

SEROEPIDEMIOLOGICAL INVESTIGATION OF FASCIOLIASIS IN NORTHERN IRAN

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ABSTRACT

Following an outbreak of fascioliasis in Anzali Harbor in 1988, 452 blood samples were collected using a multistage sampling method for hematological and serological studies. After determination of eosinophilia in the samples, antifasciolia antibodies in the sera were also determined using enzyme linked immuno sorbant assay (ELISA) and counter-current immuno electrophoresis (CCIE).

64.2% of the samples were collected from women and omitting the age effect and $P < 0.05$, the rate of infection was 1.16 times more in women than in men as shown by hypereosinophilia and ELISA test results. Age distribution of seropositivity in both sexes with $p < 0.001$ was statistically significant and the highest rate of seropositivity was seen among the below 20 age group in both sexes.

The highest expected rate of seropositivity (24.12) was observed in location six (Taleghan area), but an average and more uniform distribution was seen in other locations.

50% of the samples using ELISA techniques and 34.95% using CCIE were positive indicating that point prevalence of the disease was in Anzali Harbor.

It must be noted that all antibody positive samples showed hypereosinophilia as well and that ELISA technique was 52.6% more sensitive compared to CCIE.

Of the 50% antibody positive cases, 13.7% had only IgG, 26.5% both IgG and IgM and 37.6% only IgM showing the chronic, subacute and acute condition of the disease respectively. The remaining 22% of the cases were IgA positive. In this investigation an incidence rate of 19% was obtained using ELISA technique which was indicative of a fasciolia epidemic in the region.

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INTRODUCTION

Fascioliasis is a widespread parasitic zoonosis of many parts of the world especially where animal hus-

bandry is prevalent. Although in endemic areas, it is widely distributed among sheep and cattle, human infection is usually sporadic. Nevertheless in some parts of the world especially in seasons of heavy rain fall

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Table I. Distribution of seropositivity for fascioliasis cases in Anzali Harbor with different techniques according to age groups and sex (1989)

Sex	men				women				Total			
Method age group	ELISA	CCIE	Eos-5	Total	ELISA	CCIE	Eos-5	Total	ELISA	CCIE	Eos-5	Total
0-9	19	2	10	28	19	10	10	29	35	12	20	57
	26.03	4	16.4	17.28	12.42	9.26	9.26	10	16.81	7.59	12.12	12.61
10-19	28	25	26	73	46	24	28	79	74	49	54	162
	38.36	50	42.6	45.06	30.06	22.22	26.92	27.24	32.74	31.02	32.73	33.63
20-29	4	6	8	16	33	14	19	50	37	20	27	66
	5.5	12	13.1	9.87	21.57	12.96	18.27	17.24	16.38	12.66	16.36	14.6
30-39	9	5	8	18	30	26	18	54	39	31	26	72
	12.3	10	13.1	11.1	19.61	24.07	17.31	18.62	17.28	19.62	15.78	15.93
40-49	5	3	3	10	11	14	9	31	16	17	12	41
	6.85	6	4.92	6.2	7.19		8.65	10.69	7.08	10.76	7.27	9.07
50.	8	9	6	17	14	20	20	47	22	29	26	64
	10.95	18	9.84	10.49	9.15	18.52	19.23	16.21	9.73	18.35	15.76	14.16
Total	73	50	61	162	153	108	104	290	226	155	165	452
	100	100	100	100	100	100	100	100	100	100	100	100

infestations of epidemic proportion have occasionally been reported, such as a 500 case outbreak in Lyon (France) in 1968 and another one in Wales and West U.K. with 49 cases.

There is no history of fasciolia epidemics in Iran and only sporadic cases have been previously reported.^{1,2} In 1988 an epidemic of this disease was reported in Gilan (Anzali Harbor) with a large number of local citizens and travellers to the area attending hospitals and consulting physicians with leucocytosis and hypereosinophilia.

In a preliminary investigation 100 people were tested; all seropositive by both ELISA

(enzyme linked immuno sorbant assay) and CCIE (counter current immuno electrophoresis) methods and 75% of the 100 people were shedding fasciolia egg.³

This study was undertaken in order to investigate the sero-epidemiology of fascioliasis in the Gilan province which occurred in 1988.

MATERIALS AND METHODS

The Anzali port in Gilan province is located on the south coast of the Caspian sea with temperature variation of between 20-35°C in the summer, and dropping

Table II. Distribution of seropositivity of fascioliasis cases in Anzali Harbor according to district population and sex (1989)

Sex	men				women				Total			
Method Health districts	ELISA	CCIE	Eos-5	Total	ELISA	CCIE	Eos-5	Total	ELISA	CCIE	Eos-5	Total
1	12	0	6	24	12	2	6	26	24	2	12	62
	16.44	0	9.84	14.81	7.84	1.85	5.77	9.67	10.62	1.27	7.27	11.50
2	7	4	6	18	13	3	8	30	20	7	14	48
	9.59	8	9.84	11.11	8.5	2.78	7.69	10.34	8.85	4.43	8.48	10.62
3	9	4	9	23	17	13	10	32	26	17	19	55
	12.33	8	14.75	14.20	11.11	12.04	9.62	11.03	11.50	10.76	11.52	12.17
4	6	12	7	21	21	29	12	35	27	41	19	56
	8.22	24	11.47	12.96	13.73	26.85	11.54	12.07	11.95	25.95	11.52	12.39
5	7	11	8	18	14	13	13	27	21	24	19	45
	9.59	22	9.84	11.11	9.15	12.04	12.5	9.31	9.29	15.19	11.52	9.96
6	21	10	17	32	55	33	39	77	76	43	58	109
	28.77	20	27.87	19.75	35.94	30.55	37.5	26.55	33.63	27.22	33.94	24.12
7	8	40	7	18	12	7	10	37	20	11	17	55
	10.96	8	11.47	11.11	7.84	6.48	9.62	12.76	8.85	6.96	10.30	12.17
8	3	5	3	8	9	8	8	24	12	13	9	32
	4.11	10	4.92	4.94	5.88	7.41	5.77	8.27	5.31	8.23	5.45	7.08
Total	73	50	61	162	153	105	104	290	226	158	165	452
	100	100	100	100	100	100	100	100	100	100	100	100

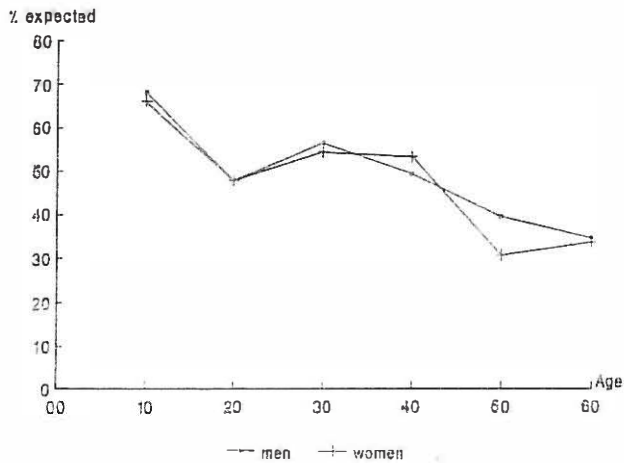


Fig.1. Distribution of age groups seropositive for fascioliasis in Anzali Harbor by ELISA technique according to positive titers (percentage of expected) and sex (1989).

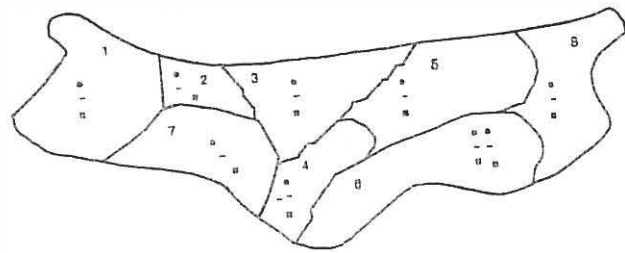


Fig.2. Distribution of seropositive cases with different techniques from each health district population in Anzali Harbor according to their total population per 1000 (1989).

Ratio of seropositivity using ELISA/District Pop.per 1000.
 average 0<--1-3
 high 00<--4-6
 Ratio of seropositivity using CCIE/District Pop.per 1000.
 average <--1-2
 high <--3-4
 Ratio of seropositivity using eosinophilia/District Pop.per 1000.
 average ■<--1-2
 high ■<--3-4

to 0°C in the winter. It has 800-1200 mm rainfall per annum and a relative humidity of 70-100%. Using a ratio of 1/200, a confidence level of 95% and precision of $d = 0.004$, it was calculated that 452 blood samples should be collected from the 95612 (reference population) inhabitants of Anzali living in 8 different local health districts. The number of samples collected from each district was proportioned to the population of that area and the method used was a multi-stage sampling method.

Giemsa staining was used for blood cell counts and the antibody level was determined using ELISA and CCIE.

Excretory-Secretory (E-S) antigens were prepared according to Santiago, et. al.⁴ The sera were diluted 1:200 in 0.01M phosphate-buffered saline (PBS) pH 7.2 for use in ELISA which was performed as described by Santiago et al.⁴ Undiluted sera were used in CCIE method as explained by Hillyer, et al.⁵

RESULTS

In the present investigation 64.2% (290/452) of the samples were collected from women and 35.8% (162/452) from men (Table I). Omitting age effect and $P < 0.05$ the rate of seropositivity as measured by antibody positivity using ELISA and CCIE techniques and high percentage of eosinophilia was 1.16 times in women compared to men which was statistically significant:

$$\frac{\text{SMR in women}}{\text{SMR in Men}} = \frac{1.05}{0.90} = 1.16$$

The rate of seropositivity varied significantly among different age groups using ELISA ($P < 0.001$) in addi-

Table III. Distribution of observed and expected seropositive cases per 1000 population in each health district, with different experimental techniques (1989)

health Districts	Pop. of each District 1986	Pop. of District reference Pop.	Infected cases (ELISA)		Infected cases (CCIE)		Eos.5%		Inf. cases (ELISA) District Pop./1000	Inf. cases (CCIE) District Pop./1000	Inf. cases (Eos.5%) / district Pop./1000
			● expected	■ observed	● expected	■ observed	● expected	■ observed			
1	8321	0.587	19	24	14	2	14	12	2.9	0.2	1.4
2	7440	0.078	18	20	12	7	13	14	2.7	0.9	1.0
3	14093	0.147	33	26	23	17	24	19	1.8	1.2	1.3
4	12998	0.136	31	27	21	41	23	19	2.1	3.5	1.5
5	14737	0.154	35	21	24	24	25	19	1.4	1.6	1.3
6	14700	0.154	35	76	24	43	25	56	5.2	3.1	3.8
7	15931	0.167	38	20	26	11	28	17	1.2	0.7	1.1
8	7392	0.077	17	12	12	13	13	9	1.6	1.7	1.2
Total	95612	1	226		158		165				

● expected ■ observed Pop. × Population Inf. × Infected

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Table IV. Distribution of seropositive cases in Anzali Harbor using ELISA and CCIE according to percentage of Eosinophilia (1989)

methods	ELISA				CCIE			
	positive		negative		positive		negative	
position	No.	%	No.	%	No.	%	No.	%
% eos.	No.	%	No.	%	No.	%	No.	%
0-5	116	41.28	165	68.72	84	29.9	197	70.1
>5	110	64.33	61	35.67	74	43.3	97	56.7
total	226	50	226	50	158	34.95	294	65.06

tion to being sex related (Figure 1). The highest number of samples were collected from location number six (24.12%) and the lowest from location number eight (7.08%) (Table II). In order to determine whether the number of seropositive cases in each district was a function of population size of that area the results obtained were compared using goodness of test which showed that the rate of seropositivity was independent of population size ($P < 0.001$) (Table III). The highest rate of seropositivity was observed in district no. six and the remaining ones had a uniform and average rate of infection (Table III, Figure 2).

50% of the samples were positive using ELISA and 34.95% with CCIE showing that the point prevalence of the disease was in Anzali Harbor (Table IV).

Comparison of antibody positivity by both serological methods and percentage of eosinophilia showed a positive correlation between the two. (i.e. all cases with hypereosinophilia were serologically positive with both ELISA and CCIE techniques) ($P < 0.001$).

Comparing the sensitivity of the two serological tests used in this study it was shown that ELISA was 52.6% more sensitive than CCIE ($P < 0.001$) (Table V). In this comparison 52.4% of ELISA positive cases were negative by CCIE technique and only 47.4% of cases were positive by both methods. According to our data the specificity of ELISA technique was 22.5% less than CCIE.

A total of 226 cases in this investigation were antibody positive of which 26.5% had both IgG and IgM related to the subacute condition of the disease,

Table V. Distribution of seropositive and seronegative cases of fascioliasis cases in Anzali Harbor according ELISA and CCIE methods (1989)

ELISA \ CCIE	ELISA		total
	positive	negative	
positive	107	51	158
negative	119	175	294
total	226	226	462

37.6% were only IgM positive and 13.7% had IgG which may be related to acute and chronic states. The remaining 22.2% were only IgA positive (Table VI). The difference between IgM and IgG positive cases was statistically significant ($P < 0.005$) so that IgM positive cases were 1.6 times more than IgG positives $IgM/IgG = 64.2/40.3 \approx 1.6$. According to the results obtained the incidence rate of fascioliasis by ELISA method was 19%.

DISCUSSION

Fascioliasis has a relatively long history in Gilan province with occasional reports of human infection.¹ However in 1988 a relatively large epidemic occurred in Anzali Harbor and the following reasons could have precipitated its occurrence.

The rise in water level of both the Caspian sea and Anzali lagoon which had caused the water to be mixed with city sewage created appropriate conditions for the parasite to infest the area. Also no effective control program had been implemented for eradication of the snail population especially *limnea* sp. the intermediary host in the life cycle of the parasite, which had increased significantly.

A large number of cattle and sheep in the Anzali area are infected and their slaughter in abattoir of the port, which has an open sewage, leads to the lagoon pollute the water. On the other hand the pellet of the sewage is used as fertilizer without prior disinfection infesting the agricultural products and people as a result. The rate of seropositivity was highest in area no. six (Taleghani area) which is nearest to the lagoon giving credence to our previous suppositions. Lack of proper hygienic precautions among teenager boys who use the lagoon for swimming, puts this age group at a higher risk of seropositivity for fasciola. Also women in this area by the nature of their daily routine housework were more in contact with the polluted water in their houses which could explain the higher rate of seropositivity observed among them. Ineffectiveness of praziquantel in treatment of fasciola causing those which had not been cured to continue shedding the parasite

Table VI. Distribution of positive immunoglobulin cases in Anzali Harbor according to IgG and IgM (1989)

IgG \ IgM	IgM		IgG		total	
	positive	negative	positive	negative	total	%
	No.	%	No.	%	No.	%
positive	60	65.9	31	34.1	91	100
negative	85	62.9	50	37.1	135	100
total	145	64.2	81	35.8	226	100

eggs, in itself was another reason for the spread of the disease.

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