

In May 1993 in United States an outbreak of an unrecognized human acute respiratory illness with high mortality was serologically associated to a new Hantaan virus: Sin Nombre Virus (SNV). The agent was isolated from the lung tissue of deer mice captured at the home of a patient with the Hantaan pulmonary syndrome (HPS)[3, 15].

In 1995 a small outbreak of human cases of HPS has been notified in El Bolson, Argentina and the Hantavirus etiology was serologically established[16]. Additional HPS cases (from 1991 to 1993) were retrospectively identified in Buenos Aires, Santa Fe and Salta provinces[17].

The purpose of our study in 1985-1987 was to determine the presence of anti-Hantaan virus antibodies among healthy laboratory workers and the general population in Argentina and neighboring countries. The results obtained established the existence of Hantavirus infection long before the demonstration of human illness in the region. Therefore a comprehensive background information on previous findings on hantavirus activity in the human population has become relevant.

Fig. 1.— Map of South America showing the location where inhabitants were found to be positive for Hantavirus infection.

Materials and methods

Population study:

To determine the presence of anti-Hantaan virus antibodies in the general population a serologic survey was carried out from 1985 to 1986 in 748 humans living in 17 Argentine provinces, as well as in Paraguay, Bolivia and Uruguay, as shown in Fig. 1. These sera were collected for a seroprevalence study of *Trypanosoma cruzi* in the general population. Age ranged from 1 to 78 years, with a mean of 38 years.

Among laboratory workers a total of 72 serum samples were collected in 1987 from 64 staff members from the Microbiology Department, School of Medicine, Buenos Aires University, and from 8 laboratory staff-members from the School of Medicine, Mendoza province, and most of them had close contact with experimental or wild rodents.

Virus:

Three serotypes of Hantavirus were used; the prototype Hantaan virus 76/118 strain, Seoul, virus 80/39 strain and Puumala virus Sotkamo strain[13].

Sera:

A total of 748 sera from normal inhabitants of Argentina, Bolivia, Paraguay and Uruguay were examined as well as 72 sera from laboratory workers (Details are shown in Tables 1 and 2).

Antibody test:

General population: antibodies against Hantaan, Seoul and Puumala viruses were assayed by indirect IFA technique[5]; antibodies against Hantaan and Seoul viruses were also assayed by plaque reduction neutralization test (PRN).

Laboratory workers: IgG and IgM ELISA antibodies against Hantaan virus[3] and PRN and IFA techniques against Hantaan and Seoul viruses were performed[14].

Results

Out of the 748 sera from the general population assayed, 20 proved positive for Hantaan virus, 16 in Argentina, 2 in Bolivia and 2 in Uruguay, pointing to a 2,7% total prevalence (Table 1 and Fig. 1). Among Hantaan virus positive sera, 16 were also reactive for Seoul virus and 2 for

TABLA 1.— *Antibody titers against Hantaviruses in 20 out of 748 sera from inhabitants of Argentina, Bolivia, Paraguay and Uruguay, 1985-1986*

Origin of sera			*Viral antigen and antibody titer*						
			Hantaan		*Seoul*		*Puumala*	No. of positive/	% of
Country Province	Age	Sex	IFA	PRN	IFA	PRN	IFA	No. of sera tested	positive sera
Argentina									
Jujuy	64	F	32		32			1/8	12.5
Salta	26	F	64		64			1/18	5.6
Chaco	43	M	64	20	256	20	64		
	52	F	64						
	26	M	64					3/39	7.6
Santiago	78	F	32		64				
del	25	F	64		64				
Estero	30	F	128		128				
	50	F	32		32			4/101	4.0
Tucumán	29	F	64						
	38	F	128		128			2/32	6.2
Misiones	32	F	64		64			1/10	10.0
Buenos	54	M	128	20	256	20	64		
Aires	23	F	128						
	27	F	64						
	51	F	128		32			4/101	4.0
Other provinces*			Neg.		Neg.			0/177	0.0
Uruguay	48	M	32		32				
	34	F	64		64			2/205	1.0
Bolivia	34	F	32		32				
	17	F	128		128			2/28	7.1
Paraguay			Neg.		Neg.			0/29	0.0
Total			20		16		2	20/748	2.7

* Formosa, Catamarca, La Rioja, Mendoza, San Luis, Neuquén, Córdoba, Santa Fe, Entre Ríos and Corrientes.

Puumala virus. In fact, IFA titers for Hantaan and Seoul viruses were quite similar. There was no correlation between positive serology and sex or age. The age of positive cases ranged from 17 to 78 years with a mean of 39 years. In Argentina the 16 sera with anti-Hantaan antibodies were detected among 309 inhabitants from: Jujuy, Salta, Chaco, Santiago del Estero, Tucumán, Misiones and Buenos Aires provinces. Anti-Hantan antibodies were not found in 177 sera from inhabitants of the following provinces: Formosa (15), Catamarca (14), La Rioja (13), Mendoza (18), San Luis (15), Neuquén (17), Córdoba (24), Santa Fe (15), Entre Ríos (23) and Corrientes (23).

Table 2 depicts that out of the 72 laboratory workers, 10 sera proved positive with 13.9% total antibody prevalence. Four positive cases had IgM antibodies indicating recent infection. None of the seropositive individuals had previous illness suggestive of classical HFRS. From the 6 positive out of the 64 staff-members of the University of Buenos Aires, 3 were animal care-takers; 2 professionals working in close contact with laboratory rats and 1 apparently non-exposed tissue culture technician. Out of the 8 laboratory work-

TABLA 2.— *Antibody titers of 10 positive sera from 72 argentine laboratory workers (13.9% of positive sera) against Hantaan and Seoul viruses by IFA, PRN and ELISA test, 1987*

Type of Job	Age	Sex	IF Ab titer (IgG)		PRN Ab titer		ELISA Ab titer HTNV	
			HTNV	SEOV	HTNV	SEOV	IgG	IgM
Professional	27	M	16	32	NT	NT	NEG	NEG
Tissue culture technician	60	F	NEG	32	NT	NT	200	NEG
Animal care-taker	40	M	64	64	NT	NT	200	NEG
Animal care-taker	59	F	16	64	NT	NT	400	800
Professional	55	F	NEG	64	NT	NT	1600	400
Animal care-taker	57	M	NEG	32	NT	NT	400	NEG
Professional	43	M	256	NT	20	2000	25600	3200
Technician	30	M	64	NT	20	200	6400	800
Animal care-taker	35	M	128	NT	20	2000	15600	NEG
Animal care-taker	50	M	128	NT	20	2000	12800	NEG

PRN: Plaque reduction neutralization, Ab: Antibody, HTNV: Hantaan virus, SEOV: Seoul virus, NT: Not tested.

ers from Mendoza province, the 4 seropositive cases were 1 professional, 1 technician and 2 animal care-takers, working in close contact with both wild and laboratory rodents.

Results presented in Table 2 indicate that these laboratory workers were infected with Seoul-type virus commonly found in laboratory rats, as shown by PRN antibody titers.

Discussion

Years before the description of human illness in Argentina we demonstrated for the first time, serological evidence of human inapparent infection with Hantavirus in the general population of Argentina and Uruguay, and in laboratory workers of Argentina. However in Bolivia, antibodies against Hantaan virus had already been reported in human serum samples as early as 1981 as well as in that study[6]. The furnished data point to a potential risk for human illnesses in those countries where subclinical human Hantavirus infection was already found. Considering the present epidemiological situation a new survey looking for the prevalence of anti-Hantaan viruses in human and rodent population of these countries has become mandatory to determine the extension of the virus activity and their importance to public health.

In 1985-1986, without recognized cases of HFRS in Argentina, Bolivia and Uruguay, we found a 2.7% prevalence of subclinical Hantavirus infection in the general population similar to those geographic areas where HFRS is endemic[2]. Other authors in 1992 identified cases of HFRS retrospectively, inferred by a history of compatible illness between 1984-1991 and confirmed later by positive Hantaan antibodies[18].

Serology in our work was performed by indirect IFA. Twenty positive individuals reacted against Hantaan virus and 16 of them against Seoul virus in similar titers. PRN performed in 2 sera among positive general population showed no significant neutralizing antibodies against Hantaan and Seoul viruses as shown in Table 1, implying that Hantavirus infection in the surveyed population might be due to a serologically different serotype. IF antibodies against hantaviruses are group specific but PRN antibodies are type specific[14]. It is worthy to mention that 9 out of 10

seropositive individuals who were given questionnaires had had close contact with urban rats and wild rodents, although none had apparent clinical illness. The source of human Hantavirus infection are wild rodent reservoirs with a persistent and asymptomatic infection. It is noteworthy that in Argentina 11% prevalence of infection was already demonstrated in wild port rats in 1983[13] and in 23% of a wild field rodent (*C. musculinus*) in 1985[14]. Our previous finding also showed that 15% of the laboratory rats assayed at the Microbiology Department, School of Medicine, University of Buenos Aires, and 70% of the experimental rats tested at the School of Medicine, Mendoza province, were infected with Seoul Hantavirus[14].

In this paper we demonstrate a high prevalence of Hantavirus infection (13.9%) among laboratory personnel working in close contact with rodents. In positive laboratory workers neutralization tests showed higher antibody titers against Seoul than Hantaan virus, indicating that subclinical infection may possibly have been acquired from infected laboratory rats. Four out of the 10 seropositive laboratory workers showed significant titers of IgM antibodies by ELISA test, indicating recent infection. ELISA antibodies were only measured against Hantaan virus, since they cross react equally to Hantaan and Seoul virus.

This paper reaffirms the necessity of a permanent surveillance system which would periodically investigate the existence and extension of emerging or reemerging infectious agents related to known or even new pathologies, in the same manner as the already established control programs.

In conclusion, our results already documented in 1985-1987 the presence of subclinical human Hantavirus infection in general population and laboratory staff in Southern countries of Latin America, where years later clinical cases of hemorrhagic fever with renal syndrome and a new lethal pulmonary disease induced by Hantavirus were recognized.

Resumen

Evidencia serológica de infección humana por hantavirus en Argentina, Bolivia y Uruguay

Con el propósito de evidenciar la presencia de infecciones por virus Hantaan, entre 1985 y 1987 se realizó una encuesta serológica en la población general de Argentina, Bolivia, Paraguay y Uruguay y en trabajadores de laboratorio expuestos a contacto con roedores en Argentina. Entre los 748 habitantes del área estudiada, 20 sueros (2,7%) resultaron positivos por inmunofluorescencia para virus Hantaan cepa 76/118, de los cuales 16 también reaccionaron frente al virus Seoul, cepa 80/39 y 2 frente al virus Puumala cepa Sotkano. De 72 trabajadores de laboratorio de Argentina, 10 sueros (13,9%) fueron positivos para los 2 primeros virus por ELISA, inmunofluorescencia y/o neutralización. En 4 de los laboratoristas se comprobó por ELISA una infección reciente con presencia de anticuerpos IgM.

Estos datos constituyen la primera evidencia de infección humana por hantavirus, en la región, cerca de 10 años antes del reconocimiento de casos clínicos de fiebre hemorrágica con síndrome renal y del síndrome pulmonar por hantavirus en Argentina.

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WE HAVE NOT YET CONQUERED INFECTIOUS DISEASE: ... we are faced with the resurgence of some «old» infections and the constant threat of the emergence of new infective agents. It appears that North America and other countries are now encountering a form of tuberculosis that is resistant to all current drugs. This may be the result of poverty and the improper use of antituberculous drugs, together with highly susceptible populations of people with AIDS. Whatever the cause, it is a clear reminder that we can never be confident about our ability to treat an infectious disease even if we possess a whole battery of drugs to which it is usually sensitive. The emergence of drug-resistant strains of malaria offers a similar story.

TODAVIA NO HEMOS VENCIDO LAS ENFERMEDADES INFECCIOSAS: ... nos vemos enfrentados con el resurgimiento de «viejas» infecciones y el riesgo constante de la emergencia de nuevos agentes infecciosos. Parecería que en EE. UU. y en otros países se encuentra una forma de tuberculosis resistente a todo tratamiento. Esto puede ser el resultado de la pobreza y del mal uso de las drogas antituberculosas, junto con poblaciones de alto riesgo como son los enfermos de SIDA. Cualquiera sea la causa, se trata de un claro recuerdo que nunca se puede confiar en nuestra capacidad para tratar una enfermedad infecciosa aun si contamos con toda una batería de drogas específicas. La emergencia de cepas resistentes de malaria ofrece una historia similar.

David Weatherall

Science and the quiet art. The role of medical research in health care.
New York: W. W. Norton, 1995, p 185