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Original Research Article

Serological analysis of mumps virus antibodies among school children in Hodeidah city, Yemen

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Keywords

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A B S T R A C T

This study is a sere-survey study aimed to Assessment the frequency of exposure to mumps virus in different age groups among children in Hodeida city, Yemen, to determine the titre of antibodies as an indicator of exposure, to detect the factors that contribute to the contract with mumps infection among the selected children and finally to assess the need of introducing the mumps vaccine within the expanded immunization program in Yemen (EIP).A cross sectional study was carried out during a period of three months, starting in 1 of January 2013 and ending at 30 of march 2013. A total of 139 children were included in this study, where males represented 52.5% and females represented 47.5 %. The children age ranged from 6 years up to 18 years, with a mean of 11.8 years. All children were not vaccinated against the mumps virus. All children included in this study came to the main Schools as Adban, Al-Sabah, Saad Bun AbyWakaas, Al.noore, Al.Khademkhaleeb, Al.Mostakhble, Al.Nahdaa, Aoothman, Al.Olafy, 30 November in the city of Hodeidah. Sera were collected from each child, separated and examined for the mumps IgG antibody by an ELISA technique.The obtained data in questionnaires as personal, clinical, predisposing factors and vaccination data were transcribed on to computer coding sheets stored and statically analyzed using SPSS version 17.

Introduction

Mumps is an acute infectious disease caused by a paramyxovirus closely related to parainfluenza virus. Although the disease is usually mild, its burden should not be underestimated. Up to 10% of mumps patients developed aseptic meningitis; a less common but more serious complication is encephalitis, which can result in death or disability; and permanent deafness, orchitis

and pancreatitis are other untoward effects that can be prevented by vaccination. As of mid-1998, mumps vaccine was routinely used by national childhood immunization programmes in 82 countries. Where high coverage has been achieved, countries have shown a rapid decline in mumps morbidity. Furthermore, in many countries encephalitis associated with mumps has almost totally vanished. Humans are the only natural hosts for mumps virus, which is usually spread by respiratory droplets. The incubation period of mumps averages 16–18 days, with a range of about 2–4 weeks (1). Infection with mumps virus is asymptomatic in one-third of cases. Nonspecific prodromal symptoms include lowgrade fever, anorexia, malaise, and headache. Classic mumps is characterized by enlargment of the parotid and other salivary glands; parotitis is bilateral in three-quarters of cases; and other salivary glands are involved in 10% of cases (2).

Mumps is prevalent worldwide, but incidence has significantly decreased due to MMR vaccination. Mumps is known as a "childhood" disease because it normally affects children under 10 years of age (3). Prior to the introduction of immunization, approximately 85 percent of adults had evidence of past mumps infection. The peak incidence in late winter and spring (4). Mumps epidemics have occurred worldwide with school-aged children generally serving as the vector for horizontal spread to household family members (5). Adults considered at high risk (e.g. Persons who work in healthcare facilities, international travelers, and students at post-high school education institutions) (6).

Serosurveys to assess mumps immunity was conducted in a number of countries prior to introduction of vaccine. Data from England and Wales, Netherlands, Singapore5 and St. Lucia6 document a steep increase in mumps antibody level from age 2–3 years; 50% of children aged 4-5 years had acquired natural antibodies; and 90% of the population aged 14–15 years was seropositive. The situation in other countries is different, with a large proportion of adults remaining susceptible to infection, such as Saudi Arabia and Poland (7,8). Mumps virus outbreaks have not been completely eliminated, even in vaccinated populations (9). Mumps virus infections in vaccines are due to either infection with a wild-type of virus following primary vaccine failure or inoculation with a relatively neurovirulent mumps virus vaccine (10.11). The important tool to assess risk for disease outbreaks is by knowing the prevalence of MMR susceptibility as measured, either by serologic markers of immunity or by surveys of vaccination coverage.

Mumps may occur in epidemics; mumps virus is the only known cause of epidemic parotitis. Diagnosis is usually clinical, based on the presence of parotitis and associated signs, symptoms, or complications. The clinical case definition is an illness with acute onset of unilateral or bilateral tender, self-limited swelling of the parotid glands, other salivary glands, or both, lasting $\geq$2 days (12, 13)). The serological diagnosis of mumps relies on a rising immunoglobulin G (IgG) (serology with samples at least 14 days apart). The presence of immunoglobulin M (IgM) in the absence of IgG is presumptive of mumps. Isolation of mumps virus from a clinical specimen in an individual with a clinically compatible illness is diagnostic of mumps (14). Mumps diagnosis was established mainly by reverse transcription-PCR in clinical samples obtained within 48 hours from the onset of disease (15). There is no specific antiviral therapy for mumps, and the basic treatment consists of supportive care (16). Ice or heat

packs applied to the neck area and acetaminophen (Tylenol) may help relieve pain. Do not give aspirin to children with a viral illness because of the risk of Reye syndrome. You can also relieve symptoms with: Extra fluids, Soft foods, Warm salt water gargles (17).In the present work has main objective that Determine the seroprevalence of mumps IgG antibodies and the vaccination to coverage (humoral immunity) among school children in Hodeidah city Yemen.

Materials and Methods

Study design and area

This cross-sectional study, was carried out during the period three months starting in 1 of January 2013 and ending at 30 of march 2013, among school students at aged 6- 18 years subjects were recruited from some selected schools in Hodeidah city, Yemen (Adban, Al-Sabah, Saad Bin AbyWakaas, Al-noore, Al- Khademkhaleeb, Al.Mostakhble, Al-Nahdaa, Aoothman, Al-Olafy, and 30 November).

Study population

A total of 139 of unvaccinated children were enrolled in this study at age ranging from 6 to 18 years from School children in Hodeidah city, Yemen, at selected Schools ,which males were represented 73 (52.5 %) and females were represented 66 (47.5 %).

Sample size and collection

Based on an expected prevalence of 10% of mumps IgG antibody a worst acceptable value of 1% for detection mumps virus antibodies in Hodeidah city, at least 139 school children were selected from a total estimation of 50,000 populations at confidence level of 99%. The collected data from the selected groups were taken through direct communication with the study subjects using a predesigned questionnaire included the personal history status of the subjects as well as history of symptoms, vaccination status Two-three ml of venous blood samples were collected from each individual by using sterile vacuum tubes with a tight-fitted cap, and left to clot , then sera were separated from the clotted blood and stored at – 20 °C until tested for mumps IgG antibody.

Method (ELISA)

The sera of the selected subjects were tested by ELISA (Enzyme linkedimmunosorbent assay) for the detection of IgG antibodies to mumps virus antigen, at Aulaqi Specialized Medical Laboratory, Sana'a city-Yemen. Labs. The ELISA test kit provides a semi quantitative or quantitative in vitro assay for human antibodies of the IgG class against mumps virus in serum or plasma .The test kit contains microtiter strips each with 8 break –off wells coated with mumps antigens .In the first reaction step diluted patient samples are incubated in the wells .in the of positivesamples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgG(enzyme conjugated) catalyzing a color reaction.

Results and Discussion

A cross sectional analytical laboratory study was carried out during a period of three months starting in 1 of January 2013 and ending at 30 of March 2013. at age groups range from 6 to 18 years with a mean age of 11.8 years and standard deviation of 3.8 years.

Children were recruited from selected schools, in Hodeidah city, Yemen. Different in variables that were tested by using chi-

square test and odds ratio was performed to evaluate the relationships between mumps virus IgG antibodies and different variables. The significant differences were indicated if P- value ≤ 0.05. All the obtained results in this study are listed in the following table1. In this table ,out of 139 subjects, 73(52.5%) were males and 66 (47.5%) were females .Regarding the age groups ,subjects were grouped into three groups .The first age group was 6-10 years ,they represented out of 57 (41.0%) , 28(38.4%) males and 29 (43.9) females. The second age group was 11-15 years. They represented out of 51(36.7), 28(38.4%) males and 23 (34.8) females .As for > 16 years, in which it count 31(22.3), 17 (23.3%) males and 14 (21.2%) females. The mean of age for males was 12.1± 3.8 years whereas in female 11.6 ± 3.9 years, the children ages ranged from 6 years to 18 years for both gender, with 12 years for median for males and 11.5 for females, and the mode for male was 15 years and for female was 7 years.

When study this table, we observed that, there was 100 (71.9%) children seropositive, there was 59 (80.8%) males and 41 (62.1%) females also of 39 (28.1%) seronegative, there was 14(20.5%) males and 25(37.9%) females. Regarding age distribution, was found that, 42(73.7%) for age group 6-10 years, 33(64.7%)for age group 11-15 years , 25(80.6%) for age group > 16 years where was seropositive and for seronegative there was 15(26.3%)for age group 6-10 years, 18(35.3%) for age group 11-15years, 6 (19.4%) for age group > 16 years. This table shows that, the seropositivity of mumps virus IgG antibodies in the males was 80.8%, it was higher than that of female which was 62.1% but for males there was associated OR of mumps equal 1.6 with statistically significant different between males and females (p=0.024,0.024 respectively) .The detected rate of seropositivity of mumps virus IgG antibodies in the age groups 6-10 years was 73.7% with no statistical significant (p= 0.88) but there is associated OR of mumps equal 1.0, and the confidence interval from 0.7-1.6 ,in the age groups 11-15 years was 64.7% with no statistical significant *(p* = 0.20), and there is not associated OR of mumps equal 0.7 and the confidence interval from 0.5-1.2, where as in > 16 years was 80.6% with no statistically significant (p=0.19) but there is associated OR of mumps equal 1.7 and the confidence interval from 0.7-3.8

This table shows the association of mumps susceptibility (no protect) with gender and age groups, It found that, there was no association of mumps susceptibility with males in which OR=0.6 and the confidence interval from 0.24-1.23, the susceptibility rate was 19.2%, this result was statistically significant in which x^2 =5.1 and p=0.024 but for females there was associated OR of mumps susceptibility equal 1.5 with confidence interval from 0.8-4.2 with susceptibility rate equal to 37.9%, this result was statistically significant in which x^2 =5.1and *p* =0.024,the crude susceptibility rate for the total was 39 (28.1%).When the age was considered, there was no association of mumps susceptibility with age group 6-10 years in which 0R=0.9 with confidence interval from 0.6-1.5 and the susceptibility rate was 26.3%, but this result was not statistically significant in which X =0.024 and *p* =0.88 For age group 11-15 years there was associated OR of mumps susceptibility equal 1.4 with confidence interval from 0.9-2.1 with susceptibility rateequal to 35.3%,, but this result was not statistically significant in which % =1.7 and p=0.20. In the third age group > 16 years there was no association of mumps susceptibility in which OR=0.6 with confidence interval 0.3-1.3 and the

susceptibility rate was 19.4%, but this result was not statistically significant in which % =1.7and *p* =0.19.

This table shows that, the relationship of seropositive for mumps IgG antibodies with clinical features. There was not statistically significant association between seropositive of mumps IgG antibodies and some clinical features such as fever, enlargement of parotid gland , lethargy, headache painful swelling of salivary gland (P =0.46, 0.52, 0.18, 0.60 and 0.82) respectively with symptoms does not predispose or protect against seropositive of mumps IgG antibodies expect lethargy (OR = 0.8,0.8,1.1,0.9,0.9) respectively, with confident interval (CI =0.6-1.1,0.5-1.1, 0.9-1.3,0.8-1.2,0.4-1.3) respectively.

In this table shows mumps virus IgG antibody were stratified into three groups; the first was 21-40 RU /ml with total subjects of 48(48.0%) and the second was 41-60 RU /ml with total subjects of 15(15.0.%); the fourth was more than 60 RU /ml with total subjects of 37 (37.0%) that considered highest one.

This table shows that. There was not association between high level of mumps IgG antibodies and males but there is association between high level of mumps IgG antibodies and females there was OR=1.2 with confident interval equal 0.8-1.8 but not statistically significant association between high level of mumps IgG antibodies and female wherewas p=0.35 .There was association between high level of mumps IgG antibodies age groups 6-10 years and > 16 years there was (OR=3.3,1.2) respectively, but there association statistically significant with age group 6-10 years& not statistically significant with age group > 16 years ,there was *(p* =0.000,0.56) with confident interval equal (1.4-7.8,0.6-2.6) respectively.

Serological survey of health population groups have been of major importance in mapping the epidemiology of mumps and documenting differences in its behavior in various part of the world. These surveys provide information on age and gender specific immunity, which identifies the needs for immunization and help to monitor the effectiveness of vaccination programs and the persistence of immunity. Specifically, a qualitative understanding of the factor affecting mumps immunity enables the setting of targets for vaccination program to eliminate mumps in Yemen the (aims of this study).

Despite the lake of a mumps vaccination program aimed at children in Yemen. The crude sero-prevalence rate of mumps antibodies among childhood age in Hodeidah city in Yemen was very high 71.9% this indicates that these children have a close and prolonged contact with mumps and suggests that wild mumps virus is circulating in the community and endemic in Hodeidah city, Yemen.

This result of total seroprevalence similar studies in Isreali was 77.0% (18). The highest seroprevalence of mumps IgG antibodies was reported from studies conducted in Iranian was 80.2% (19) and Turkey was 89.2% (20), this low prevalence in Hodeidah city could be explained by that the wild virus is less frequently circulated in our communities, comparing with Turkey and Iranian .On the other hand the lowest seroprevalence findings were encountered among studied group in Palestinian refugee camps of the West Bank was 68.1% (21), in Sana'a city, Yemen was 59.8% (22).

In our study the prevalence rates of mumps among different age groups were rate in age group 6-10 years was 73.7% then dropped to about 64.7% in age group 11-15 and elevated to 80.6% the prevalence rate in age

group >16 years, (Table 2). This result is similar to those in Jorgen study that mumps occurred in all age groups in both gender (23).Although the normal pattern of immunity to mumps usually acquired between age 5-14 years, with maximal acquisition of humoral antibody occurring between 4 and 7 years of age (24).

The major morbidity and immunity from mumps occur as age, and gender specific hazards with peak risk in post pubertal males (21). Similarly in our result the prevalence rate of mumps antibodies was higher among males, where it count 80.8%,comparing with 62.1% among females, these data showed that females are more likely to lake antibodies against mumps which susceptibility rate 37.9% (table 2 &.4), and this make them more susceptible to infection with mumps because of the lower immune state in young females, but also 19.2% of our males had high risk of post pubertal males complication of mumps, it is crucial that steps be taken to address this issue. Similarstudy have been done in Isreali who reported that the highest in the males was 76.7%. However lowest seroprevalence findings were encountered among studied group in Sana'a city,Yemen was 64.5% among males 55.8% among females(22).

Infection of mumps and complication of meningitis /encephalitis and orchitis would occur in large number in the event of an epidemic. It's known that mumps outbreaks in largely unimmunized community can lead to high rate of mumps complication among post pubertal males (25), where fatality ratio for mumps reach 4/10,000 (26) and all mumps encephalitis cases are fatal (27). Reasons for the higher rates of sero-negativity in females in Hodeidah are unclear; there may be different levels of outdoor activity (e.g. girls spending less time outdoors than boys) or different levels of family crowding between the houses.

Mumps a well-known common childhood disease characterized by swelling of the parotid glands, salivary glands and other epithelial tissues, causing high morbidity and in some cases more serious complications such as deafness (28). In our study only 38 of children from 100 who were positive for mumps IgG, had history of typical presentation of mumps (enlarged of parotid gland) (Table 5). This result is explained by the fact of that mumps virus usually show to be a rather frequent cause of subclinical and mild unnoticed infections particularly among children (29).This result is similar to that reported byTan *reported that about*87% of infected children had sub clinical and mild infections (20) and also the Turkey study 61.7% of the sero-positive have asymptomatic or subclinical mumps infection without demonstrable parotitis. This also shows us the importance of serologic test for evaluating the mumps prevalence in a population (19).

The host immune response to mumps virus was the subject of many reviews. Humoral as well as cellular mechanisms undoubtedly play roles in cleaning virus infection, but this is complex process because the exact time course of the development of specific cellular immunity or humoral immunity following natural or live vaccine induced mumps is not completely established. However detection of antibodies amount in circulation of exposed or infected persons were found to be a good indicator for the course of the disease (26).In our study 37.0% of the seropositive showed high level of neutralizing circulation antibodies (>60 RU/ml) (table 6). These antibodies have a role in protecting these children from re-infection (19) and this appears to be true by the finding of Edmunds et al where they

found that the presence of high level of IgG antibodies was protected from infection after exposed this subjects to mumps virus (30). Although a rising titer of IgG also indicates repeated exposure to the mumps virus. A 48.0% of seropositive children have low level of antibodies, and this subject under high risk of re-infection if they exposed to the virus. These findings suggest that a large proportion of these children are at risk in our population, and that mumps or MMR vaccination at this age may be most beneficial. We recommend that mumps immunization of these children is important until an EPI for mumps with high coverage can be sustained.

Table.1 Distribution of studied group according to age and sex

Age in years	**Male**		**Female**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
6-10	28	38.4	29	43.9	57	41.0
11-15	28	38.4	23	34.8	51	36.7
> 16	17	23.3	14	21.2	31	22.3
Total	73	52.5	66	47.5	139	100
Mean of age/years	12.1		11.6		11.8	
S.D/years	3.8		3.9		3.8	
Median/years	12		11.5		12	
Mode/years	15		7		17	
Min/years	6		6		6	
Max/years	18		18		18	

Table.2 Seroprevalence of mumps IgG antibodies among studied group

Variables	**Positive**		**Negative**		**Total**	
	No.	**%**	**No**	**%**	**No**	**%**
Sex						
Males	59	80.8	14	19.2	73	52.5
Females	41	62.1	25	37.9	66	47.5
Age group/ years						
6-10	42	73.7	15	26.3	57	41.0
11-15	33	64.7	18	35.3	51	36.7
>16	25	80.6	6	19.4	31	22.3
Total	100	71.9	39	28.1	139	100

Table.3 The seroprevalence of mumps IgG antibodies among studied group according to sex and age groups

Variables	Positive IgG n=100		Statistical analysis			
	No.	%	OR	CI	%2	*P*
Sex						
Male n=73	59	80.8	1.6	1.0-2.4	5.1	0.024
Female n=66	41	62.1	0.7	0.5-0.9	5.1	0.024
Age group/ years						
6-10 n=57	42	73.7	1.0	0.7-1.6	0.024	0.88
11-15 n=51	33	64.7	0.7	0.5-1.2	1.7	0.20
>16 n=31	25	80.6	1.7	0.7-3.8	1.7	0.19
Total	100	71.9				

OR Odds ratio >1 at risk. CI Confidence intervals. X^2 Chi-square ≥ 3.84 (significant).
P probability value ≤ 0.05(significant).

Table.4 The susceptibility rate of mumps among different sex and age groups of children in Hodeidah city, Yemen

Variables	susceptibility n =39		Statistical analysis			
	No.	%	OR	CI	X2	*P*
Sex						
Males n=73	14	19.2	0.6	0.24-1.23	5.1	0.024
Females n=66	25	37.9	1.5	0.8-4.2	5.1	0.024
Age group/ years						
6-10 n=57	15	26.3	0.9	0.6-1.5	0.024	0.88
11-15 n=51	18	35.3	1.4	0.9-2.1	1.7	0.20
> 16 n=31	6	19.4	0.6	0.3-1.3	1.7	0.19
Total	39	28.1				

OR Odds ratio >1 at risk. CIConfidence intervals. X^2 Chi-square > 3.84 (significant). ***p*** probability value < 0.05(significant

Table.5 The relationship between history of signs and symptoms of mumps virus infection and seropositive of mumps IgG antibodies

Signs	Seropositive of mumps IgG antibodies n=100		Statistical analysis			
	No.	%	OR	CI	%2	*P*
Fever n=109	**76**	**69.7**	**0.8**	**0.6-1.1**	**0.55**	**0.46**
Enlargement of parotid gland n= 51	**38**	**74.5**	**0.8**	**0.5-1.1**	**0.43**	**0.52**
Lethargy n=95	**71**	**74.7**	**1.1**	**0.9-1.3**	**1.81**	**0.18**
Headache n=105	**76**	**72.4**	**0.9**	**0.8-1.2**	**0.30**	**0.60**
Painful swelling of salivary gland n=26	**19**	**73.0**	**0.9**	**0.4-1.3**	**0.05**	**0.82**

OR Odds ratio >1 at risk. **CI** Confidence intervals. X**2** Chi-square ≥ 3.84 (significant).
P. probability value ≤ 0.05 (significant).

Table.6 Distribution of seropositive group according to mumps IgG antibodies levels detecting

Levels IgG antibodies RU /ml	Studied group n=100	%
21-40 RU /ml	48	48.0
41-60 RU /ml	15	15.0
> 60 RU / ml	37	37.0

Table (7): The relationship between sex and age groups with high level of mumps IgG antibodies

Variables	High level of antibodies n= 37		Statistical analysis			
	No.	%	OR	CI	X2	*P*
Sex						
Males n=73	**17**	**23.3**	**0.8**	**0.6-1.2**	**0.9**	**0.35**
Females n=66	**20**	**30.3**	**1.2**	**0.8-1.8**	**0.9**	**0.35**
Age group/ years						
6-10 n=57	**22**	**43.9**	**3.3**	**1.4-7.8**	**14.7**	**0.0000**
11-15 n=51	**3**	**9.8**	**0.5**	**0.3-0.7**	**11.7**	**0.001**
> 16 n=31	**7**	**22.6**	**1.2**	**0.6-2.6**	**0.30**	**0.56**
Total	**32**	**26.6**				

OR Odds ratio >1 at risk. **CI** Confidence intervals. X**2** Chi-square ≥ 3.84 (significant). **P** probability value ≤ 0.05 (significant)

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