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Pediatric hydatidosis in the south-east of the Buenos Aires province, Argentina

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ABSTRACT

Hydatidosis is a zoonosis produced by the metacestode *Echinococcus* spp. The aims of this research are: to contribute to the knowledge of pediatric hydatidosis in the south-east of Buenos Aires province, to study its evolution from 1993 to 2002 at the Regional Maternity and Pediatric Hospital "Dr. Victorio Tetamanti", to determine the strains involved and to discuss the importance of the disease. The clinical records of diagnosed and/or operated patients were reviewed with regard to the hydatid disease. The strain was determined by using PCRs with Eg1 121a/122a primers. Forty-four cases were analyzed. Fifty nine point one per cent of the patients were boys. The mean age was 8 SD=3.8 years. Sixty one point four per cent had urban residence. Ultrasonography was used in 61% of the cases. The hepatic location was most frequently seen and the liver/lung ratio was 1.25. Ninety point nine per cent of patients received surgical treatment. Albendazole was used in 52% of cases. The average hospitalization time was 11 days. The GI/G2 strain group was determined. This report is the first one of its kind in the studied region. The permanence of hydatidosis in the region depends on the natural transmission of the parasite in the absence of control and prevention measures. The health authorities should implement strategies of prevention and control in the study area.

Key words: pediatric hydatidosis; *Echinococcus granulosus*; epidemiology; zoonoses; Argentina

RESUMEN

Hidatidosis pediátrica en el sudeste de la provincia de Buenos Aires, Argentina. Los objetivos de este trabajo fueron: contribuir al conocimiento de la hidatidosis pediátrica en el sudeste de la provincia de Buenos Aires, estudiar su evolución desde 1993 hasta 2002, establecer la o las cepas involucradas y discutir la importancia de la enfermedad. Para ello se revisaron las historias clínicas de los pacientes pediátricos con diagnóstico de hidatidosis asistidos en el Hospital Interzonal Especializado Materno Infantil "Dr. Victorio Tetamanti" durante ese período. Se analizaron 44 casos, el 59,1% de ellos correspondió a varones. La media de edad fue de 8 años (SD=3,8 años) y el 61,4% de los niños afectados eran de residencia urbana. Se empleó ultrasonografía como método diagnóstico en el 61% de los casos. La localización hepática fue la más frecuente y la relación hígado/pulmón fue 1,25. El 90,9% recibió tratamiento quirúrgico. Se utilizó albendazol en el 52% de los pacientes. El tiempo de hospitalización tuvo una mediana de 11 días. Las cepas se determinaron mediante PCR con los cebadores Eg1 121a/122a. Se determinó la presencia de cepas del grupo G1/G2, dato informado por primera vez en humanos para la región de estudio. Se concluyó que la permanencia de la enfermedad en la región depende de la transmisión natural del parásito en ausencia de medidas de control y prevención. Por consiguiente, las autoridades de salud deberían implementar estrategias de prevención y control en dicha zona.

Palabras clave: hidatidosis pediátrica; *Echinococcus granulosus*; epidemiología; zoonosis; Argentina

INTRODUCTION

The cestode *Echinococcus granulosus* is the agent of hydatid disease, one of the major zoonoses affecting man as well as domestic and wild animals. This parasite requires two mammalian hosts for the completion of its life cycle. The hermaphroditic adult commonly develops in the dog's intestine, whereas the metacestode larva (hydatid cyst containing protoscoleces produced by asexual multiplication) develops in the viscera of many ungulates and man. The hydatid disease has spread to all continents. South America is one of the most affected regions with 2,000 new human cases per year (7).

In Argentina, hydatid disease is widespread, being *Echinococcus granulosus* the involved species. Between the years 1997-2001, 294 new cases were reported, with an annual incidence rate in Buenos Aires province of 0.3/100 000 in the year 2000 and 0.4/100 000 in 2001 (27). In this province, located in the Pampa's region, there are two areas: a low endemic area in the north and a high endemic area in the south. Forty seven point five per cent of the rural population inhabits the high endemic area, where there is one dog per inhabitant (8).

E. granulosus occurs as a series of genetic variants or strains which differ in a wide variety of criteria that impact on the epidemiology, pathology and control of cystic hydatid disease (35, 36). At the present time, 10 distinct genotypes have been identified within *E. granulosus* (G1: the common sheep strain, G2: the Tasmanian sheep strain, G3: the buffalo strain, G4: the horse strain, G5: the cattle strain, G6: the camel strain, G7: the pig strain, G8: the cervid strain, G9: the genotype identified in a human cyst in Poland and G10: the Fennoscanadian cervid strain (7, 24, 37). In Argentina, 5 genotypes have been identified: G1, G2, G5, G6 and G7 (32) and, particularly in Buenos Aires province, genotypes G1 in human, cattle and pig; G6 in human and G7 in pig (4, 11, 18, 31).

Epidemiological studies of hydatidosis carried out in the south-eastern region of Buenos Aires province, showed a worrying situation in health centres in Mar del Plata city, due to the great quantity of diagnosed cases during 1992-2003 (4, 6, 9, 10). The disease is endemic in this region since, besides the human cases, there is a prevalence in cattle and pig of between 12% and 16%, respectively. Furthermore, *E. granulosus* antigens were also detected in dogs' feces in fields and squares in General Pueyrredón district (4-6).

As a result, considering that at present, the region is not included under the activities of the province's control programme, the hydatid disease is a great public health concern in General Pueyrredón district and neighbouring areas.

The main objectives of this study were to contribute to the knowledge of pediatric hydatidosis in the south-east of Buenos Aires province; to study its evolution at the Regional Maternity Pediatric Hospital "Dr. Victorio Tetamanti" (HIEMI) in Mar del Plata city during 1993-2002; to determine the *E. granulosus* strains involved and to discuss the importance of the disease in the population studied.

MATERIAL AND METHODS

Studied population

The study area belongs to Mar del Plata city, the principal city in General Pueyrredón district, located on the seacoast in the south-east of Buenos Aires province, Argentina (38° S/57°33´ O).

A retrospective and descriptive study was carried out at the HIEMI, which is a 300-bed hospital and a reference centre for the 16 districts that are included in the 8th Sanitary Region of the Province.

Clinical records of all the patients who had been diagnosed or had undergone surgery for hydatidosis between1992 and 2002 were analyzed. A data file was completed for each patient containing personal information, medical background, diagnostic methods and the number and location of cysts present. All the information was analyzed with the Epi 6 microcomputer software for handling epidemiologic data (version 6, CDC, Atlanta, U.S.A). The Epitable software was used in order to calculate χ^2. The Kruskal Wallis test was used to compare the mean diameters for the diagnosed cysts. Statistical significance was assessed at $p \leq 0.05$.

In order to determine the patient's origin, their place of residence was considered, as well as any time of their lives spent in a rural area.

E. granulosus strains determination

Total *E. granulosus* genomic DNA was obtained from 70% ethanol-preserved protoscolex or germinal layer isolates by conventional techniques (17). Parasitic material from three cysts was provided by the Service of Surgery (HIEMI). Strain determination was carried out using molecular markers.

Primers described by Abbasi *et al.* were used to amplify repeated fragments from the *E. granulosus* genome and to distinguish between strains or groups of strains by comparison of the bands obtained in the PCR with typified controls (1, 29). PCR conditions were the same as those described by Naidich *et al.* (29). PCR products were separated in 1.5% agarose gels (25). The bands were visualized with ethidium bromide solution under UV.

RESULTS

During this descriptive and retrospective study, 44 hydatid cases were identified and analyzed at the HIEMI. The frequency by sex was similar (boys 59.1% tendency X^2 p=0.23). The distribution by age is shown in Figure 1. It ranged between 2 and 15 years with a mean age of 8 SD=3.8 years and a median of 7 years. Ninety three point eight per cent of the children aged > 5 years old went to school.

The distribution of cases according to place of residence showed that 61.4% of the patients had urban residence, 29.5% had rural residence and 4.5% had peripheral residence. Four point six of the cases could not be

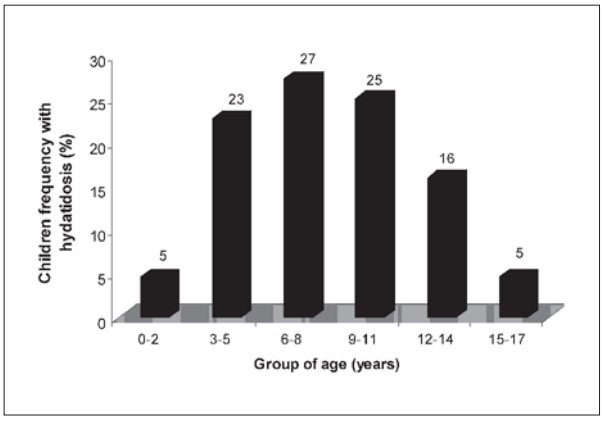

Figure 1. Distribution of the 44 hydatidosis cases according to age.

determined. Table 1 shows the distribution of cases according to origin and age-groups. The largest number of cases belongs to General Pueyrredón district (36.4%), followed by Mar Chiquita and Maipú (Table 1). All the children lived within the 8th Sanitary Area. New cases were reported during all the years of study (except for 1993) (Figure 2).

The methods used for diagnosing the disease are shown in Table 2. Ultrasonography alone or combined with other diagnostic techniques was the chosen method for 84.1% of the patients. In 50.0% of the cases, the computerized axial tomography in combination with ultrasonography was used. Immunological double-diffusion techniques (DD5), latex or immunofluorescence tests were used in 43.2% of the patients.

The cyst location is shown in Table 3. The average ratio liver/lung infection was 1.25. Among age groups, the pulmonary location prevailed in the 12-14 year old group whereas in the 6-8 year-old and 9- 11 year-old groups, the hepatic location prevailed (Table 3).

Cyst size average was 6.2 SD = 2.8 cm (2.5-18 cm; n = 49). Statistically significant differences were not found when comparing cysts diameter and location, the averages were 6.4 SD = 2.9 cm (3-12.5 cm; n = 15) and 6.1 SD

Table 1. Distribution according to procedence and age-groups for the 44 hydatid cases diagnosed at HIEMI.

District	Groups of age (years)						N	%
	0-2	3-5	6-8	9-11	12-14	15-17		
General Pueyrredón							16	36.4
Urban		4	1	1	3	1	10	
Rural		1		1	1		3	
Peripheral	1			2			3	
Mar Chiquita							7	15.9
Urban		1	2	1			4	
Rural		1	1		1		3	
Maipú							4	9.1
Urban			1	1			2	
Rural	1			1			2	
General Madariaga							3	6.8
Urban					1		1	
Rural		1	1				2	
Balcarce							3	6.8
Urban			1	1			2	
Rural				1			1	
Ayacucho							2	4.5
Urban		1					1	
Not indicated						1	1	
Lobería							2	4.5
Urban			1				1	
Not indicated			1				1	
Necochea							2	4.5
Urban		1			1		2	
General Alvarado							1	2.3
Urban				1			1	
General Guido							1	2.3
Rural			1				1	
San Cayetano							1	2.3
Urban			1				1	
Tandil							1	2.3
Urban			1				1	
Villa Gesell							1	2.3
Urban				1			1	
Total	2	10	12	11	7	2	44	100

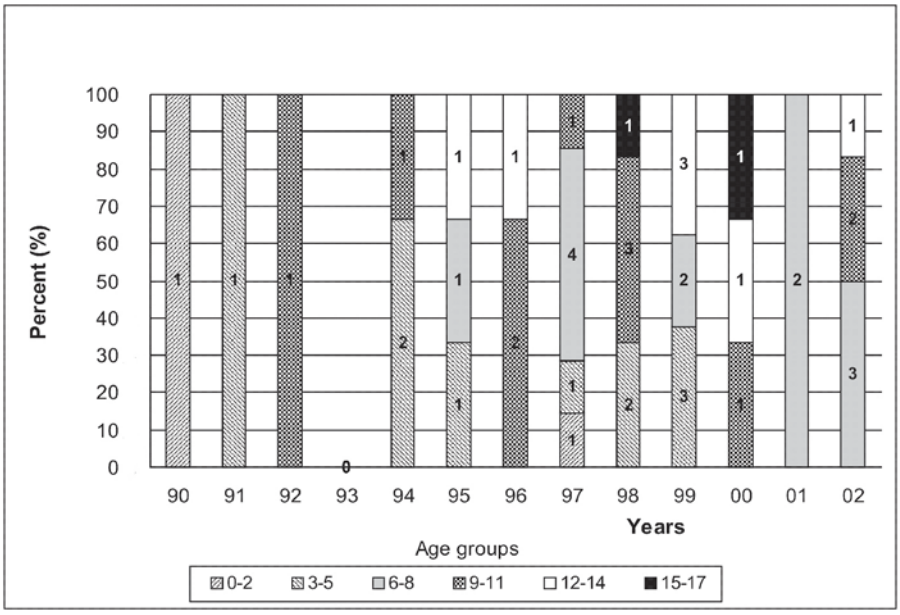

Figure 2. Percentage of patients with hydatidosis in each age-group in the period of study. The numbers in the bars indicate number of cases.

Table 2. Diagnostic techniques.

Type	Diagnostic N°	%
CT + US + other	16	36.4
US + other	10	22.7
CT + US	6	13.6
CT + other	6	13.6
US	5	11.4
RX + other	1	2.3
Total	44	100

US: ultrasonography, CT: computed tomography, RX: X Rays, other: includes the combination of one or more of the following techniques: RX, DD5, indirect haemagglutination or immunofluorescence.

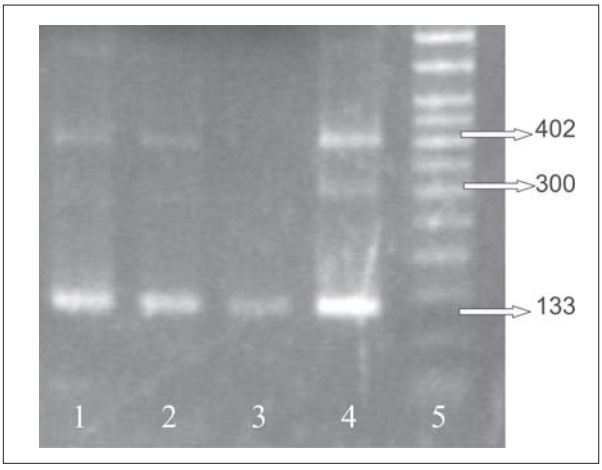

Figure 3. Electrophoresis in 1.5% agarose gel showing the PCR products with Eg1 121a/122a primers. Lanes: (1) Sample 1, (2) Sample 2, (3) DNA pattern from G5 strain, (4) DNA pattern from G1/G2 strain group, (5) Molecular size marker 50 bp.
Legend: The arrows show bands with their respective bp size. Lanes 1 and 2 corresponded to the G1/G2 pattern.

= 2.9 cm (2.5-18 cm; n = 34) for pulmonary and hepatic locations, respectively. No significant differences were registered for cyst diameters according to age-groups for hepatic and pulmonary locations. Furthermore, comparison per locations for every age-groups was not significantly different.

The most frequent signs and symptomatology in children with hepatic hydatidosis were: abdominal pain (40%), abdominal tumor (30%) and vomiting (15%); coughing (50%) and vomiting (12.5%) in patients with pulmonary hydatidosis. With regard to the amount of cysts, 24 patients (54.5%) presented only one cyst, 13 patients (29.5%) presented two, 4 (9.1%) three and 3 (6.8%) presented multiple cysts.

Out of the 44 patients, 40 (90.9%) underwent surgery. The mean number of operations was 1.04 per patient and ranged between 1 and 2 operations. Five patients were re-infected (11.3%, n = 5/44). Among the patients under-

Table 3. Distribution for the 44 hydatid cases by group of age, cyst location and liver and lung relation.

Group of age (years)	Location										Relation
	Liver		Lung		Lung-liver		Liver-neck		Total		Liver/lung
	N°	%	N°	%	N	%	N°	%	N°	%	
0 – 2	2	100	0	0	0	0	0	0	2	100	–
3 – 5	4	40	2	20	4	40	0	0	10	100	2
6 – 8	5	41.7	5	41.7	1	8.3	1	8.3	12	100	1
9 – 11	6	54.5	3	27.3	2	18.2	0	0	11	100	2
12 – 14	2	28.6	5	71.4	0	0	0	0	7	100	0.4
15 – 17	1	50	1	50	0	0	0	0	2	100	1
Total	20	45.5	16	36.4	7	15.9	1	2.3	44	100	1.25

going surgery, 21 patients received pre- and post-surgery medical treatment, with albendazole (52.5%, n = 21/40). Of the 4 non-operated children, two received pharmacological treatment with albendazole whereas the other 2 children were not treated.

As regards the length of hospital stay, forty-three patients (97.7%) were hospitalized for different periods ranging from 1 to 63 days. The mean was 11 days, and there was a displacement of the curve to the right (Per 25 = 7, Per 75 = 19.5) (data not shown). During the study period, all children were still alive.

With respect to other cases of the disease in the family, it was known that 20% of 35 children had affected relatives. The information about the rest of the patients is unknown.

The strain group determined from DNA analyses was (G1/G2) (sheep strain/ Tasmanian sheep strain) (Figure 3). The cysts belonged to a 6-year-old girl and the other two belonged to a 6 and 11-year-old boys. All children had urban residence in General Pueyrredón, Maipú and General Alvarado, respectively.

DISCUSSION

This study describes pediatric hydatidosis cases at the HIEM during 10 years. The existence of very young children with hydatidosis and the new cases registered every year allow us to assume that the disease is being actively transmitted in the south-east of Buenos Aires province, because these young children have little probability of moving to places traditionally identified as endemic. It is also evident that the Programme for Control and Prevention for the province is not being implemented in the region under study.

At a certain period of time, the incidence values can increase or decrease and they might be used as indicators of the efficiency of control programmes. This fact is shown by Larrieu *et al.* and Jiménez *et al.* in their works when they report the decrease in hydatidosis incidence in the province of Río Negro (Argentina) and in La Rioja (Spain), respectively (16, 20). The abovementioned indicates the progress achieved in control programmes that were designed for and are being implemented in these regions. An increase in incidence of pediatric cases was registered in Kyrgystan and Kasakhstan, two countries that had belonged to the Soviet Union, and had experienced important political changes. Cold-storage plants with veterinary supervision had become bankrupt, rural dog treatment together with the big fields with killing of animals and official disposal of entrails or carcasses had been suspended. These factors had contributed to the re-emergence of the disease (34, 38, 39). The authors propose that the increase in the values is related to the changes that had ocurred after the independence of these countries.

The hydatid disease is habitually considered a rural disease because of the characteristics of the parasite life cycle which involve domestic herbivores and omnivores (sheep, cattle, pigs, dogs, etc.) (26). Nevertheless, in this study the highest percentage of patients live in urban areas. The reason for that could have been a population movement from farms to cities (15). Even so, it is also possible that urban people might have been in contact with *E. granulosus* eggs in the city. Previously we have reported the presence of *E. granulosus* antigens in soil samples from squares and dogs' feces in Mar del Plata city (4, 5).

With regard to diagnostic methodologies, ultrasonography (US) alone or in combination with other techniques, such as computer tomography (CT) and immunological techniques were the most used. The US is widely recognized for its low operative cost, its speed in obtaining results, and its high sensibility and specificity for the detection of this disease (2, 14, 21, 22). In this health centre it was generally combined with other techniques.

The liver/lung ratio found in this study is lower than that reported by Larrieu and Frider, who in a bibliographi-

cal review considering 9,770 hydatidosis patients from Uruguay, Argentina, Tanzania, New Zealand, Israel, Jordan, Australia, Bulgaria, Turkey and Iran informed a general ratio of 2.5:1, which included values ranging from 0.89:1 to 12:1 (23).

In many cases, the resistance shown by the liver tissue surrounding the cyst determines a slow growth or even avoid growth for many years. Therefore, this explains the high percentage of hepatic cysts that remain asymptomatic while supporting a balance parasite/host during the host's life (12). On the other hand, the lungs show low-resistance to the growth of the hydatid cyst due to their elasticity. This situation allows a proportional increase of the cyst size, and its consequence is the manifestation of clinical symptomatology in a great percentage of the total cases (33). Studies of longitudinal follow-up by US in patients with asymptomatic hydatid cysts, have demonstrated that in a period of 14 years, 67% of the carriers persist without developing symptoms (13). In general, the registered symptoms refer to the cyst location, which is unspecific for the disease (12). The symptomatology found in this work is unspecific and frequent at pediatric age and also occurs in many clinical entities. This situation might require an imaging study arriving at different diagnoses.

The major frequency of unique cysts detected in this study matches those mentioned by other authors (12, 30). Cyst diameter does not show association with cyst location and patient's age, which coincides with the characteristics of the disease as: the viability of eggs when the definitive host consumes them, the host´s resistance to cyst development, the rate of symptomatology development and its subsequent detection. In agreement with this, the development of the cyst can be very fast (5 to 10 cm in a few years), and generate serious symptoms including the carrier's risk of death. However, it can behave in a benign way, growing less than 2 to 7 cm and not producing major symptoms (13, 19, 21).

Regarding treatment of the disease, this study shows that surgery was the chosen treatment in 91% of the cases and that the use of the pre- and post-surgical chemotherapeutical treatment is relatively high in this health centre. The hydatid disease has traditionally been considered to require surgical resolution. Nevertheless, alternative methods have been developed over the last years like chemotherapeutical treatment with albendazole at a dose of 10 mg/kg during 120 days (3, 19, 23, 28).

The results obtained in the present study regarding therapeutic treatment and length of hospital stay, evidence the high cost that the disease implies for the institution (hospital stay, pre- and post-surgical costs, medical costs, etc.) and social costs such as long stays in surgical centres, often far away from the host´s address, and loss of physical capacities (19). The fact that 20% of patients have relatives affected by the disease should indicate that there are cultural habits in the families that promote the transmission of the disease.

The determination of strain group G1/G2 (sheep strain/Tasmanian sheep strain) of *E. granulosus* by molecular techniques is the first one reported in the south-east region of Buenos Aires province with regard to human cases. This contributes to our previous reports where we found hydatid cyst G1 strain in cattle and pigs from this region (4, 5, 11). Given the number of new cases and the vulnerability of the youngest children observed over the study period, added to the surveys previously carried out in other health centres in the same region, we can confirm that this zoonosis is a public health concern in General Pueyrredón district, in the 15 remaining districts that belong to the 8th Sanitary Region and in the southeast region of Buenos Aires province (4-6, 8-10). This situation shows that the maintenance of the disease in the region depends on the natural transmission of the parasite, in the absence of control and prevention measures. Therefore the health regional and municipal authorities possess basic information to implement strategies of hydatidosis prevention and control in the region of study.

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