

HISTOPLASMIN AND PARACOCIDIIDIN SKIN REACTIVITY IN INFANTILE POPULATION OF NORTHERN ARGENTINA (1)

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SUMMARY

In order to estimate ages at which etiological agents of systemic mycoses initiate infection, histoplasmin and paracoccidioidin skin tests were performed in 344 children of both sexes, between 2 and 15 years old. They were selected from a statistically significant population sample Gral. San Martín city (Northeast Argentina). Tests were read 48h after injection and considered positive if a 5 mm or larger induration was present. Circulating antibodies were also evaluated by agar gel immunodiffusion. The overall infection rate for *H. capsulatum* was 9.2%, belonging to children from 4 to 14 years old, without significant differences among sexes. Five children from 2 to 14 years old were positive to paracoccidioidin (1.6%). None of the children had specific antibodies neither signs of active mycosis. Results show *H. capsulatum* infection can be found from age 4, while for *P. brasiliensis* the lower limit was two years old. These findings may contribute to better knowledge on infantile fungal infection in a geographical region where no previous references can be found.

KEYWORDS: Histoplasmosis; Paracoccidioidomycosis; Skin Tests.

INTRODUCTION

Even though many techniques have been developed to evaluate cell mediated immunity, skin tests are still used as a simple and easy way to provide information on various infections and to indicate whether afferent, central or efferent pathways of immune response, as well as inflammatory reaction, are acting properly⁹.

Human beings develop humoral and cellular types of immunity during fungal infection. The first response is useful for serological diagnosis and for monitoring both evolution of the disease and response to treatment, but it does not help to avoid agent dissemination and tissue damage. On the contrary cellular immunity represents a significant defense mechanism against pathogenic fungi causing systemic mycosis^{9,10}.

An important characteristic of some systemic fungal infection is the particular geographical distribution of etiological agent. *Histoplasma capsulatum* is worldwide present, but in the American continent *capsulatum* is the only variety reported while *var. duboisii* is predominant in Africa^{8,13,15}, and, *Paracoccidioides brasiliensis* is restricted to Latin America, with endemic areas from Central America down to Northern Argentina, with the exception of Nicaragua, Guyanas, Suriname and Chile^{21,24}.

In Argentina paracoccidioidomycosis has been found in the north half of the country, particularly in the Northeast border where it constitutes a public health problem¹. Although skin test surveys carried out on healthy adults from two different regions of the country

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showed a positivity of 25%, actual prevalence of *P. brasiliensis* infection for the whole Argentine endemic area has not been established³.

It is well known that active forms of systemic mycoses commonly appear around the third decade of life, but there is limited information on infantile infection, particularly on *Histoplasma* and *Paracoccidioides* infection in Argentina.

In order to estimate ages at which etiological agents of these mycoses initiate infection, skin test and agar gel immunodiffusions were performed on infantile population of General San Martín, a small town located in the Province of Chaco, 1,100 km north from Buenos Aires.

MATERIALS AND METHODS

Population survey: A statistically significant sample of 344 persons, from urban and suburban areas, aged from 2 to 15 years were selected from school children of General San Martín city. Rural population was not included. Survey was done on a population of approximately 20,000 inhabitants, located in Province of Chaco, in the northeast border of Argentina (27°10'S – 58°59'W)

The area is characterized by an average annual temperature of 21° C an average dampness of 73% and 1350 mm³ annual rainfall. People mainly work in growing cotton or vegetables on small farms. Poultry yards are present in most of urban houses as well as birds and domestic animals. These conditions define a particularly suitable region for endemic histoplasmosis and paracoccidioidomycosis.

Skin tests: Children's skin reactivities, with parents authorization, were studied by intradermal injection of 0.1 ml of Histoplasmin (HPN) and Paracoccidioidin (PCN) diluted 1:50 in saline solution, in the flexor surface of each forearm. Antigens used were prepared at the

Mycology Center of Buenos Aires University according to previous reports^{17,18}. They were obtained from cellular lysates of disrupted yeast phase of strains Pb 12499, Pb 19333 and Hc 992-73 and kindly provided by Prof. Dr. R. Negroni. Tests were read 48h after injection and a 5 mm or greater induration was considered positive^{6,16}. Positive reactions were considered indicative of past, intercurrent or subclinic infection by *H. capsulatum* or *P. brasiliensis*.

Serology: Before skin tests were performed, blood samples were taken for serology. Agar gel macro immunodiffusion tests and counterimmunoelectrophoresis with fungal antigens were carried out according to standard methods recommended by the Pan American Health Organization¹⁹.

Clinical evaluation: By means of medical examinations and questionnaire information was performed in order to find out health and nutritional status of children and signs of active systemic mycosis.

RESULTS

From 344 children studied, only 315 skin tests readings could be performed. From these, 29 (9.2%) were positive to HPN (13 males and 16 females). Ages of HPN reactors varied from 4 to 14 with an average of 10 years (Table 1). Mean induration diameter for this antigen was 10.0 mm with a maximum of 20.0 mm (two cases).

Five children (1.6 %) were positive to PCN, 3 males and 2 females, aged from 2 to 14 years with an average of 10 years. Mean induration diameter was 8.0 mm, with a maximum of 10 mm. They all belong to the urban group and did not show epidemiological differences with other children (Table 2).

Distribution of induration diameters for both antigens is shown in Figure 1. Mean age of negative children

TABLE 1
Histoplasmin reactors in San Martín City (Argentina), according to age and sex

Age groups (years)	Males		Females		Total	
	No.	%	No.	%	No.	%
2 – 4	1/42	2.4	0/47	0.0	1/89	1.1
5 – 7	3/39	7.7	3/37	8.1	6/76	7.9
8 – 10	4/38	10.6	3/33	9.1	7/71	9.9
11 – 13	6/30	20.0	6/26	23.8	12/56	21.4
14 – 15	0/8	0.0	3/15	20.0	3/23	13.0
Total	14/157	8.9	15/158	9.5	29/315	100.0

TABLE 2
Paracoccidioidin reactors among infantile population of San Martín City (Argentina)

Patient N°	Sex	Ages (years)	Induration diameters (mm)	
			PCN	HPN
33	F	14	10	0
17	F	7	10	10
56	M	12	10	0
68	M	9	5	0
103	M	2	5	0

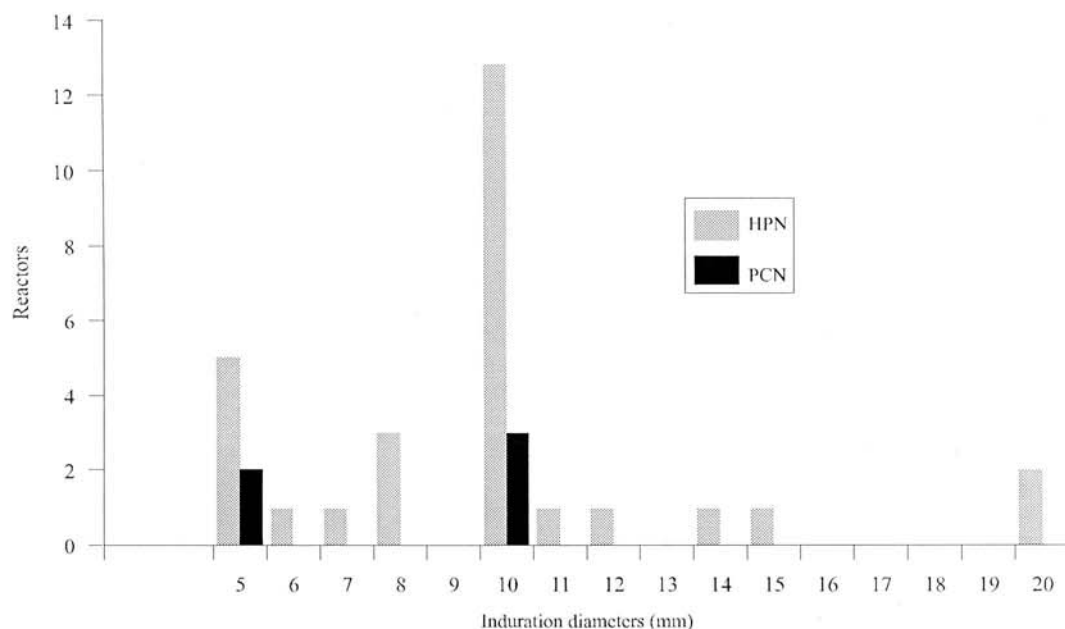


Fig. 1 – Distribution of HPN and PCN induration diameters among reactors from San Martin City (Argentina).

(Non Reactors Group) was 6. Only one girl showed positive reaction to both skin tests. All serological tests were negative and none of the children showed signs of active mycosis.

DISCUSSION

Population surveys by skin testing is a simple, adequate and low costing strategy to evaluate prevalence of regional pathogens⁷.

In Argentina many surveys have been done to evaluate *H. capsulatum* infection rates, mostly focus on adults with wide variation in results^{4,12,20,22}. However, little information exists on infantile infection. CARRILLO⁵ studied in Jujuy (Northwest Argentine border) 1508 children, 6 to 15 years old, and reported a mean prevalence of 30%, without differences among sexes.

RUBINSTEIN²³ evaluated 790 children up to 12 years old from suburban areas of Buenos Aires finding a prevalence of 17.8%.

There is not any reference in the Province of Chaco about surveys on infantile population. In this work we found a mean infection rate of 9.2% with an increasing number of reactive children according to age, such as is

known to occur in other population²⁴. This was also observed when we compared mean ages of Reactor and Non Reactor groups. There were not significant differences among infection rates of males and females. Skin reactivities were mostly very intense, 19 children out of 29 showed 10 mm or larger indurations suggesting the possibility of strong exposition to the agent. Nevertheless, histoplasmosis cases are rarely reported in this area, which may be due to low pathogenicity of local varieties of fungus.

Different portal of entry have been mentioned for *P. brasiliensis*, but presently it is accepted that fungus enters the host body through respiratory tract¹⁴.

Infection may stay asymptomatic during long periods of time, by means of a quiescent host-parasite relationship, but any alteration in the balance between host factors, fungal virulence and environmental conditions make infection progress to development of disease¹⁰.

Since skin tests to *P. brasiliensis* were first described, efforts have been made to establish real prevalences of human infection. Even though difficulties have been reported in comparison of results due to heterogeneity of antigens, existing data clearly indicate that skin tests are positive in subclinical and chronic forms of

disease. On the contrary, in acute or subacute forms (juvenile type) skin tests are negative and patients tend to develop a good humoral immune response¹⁰. Although crossed reactions between *P. brasiliensis* and other fungus, particularly *H. capsulatum* have been described, specific immune response is recognized because homologous antigen produces larger indurations than heterologous².

Cases of paracoccidioidomycosis are not very frequent in Argentina, but mostly they come from Province of Chaco, being notified 140 patients until 1993¹¹.

BOGADO⁴ reported an infection rate of 10.9% for adult population while among woodcutters the figure was 20.0%. In our survey there were 5 children with positive skin reaction to PCN.

Considering that in one girl – Patient 17 – crossed reaction between antigens could exist, and that none of the children showed clinical signs of disseminated disease and that serological tests were all negative, results indicate at least 4 children were infected by *P. brasiliensis* or carried on an asymptomatic form of paracoccidioidomycosis.

Results obtained indicate that *H. capsulatum* infection can be found from age 4, while for *P. brasiliensis* the lower limit was 2.

This may contribute to a better understanding on infantile infections caused by fungi, particularly in an geographical area where no previous references could be found.

RESUMO

Reatividade cutânea à histoplasmina e paracoccidioidina na população infantil do norte da Argentina

Para avaliar as idades em que os agentes etiológicos das micoses sistêmicas iniciam a infecção, testes cutâneos da histoplasmina e paracoccidioidina foram realizados em 344 crianças de ambos os sexos, com idades entre 2 e 15 anos. Foram selecionadas de amostra estatisticamente significante da população da cidade de General San Martín (Nordeste da Argentina). Os testes foram analisados 48 horas após a injeção e considerados positivos se uma endureção de 5 mm ou mais estivesse presente. Anticorpos circulantes foram também avaliados pela imunodifusão em gel de agar. A média de infecção para o *H. capsulatum* foi de 9,2% e pertencia às crianças de 4 a 14 anos, sem diferença significativa entre os sexos. Cinco crianças de 2 a 14 anos foram positivas para paracoccidioidina (1,6%). Nenhuma das crianças tinha anticorpos específicos nem sinais de micose ativa.

Resultados mostram que infecção pelo *H. capsulatum* pode ser encontrada a partir da idade de 4 anos, enquanto para o *P. brasiliensis* o limite inferior de idade foi de 2 anos. Estes achados podem contribuir para o melhor conhecimento sobre a infecção infantil por fungos, numa região geográfica onde não foram encontradas referências anteriores.

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REFERENCES

1. ACOSTA, L. – Paracoccidioidomycosis. In: GORODNER, J.O., ed. *Infectología*. Buenos Aires, López Libreros, 1989. p. 526.
2. ALBORNOZ, M. de – Paracoccidioidomycosis-infección. In: DEL NEGRO, G.; LACAZ, C.S. & FIORILLO, A.M., ed. *Paracoccidioidomycose. Blastomicose sul-americana*. São Paulo, Sarvier Edusp, 1982. p. 94.
3. BAVA, A.J.; NEGRONI, R.; ROBLES, A.M.; ARECHAVALA, A. & BIONDIN, M. – Estudio de algunas características epidemiológicas de 194 casos de paracoccidioidomycosis diagnosticados en el Hospital Muñiz. *Rev. argent. Micol.*, 13: 15-22, 1995.
4. BOGADO, M.M.; CAMARGO, N.P.; FRANCHISENA, E.M. et al. Encuesta con paracoccidioidina en la Prov. de Chaco (Rep. Arg.). *Rev. argent. Micol.*, 8: 21-23, 1985.
5. CARRILLO, L.; ROMANO, F.; ALTAMIRANO, F. & SISTI, P. – Pruebas cutáneas con histoplasmina en Jujuy. *Acta bioquím. clín. lat-amer.*, 18: 571-578, 1984.
6. DIOGENES, M.; GONÇALVES, H.; MAPURUNGA, A. et al. – Reações a histoplasmina e paracoccidioidina na serra do Pereira (Estado do Ceará – Brasil). *Rev. Inst. Med. trop. S. Paulo*, 32: 116-120, 1990.
7. DRUTZ, D. & GRAYBILL, J. – Enfermedades infecciosas. In: STITES, D.; FUDEMBERG, H.; STOBO, J. & WELLS, J., ed. *Inmunología básica y clínica*. Mexico, Manual Médico Moderno, 1985. p. 607-654.
8. DUNCAN, J.T. – Tropical African histoplasmosis. *Trans. roy. Soc. trop. Med. Hyg.*, 52: 468-474, 1985.
9. FAINBOIM, L. & SATZ, M.L. – *Introducción a la inmunología humana*. 3. ed. Buenos Aires. Edición del autor, 1995. p. 233-247.
10. FRANCO, M. – Host-parasite relationships in paracoccidioidomycosis. *J. med. vet. Mycol.*, 25: 5-18, 1986.
11. GIMENEZ, M.F.; ESCOBAR, E.; GUNIA, M. & GIMENEZ, M.M. – Vigilancia epidemiológica en paracoccidioidomycosis. *Rev. argent. Micol.*, 15: 19-21, 1992.
12. GONZALEZ MONTANER, L.J. – Histoplasmosis. In: GORODNER, J.O., ed. *Infectología*. Buenos Aires, López Libreros, 1989. p. 545.
13. GOODWIN, R.A. & DES PREZ, R.M. – Histoplasmosis. *Amer. Rev. resp. Dis.*, 117: 929-956, 1978.

14. KWON-CHUNG, K.J. & BENNETT, J.E. – **Medical mycology**. Philadelphia, Lea & Febiger, 1992, p. 596.
15. MARESCA, B.; ALI, A.; KOBAYASHI, G. & SACCO, M. – Incidence of histoplasmin skin test reactivity in Somalia. **Mycopathologia (Den Haag)**, **98**: 77-81, 1987.
16. NAIFF, R.; BARRET, T.; ARIAS, J. & NAIFF, M. – Encuesta epidemiológica de histoplasmosis, paracoccidioidomicosis y leishmaniasis mediante pruebas cutáneas. **Bol. Ofic. sanit. panamer.**, **104**: 35-50, 1988.
17. NEGRONI, R.; ELIAS COSTA, M.R.; BIANCHI, O. & GALIMBERTI, R. – Preparación y estudio de un antígeno celular de *P. brasiliensis* útil para pruebas cutáneas. **Sabouraudia** **14**: 265-273, 1976.
18. NEGRONI, R.; ELIAS COSTA, M.; GOLFERA, H. & ARECHAVALA, A. – Estudio de los antígenos de la fase levaduriforme de *H. capsulatum* para pruebas cutáneas. **Sabouraudia**, **17**: 155-161, 1979.
19. PAN AMERICAN HEALTH ORGANIZATION – **Manual of standardized serodiagnostic procedures for systemic mycosis**. Washington, 1975, p. 3-8.
20. PEIRETTI, Y. & CARRETERO, R. – Sensibilidad a la histoplasmina y a la coccidioidina en la población de Mendoza. In: **JORNADAS SOCIEDAD ARGENTINA DE MICOLOGIA**, 7, CORDOBA, 1975, p. 8.
21. RIPPON, J.W. – **Micología médica. Hongos y actinomicetos patógenos**. México, Interamericana-McGraw Hill, 1990, p. 411-567.
22. RODRIGUEZ DE MARENGO, Z.; ARAUJO, A.; NEGRONI, R. et al. – Encuesta epidemiológica de histoplasmosis en la Prov. de Salta. **Rev. argent. Micol.**, **2**: 5-10, 1979.
23. RUBINSTEIN, P.; NEGRONI, R.; ROBLES, A.M. & REY, D. – Catastro serológico en 193 casos de reactividad a la histoplasmina. In: **JORNADAS SOCIEDAD ARGENTINA DE MICOLOGIA**, 5., Rosario, 1971, p. 12.
24. SAN BLAS, G. – Paracoccidioidomycosis and etiologic agent *Paracoccidioides brasiliensis*. **J. med. vet. Mycol.**, **31**: 99-113, 1993.

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