Summer 1974, Vol. 3, No. 2

VIRAL ANTIBODIES IN PRESCHOOL CHILDREN FROM THE CASPIAN AREA, IRAN*

S. Saidi**

ABSTRACT

One hundred sera from children 1 - 6 years of age, representative of a large serum collection, were tested for the prevalence of antibodies against 15 different viruses. Hemagglutination-inhibition (HI) antibodies were found in 68% for measles, 61% for rubella, 75% for influenza A2/Hong Kong/68, 16% for influenza B/Md./59, 0% for group A arboviruses, 10% for group B arboviruses, 3% for phlebotomus fever group and 4% for Congo-Crimean hemorrhagic fever (C-CHF) group of arboviruses. Poliomyelitis-neutralizing antibodies for type 1, 2 and 3 were 90%, 85% and 84%, respectively. Antibody to EB virus was detected in 84% of the sera by immuno-fluorescence. None of the sera were positive for hepatitis-B antigen or antibody by immuno-precipitation test. The prevalence of some viral antibodies found in this survey are compared with results obtained from surveys in other parts of the country.

INTRODUCTION

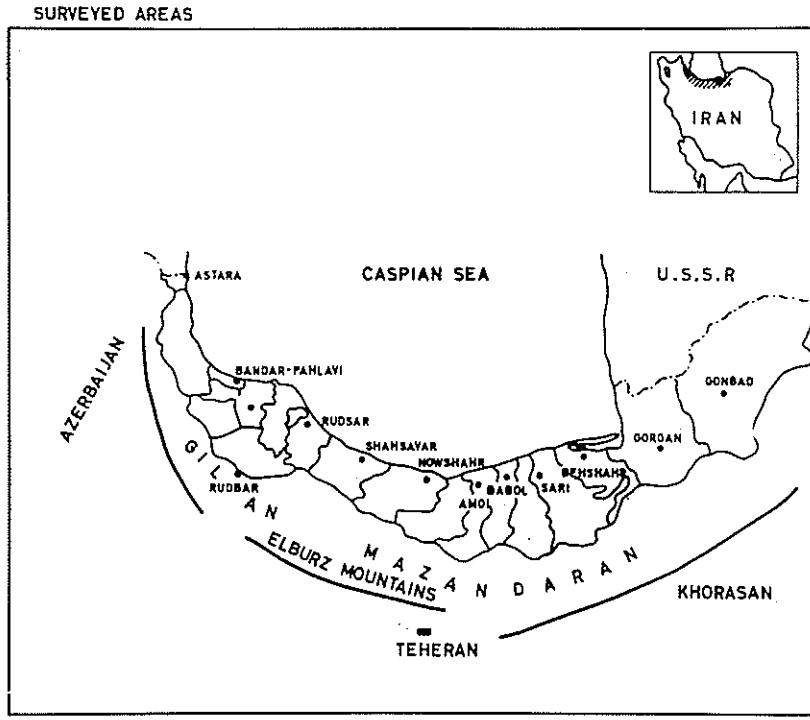
Information on the prevalence and intensity of various viral infections has not yet been collected from all parts of the country, and previous surveys have not entirely covered the same spectrum of viruses. This paper reports on a seroepidemiological survey of preschool children from the Caspian areas of Iran for the prevalence of 15 different viral antibodies.

* This study was supported in part by funds of the School of Public Health and Institute of Public Health Research, University of Teheran, and in part by the Public Health Research Project of the Ministry of Health and the Plan Organization.

** Department of Epidemiology & Pathobiology, School of Public Health and Institute of Public Health Research, University of Teheran, P.O. Box 1310, Teheran, Iran.

MATERIALS AND METHODS

Sera: Blood specimens were collected from May 1970 to September 1971 from more than 4,000 individuals of different age groups living in the northern parts of the country and central plateau, for a serological survey of arboviruses. One hundred sera from children 1 to 6 years of age were selected at random from serum samples obtained from the Caspian region (Guilan and Mazandaran Provinces). This region has a subtropical climatic characteristics (1). The sera were kept at —20° prior to examination, except for a few days at 5°.



Reagents: The viral antigens and the reference sera were kindly supplied by the Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Antibody Assay: HI tests were carried out using the microtiter method of Sever (2), 4-8 hemagglutination units (HAU) of each antigen and the proper erythrocytes. Neutralization tests for polio viruses were done in tube culture. Detailed serological procedures for different viruses are described in each section.

Measles: HI tests were carried out as described previously (3) using kaolin treatment of sera, grivet monkey erythrocyte and 4 HAU of antigen in the test system.

Rubella: HI tests were done as described earlier (4). 4 HAU of antigen and pigeon red blood cells were used.

Influenza: Sera were treated with receptor-destroying enzyme (RDE) overnight, inactivated at 56° for 30 minutes and tested at various two-fold dilutions. 4 HAU of antigen and chicken erythrocyte were employed. The influenza strains used were A2/Hong Kong/68 and B/Md./59.

Arboviruses: HI antibodies were measured by the method of Clarke and Casals (5), using kaolin treatment of sera, 8 HAU of each antigen and goose erythrocytes. The following antigens were tested: group A— Sindbis and Chikungunya; group B— West Nile and Dengue 2; Phlebotomus fever group-strain I-58; and Congo-Crimean hemorrhagic fever group— strain IbAr 10200.

Polio Virus: Neutralization tests were carried out in vero cells, using 100 TCD 50 of each strain of polio virus and a single serum dilution of 1:10.

Epstein Barr (EB) Virus: Antibodies were determined by indirect immunofluorescence (IFA) method of Henle and Henle, and Evans and coworkers (6,7), using a single serum dilution of 1:5.

Hepatitis-B Antigen and Antibody (HBAg/Ab): Immunoprecipitins were measured by agar gel double diffusion precipitation test according to the technique described previously (8).

FIG. 1
PREVALENCE OF ANTIBODY TO VARIOUS VIRAL INFECTIONS BY AGE GROUP

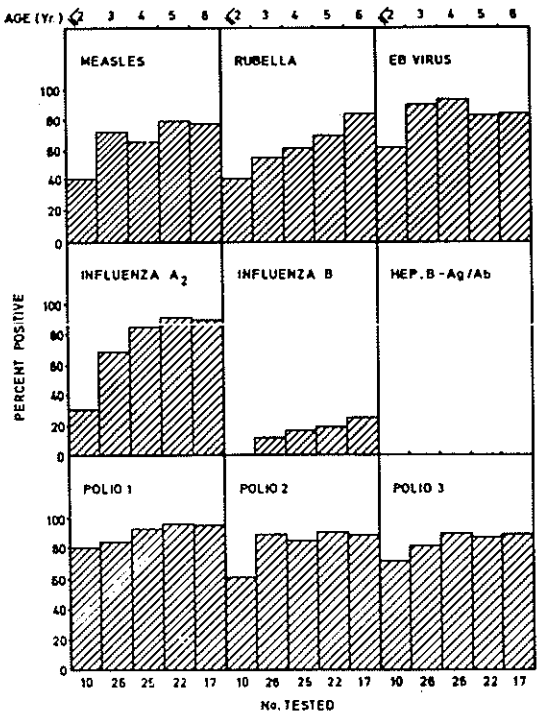
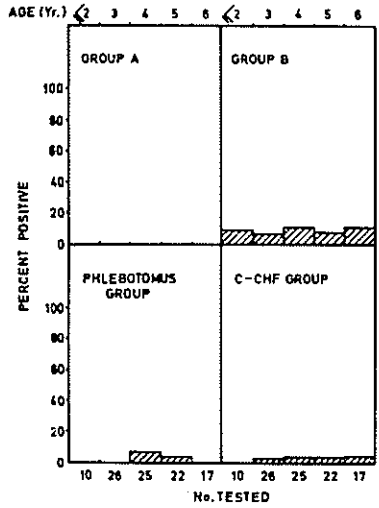


FIG. 2
PREVALENCE OF ANTIBODY TO ARBOVIRUSES BY AGE GROUP



RESULTS

The age and sex distribution of the study group is shown in Table 1. Since the pattern of antibodies for the viruses tested was similar for 57 males and 43 females tested and the number in each group was small, data has been presented for the entire group. The prevalence of antibodies against different viruses tested are summarized in Figures 1 and 2.

Measles HI antibodies were detected in 68% of the sera. Antibody

TABLE 1
THE AGE AND SEX DISTRIBUTION OF THE STUDY GROUP

AGE (Yr.)	SEX		TOTAL
	MALE	FEMALE	
≤2	5	5	10
3	15	11	26
4	12	13	25
5	14	8	22
6	11	6	17
TOTAL	57	43	100

TABLE 2
THE AGE DISTRIBUTION OF ANTIBODIES TO VARIOUS VIRAL INFECTIONS

AGE (Yr.)	No.TESTED	PERCENT POSITIVE WITH:					
		MEASLES	RUBELLA	EBV	INFLUENZA A_2	INFLUENZA B	HEP•B•Ag/Ab
⩽2	10	40	40	60	30	0	0
3	26	69	54	88	69	11.5	0
4	25	64	60	92	84	16	0
5	22	77	68	81	90	18	0
6	17	76	82	82	88	29	0
TOTAL	100	68	61	84	75	16	0

was present in all age groups and the proportion of immunes increased with age from 40% at 2 years of age to 76% at 6 years (Table 2). Rubella HI antibodies were found in 61% of the sera. More than 50% of the children were sero-positive at the age of 3 and the percentage reached 82% at the age of 6 years (Table 2).

Of the two strains of influenza virus used in the test, antibody was found in 75% to influenza A2/Hong Kong/68, while only 16% of the samples were sero-positive to influenza B/Md/59, suggesting that influenza B virus had not been widespread in this community whereas influenza A2 had been common (Table 2). The distribution of HI titers for measles, rubella and influenza viruses is demonstrated in Table 3.

The distribution of HI antibodies with a titer of 1 :20 or greater among six arbovirus antigens employed in the test is shown in Table 4. In all, 17 antibody positives were noted, 10 of which reacted with West Nile, 3 with the strain I-58 of phlebotomus fever group and the remaining 4 were sero-positive

TABLE 3
PERCENT DISTRIBUTION OF HI TITERS TO INFLUENZA, MEASLES, AND RUBELLA

ANTIGEN	⩽1:10	1:10	1:20	1:40	1:80	⩾1:160
INFLUENZA:						
A_2	13	12	32	25	8	10
B	32	52	10	3	1	2
MEASLES	32	27	17	15	5	4
RUBELLA	39	3	4	14	20	20

TABLE 4

PREVALENCE OF ANTIBODIES AGAINST ARBOVIRUSES BY AGE GROUP - PERCENT POSITIVE AT 1:20

AGE (Yr.)	≤2	3	4	5	6	TOTAL
No. TESTED	10	26	25	22	17	100
GROUP A:						
SINDBIS	0	0	0	0	0	0
CHIKUNGUNYA	0	0	0	0	0	0
GROUP B:						
WEST NILE	10	7.5	12	9	12	10
DENGUE 2	0	0	0	0	0	0
PHLEBOTOMUS:						
I-58	0	0	8	4.5	0	3
C-CHF:						
IbAr 10200	0	4	4.5	4.5	6	4

for the strain IbAr 10200 of C-CHF group of arboviruses. None of the sera reacted with group A antigen or Dengue 2. Sero-positives were generally low in titer (1 :20 or 1 :40) except for two children who had a titer of 1 :80 to West Nile.

All three types of polio virus neutralizing antibodies were frequently found in all age groups (Table 5). The immune rates were 90%, 85% and 84% for types 1, 2 and 3 of polio virus, respectively. Since these children had not received polio vaccine, the high prevalence of immunity is probably due to

TABLE 5

THE AGE DISTRIBUTION OF ANTIBODIES TO POLIO VIRUS TYPES 1, 2 AND 3

AGE (Yr.)	No. TESTED	PERCENT POSITIVE AT 1:10		
		TYPE 1	TYPE 2	TYPE 3
≤2	10	80	60	70
3	26	84.5	88	80
4	25	92	84	88
5	22	95	90	86
6	17	94	88	88
TOTAL	100	90	85	84

natural infection in childhood; however, the accuracy of the vaccination histories of the children is questionable.

For EB virus, antibodies were present in a large proportion of sera tested in each age group. The overall immunity was 84% and the highest rate was 92% at the age of 4 years (Table 2). Immuno-precipitation lines were not detected in the sera tested against the HBAg or HBAb using a double diffusion precipitation test in agarose (Table 2).

DISCUSSION

The prevalence of antibodies against the acute viral infections tested was rather high in preschool children from the Caspian region, except for arboviruses and hepatitis-B virus. The high prevalence of HI antibodies to measles and rubella in all age groups indicates the widespread distribution of these acute viral infections early in life. This confirms our previous findings (9,10). Similarly high prevalence rates were found for EB virus antibodies in all age groups, with an overall immunity of 84%. This frequency is close to the findings in other tropical and subtropical areas of the world (7,11,12).

Most of the children had antibodies to influenza A2, suggesting the effective spread of the 1968-1969 influenza outbreak. The rate of influenza B antibodies in this community was low both in incidence and in titer, and only 16% of the children were sero-positive at a low titer (Tables 2 & 3). The prevalence of HI antibodies to influenza A2 and B, in all age groups in Teheran, has been reported 74 and 32 percent respectively (13).

The high prevalence of sero-positives to poliovirus at an early age might be due to the low standard of hygiene in areas where blood samples were obtained. More than 80% of the children had antibody to polio virus types 1, 2 and 3 by the age of 3 years. This rate is much higher than the 50% immune rate obtained from the children of the same age group in villages in the vicinity of Teheran (9). The prevalence of antibodies to all 3 types of polio virus in Isfahan heas been reported 92 percent (14).

The activities of several arboviruses of group A, B, phlebotomus fever and C-CHF groups have been demonstrated in other studies (9,15,16). In the present study, none of the sera reacted with Sindbis or Chikungunya of group A, and Dengue 2 of group B. A positive reaction was detected in 10% of the sera with West Nile virus, 3% with strain I-58 of phlebotomus fever group and 4% with C-CHF group of arboviruses. The immune rates are shown to be much higher in the older age groups in the Caspian areas of Iran, especially for group B and C-CHF group of arboviruses (15,16).

Hepatitis-B antigen or antibody was not detected in this survey. In a previous survey we found a carrier rate of 1.4% for HBAg in an apparently healthy population of the older age group in Rudsar, Guilan (17).

The present survey was done on the basis of availability of serum samples and reagents. Additional surveys of this type on different age groups from

various parts of the country are desirable to provide data on the prevalence of viral infections in Iran, and to evaluate public health measures in each community.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. R. W. McCollum, Dr. J. Casals, Dr. A. S. Evans, Dr. F. L. Black, and Dr. H. Leibhaber, the staff members of the Department of Epidemiology and Public Health, School of Medicine, Yale University, New Haven, Conn., USA for the supply of most of the reagents used in this survey.

REFERENCES

1. Kmet, J. and Mahboubi, E. (1972). Esophageal cancer in the Caspian Lithoral of Iran. Science, 175: 846-853.
2. Sever, J.H. (1962). Application of a microtiter technique to serological investigations. J. Immunol. 88: 320-329.
3. Black, F.L. and Rosen, L. (1962). Pattern of measles antibodies in residents of Tahiti and their stability in the absence of re-exposure. J. Immunol. 88 : 725-731.
4. Halonen, P.E., Ryan, J.M. and Stewart, J.A. (1967). Rubella hemagglutinin prepared with alkaline extraction of virus grown in suspension culture of BHK-21 cells. Proc. Soc. Exp. Biol. (N.Y.), 125 : 162-167.
5. Clarke, D.H. and Casals, J. (1958). Technique for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. 7 : 561-573.
6. Henle, G. and Henle, W. (1966). Immunofluorescence in cells derived from Burkitt's lymphoma. J. Bact. 91: 1248-1256.
7. Evans, A.S., Niederman, J.C. and McCollum, R.W. (1968). Sero-epidemiologic studies of infectious mononucleosis with EB virus. N. Eng. J. Med. 279 : 1121-1127.
8. LeBouvier, G.L. and McCollum, R.W. (1970). Australia (Hepatitis Associated) antigen : Physiological and immunological characteristics. Adv. Virus Res. 16 : 357-396.
9. Naficy, K. and Saidi, S. (1970). Serological survey on viral antibodies in Iran. Trop. Geog. Med. 22 : 183-188.
10. Saidi, S. (1972). Epidemiological survey of rubella immunity in Iran. Bull. WHO. 46 : 563-565.
11. Pereira, M.S., Black, J.M. and Macrae, AD. (1968). EB virus antibody of different ages. Brit. Med. J. 4: 526-527.
12. Anderson, N. and Mufson, A. (1972). Viral antibodies among Turkana people of Northern Kenya. Trop. Geog. Med. 24: 168-177.
13. Kiai, F.M. and Farrohi, K. (1973). The prevalence of influenza viruses

in Tehran. J. Faculty of Med. Tehran. 6 : 173-178.
14. Balassanian, N. (1970). Serological and virological studies with live triple polio virus (Sabin) in Isfahan, Iran. Vol. Abst. VII Middle Eastern-Mediterranian Pediat. Cong. Tehran. Sept. 13-16, p. 30.
15. Saidi, S., Casals, J. and Faghih, M.A. (1973). Antibodies against Crimean hemorrhagic fever-Congo (CHF-C) virus in Iran. Vol. Abst. 9th Int. Cong. Trop. Med. Malar. Athens, Oct. 14-21, p. 35.
16. Saidi, S. and Casals, J. (1974). Survey for antibodies against arboviruses in Iran (in preparation).
17. Saidi, S., Farrohi, K., et al (1972). Hepatitis-B antigen in Iran : Frequency and subtype. Lancet. 7791(2) : 1377-1378.