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Seroepidemiology of fascioliasis in school children in Mexico City.

Original Article

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SUMMARY.

The aim of this investigation was to determine the prevalence of *Fasciola hepatica* by the indirect haemaglutination test (IHAT) and by coprological and epidemiological analyses of samples from asymptomatic children in Mexico City. A blood sample and three feces samples were collected from 331 children between 6 and 13 years old. The immunological analysis was effected by IHAT using raw extract of the adult worm *Fasciola hepatica* antigen. Fecal material was analysed using a simple sedimentation series of three. Six epidemiological variables were analysed.

Results. Five sera were positive; seroprevalence was 1.5%. The coprological study was negative to *F. hepatica*. No statistical relationship between seropositivity and consumption of vegetables (χ^2=0.42, p=0.84) was found. However, a statistically significant relationship (p=0.002) between parasites and age was found. This finding coincides with the age of the seropositives. No association between seropositivity and housing, drinking water and drainage, water supply, or consumption of raw vegetables (χ^2=6.0, p=0.11) was found; the association with the place of origin of parents was also negative (χ^2=1.85, p=0.17).

Conclusions. Although Mexico City is not an endemic zone and the proper conditions for fascioliasis development are not present, it is important to recognize that the existence of residents from multiple origins represents risks for this, as we were able to show. Specifically, school age is the identified risk factor.

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Key words: fascioliasis, *Fasciola hepatica*, seroepidemiology, children.

RESUMEN.

Seroepidemiología de la fasciolosis en escolares de la Ciudad de México.

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El objetivo de esta investigación es conocer la prevalencia de infección por *Fasciola hepatica*, mediante examen serológico, coprológicos y epidemiológico en una muestra de niños aparentemente sanos residentes en la delegación Coyoacán de la Ciudad de México. En la búsqueda de anticuerpos anti-*Fasciola hepatica*, se analizaron los sueros de 331 niños de 6 a 12 años de edad mediante la prueba de hemoaglutinación indirecta (HAI). Se realizó examen coprológico de sedimentación simple en serie de tres a todos los participantes. El estudio epidemiológico incluyó 6 variables.

Resultados. Cinco de los niños fueron seropositivos a *F. hepatica* con una seroprevalencia de 1.51%. El estudio coprológico no reportó huevos de *F. hepatica*. El análisis estadístico entre seropositividad y consumo de berros, alfalfa, y lechuga no resultó significativo ($\chi^2 = 0.42$, $p = 0.84$). Sin embargo se encontró una relación estadísticamente significativa entre los parásitos y la edad de los sujetos. Este hallazgo coincide con la edad de los seropositivos. No se encontró asociación entre seropositividad y vivienda, agua potable, drenaje, abastecimiento de agua o consumo de verduras crudas,($\chi^2=6.00$,$p = 0.11$) la asociación con el origen de la familia también fue negativo ($\chi^2= 1.85$, $p = 0.17$).

Conclusiones. Aunque la Ciudad de México no es una zona endémica, y no tiene características adecuadas para la fasciolosis es importante reconocer que la existencia de residentes de origen múltiple presenta riesgos como pudimos demostrar y es precisamente en la edad escolar la que se tiene como factor de riesgo demostrado.

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Palabras clave: fasciolosis, niños, *Fasciola hepatica*, seroepidemiología.

INTRODUCTION.

Fascioliasis is a parasitic zoonosis caused by the trematode of Fasciola and is transmitted through the food chain. It affects the liver and bile ducts of herbivorous animals and, occasionally, of man (1). It is endemic in sheep, goat, and cattle rearing countries where it is the cause of considerable economic losses (1-3). Human fascioliasis, on the other hand, is more limited. The symptoms depend on the number of parasites and their location in the body. They may vary from life-threatening to asymptomatic (1-4).

Man acquires fascioliasis by the accidental ingestion of the metacercariae present in water or infected food. When these reach the duodenum, they deposit their cysts and liberate a young parasite which perforates the wall of the intestine by enzymatic action and it migrates through the peritoneal wall to the liver. It establishes itself in the bile ducts where it remains until it becomes an adult and begins laying its eggs at between two and four months after the beginning of the infection (1-5).

The prevalence of this disease in man in Mexico is still unknown because few studies have been carried out (1). Diagnosis depends on the identification of the parasite or its eggs in feces, or in bile obtained by a duodenal probe, or by the detection of antibodies in the blood (1-8).

Given the lack of epidemiological studies of this disease in Mexico, the aim of this investigation was to discover the prevalence of infection by *Fasciola hepatica* and the risk factors associated with the disease. To this end, immunological, coprological, and epidemiological studies were carried out on a group of asymptomatic children in the Coyoacan area in Mexico City.

MATERIAL AND METHODS.

The Coyoacan area is situated between 19º 22'N and 19º 16' S latitude and 99º 06'E and 99º12'W longitude. It is at 2240 m above sea level. Its surface area is 5400 hectares, and is completely urbanized. It has a temperate subhumid climate: minimum temperature 8ºC, maximum 24ºC with rain in summer and autumn (9). A primary school was selected whose pupils were the children of

parents born in states in Mexico where cases of human fasciola had been reported. Meetings were held at the school at which information about parasites was given to teachers, pupils, and parents. The aims of the study were explained and an invitation to participate was issued. Three clean flasks, labeled with the name, grade and group of the pupil, were provided for each participant to facilitate the collection of fecal material during three consecutive days. The samples were conserved in 10% formol using an isotonic saline solution at 0.85%

A questionnaire was applied in which general information about the children and the possible presence of epidemiological factors associated with *F. hepatica* was solicited. Data collected included: identification number, name, age, sex, school grade, geographical origin of the parents, present address, characteristics of the dwelling, presence or absence of piped water and main drainage. Family diet (frequency of consumption of raw vegetables such as cress (*Nasturtiun officinale*), lettuce (*Lactuca virosa*), alfalfa (*Medicato sativa*) or garden vegetables such as chard, celery, broccoli, coriander, spinach, and parsley) and sources of supply such as a covered market, a weekly market ("tianguis") or a supermarket were also taken into account.

The samples were taken in the infirmary of the school using vacutainer syringes. 5ml of blood were obtained from the vein of the forearm of each participant, leaving it to coagulate at room temperature before being taken to the Immunoparasitology Laboratory of the Department of Microbiology and Parasitology of the Faculty of Medicine of the National University of Mexico (UNAM) where the immunological study was to be carried out.

Adult worms of *F. hepatica* were collected by dissecting the bile ducts of the livers of cattle infected with the trematode. The antigen was obtained using the saccharose-acetone antigen extraction technique (10).

The positive control serum was obtained by immunizing a group of 5 male New Zealand rabbits weighing 2500g with the complete antigen of *F. hepatica*. Using the IHAT test, their immunological development was monitored for specific antibodies. When dilution a title of 1: 4096 was reached, blood was extracted to obtain the serum. The negative control serum was obtained in the same way from rabbits with a negative reaction to the antigen of *F. hepatica*.

The sera were processed using the (previously standardized) complete antigen of *F. hepatica* by means of IHAT (11-12). The reading was carried out two hours after the test. In the light of previous experience, positive dilution was considered to be the reaction which showed dilution titles of 1:32 or higher.

Analysis of fecal material The feces were processed in the Parasitology Laboratory of the Metropolitan University (UAM), Xochimilco. All the samples were analysed using the method of simple sedimentation. Samples stained with lugol were observed with a Carl Zeiss microscope using 10x and 40x magnification.

The results of the immunological and coprological analyses together with the epidemiological questionnaire were processed and codified. A data base was constructed using SPSS (Statistical Package for the Social Sciences).

The consent of the parents or tutors of the children was obtained. The aims, benefits (free diagnosis) and risks of the investigation were explained.

RESULTS.

The study population consisted of 331 children, 51.3% male and 47.7% female, between the ages of 6 and 13. Their average age was 7.8 and the median and mode was 7 (see Table 1). They were divided for the purposes of the investigation into 3 age groups, of which the largest was that from 6 to 7 years which contained 185 individuals (55.6%). In Table 2 the distribution of the sample

Table 1
Distribution of the study population by age and sex

Age Group	Males	%	Females	%	Total	%
6 - 7	96	55.5	88	55.7	184	55.6
8 - 9	50	28.9	43	27.2	93	28.1
10 - 12	27	15.6	27	17.1	54	16.3
Total	173	100.0	158	100.0	331	100.0

by age and reaction titles is presented. It will be noted that only 5 sera showed positive titles, with a seroprevalence of 1.51%. Of these, three sera corresponded to the children between 6 and 7 years of age. The dilution titles in these cases were 1:32, 1:64, and 1:256. The average age of these children was 6.6 years.

Although no significant relationship (p=0.84) was found between the consumption of cress, lettuce, and alfalfa and the infection (see Table 3) it should be observed that, of the 5 children shown to be seropositive to *F. hepatica*, four had consumed these vegetables.

Perhaps the most interesting finding of this investigation is to be found in Table 5. A statistically significant relationship (p=0.002) between the species of the identified parasites (see below) and the age of the study population was found. Moreover, these data coincide with the age of those subjects who were found to be seropositive (6-7 years old).

In Table 4 the distribution of the study population is shown by age, gender, place of origin and seropositivity. It can be observed that 50.2% of the children stated that they were natives of Mexico City and 49.8% from different states in the interior of the Republic. If the places where seropositivity, place of origin, gender, and age intersect are examined, it will be noted that, of the seropositive subjects, only one –a male- is native of Mexico City. The other four are girls who are the daughters of immigrants from other parts of the country.

In the statistical analysis of the epidemiological data, associations between seropositivity to *F. hepatica* and a series of

Table 2
Distribution by age group and seropositivity to *F. hepatica* of the children analyzed by IHAT

	Dilution titter in IHAT							
Age	Negative	1:2 – 1:4	1:8	1:16	1:32	1:64	1:256	Total
6 - 7	4	113	49	13	3	1	1	184
8 - 9	2	68	21	2	0	0	0	93
10 -12	0	28	22	4	0	0	0	54
Total	6	209	92	19	3	1	1	331

Table 3
Distribution by seropositivity and consumption of vegetables (cress, lettuce, and alfalfa)

Consumption of vegetables	Seropositives		Seronegatives		
	No.	%	No.	%	TOTAL
Yes	4	80	248	76.1	252
No	1	20	78	23.9	79
TOTAL	5	100	326	100	331

N. B. The χ^2 calculated is: 0.42, with a p= 0.8

Table 4
Distribution of the study population by place of origin, age, sex, and seropositivity to *F. hepatica*.

State (of the Republic)	Mexico City				Other states				Total
	Seropositive	%	Negative	%	Seropositive	%	Negative	%	
Sex									
Males	1	100.0	80	48.5	0	0.0	87	54.0	168
Females	0	0.0	85	51.5	4	100.0	74	46.0	163
Total	1	100.0	165	100.0	4	100.0	161	100.0	331
Age									
6 - 7	1	100.0	87	52.7	4	100.0	92	57.1	184
8 - 9	0	0.0	56	33.9	0	0.0	37	23.0	93
10 - 12	0	0.0	22	13.3	0	0.0	32	19.9	54
Total	1	100.0	165	100.0	4	100.0	161	100.0	331

other variables were hypothesized. However, no significant correlation was found to link it with kinds of dwelling, presence or absence of piped drinking water and main drainage, consumption of raw vegetables, source of supply of diet or place of origin of parents.

The coprological analysis did not find *F. hepatica* eggs. However, it seems important to mention that 37% (122 cases) of the study population (n=331) showed at least one kind of parasite. Of these, 7% harbored between 2 and 3 species and 1 individual 4. In this subpopulation (n=122) the parasites that were found were: *Giardia duodenalis* 24%, *Ascaris lumbricoides* 16.7%, *Entamoeba histolytica, Hymenolepis nana* 4.7%, and *Enterobius vermicularis* 2% (see Table 6). The statistical relationship between these parasites and seropostivity was not significant (χ^2=0.56, p=0.45).

Finally, Table 5 shows the statistical relationship between the parasites found and the age of the subject.

DISCUSSION.

It is difficult to diagnose fascioliasis because of its infrequent occurrence and the fact that its symptoms are easily confused with those of other illnesses. Excellent examples of this may be seen in the outbreaks of fascioliasis which occurred in Cuba in 1983 and Iran in 1989. These were initially diagnosed as larva migrans visceral caused by *Toxocara canis*. In both countries, diagnosis combined immunological tests with clinical and parasitoscopic studies (1).

The results in this study contribute to the epidemiological information available because studies of the disease in Mexico are rare. It was

Table 5
Distribution of the study population who showed the presence of parasites in the analysis of feces.

	IHAT				
	Positive	%	Negative	%	Total
Parasited					
6 - 7	55	45.1	129	61.7	184
8 - 9	48	39.3	45	21.5	93
10 - 12	19	15.6	35	16.7	54
Total	122	100.0	209	100.0	331

N.B. the χ^2 calculated is 0.126 with p=0.002

Tabla 6
Percentage distribution of the 122 children harboring parasites (who consume vegetables) by age group

Parasite	Age 6 - 7	8 - 9	10 -12	Total
A. lumbricoides	8.0	7.3	1.3	16.7
E. coli	18.7	20.0	7.3	46.0
E. histolytica	0.0	3.3	1.3	4.7
E. vermicularis	0.7	0.7	0.7	2.0
Enteromonas. sp	0.0	0.7	0.0	0.7
G. lamblia	11.3	8.7	4.0	24.0
H. nana	2.7	0.7	1.3	4.7
I. büestchlii	0.0	0.7	0.7	1.3
Total	41.3	42.0	16.7	100.0

N.B. The χ^2 calculated is 0.56 with a p=0.45

disappointing to find that the correlation with particular vegetables or place of origin was not statistically significant. A study in San Cristobal de las Casas, Chiapas (in the south of Mexico) found a seropositivity of 5.75% (13). This is a city which has many features in common with Mexico City.

The risk of acquiring fascioliasis in Mexico City persists since local market gardening is carried out in areas where the system of irrigation uses untreated water and the distribution points for the vegetables are weekly markets (tianguis) which are not subject to health control. The city also has quite a large area of lakes and canals within its limits where cress and other vegetables are cultivated. Zones where *F. hepatica* are endemic are only 30 miles from the city.

In areas where it is endemic infantile fasciolasis is mostly found in children between 8 and 11 years of age with a marked preference (9 to 1) for boys (7, 14, 15). In this study, a slightly greater degree of serological reaction to the antigen of *F. hepatica* was found in girls (in a proportion of 4 to 1). Here the age group with greatest seropositivity was that of the seven year olds. The seropositive frequency reported here (1.51%) is similar to that found, for example, in Region VII of Chile (16) and is much less than that found is areas where the disease is endemic (10%-15% or more). (15, 17, 18)

Immunological fascioliasis tests are of great usefulness for the collection of epidemiological information. They are also important as auxiliary tools in clinical diagnosis as they can detect specific antibodies with great precision. This is the case, above all, when they are carried out during the periods of invasion or migration of the parasite, when the symptoms shown by the patient may lend themselves to various interpretations. Obviously, when fascioliasis is well established in the patient, these tests (together with coprological analysis) are the basis of a more trustworthy diagnosis.

As for the negative results of the coprological studies, these are unsurprising since positive serological analysis and negative coprological studies have been reported frequently (19). Nevertheless, deficient hygiene is amply shown by the number and variety of cysts and eggs of other intestinal species found in this population.

CONCLUSIONS.

Mexico City -highly urbanized- is not an ideal environment for the transmission of fascioliasis. However the possibility of contracting the infection somewhere close to the city cannot be ruled out. Therefore, seroepidemiological studies should be carried out both in the lake area of the city and in

the neighboring states. Above all, the investigations should take into account the possible infection of particular age groups because the present study seems to suggest that this might be a fruitful line of research. It goes without saying that periodic coproparasitic analyses and preventive hygiene measures are of utmost importance.

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