

SERO - EPIDEMIOLOGY OF RUBELLA
VIRUS INFECTION IN
BAHRAIN

By

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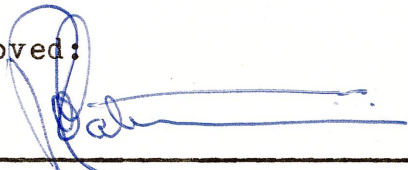
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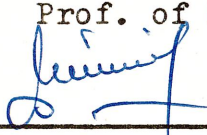
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TO MY FATHER
with my great gratitude and love

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ABSTRACT

This serological survey was carried out using the single-radial-hemolysis test (SRH), with a view to study the distribution of rubella antibodies in children and women of childbearing age in Bahrain. Specimens were collected during 1981, from different age groups including children and adult females.

It was observed that rubella infection could occur in young children and its incidence rose steadily from school age to adolescence leaving 13.2% of the adults without antibody. Among 167 school children of both sexes 34.1% were seronegative, indicating susceptibility to rubella. Females from schools in the age groups 15-18, 19-24, 25-42 showed 15.5%, 10.0%, 6.6% respective susceptibilities. The same pattern of rubella antibody was found among the females of the College of Health Sciences. 14.2% and 9.0% susceptibilities were recognized in the 15-18, 19-24 age groups. Slightly less susceptibility (10.2%) was noted among pregnant women attending the Ante-natal clinic for follow-up.

The SRH employed in this study, correlated well with the HI method for detecting rubella antibody, and was to be simple, reliable, easy to perform and well suited for screening of a large number of sera.

The results of this study suggest that rubella is endemic in Bahrain. Thus we stress the importance of awareness of females of childbearing age of the possibility of acquiring acute rubella infection with its subsequent complication specially in the first trimester of pregnancy and urge the vaccination of susceptible females prior to marriage.

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I N T R O D U C T I O N

INTRODUCTION

Rubella or German Measles, one of the common childhood exanthems, occurs mostly in school-age children. Its clinical manifestations may range from inapparent infection to a characteristic clinical picture of rash, lymphadenopathy, and low grade fever. In adults, however, the disease may be more severe, with polyarthralgia and polyarthritis being present so commonly as to be considered typical of the disease.

The description of congenital rubella syndrome, (CRS), following rubella infection early in pregnancy, established a much more serious significance to this relatively mild exanthematous disease. It was noted that congenital malformations occur in about 50% of infants whose mothers had clinical rubella during the first month of gestation, 22% during the second, 6% during the third and about 1% during the fourth month (Sever and Witte, 1968). Recently it has been shown that the fetus can be infected at any time during pregnancy, but the risk varies at different stages (Cradock-Watson, 1980). In addition to fetal abnormalities, spontaneous abortion and fetal death following maternal rubella were observed.

Worldwide seroepidemiologic studies have shown that about 80-85% of women of childbearing age are immune, presumably by clinical or subclinical natural infection with rubella virus. The exception of this being islands and isolated populations, where the rate of susceptibility may approach 50% (Sever, 1965 - Rawls et al., 1967 - Dowdle et al., 1970).

Since the introduction and widespread use of live attenuated rubella vaccine, the reported incidence of rubella in children has decreased. However, during the same period, there has been an increase in the proportion of cases among older people. Outbreaks of rubella among young and older adults are being reported with

greater frequency (Charetien et al., 1976 - Morbid. Mortal. Wkly Rep., October 31, 1980). Some of these outbreaks have occurred in hospital settings and obstetric clinics (Morbidity Mortality Wkly Rep., July 20, 1979 - Strassburg et al., 1981).

Bahrain, being an island, a high rate of susceptibility might be expected. The potential danger of a rubella epidemic with congenital aftermaths has made the need for a control program obvious. However, before embarking on an extensive rubella vaccination program, a seroepidemiological study should be conducted to determine the proportion of women of childbearing age susceptible to rubella. Of the various tests employed, the single-radial-hemolysis (SRH) test has proven to be the most simple, specific test that can be carried on a large scale for detection of rubella antibody.

This study employed the SRH test developed by Skaug et al., (1975), and modified by the Central Public Health Laboratories, Colindale, England, for screening of sera from school children of both sexes, females of childbearing age and pregnant women attending the Ante-natal clinic of Salmaniya Medical Center to determine the prevalence of antibodies to rubella virus in Bahrain.

L I T E R A T U R E R E V I E W

LITERATURE REVIEW

I. THE STATE OF BAHRAIN:

1. General Information:

The state of Bahrain comprises an archipelago of 35 low lying islands, situated about half-way down the Arabian Gulf, some 30 km. off the east coast of Saudi Arabia, and 27 km. north-west of the State of Qatar. The total area is about 670 km. with the largest island, Bahrain, accounting for the major part of the State. The climate is characterised by high summer temperatures, scanty and irregular rainfall, and high relative humidity (Kabeel, 1975).

The population was established at 358,857 by the April, 1981, census (To be published). This has shown a population increase of 3.1% per year, since the last census taken in 1971. The average population density was estimated to be 563/km². Nearly 80% of the community are urban. Approximately 41.5% of the population are under the age of 15. This high percentage of young people illustrates the present great need for expatriate support. Thus, of the total population, 32.4% are non-Bahrainis, representing in the main expatriates from other Arab states, Indians, Pakistanis and Europeans.

The steady growth of Bahrain's health services, both preventive and curative, supported by vigorous Public Health and Health Education Programs, has resulted in the fall of infant mortality rate (25.2 per thousand), a rise in life expectancy at birth, and probably the lowest maternal mortality rate in the region (0.5 per thousand). A negligible incidence of trachoma, malaria and other endemic diseases has also been achieved (The Public Health Annual Report, 1980).

2. Immunization:

Surveillance and control measures with regard to the major communicable diseases of importance in Bahrain are continuously being strengthened. Preventive vaccination is available and immunization is actively promoted. With the advent of the expanded program of immunization, the incidence of six target diseases-diphtheria, tetanus, pertussis, poliomyelitis and tuberculosis-should be minimal in the near future. DTP and oral poliomyelitis immunizations start at two months of age. A separately administered school Immunization Program calls for three doses of poliomyelitis vaccine, one dose of BCG and two doses of tetanus toxoid, during the first year of primary school. Pregnant women are given two doses of tetanus toxoid at six weeks apart starting after the 16th week of pregnancy. This is repeated in each subsequent pregnancy. Cholera and yellow fever vaccines are usually given for ship crew and international travellers (The Public Health Annual Report, 1980).

An evaluation on immunization activities was conducted jointly between 27 November and 12 December, 1980, by the Ministry of Health of Bahrain and a five member international team of the WHO. This study showed that the coverage with DTP and polio has reached highly satisfactory levels. The measles coverage has lagged because acceptance of measles vaccine by mothers has been less satisfactory than with other vaccines. However, considerable improvement in measles coverage has been observed during 1980 (Wkly Epidem. Rec., 1981). (Table I).

Year	DTP					Poliomyelitis				Measles	No. of registered births
	1st	2nd	3rd	Booster		1st	2nd	3rd	Booster		
1975	9665	8597	7389	3424		9634	8606	7453	3507	2458	7909
1976	10030	8399	7213	3202		10274	8392	7244	3058	1668	9105
1977	12668	10755	9088	4736		16709	10945	9726	4850	3102	9001
1978	13299	12207	10983	5438		13513	12264	11308	6044	5463	9762
1979	11432	10007	8923	5161		11472	9973	8973	5336	4406	9985
1980	8439	8014	6910	4852		9149	8175	7674	4967	5943	8165
° January through October.											

TABLE I: Vaccination coverage of childhood immunization, done in Health Centers, Bahrain, 1975-1980 (Wkly Epidem. Rec. 198, 56.

3. Rubella in Bahrain:

Between the years 1971 and 1980 the highest number of rubella cases were noted in 1974 and 1980, corresponding to 437 and 836 cases respectively. Since the specific diagnostic procedures for congenital abnormalities are lacking, all such abnormalities are grouped together with no specific reference to congenital rubella. However, 3.4% of the total cataract cases admitted to the ophthalmology department in 1980, were congenital cataract cases (The Public Health Annual Report, 1980).

II. THE RUBELLA VIRUS:

Rubella virus has been the object of several investigations since its isolation in 1962 by Parkman et al., and, Weller and Neva.

It has been classified as a Togavirus despite the differences in its structural proteins and genetic material from the other members of the family (Fenner, 1975). Electron microscopic studies have shown rubella virus to be a spherical particle with a diameter of 60-70 nm. icosahedral core enclosed in a tightly fitting envelope. It has surface projections or spikes of 6 nm. in length (Oshiro et al., 1969).

Studies on the nucleic acid content of the virus have shown that rubella virus is a single stranded ribonucleic acid (RNA) virus. Purified virus preparations revealed no trace of deoxyribonucleic acid (DNA). (McCarthy and Taylor-Robinson, 1967). Rubella virus is relatively sensitive to heat. At 4°C the virus infectivity titer is relatively stable for one week and at -60°C the virus can retain its titer for many years. Extremes of pH may inactivate the

virus which has a relatively narrow optimal pH range, between 6.8 and 8.1 (McCarthy and Taylor-Robinson, 1967). Chemical, such as ether, acetone and chloroform, destroy the infectivity of the virus within 10 minutes (Parkman et al., 1964).

Only a single serotype of rubella virus is known. No serologic relationship exists between rubella and any other known virus group. Two antigenic components of rubella virus were described: hemagglutinin, associated with the viral envelope, (Stewart et al., 1967), and, complement fixing antigen associated with both the envelope and RNA core component (Sever et al., 1965 b).

A wide variety of cell types support the replication of rubella virus. The growth is usually indicated by interference, cytopathogenicity or plaque formation depending on the type of cell involved and the environmental variables (Lennette and Schmidt, 1980).

III. EPIDEMIOLOGY OR RUBELLA:

1. In general populations:

Rubella has a worldwide distribution, with a cyclic nature of epidemics occurring every 6-9 years (Witte et al., 1969). The major rubella epidemic that struck the United States in 1964, resulted in congenital rubella anomalies in nearly 20,000 infants. The pattern of susceptibility to rubella was found to be quite similar in different areas of the world. Generally about 12%-20% of any population is susceptible to infection with rubella virus; the rest is immune by past clinical infection or subclinical natural reinfection. An increasing incidence of rubella immunity with age has been reported; approximately 50% of the population have antibody by age of 6-8 years, the percentage increases to

of the cases reported were among greater than 15 years old and the highest incidence rate was for the less than 5 years old. This shift probably resulted from the fact that the young children who were targeted for vaccination in 1969 moved into older age group and that over the past 2-3 years efforts increased in vaccinating adolescents and young adults (Morbidity and Mortality Weekly Report, Oct. 23, 1981).

The incidence of congenital rubella syndrome (CRS) was estimated to range from as high as 4-30 cases/1000 live birth during epidemic period to less than 0.5 cases/1000 birth during non-epidemic period (Alford et al., 1975). The data of the Center for Disease Control (November, 1981), shows that, while the total number of cases has decreased greatly over the past decade, no change has been noted in the incidence of CRS.

Strassburg et al., (1981), reported an outbreak of rubella that occurred among the medical staff in an Obstetric Service of a Los Angeles hospital in which a large number of pregnant women were exposed. Some 15 cases of suspected rubella were determined. The immunity status of 231 pregnant women who may have been exposed to any one of the 15 cases, was reported to be 75% whereas the corresponding rate in employees was 80.8%. A post-exposure illness survey revealed that 15% of 130 women reported various rash-like illnesses after their visit to the hospital.

2. In Island Populations:

Rawls et al., (1969), and Bodwel et al., (1970), have proposed that a certain minimum population density is necessary to maintain rubella disease in epidemic form. Theoretically, this minimal level is not reached on the islands and the disease must be periodically reintroduced from mainland areas.

In Egypt, where the population density is known to be very high,

the rate of seropositivity was found to be 100% in female students in the group aged 10-15 years, in housewives of childbearing age and in a group of medical staff, including doctors, nurses and laboratory assistants (Khalifa et al., 1973).

A WHO collaborative study on 30 to 46 serum samples in each of the age groups, 6-8 years, 10-12 years, 17-22 years, 23-29 years and 30-35 years that were collected as representative of the female population in each area of the nine collaborating WHO centers: Prague, Czechoslovakia; Copenhagen, Denmark; London, England; Lyons, France; Melbourne, Australia; Tokyo, Japan; Ottawa, Canada; Atlanta, and Houston, U.S.A. The pattern of antibody development was found to be similar in all European cities, Australia, Canada and U.S.A. About half of the females had been infected with rubella by the time they reached the age of 6-8 years; the number possessing antibody increased over the following years and by the age of 17-22 years, 80-88% of the sera tested were positive. The percentage remained constant thereafter. Contrary to these, there were many rubella-negative individuals in Japan and only 38% of the individuals tested were found to have antibody. Samples from Jamaica and Trinidad showed that the number of persons past the age of 12 years with antibody, was significantly lower, indicating a large degree of susceptibility. A high rate of susceptibility was reported in Hawaii (Sever, 1965). Serum samples from 228 pregnant women in Hilo, Honolulu and Hawaii, were tested for rubella antibody. A 60% susceptibility to rubella infection was found among them. It was noted that in spite of the extensive travel which occurs between the mainland of the U.S. and the state of Hawaii a large reservoir of susceptible pregnant women exist in the island population.

The conditions shared by all these areas which might account for the low incidence of rubella infection are not apparent. The groups do not represent a common ethnic or racial origin. These areas share somewhat similar climatic conditions. However, the data

by Rawls et al., (1967), do not support a consistent correlation between antibody ratio and climatic condition. There seems to exist some correlation between population density and the level of antibody in the female population. The opportunity for reintroduction of rubella into islands' populations would seem to be less than in continental mainland communities.

By studying an epidemic of rubella, Hattis et al., (1973), described some factors concerning the possible nature of rubella transmission which may permit an explanation of epidemiological variation of rubella throughout the world. It has been shown that during an uncomplicated rubella infection, the average individual may only have minimal contagious potential. Others may have substantially greater potential for spread, and these are known as "spreaders". These, when exposed to a large population, may be expected to infect high proportion of susceptible persons. Rubella virus transmission will be maintained in a population only when spreader-to-spreader contact occurs. Furthermore, the virus will be transmitted more readily when the ratio of spreaders to non-spreaders is relatively high. In addition, the presence of previous upper respiratory infections or their residue may increase the proportion of spreaders among persons infected with rubella.

Honeman and Menser, (1974), admitted that ethnicity is a significant factor in the epidemiology of rubella, since there are marked differences in the HL-A profile among ethnic groups. These antigens are coded for by genes located in the Major Histocompatibility Complex (MHC). They may be responsible for the variation in susceptibility to infectious agents observed in different ethnic groups. By correlating the percentages of young adults with serological evidence of previous rubella infection with the mean gene frequency of HL-A 1 & 8 for 28 different population groups, all over the world, it has been shown that the presence of genes of low frequencies of HL-A 1 & 8 was an important factor in the increasing susceptibility to rubella.

IV. THE IMMUNOLOGICAL RESPONSE IN RUBELLA:

1. Postnatally acquired rubella:

Immunity to rubella is associated with the development of specific antibodies that appear soon after the onset of illness and disappearance of rash (Figure 1). Neutralizing antibodies were found to appear in the blood within 2 to 3 days after the rash. The titer reached a peak level 21 to 28 days later, and persisted unaltered for more than 15 months (Sever et al., 1966 - Dudgeon, 1967). Hemagglutination inhibition (HI) antibodies were found to appear early in the course of infection, usually within 2 to 3 days after the onset of rash. These antibody titers reached a peak within two weeks of onset and persisted for many years (Stewart et al., 1967). Complement fixing (CF), and, fluorescent antibodies (FA), appeared to be somewhat different. CF antibodies became detectable a week or more after rash onset; FA antibodies were detected within one day after the onset of rash. However, they both declined gradually to undetectable levels within 15 months of rubella infection (Sever et al., 1966).

Vesikari and Vaheri, (1968), using 2-Mercaptoethanol treated-serum, proved that the first class of antibody to appear following rubella infection is specific IgM. These globulins gradually decline and become undetectable approximately 25-30 days following the rash after which only IgG globulins are detected. Desmyter et al., (1970), studied the IgM antibody response in rubella during pregnancy. As in non-pregnant subjects, rubella virus antibody activity was detected in the IgM fraction for only the first month after the onset of rash. Ankerst et al., (1974), taking the advantage of the ability of Staphylococci of the Cowan I strain to absorb IgG immunoglobulins from human sera,

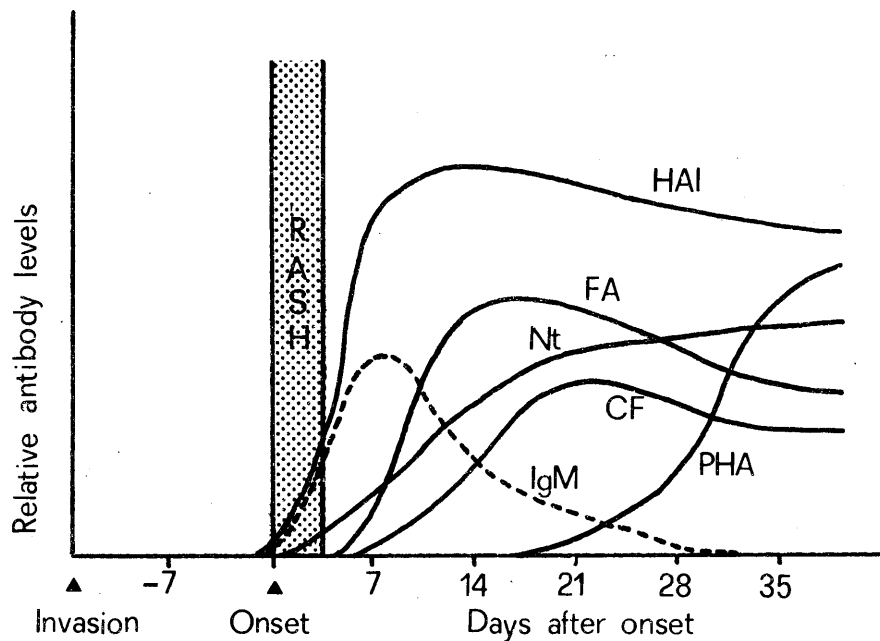


Figure 1: Immune response in acute rubella infection.

HAI= heamagglutination inhibition.

FA = immunofluorescence.

Nt = neutralization.

CF = complement fixation.

PHA= passive heamagglutination.

IgM= immunoglobulin M.

(Lennette, E.H., & , Schmidt, N.J., 1979.

DIAGNOSTIC PROCEDURES FOR VIRAL, RICKETTSIAL & CHLAMYDIAL INFECTIONS. 5th edition, p. 727).

proved that only IgG antibodies are long standing following rubella infection. They showed that in the group of patients with remote rubella, all HI activity was removed after absorption with Staphylococcus Cowan I strain, whereas HI antibody titers remained unchanged in those with a recent rubella infection. The same findings were described by Craddock-Watson and Ridehaly, (1979). They reported that the immunofluorescence technique revealed a simultaneous increase of IgG, IgA and IgM within 3 days after the onset of rash. IgM and IgA antibodies displayed a similar pattern of response; both reached the maximum titer of response during the second week but then declined and were not detected after 28 days. IgG antibody reached a maximum about two weeks after the rash and persisted.

Harcourt et al., (1980), studied rubella specific serum and nasopharyngeal antibodies in volunteers with naturally acquired immunity and after intranasal challenge. A four-fold or greater rise of rubella specific IgA and IgG were detected in 50% of these volunteers. None of them developed rubella specific IgM. In contrast to the findings of Plotkin et al., (1973), and Schluederberg et al., (1978), that the local antibody plays an important role in protection of mucosal surfaces against rubella reinfection, it has been shown that the presence of local IgA even at a level of 1:40 did not always confirm protection. It is possible that these antibodies lacked sufficient neutralizing activity or that they were broken down in the respiratory tract by proteolytic enzymes produced by certain bacteria, which cleave human IgA dimers and so greatly reduce its binding capacity.

Partridge et al., (1981), showed that the previous detection of high titers of rubella specific antibodies do not confirm protection against rubella reinfection. They described a case of CRS in an infant whose mother had had high titer of rubella specific antibody some months before pregnancy. Congenital rubella was virologically and serologically confirmed at 4 months.

Conflicting results regarding cell-mediated immune response following natural rubella infection have been reported. Kauffman et al., (1974), document the effect of experimentally induced rubella infection on several parameters of CMI in humans. Response to phytohemagglutinin was not changed during rubella infection. T - lymphocyte numbers in peripheral blood were not influenced by the viral illness. However, dermal hypersensitivity was markedly impaired in all volunteers during the height of illness. This diminished skin test response during rubella might have reflected viral interference with macrophage/monocyte function rather than lymphocyte function, or, it might have been related to dermal changes associated with this exanthem.

Niwa and Kanoh, (1979), performed several immunological tests on eighty five patients with rubella. There was a decrease in total leucocytes, neutrophils and T - cells, an increase in IgG and the complement components, and a marked insensitivity to dinitrochlorobenzene (DNCB) and purified protein derivative (PPD).

Arneborn et al., (1980), described the immunosuppression and alteration of T - lymphocyte subpopulation using the live attenuated vaccine strain as a challenge virus. Suppression of lymphocyte stimulation by PHA was demonstrated 7 to 10 days after rubella administration and was accompanied by an increase in the proportion of T_G cells (T cells that have receptors for the F_C portion of IgG and believed to have a suppressor effect on B - cell response in vitro). However, there was no direct proof that the T_G cells caused the immunosuppression.

2. Congenital Rubella:

Congenitally infected children are capable of synthesizing rubella specific antibodies quite normally and they begin to do so

even before birth (Figure 2). However, these antibodies apparently have little or no direct role in the termination of infection.

Alford, (1965), showed that sera from infants with congenital rubella contain soon after birth, specific IgM antibodies made by themselves since IgM does not cross the placenta, as well as IgG antibodies, passively acquired from the mother. The former antibodies persist but the latter wane, and are only subsequently replaced by the child's own antibodies. This pattern differs from the normal development of immune competence only in the early IgM response. Both IgM and IgG were found to be neutralizing antibodies.

Sever et al., (1966), reported a slight decrease or loss of detectable Nt, CF and FA antibodies in CRS infants during the period of one to five months, which indicated that, inspite of the chronic congenital infection of these infants, the antibody response is not rapid and does not reach maximum levels until after the 6th month.

Hancock et al., (1968), have shown that humoral and cellular defects are not always found in congenital rubella infants. When found, they may be incomplete or transient disappearing later in infancy, when rubella virus infection is no longer present.

Cradock-Watson et al., (1980), studied the antibody response in infants whose mothers had had rubella at various times after the first weeks of pregnancy. When maternal rubella occurred at 12-13 weeks of pregnancy, specific IgM antibodies were tested soon after birth and were detected in 56% of the samples tested. The proportion fell progressively to 12% after maternal rubella at 24-28 weeks, rose to 19% after rubella at 28-36 weeks and then to 58% when the illness occurred during the last month of pregnancy. In all cases IgM antibody was detected in 29%. The fetus can thus be infected at any time during the second and third trimesters of pregnancy, but the risk varies at different stages. The figures for the prevalence

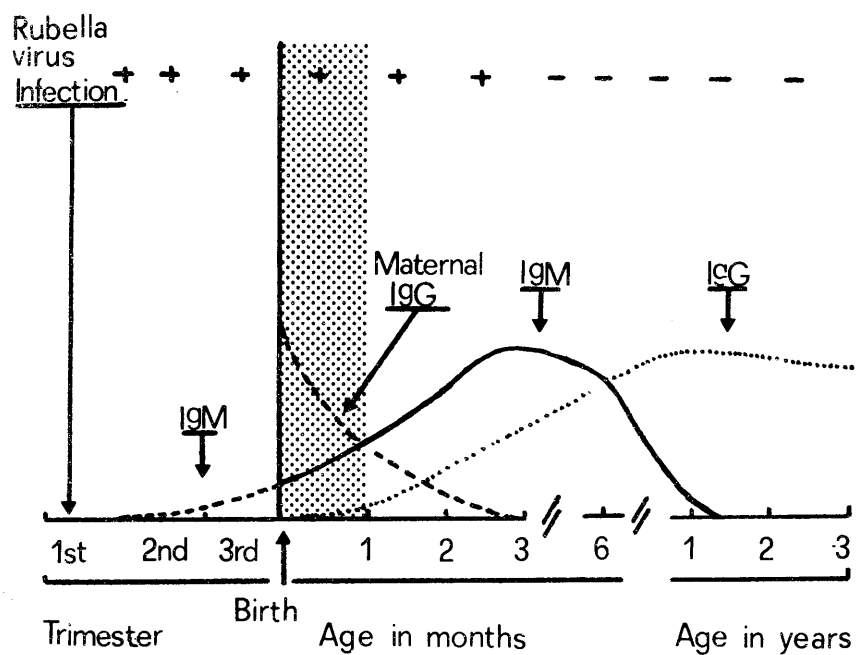


Figure 2: The pattern of viral excretion and antibody response in congenital rubella.

IgG = immunoglobulin G.

IgM = immunoglobulin M.

(Fenner F., and D. White, 1976 . MEDICAL VIROLOGY. 2nd edition, p. 443).

of IgG antibodies were greater throughout. After maternal rubella, at 12-16 weeks, IgG antibodies persisted in 71% of infants, in 28% after rubella at 24-28 weeks and then increased progressively to 94% after rubella during the last months. In all cases IgG antibodies persisted in 49% of infants.

The persistence of virus in the presence of antibodies may point to a defect in the cellular immune mechanism. Olson et al., (1967), showed that the peripheral lymphocytes obtained from babies with CRS, did not undergo in vitro blast transformation in the presence of PHA, however, these babies were able to express delayed hypersensitivity.

Witte et al., (1968), have shown that even after the disappearance of the rubella virus, the lymphocytes of patients who have had intrauterine rubella infection may be so modified that they are not capable of responding to certain antigenic stimuli such as, vaccinia, diphtheria toxoid or candida skin test.

Elena et al., (1979), correlated the impaired cell-mediated immune response with congenital rubella with gestational age at time of infection. Lymphocyte transformation, interferon and leukocyte migration inhibition factor synthesis were assessed in 20 children with congenital rubella. Lymphocyte transformation after PHA stimulation and response to purified rubella virus were two times lower in congenital rubella children than in immune controls. Leukocyte migration inhibition factor production was detected in all immune controls but in none of the congenital rubella infected children. The impairment of CMI, both after PHA or rubella virus stimulation, was more severe in the children infected in the first two months than in the later stages of gestation. This may be attributed to a direct teratogenic effect of the rubella virus on the not fully differentiated immune system. The impairment of the CMI response may be the result of one or more of the following: a decrea-

sed number of T - cells, the presence of an immunosuppressive factor or an inability to produce or maintain a sufficient quantity of specific rubella antibody, the dependance of lymphoblast transformation as well as LMIF & IF production of the quantity of IgG anti-rubella antibody was demonstrated in their study.

V. DIAGNOSIS OF RUBELLA:

Three distinct situations commonly necessitate the diagnosis of rubella infection:

- Non-immunized women coming into contact with possible rubella during the first trimester of pregnancy, in order to avoid any unnecessary abortion.
- Newborn babies born of a mother with a history of rash during early pregnancy.
- Babies whose appearance gives rise to suspicion of the CRS.

1. Isolation in Tissue Culture:

Virus isolation has only limited applicability for routine rubella diagnosis because of the lability of the virus at ambient temperature and the expense and time involved.

Parkman et al., (1962), described the isolation of rubella virus in primary cultures of African Green Monkey kidney (AGMK). The virus was detected in these cells by interference with CPE of echovirus type II. RK - 13, rabbit kidney cell lines, have been used successfully for the isolation of rubella virus. Cytopathogenic effect is produced but is not always clear on primary isolation and tissue culture fluids may have to be passed several times for full detection of virus (Best and Banatvala, 1967). A number of other cell lines have been used for isolating rubella

virus, but they are not as sensitive as AGMK, RK - 13 and Vero cells for primary isolation of rubella virus.

A nose or a throat swab is the specimen which most likely yields virus. In the case of congenital rubella, the virus may also be readily isolated from blood, CSF, urine, cataract material and lens fluid, autopsy tissue, placenta, amniotic fluid and fetal tissues from spontaneous or surgical abortion or virtually any organ (Dudgeon, 1967 - Partridge et al., 1981).

Identification of the isolates:

Neutralization test: indicated by the interference of rubella virus with the cytopathic effect of a challenge virus in primary culture of AGMK cells (Parkman et al., 1962).

Hemagglutination inhibition test: indicated by titrating the hemagglutinin antigen detected in the isolate, against known immune and non-immune sera. Inhibition of agglutination by the immune serum confirms rubella.

Immunofluorescence: Schmidt et al., (1966), have used FA test to identify rubella isolates, through the treatment of the isolates with immune rabbit serum, and fluorescein-conjugated anti-rabbit globulin. Fluorescence of the cells indicate rubella virus.

2. Serologic Diagnosis:

In practice, the laboratory diagnosis of rubella depends upon demonstrating a four-fold or greater rise in antibody titer from the acute serum to the convalescent one. Kangro et al., (1978), showed that the detection of rubella specific IgM antibody has a confirmed role in the diagnosis of both acquired and congenital rubella. Specific IgM determination avoids the necessity of appro-

priately timed paired sera and in babies with suspected congenital rubella such determination avoids the delay of waiting 9-12 months to see if rubella antibody persist.

A wide range of techniques have been applied for the detection of IgM. However, most of them involve the detection of IgM in the absence of both IgG and rheumatoid factors, since their presence may lead to false-positive results. Physical separation of IgM from IgG by sucrose density gradient ultracentrifugation followed by HI assay for the fractions, has been generally considered the procedure of choice, because of the proven reliability of this technique by many workers (Schmidt and Lennette, 1980). Care must be taken to remove non-specific inhibitors of rubella hemagglutinin before testing (Stewart et al., 1967 - Al-Nakib and Lilley, 1978).

Several other test methods have recently been introduced such as FAT, ELISA, RIA, however, they have not yet become reliable diagnostic tests. They are more applicable to research laboratories than to diagnostic laboratories (WHO Technical Report Series, 1981).

VI. RUBELLA CONTROL:

The obvious solution to congenital rubella problem lies in the active immunization of potential mothers. Prior to immunization, surveillance tests to recognize the susceptible individual is essential.

1. Surveillance tests for rubella:

Rubella antibody can be detected by a number of methods, all of them employ the detection of residual antibodies of IgG class, that mostly persist for the lifetime of the patient.

Neutralization test: Interference of rubella virus with CPE of a challenge virus in primary culture of AGMK cells, indicates the presence of specific rubella antibodies (Parkman et al., 1962). Neutralization antibodies are considered to be the protective ones (Dudgeon, 1967). However, the tests used to demonstrate these antibodies, although sensitive and specific, have presented problems of being expensive and time-consuming and have therefore been of little use (Stewart et al., 1967).

Complement Fixation test: Indicated by the ability of the rubella virus-specific complex to fix complement in the presence of an indicator system (Sever et al., 1965). This test is of no use for surveillance purposes due to the finding that CF antibody titers decline to undetectable levels after 15 months following rubella infection (Sever et al., 1966).

Hemagglutination inhibition test (HI): The HI antibodies appear early in the infection and persist for many years. Their presence is indicated by the inhibition of hemagglutination by rubella virus and certain red blood cells (Stewart et al., 1967). The test has been used as a standard method for diagnosis, seroepidemiology and immuno-surveillance. It is relatively simple but involves pre-treatment of the sera to remove non-specific inhibitors, and absorption to remove agglutinins to the erythrocytes. Although the test is sensitive, some difficulty arises in interpreting the significance of low HI titers, because of the interference of the residual non-specific inhibitors (Bradstreet et al., 1978). Low density lipoproteins (LDL) seem to include the most potent inhibitors. Activity may also be found in the high density lipoproteins (HDL) and very low density lipoproteins (VLDL) fractions of sera (Traavik et al., 1981). The ideal procedure for exclusion of non-specific inhibitors of rubella HA should remove lipoprotein completely with a minimum loss of immunoglobulins. None of the methods available are satisfying such demands. They had compared four methods for

exclusion of non-specific serum inhibitors: aerosil, kaolin, heparin/ MnCl_2 and dextran sulphate/ CaCl_2 methods. Their results suggested that low rubella HI titers could be looked upon with some confidence after kaolin and aerosil treatment, the other methods left non-specific inhibitors in a majority of the sera. The four methods investigated gave similar results with regard to removal of immunoglobulins.

Enzyme-linked immunosorbant assay (ELISA): applied by Voller and Bidwell, (1975), for the detection of specific rubella antibodies. The test has proved to be simple, specific with a 95% correspondence rate with the HI test. However, the test needs further investigation to be suited for routine laboratories.

Solid-phase radioimmunoassay (RIA): described by Chiekosugishita et al., (1978), for the detection of rubella IgG antibodies. Titers by RIA were approximately ten-fold higher than by HI, thus it is particularly useful in assessing the true immune status of those with apparently low levels of HI antibodies. Also, RIA has the added advantage that pretreatment of sera to remove inhibitors of HA and red cell agglutinins are unnecessary. However, similar to ELISA, the test needs further investigation to be suited for routine laboratories.

Single-radial-hemolysis (SRH) test: Schild et al., (1975), described the use of SRH for influenza virus. It is based on the principle that erythrocytes which are coated with hemagglutinating antigens will be lysed by hemagglutinating antibodies in the presence of complement. This application of this method to the detection of antibodies to rubella virus has been described by Skaug et al., (1975). It was found to be as specific as the HI test for the demonstration of antibodies against rubella virus hemagglutinin. The test showed practical advantages over HI. The serum can be tested undiluted and needs only prior heat inactivation to re-

move complement and any heat labile substances that may lyse erythrocytes. Chick, pigeon, sheep, trypsinized human "O" cells, all were found to give reaction in the SRH. Russell et al., (1978), found that the use of sheep erythrocytes did not appear to reduce the specificity of the technique, although, some human sera may contain antibodies that react with sheep cells. In their study no problems have been encountered with a number of sera obtained from cases of infectious mononucleosis, containing heterophile antibodies, which could agglutinate sheep cells in the SRH gel. Kurtz et al., (1980), found that sheep cells were particularly suitable for rubella SRH, as well as being readily available to diagnostic laboratories. They were not prone to non-specific lysis as a result of storage or the presence of complement incorporated in the gel. It was also found that the amount of hemagglutinin required to sensitize sheep cells for the SRH, was no more than for the red cells of other species.

Clarke et al., (1977), showed that the SRH test does not detect the early IgM antibody response in the acute phase of illness. With fractionated sera, those fractions containing rubella antibodies in the 19S region, were consistently negative in the SRH test, while those in the 7S region always gave positive results with a clear zone of hemolysis. The reason for this failure to react was unknown.

Appleton and Macrae, (1978), proved that the results of the SRH test are not altered by the presence of non-specific serum inhibitors or of erythrocyte agglutinins. Even the hemagglutination inhibitor in bacterially contaminated samples did not affect the test.

Russell et al., (1978), found that the rheumatoid factor may interfere in the SRH. Some sera that were SRH negative but had HI titers 1:16, to, 1:32, were found to contain "rheumatoid factor".

These sera did not contain detectable amounts of anti-rubella IgM, therefore, it was possible that rheumatoid factor interfered in the rubella SRH test. A common feature was observed in most of the sera containing both rubella HI activity and rheumatoid factor. Immediately surrounding the well was an area without hemolysis, but with a halo of lysis 2-3 mm. from the edge of the well. The nature of this phenomenon was not known. It is possible that in sera with low HI titers the small zones of hemolysis would be obscured. These sera were recorded as positive by the SRH. Gee *et al.*, (1978), admitted that 2-Mercaptoethanol was effective in removing the interfering factor, but it is doubtful whether pretreatment of all sera with 2-Mercaptoethanol can be justified. Heating sera at 60°C for 20 minutes reduced the interference and involved no extra effort, so it was wise to inactivate all sera in this way. In general, it was found that the interfering effect of rheumatoid factor in sera being investigated, was unlikely to be a serious problem.

2. Rubella immunization:

Rubella vaccine strains:

Three live attenuated vaccines have been in general use since 1969.

The HPV-77 was developed in the United States by passage of rubella virus 77 times in cultures of Green Monkey kidney cells, and then several times in duck embryo cells (Parkman *et al.*, 1966). Three derivatives with comparable efficiency to the original HPV-77 virus strain were introduced. They were designated: HPV-80, from Green Monkey kidney cells with three additional passages, the HPV-77:DE-5, with 5 passages in duck embryo cell cultures and the HPV-77:DK-12, with 12 passages in dog kidney cell cultures (Detels *et al.*, 1969).

The Cendehill vaccine was developed in Belgium by fifty-one passages of rubella virus in primary cultures of rabbit kidney cells (Prinzie et al., 1969).

The RA 27/3 vaccine was developed in the United States through the attenuation of Wistar RA 27/3 strain by serial passages in WI - 38 human diploid fibroblasts (Plotkin et al., 1969).

Rubella vaccine efficiency:

Although vaccinees shed virus from the pharynx at times for two or more weeks after vaccination, no evidence of transmission of infection to susceptible contacts was reported (Dudgeon et al., 1969 - Cherry et al., 1969 - Plotkin et al., 1973).

The three rubella vaccines were found to induce specific rubella antibodies in approximately 95% of vaccinated individuals, in similar patterns, but 4-8 fold lower than those following natural rubella infection (Dudgeon et al., 1969).

McCollum et al., (1969), studied the protective efficacy of rubella vaccine (HPV-77:DE-5) in a community trial, during a period of natural rubella activity. The overall antibody conversion rate of 97.6% was an indication that the vaccine was highly effective under field trial conditions. This efficacy was also supported by the disappearance of clinical rubella among vaccinees while still occurring at fairly steady rates among unvaccinated control groups. Rachelefsky and Herrmann, (1974), described an epidemic of rubella that occurred nine months after completion of a community-wide rubella vaccination program for children under 12 years of age; 84% of the 1,039 reported cases were in unvaccinated teenagers. By surveying 830 pregnant women and their newborn infants, they showed that rubella-vaccine induced immunity sufficient to reduce the occurrence of total infection and congenital malformations, but not adequate enough to prevent epidemics.

Plotkin et al., (1973), compared the RA 27/3 vaccine with both HPV-77:DE-5 and Cendehill vaccines. They showed that the RA 27/3 vaccine is immunogenic both intranasally and subcutaneously and the humoral antibody response to this vaccine administered either way is characterized by HI, CF, Nt and precipitating antibody response comparable to those of natural infection, but in a fashion superior to the other vaccines. The HI titers in the immediate period after administration of RA 27/3 vaccine were two to four-fold higher than those following Cendehill or HPV-77:DE-5 vaccination.

Craddock-Watson and Ridehalgh, (1975), showed that following RA 27/3 vaccine administration, all three classes of antibodies increased simultaneously after a delay of about 2 weeks, which may be regarded as the incubation period of vaccine induced infection. IgA and IgM antibodies reached peak titers between 21 and 28 days after vaccination and then declined to undetectable levels within 9 weeks. IgG antibodies reached a maximum 4-6 weeks after vaccination and persisted, but titers were about four to eight fold lower than those following natural infection.

Grinller, (1976), suggested that high levels of Nt antibodies in addition to local IgA secretory antibodies must be a key factor in the resistance of RA 27/3 vaccinees to reinfection. Individuals immunized with the RA 27/3 strain have had a lower rate of reinfection than those immunized with the other two vaccine strains. Intranasal or subcutaneous immunization with RA 27/3 vaccine, subcutaneous administration of Cendehill vaccine (Plotkin et al., 1973), and parenteral immunization with HPV-77 vaccine (Detels et al., 1969), have been found to be highly effective in inducing specific serum antibody response in susceptible subjects.

Induction of nasal antibodies was found to be correlated with

the resistance to reinfection with wild rubella virus. Secretory IgA antibody response was found to be regularly detected after intranasal RA 27/3 administration, frequently but irregularly detected after subcutaneous RA 27/3 administration and undetected after subcutaneous Gendehill administration (Plotkin et al., 1973).

Ehrenkrouz et al., (1975), evaluated the new measles-mumps-rubella combined live virus vaccine. They admitted that the sero-conversion rates, and the geometric mean antibody titers were no different from those usually found following the use of the same virus strains as monovalent vaccines, which indicates that the three vaccines are as effective when given together as when used individually.

Vaccine usage:

The goal of rubella vaccination programs is to prevent maternal rubella and subsequent fetal abnormalities. Kurgman, (1981), suggested that the decrease in the incidence of congenital rubella syndrome will require the vaccination of women currently of child-bearing age in conjunction with continued vaccination of young children. The combined approach seems to be necessary, since the experience in the United Kingdom (Lancet, 1979), indicates that selective vaccination of 11-14 year old girls and postpubertal women at high risk of rubella does not result in a rapid reduction in reported cases of rubella in women of childbearing age or in the incidence of CRS.

The Center for Disease Control, (1980), recommended that further reductions in the incidence of rubella and CRS can be accomplished by vaccinating all children greater or equal to 12 months of age, by rigorous follow-up vaccination of susceptible persons identified through premarital or other serology screening programs,

by requiring incoming college students to provide proof of rubella immunity, by vaccinating susceptible women in the immediate post-partum period and by vaccinating persons attending family-planning clinics.

In addition, hospitals should initiate programs to ensure a high level of immunity among employees at risk of acquiring or transmitting rubella in hospitals or clinics (Strassburg et al., 1981).

The teratogenic potential of rubella vaccines is unknown. Bernstein and Orga, (1980), studied the fetal aspects of immunization with RA 27/3 live attenuated rubella virus vaccine during pregnancy. Infectious rubella virus was not recovered from any of the placental or fetal tissues obtained after intranasal or subcutaneous maternal immunization. No specific HI or IgM was detected in the fetal tissue of vaccinated subjects. However, the risk of fetal damage has to be assumed if the vaccine is given to a rubella susceptible woman during pregnancy. Females of childbearing age should avoid rubella immunization during or shortly before pregnancy (Morbidity and Mortality Weekly Report, Feb. 6, 1981).

M A T E R I A L S & M E T H O D S

MATERIALS & METHODS

I. THE STUDY POPULATION:

This consisted of 604 normal individuals aged 6-42 years: 167 were school children of both sexes (6-14 years old), 437 were females of childbearing age including; 282 secondary school students and teachers (15-42 years old), 67 females from the College of Health Sciences (16-24 years old), and 88 pregnant women attending the Ante-natal clinic of Salmaniya Medical Center for follow-up.

Sera collection:

During 1981, blood was withdrawn from individuals attending schools of different levels in different areas of Bahrain, the College of Health Sciences, and the Ante-natal clinic, by the Public Health Laboratory in Bahrain, for an independent project. With the kind permission of Dr. S. Dutta (head of the Public Health Laboratory), a number of sera (604), were selected and classified according to Table II.

Full data concerning age, area of residence, profession and nationality were obtained from the Public Health Laboratory registration book.

All sera were given serial numbers and stored in the deep freeze at -20°C in screw-capped and labelled test tubes.

II. THE ANTIGEN TITRATION:

This followed the procedure described in LABORATORY DIAGNOSIS OF VIRAL DISEASES. H. Ballew, F. Forrester, H. Lyerla, W. Velleca,

Place of sample collection	Age groups						Total
	6-10	11-14	15-18	19-24	25-42		
Elementary schools:Manama	83						
Intermediate school:Manama		84					
Secondary schools: Manama			73	7	9		89
Muharraaq & Hidd			82	9	12		103
Essa Town & Riffa			77	4	9		90
College of Health Sciences			56	11			67
Ante-natal clinic						88	
T O T A L	83	84	288	31	30	88	604

TABLE II: Classification of the study population according to age and
the place of sample collection.



and B. Bird. (1977). U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Atlanta, Georgia.

A. REAGENTS:

1. Rubella Hemagglutinin:

The lyophilized hemagglutinin antigen, was obtained in small vials (Standard Laboratory, C.P.H., Colindale, London). It was reconstituted with 1 ml. sterile distilled water and stored at 4°C to be used in the SRH test.

2. HSAG (HEPES-Saline-Albumin-Gelatin) diluent:

a. HEPES saline 5X stock solution:

HEPES (N-2-Hydroxyethyl piperazine N-2-ethanesulfonic acid) (CALBIOCHEM, ULTROL)	29.80g.
Sodium chloride, NaCl (MERCK)	40.95g.
Calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (MERCK)	0.74g.
Distilled water	1.00l.

The constituents were dissolved in 900 ml. distilled water. The pH of the solution was adjusted to 6.5 by adding 1 N. NaOH, then distilled water was added to bring volume of solution to 1000 ml. The solution was sterilized by filtration through millipore membrane 0.22 u pore size and stored at 4°C.

b. Bovine serum albumin 2X stock solution:

Bovine albumin powder (SIGMA).....	20g.
Distilled water.....	1.0l.

The bovine albumin was dissolved in 900 ml. water and the volume was adjusted to 1000 ml. by adding distilled water. Sterilization was done by filtration through millipore membrane 0.22 μ pore size and stored at 4°C.

c. Gelatin 10X stock solution:

Gelatin (DIFCO).....	25mg.
Distilled water	1.0l.

The gelatin was dissolved in water. The solution was sterilized by autoclaving for 15 minutes at 120°C and stored at 4°C.

d. HSAG diluent:

HEPES saline 5X stock solution.....	200ml.
Bovine serum albumin 2X stock solution.....	500ml.
Gelatin 10X stock solution.....	100ml.
Sterile distilled water.....	200ml.

The solutions were combined at 25°C, at which the pH was supposed to be 6.2 ± 0.05 . 1.0 N. NaOH was used to adjust pH below 6.2. pH above 6.2 was adjusted with 1.0 N. HCl. The solution was stored at 4°C, to be used within 2 months, if kept sterile.

3. Dextrose-Gelatin-Veronal (DGV) buffer:

Veronal (Barbital) (BRITISH DRUG HOUSES. B.D.H.).....	0.58g.
Gelatin (DIFCO).....	0.60g.
Sodium barbital (B.D.H.).....	0.38g.

CaCl ₂ (anhydrous) (MERCK).....	0.02g.
MgSO ₄ .7H ₂ O (MALLINCKRODT).....	0.12g.
NaCl (MERCK).....	8.50g.
Dextrose (DIFCO).....	10.00g.
Distilled water.....	1.00l.

The veronal and gelatin were dissolved in 250 ml. of water by heating. This solution was combined with the other constituents and sterilized by filtration.

4. Alsever's solution - pH 6.1:

Dextrose, anhydrous (DIFCO).....	20.50g.
Sodium citrate, Na ₃ C ₆ H ₅ O ₇ .2H ₂ O (MALLINCKRODT).....	8.00g.
Citric acid, C ₆ H ₈ O ₇ .H ₂ O (MERCK).....	0.55g.
NaCl (MERCK).....	4.20g.
Distilled water.....	1.00l.

Constituents were combined and the pH was adjusted to 6.1, sterilization was done by filtration (millipore).

5. Chick cells:

One to three day-old chick red blood cells were collected in Alsever solution. The erythrocytes were washed three times in DGV buffer, centrifuging at 600 x g for 10 minutes. The buffy coat was carefully removed after each washing. Then 0.25% cell suspension was prepared in HSAG (chick cells could not be used for more than two weeks).

B. EXPERIMENTAL PROCEDURE:

The antigen titration was done in a plastic disposable "U" microplate (Cooke Engineering Co.). A 1:14 dilution of the rubella antigen was prepared by adding 0.1 ml. of the antigen

to 0.3 ml. of HSAG diluent and allowed to stand at room temperature for 15 minutes. 0.025 ml. of HSAG diluent was added to wells "2" through "9" in each of 3 rows. Then 0.05 ml. of the diluted antigen were added to the first well in each of the three rows. 0.05 ml. of HSAG diluent were added to three wells for erythrocyte control. Serial two-fold dilutions (1:4 - 1:1024) of the antigen in all three rows were carried out using a 0.025 ml. microtiter loops, and 0.025 ml. of HSAG diluent were added to each well of diluted antigen. The plate was placed in the refrigerator for 15 minutes, then on a mechanical shaker and 0.05 ml. of cold 0.25% baby chicken erythrocytes was added to each well of diluted antigen and to the three erythrocyte controls. The plate was sealed with cellophane tape and incubated at 4°C for one and half hours. Then it was removed and allowed to stand at room temperature for 15 minutes.

The antigen titer was recorded as the highest dilution of antigen causing complete agglutination of the cells. This titer was found to be 1:64.

III. SINGLE RADIAL HEMOLYSIS TEST:

Following the procedure of the Central Public Health (C.P.H.) Laboratories, Colindale, England. (Adapted from Bradstreet et al., 1978).

A. REAGENTS:

1. The CFB buffer (Oxoid BR 16):

Barbital, 5,5-Diethylbarbituric acid..... (B.D.H.)	0.575g.
Sodium chloride, NaCl (MERCK).....	8.500g.
Magnesium chloride, $MgCl_2 \cdot 6H_2O$ (MALLINCKRODT)	0.168g.
Calcium chloride, $CaCl_2$ (MERCK).....	0.028g.
Sodium barbital, Na 5,5-Diethylbarbiturate..... (B.D.H.)	0.185g.
Distilled water.....	1.00 l.

The constituents were dissolved in warm distilled water, by continuous shaking, the pH of the buffer was adjusted to 7.2 and the solution was sterilized by autoclaving at 110 lb. pressure, 120°C for 15 minutes, and kept at 4°C.

2. The 1% agarose:

2 g. of agarose (SIGMA) were dissolved in 200ml. of CFB. Portions of 10 ml. were distributed in 20 screw-capped test tubes, autoclaved at 110 lb. pressure, 120°C for 15 minutes and kept at 4°C.

3. Sheep erythrocytes:

Sheep erythrocytes collected in Alsever's solution were

washed three times in CFB, and their concentration was adjusted to 15%.

4. Complement:

Lyophilized guinea pig complement (Behring Institute) was reconstituted with 5 ml. diluent to give a dilution of 1:30 and stored in 1 ml. vials at -20°C to be used within 2 months.

5. Antibiotics:

Penicillin (HOECHST) and Streptomycin (SPECIA) were reconstituted with distilled water, and stored at 4°C.

6. Positive control serum:

Anti-rubella reference reagent 80/1 was obtained in small vials from the Division of Microbiological Reagents and Quality Control, C.P.H. Laboratory, Colindale, London. It was reconstituted with 0.5 ml. of sterile distilled water and stored in the refrigerator at 4°C to be used within seven days without heat inactivation. Each vial contained 7.5 i.u./0.5 ml. of rubella antibodies as measured by the SRH technique.

B. EXPERIMENTAL PROCEDURE:

10 ml. of agarose was melted by steaming, and then held in 44°C waterbath.

Sheep erythrocytes were sensitized by adding 0.3 ml. of the 15% sheep cell suspension to an equal amount of rubella hemagglutinin (titer 1:64) in a universal tube. The mixture was left at room temperature for 30 min., to allow adsorption of the antigen on the cells. Then the tube was filled with CFB and centrifuged at mark 6

(2000 rpm.) for 10 min. The supernatant fluid was discarded and the red cells were resuspended in 0.5 ml. CFB containing 50 units of penicillin and 50 ug/ml. of streptomycin. 0.5 ml. of undiluted guinea pig complement was added and the tube was held in the water-bath at 44°C for 10 seconds. Then 10 ml. melted pre-cooled agarose was poured into it, and the contents were immediately poured into a round plastic petri dish on a levelled table. A control plate containing unsensitized sheep cells was prepared in a similar manner.

The plates were allowed to set for 10 min. at room temperature and then kept in a humid box, at 4°C (for from 30 min. to 5 days).

The test and control plates prepared were sufficient for the testing of 20 sera.

Within the area of the plate, 20 wells of 2 mm. diameter were punched in the agarose, and the agarose plugs were then removed by a pasteur pipette connected to a suction apparatus. The sera to be tested were inactivated at 56°C for 30 min. and 5 ul. of each serum was added with a microcapillary tube, in corresponding wells of test and control plates. Positive and negative control sera were included with each test and control plates. Plates were then incubated in a humid box at 37°C for 18 hours. The diameter of the hemolyzed zone around each well was measured in mm. For future reference, the plates were fixed by flooding them with 10% formalin for 2 hours (See photograph in Fig. 3).

C. INTERPRETATION OF RESULTS:

In most of the SRH test plates, the standard positive control serum gave diameters of 7.5 mm. corresponding to an antibody content of 15 i.u./ml. This was considered as the minimum immune titer (MIT) which gives protection against rubella infection

(Bradstreet et al., 1978). Serum samples giving diameters equal to or larger than that of the control serum (15 i.u./ml. contained sufficient rubella specific antibodies and were considered to be immune. Those giving diameters smaller than that of the control serum, and those with no zone of lysis were considered to be rubella non-immune.

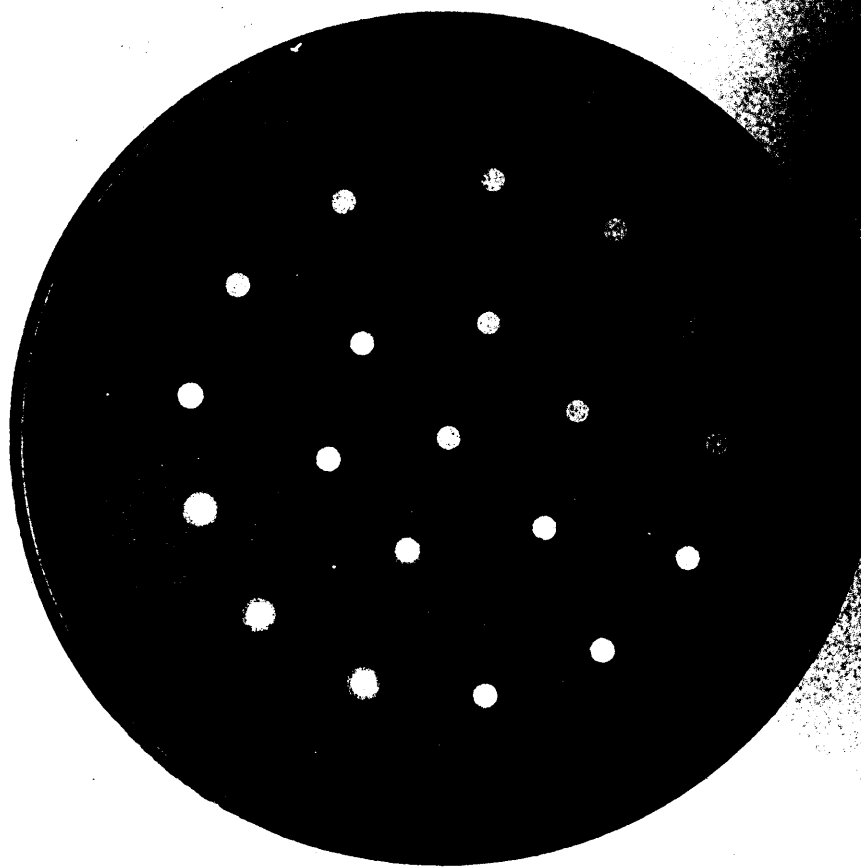
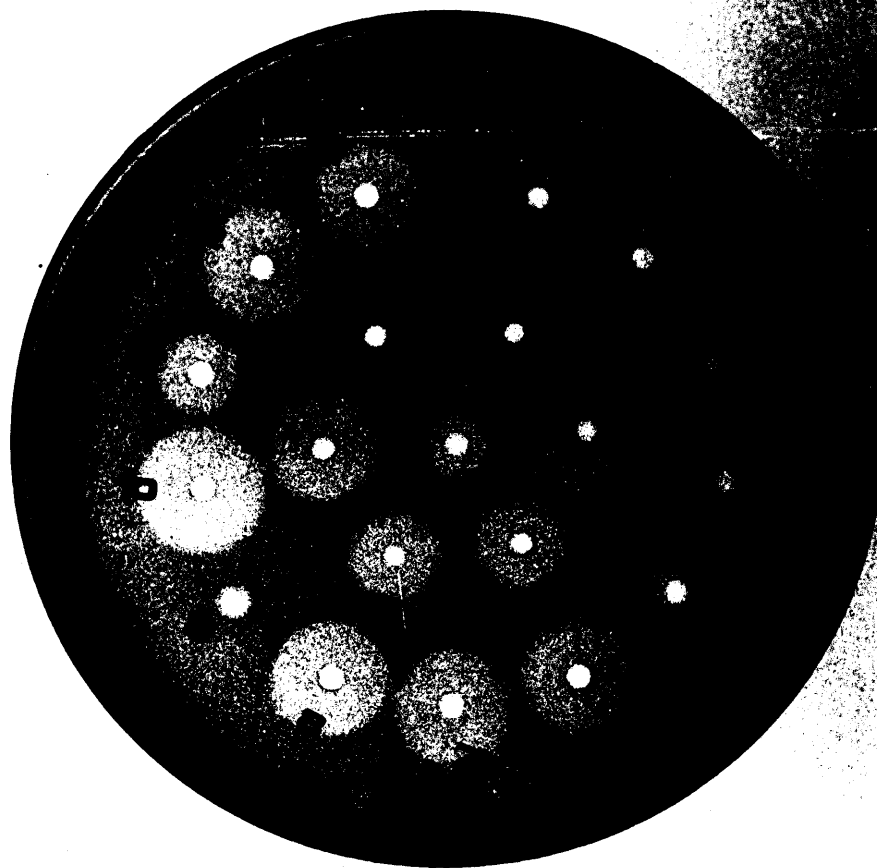
IV. HEMAGGLUTINATION INHIBITION TEST:

The test was performed in Bahrain, with the cooperation of the Public Health Laboratory, using the modified rubella HI test, Trypsinized human "O" procedure and following the procedure described in Flow Laboratories Inc., modified from the "Immunology Series No. 5, January, 1974. Center for Disease Control, Atlanta, Georgia, 30333.

Figure 3: Single-radial-haemolysis plates.

T: Test plate showing haemolysis zones in rubella positive sera, and no haemolysis in rubella negative sera.

C: Control plate with no zones of haemolysis.



R E S U L T S

RESULTS

I. THE SUSCEPTIBILITY TO RUBELLA OF 604 INDIVIDUALS BY SRH:

A steady rise with age in the percentage of protected women was noted. Of 604 individuals tested, 115 (19.0%) were susceptible to rubella (Table IV). 167 were school children and of these 57 (34.1%) were susceptible. Among the 437 females of childbearing age 58 (13.2%) showed susceptibility.

Table V shows the susceptibility of 167 school children tested. Out of 83 children in the first age group 6-10, 46 were females, with 41.3% susceptibility compared to 40.5% susceptibility among 37 males in the same age group. In the age group of 11-14 years old, 51 were females, 33 males with 27.4% and 27.2% respective susceptibility.

Female students were divided into 15-18, 19-24, 25-42 age groups. The first age group 15-18, consisted of 232 samples with 15.5% susceptibility. In the second age group 19-24, 20 samples were tested, with 10.0% susceptibility. The last age group 25-42, consisted of 30 females with 6.6% susceptibility (Table VI).

Table VII shows the susceptibility to rubella among female students and staff in the College of Health Sciences. 56 were in the age group of 15-18 years old and among them 14.2% were susceptible, 11 individuals between ages 19-24, showed 9.0% susceptibility.

The immune status of 88 pregnant women, is shown in Table VIII. 9 samples were seronegative, giving a total susceptibility of 10.2%. 8.6% susceptibility was among Bahrainis compared to 15.3% non-Bahraini individuals.

The total susceptibility to rubella among women of child-bearing age is summarized in Table VIII, the highest susceptibility was found in the age group 15-18, where it was 15.2%. A decrease in susceptibility was shown with age; 9.6%, 6.6% of susceptible individuals were found in the age groups 19-24, and 25-42 years old. Figure 4 indicates that the most susceptible age group in all females of childbearing age tested is between 15-18.

Table IX shows the prevalence of rubella specific antibody in 288 females aged 15-18 and grouped according to the place of residence in Bahrain.

No significant difference was noted in the susceptibility to rubella in the three areas tested.

II. CORRELATION OF HI AND SRH TESTS:

54 sera were tested for rubella antibody by both the SRH and HI techniques. The HI titers, the ranges with the mean of diameters and the number of sera corresponding to them are shown in Table XII. Two samples with HI titers less than 1:8 were also negative in the SRH test. A good correlation was noted between the HI titers and the zones of hemolysis obtained by 52 positive sera when tested by the SRH. Each HI titer was found to correspond to a range of diameters of lysis.

The correlation between the HI and the SRH tests of 52 successive positive samples is shown in Figure 5. The results of the SRH test were plotted against the reciprocal of the HI titer, and a straight line was obtained.

T A B L E S

Age groups	Number of samples tested	Number of seronegatives	% susceptibility
6-10	83	35	42.1
11-14	84	22	26.1
15-18	288	44	15.5
19-24	31	3	10.0
25-42	30	2	6.6
Unclassified	88	9	10.2
TOTAL	604	115	19.0

TABLE III: Age susceptibility as demonstrated by reactivity to rubella antigen in the SRH test, among school children and women of childbearing age in Bahrain, between ages 6-42 (1981).

Age groups	Number of samples tested	% susceptibility	
		Females	Males
6-10	83	41.3	40.5
11-14	84	27.1	25.3
TOTAL	167	34.3	33.0
		34.1	

TABLE IV: The susceptibility to rubella of 167 school children, between ages 6-14 (1981).

Age groups	Number of samples tested	Number of seronegatives	% susceptibility
15-18	232	36	15.5
19-24	20	2	10.0
25-42	30	2	6.6
TOTAL	282	40	14.1

TABLE V: The susceptibility to rubella of 282 females of childbearing age, from schools, between ages 15-42 (1981).

Age groups	Number of samples tested	Number of seronegatives	% susceptibility
16-18	56	8	14.2
19-24	11	1	9.0
TOTAL	67	9	13.4

TABLE VI: The susceptibility to rubella of 67 female students and staff from the College of Health Sciences, between ages 16-24 (1981).

Nationality	Number of samples tested	Number of seronegatives	% susceptibility
Bahraini	69	6	8.6
°Non-Bahraini	19	3	15.3
TOTAL	88	9	10.2
° Non-Bahrainis include Indians, Europeans & Americans			

TABLE VII: The susceptibility of 88 pregnant women, attending the Ante-natal clinic of the Salmaniya Medical Center (1981), according to their nationality.

Age groups	% susceptibility			Total susceptibility
	Females of the schools	Females of the C.H.S	Pregnant women	
15-18	232	56	-	15.2
19-24	20	11	-	9.6
25-42	30	-	-	6.6
Unclassified			88	10.2
TOTAL	282	67	88	13.2

TABLE VIII: The age susceptibility to rubella of females of childbearing age in the different groups tested.

Area	Number of samples tested	Number of seronegati- ves	% suscepti- bility
(a) Manama	91	12	13.1
(b) Muharraq	101	14	13.8
(c) Essa Town	96	18	18.7
TOTAL	288	44	15.2
P value between (a) and (b) is more than 0.05. P value between (b) and (c) is more than 0.05. P value between (a) and (c) is more than 0.05.			

TABLE IX: Percentage of susceptibility to rubella in 288 females in the age group 15-18, in relation to the area of residence in Bahrain.

Serum number	Diameter of lysis with undiluted sera (in mm.)	HI titer °
1	No lysis	Less than 8
2	6.5	"
3	7.5	8
4	8.0	"
5	8.3	"
6	8.3	"
7	8.4	"
8	8.5	"
9	8.9	"
10	9.3	16
11	9.6	"
12	9.6	"
13	9.7	"
14	9.9	"
15	9.9	"
16	10.0	"
17	10.4	32
18	10.9	"
19	11.2	"
20	11.2	"
21	11.2	"
22	11.3	"
23	11.3	"
24	11.4	"
25	11.8	"
26	11.9	"
27	12.0	"
28	12.0	64
29	12.5	"
30	12.8	"
31	12.9	"
32	12.9	"
33	12.9	"
34	13.0	"
35	13.2	"
36	13.4	"
37	13.5	"
38	13.0	128
39	13.7	"
40	13.7	"
41	13.9	"
42	14.0	"

(continued)

Serum number	Diameter of lysis with undiluted sera (in mm.)	HI titer
43	14.3	128
44	14.3	"
45	14.3	"
46	14.5	"
47	14.8	"
48	14.5	256
49	15.4	"
50	15.5	"
51	15.6	"
52	15.6	"
53	15.7	"
54	15.9	"
• HI titer: reciprocal of the dilution.		

TABLE X: Comparison between rubella HI titer and zone of lysis, in 54 sera tested by the HI test in cooperation with the Public Health Laboratory, Bahrain.

Number of samples tested	HI titer (a)	Range of diameters (in mm.)	Mean Zone Diameter ($\pm$ SD) (b)
2	Less than 8	No lysis	-
7	8	(c) 7.5-8.9	8.3 $\pm$ 0.42
7	16	9.2-10.0	9.7 $\pm$ 0.27
11	32	10.4-12.0	11.3 $\pm$ 0.45
10	64	12.0-13.5	12.9 $\pm$ 0.43
10	128	13.0-14.8	14.0 $\pm$ 0.47
7	256	14.5-15.9	15.4 $\pm$ 0.41
(a) HI titer : reciprocal of the dilution. (b) Mean Zone Diameter: Diameter of lysis in mm., in the SRH test. (c) Standard positive control serum: 7.5 mm. of lysis.			

TABLE XI: Ranges of diameters in 54 sera, in relation to each HI titer.

F I G U R E S

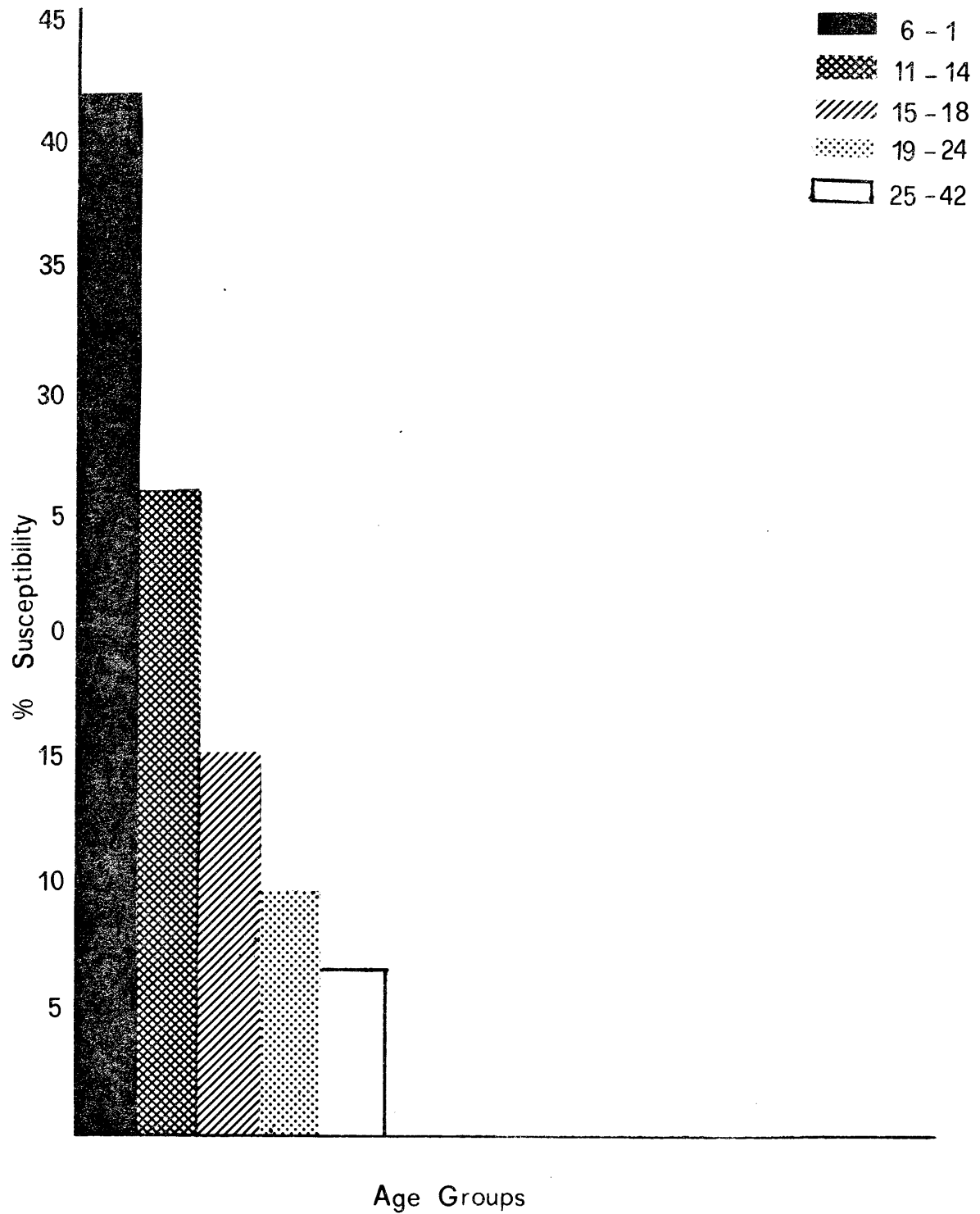


Figure 4: Susceptibility to rubella among different age groups.

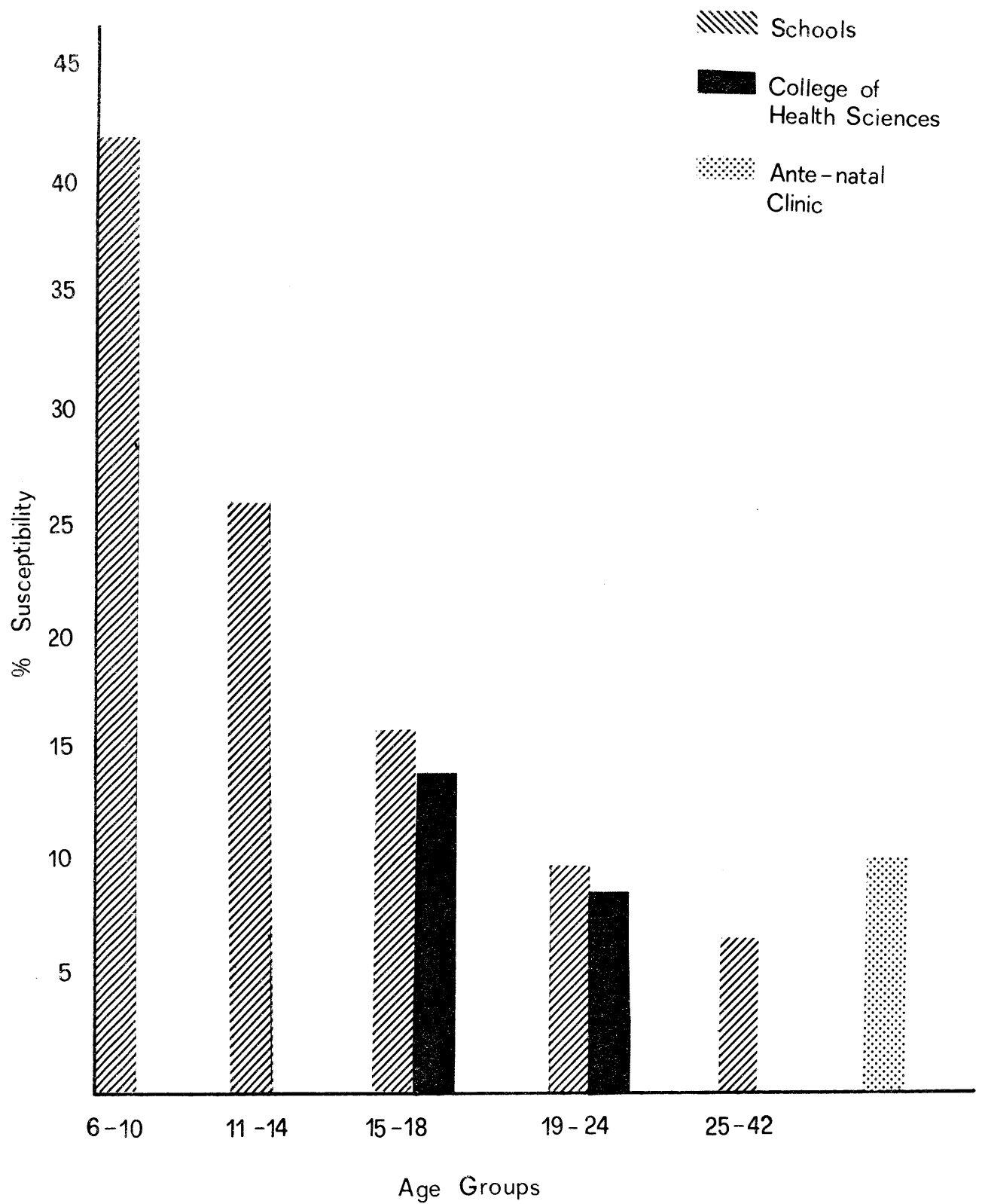


Figure 5: Susceptibility to rubella among different test groups.

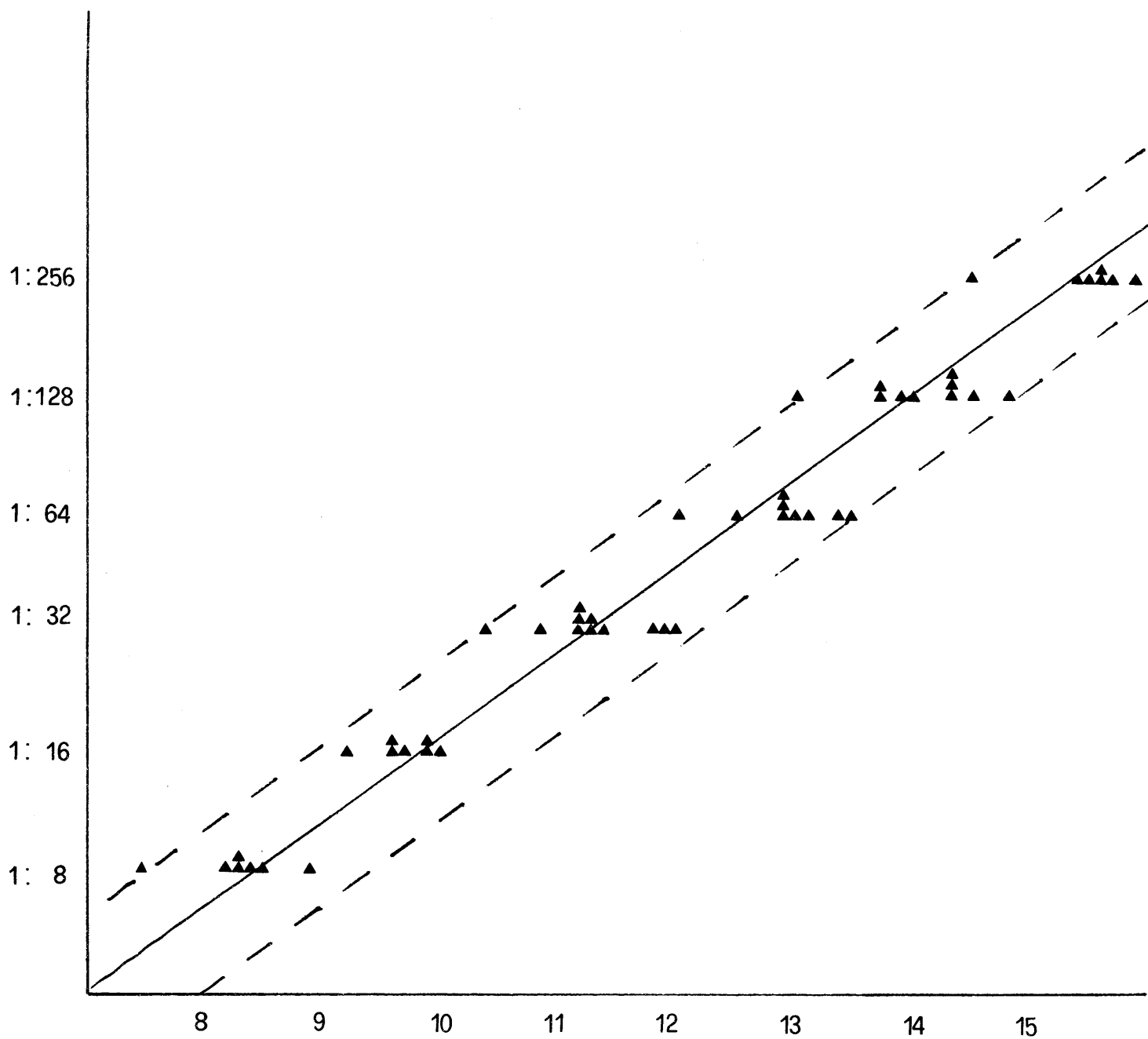


Figure 6: Correlation between 52 positive results obtained by the HI and the SRH test. The solid line indicates the same antibody titer in both test, and the dotted lines indicate limits of $\pm$ one titer step.

D I S C U S S I O N

DISCUSSION

The marked variation in epidemic potential of the rubella virus, in different population groups, is difficult to explain. In most island populations, it has been found that epidemics of rubella are less common than expected, and in some, the virus fails to become endemic, inspite of high population densities. These results indicate that in contrast to other islands, rubella is endemic in Bahrain. Out of 604 individuals studied by the SRH, 19% were found to be susceptible, of these 13.2% were females of childbearing age. These figures are comparable to the worldwide rates of susceptibility to rubella described by Rawls et al., (1967); they are considered relatively low in comparison to the 50% rate observed in other island population. The reasons for the epidemiological variation in different populations were not fully understood.

A number of possible reasons have been investigated. Cockburn, (1969), and Dowdle et al., (1970), suggested that the population density and the geographical location were more likely to influence the spread of rubella virus than ethnic factors, since island populations are of mixed ethnic origin. Rawls et al., (1967), suggested that the minimum population required to maintain the endemicity of rubella is greater than that required for measles. This is probably because closer personal contact is required for the transmission of rubella than for measles. The population density factor is the most likely to explain the relatively high prevalence of immunity among Bahrainis in comparison to those of other island populations. Bahrain is a small densely populated country ($563/\text{km}^2$), with a high rate of population movement, specially among the non-Bahraini section of the community. Perhaps this, in addition to the large family size, common in Bahrain, could encourage the widespread dissemination of rubella virus in the community with consequent development of immunity in a high proportion of the population.

The geographical location of Bahrain is another important factor, that may influence rubella epidemiology. Bahrain forms the link between East and West for transit aircraft of many countries. The number of transit passengers at Bahrain International Airport in 1978 was 956,723 which is nearly double the number registered in 1974 (Statistical Abstract, 1978). This high rate of movement to-and-from Bahrain will encourage the introduction and dissemination of the virus in Bahrain, and might in addition to the density factor explain the epidemiology of rubella in Bahrain.

Honeyman and Menser, (1974), showed that there was an association between the presence of low genes frequencies of HL-A1 and A8 and increased susceptibility to rubella, which indicate that ethnicity might be a significant factor in the epidemiology of rubella throughout the world, since marked differences exist in the HL-A profile among ethnic groups. These antigens are coded for by genes located in the MHC. They may be responsible for the variation in susceptibility to infectious agents observed in different ethnic groups.

Hattis et al., (1973), have shown that during an uncomplicated rubella infection the average individual may only have minimal contagious potential. Others may have substantially greater potential for spread, "spreaders". These, when exposed to a large population, may be expected to infect high proportion of susceptible persons. Honeyman and Menser, (1974), postulated that these "spreaders" possessed the HLA A1 & A8 haplotypes. The mean gene frequency of HLA A1 & A8 in a population would then be related to the number of potential spreaders and consequently the extent and rate of transmission of virus in that population. The failure of rubella virus to become endemic inspite of high population densities, would seem to be due to the low HLA A1 & A8 gene frequencies, causing a sparser distribution of spreaders in a population which is genetically less susceptible.

If this postulated hypothesis is true, we can apply it to the present study and say that the frequency of HLA 1 & 8 genes in Bahrain is high and so genetically Bahraini population is more susceptible to infection than other island populations. This may be supported by the finding that in Kuwait, which belongs to the same ethnic group of Bahrain, the susceptibility to rubella among Kuwaiti females of childbearing age was found to be very low (5.2%); (Hathout et al., 1978). Further work is needed to confirm this in Bahrain.

Climate may also have an influence on rubella virus transmission. It has been shown that in some tropical islands, the transmission of rubella is interrupted during the hottest summer months. This may not be the case in Bahrain. The steady rise with age in the percentage of protected women seen in my study, suggested a relatively high rate of exposure and a continuous circulation of the virus in the community.

The accurate detection of susceptible females that need to be immunized was found to be influenced by the method employed for screening. Mortimer et al., (1981), showed that a significant proportion of those women currently reported as susceptible to rubella, were immune and that they were recommended for immunization unnecessarily. There would be a considerable benefit in defining more accurately the group of women who need to be immunized, particularly in reducing the number who might be inadvertently given rubella vaccine when they were pregnant.

Of the methods of serotesting available, the HI test has been widely used for detection of antibodies to rubella virus, since 1967, (Stewart et al., 1967). This test, although sensitive and specific, may give false positive reactions. Low titers obtained are difficult to interpret, because of the possible interference of residual non-specific inhibitors. On the other hand, the SRH test, introduced by Schild et al., (1975), was unaffected by the

non-specific inhibitors and any zone was an indication of specific antibody (Gee et al., 1978).

In the present study the SRH test was used and was found to be simple, convenient, reliable and well suited to rapid screening of large numbers of sera (Skaug et al., 1975 - Gee et al., 1978 - Russell et al., 1978). Only 1 ml. of rubella hemagglutinin antigen and 3 ml. of complement were sufficient to screen 60 sera. It required small quantities of serum which did not need pretreatment to remove the non-specific inhibitors or naturally occurring agglutinins. Thus, fewer red cells are needed than for the HI test. Furthermore, the test is simpler to perform and less time-consuming than the HI test.

One shortcoming of the SRH test is that it does not detect the early IgM antibody response in the acute phase of the illness. Schild et al., (1975), showed that the immunoglobulin class active in the SRH test is IgG, with a sedimentation coefficient of 7S.

Chick, pigeon, sheep, trypsinized human "O" cells, all were found to give reaction in the SRH. In this test, sheep cells were perfectly satisfactory, even though sensitization was not carried out by chemical means, described by russell et al., (1978).

Several factors have been found to affect the reproducibility of the SRH test. the titration of each patch of antigen was essential. So much delay was encountered in this work, because of the use of antigen from different commercial sources. Some of them failed to react in the SRH test and some gave diffuse zones of lysis. This might be attributed to the drop in titer or impurity of this antigen. The incorporation of complement with the agarose before the addition of sera into the wells was done to avoid the development of the "target" effect with a ring of lysed cells at the perimeter of the zone and virtually intact cells around the

wells (Appleton and Macrae, 1978). To obtain reproducible zone sizes, it was essential to fill the wells to the level of the agar surface. In the test, this was done most satisfactorily with disposable microcapillary tubes. The diameter of hemolysis was measured after 18 hours of diffusion at 37°C since this was found to be the optimal time at which all antibodies were allowed to diffuse (Skaug et al., 1975). Diffuse perimeters made measurements impossible in any readings done at times greater than 18 hours. Occasionally, when gels were left at room temperature after incubation, some non-specific zones gradually developed. To avoid this possibility, gels should be read soon after removal from the incubator. In case of delay, 10% formalin was used for fixation.

In this study, a good correlation was obtained between the HI and the SRH tests, confirming the observations made by Skaug et al., (1975), Clarke et al., (1977), Appleton and Macrae, (1978), Gee et al., (1978), Russell et al., (1978), and Bedrossian, (M.S. Thesis, 1981). The SRH was found to involve several practical advantages. Firstly, the serum can be tested undiluted. Furthermore, in contrast to the HI test, it was not necessary to remove the non-specific inhibitors of hemagglutination. The pretreatment was limited to heating the serum at 56°C for 30 min. in order to inactivate complement and any heat labile substances that may lyse erythrocytes (Skaug et al., 1975). Appleton and Macrae, (1978), have found that even agglutinin inhibitors in a bacterially contaminated samples, did not affect the SRH test, whereas this contamination was found to lead to the appearance of non-specific inhibitors of rubella hemagglutinin, that could not be removed by heparin/MnCl₂ pretreatment (Pattison et al., 1976). Moreover, storage of the sera may also give false-positive results in the HI test due to the failure of heparin/MnCl₂ to adequately remove lipoproteins altered by storage (Pattison et al., 1976). In this study some sera which had been stored for a minimum of 6 months were tested and these showed no problem in the SRH test. As a third advantage, Kurtz et al., (1980), have found the SRH test

to be slightly more sensitive than the HI test, as it was possible to measure a hemolysis zone corresponding to an antibody concentration of 5.6 i.u./ml., an amount of antibody below the threshold detectable by the routine HI method. They found that at low antibody titers, the correlation between the two tests could be inexact as a result of variations in sensitivity between them. However, by using the zone size of the 15 i.u./ml. standard as the lower limit representing immunity, discrepancies between the tests were avoided. Mortimer et al., (1981), concluded that, as the SRH test is not only easier to perform but also less liable to incorrect interpretation, it should replace the HI test as the method of choice for rubella antibody screening.

In this work, it was found that 13.2% of females of childbearing age tested by the SRH method, were susceptible to rubella. Female students showed a 15.5% susceptibility and 8.6% of the Bahraini pregnant women tested were found to be susceptible. Therefore, the danger of bearing congenital rubella babies is still present. Furthermore, 13.4% of females of the College of Health Sciences were non-immune and this may illustrate the threat of nosocomial infections to them, since most of these females will be or have been already enrolled for working in the different hospitals and Health Centers.

These results illustrate the importance of surveillances and the subsequent immunization of females found to be susceptible to rubella, to avoid the congenital malformations when susceptible women contract the disease during pregnancy.

Although the rate of susceptibility among females of childbearing age in Bahrain is similar to the rate found in U.K. & U.S.A. (Rawls et al., 1969), a similar large-scale vaccination programs for children may be uneconomical and perhaps unnecessary. The herd immunity approach followed in the U.S.A. to eradicate rubella, seems to be a

failure. Epidemics continue to occur despite the previous rubella vaccination programs involving one year-old children, preschoolers, and children 5 to 9 years of age.

The occurrence of the epidemics was not attributed to vaccine failure to elicit an immune response. The main cause of the epidemics was the failure of the herd immunity concept. Although children remained immune, rubella spread among susceptible adolescents (Center for Disease Control, Nov. 1980).

The British rubella vaccination program was at first directed principally towards school girls aged 11-14 and women at high risk, such as nurses, school teachers, and doctors. However, this program was unlikely to reduce the incidence of congenitally acquired rubella until the mid-1980's (Lancet, June 23, 1979).

It has been found that there are certain problems which must be overcome if a rubella vaccination campaign is to succeed. Firstly, it is not always possible to vaccinate all female children or even females of childbearing age, and "pockets" of individuals, who have not been vaccinated may occur. Secondly, the vaccine failure rates must be taken into account, since it is known that a small percentage of those who receive rubella vaccines fail to develop antibodies. Thirdly, vaccinees may develop low levels of antibodies in comparison with the naturally occurring disease (Plotkin, 1973), which in children may decline to undetectable levels by the time these individuals reach childbearing age and so lead to a large population of susceptible women, when compared with the current natural adult immunity. Furthermore, in Bahrain, approximately 32.4% of the population are expatriates, many of whom arrive just before or during childbearing age and would escape a childhood vaccination program.

Even with the high percentage of susceptibility, (34.1%), found among school children tested, it is clearly seen that it is more rea-

sonable to provide protection to non-immune females during adolescence, since rubella infection in children is a mild, short-lived disease, with a low rate of complication and that the primary purpose of vaccination is the prevention of fetal sequels, associated with infection during pregnancy.

For all these reasons in addition to the possibility for potential hazard of inadvertent vaccination of pregnant women during a large vaccination campaign, and to provide the chance for all females to be naturally immunized with the wild rubella virus circulating in the community, it was felt that a vaccination policy similar to that proposed for Kuwait by Hathout et al., (1978), is the most suitable policy to be followed in Bahrain.

In Bahrain, there are about 122 marriages per month (Statistical Abstract, 1978). Since this experience shows that rubella antibody testing is simple, and that one person can easily handle 200 samples per week, it would be within the scope of the laboratory to screen 400 or more samples per month for rubella antibodies, as a routine pre-marital test. Rubella vaccination can then be offered to those found to be seronegative. This program would obviate the necessity of organizing an extensive vaccination campaign among school children, with its possible risks and considerable expense. Women shortly to be married, must of course be given a reliable contraceptive measure for two or three months following vaccination.

With the cooperation of the Health Education section of the Public Health Directorate, this policy can be followed most satisfactorily through the awareness of females of childbearing age of the possibility of acquiring acute rubella infection with its subsequent complication, specially in the first trimester of pregnancy.

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