



ESTIMATED HUMORAL IMMUNE STATUS AGAINST BORDETELLA PERTUSSIS AMONG MILITARY RECRUITS IN SANA'A, YEMEN

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ABSTRACT

Back ground and Objective: Pertussis is a major cause of morbidity and mortality in infants and children worldwide in spite of the availability of a good protected vaccine. The incidence or prevalence rate among children or adults in Yemen are unknown. To determine the level of IgG antibodies of *Bordetella pertussis*.

Methods: Blood samples were collected from participated military recruits and commercially available Enzyme-linked Immunosorbent Assay was used. Cross-sectional study was conducted on 188 military recruits, between March and October 2013.

Results: The prevalence of *B. pertussis* protective rate among different age groups of military recruits was 92.6%. The highest rate of antibodies 100% was among age group below 20 years followed with 96.3% for >35 years and the lowest protective rate was 89.5% for age group 25-29 years old. 92.9% protective rate for age group 30-34 years. While the seronegative rate was 7.4% among all age group. Meanwhile, the levels of *B. pertussis* IgG antibodies were 38.8% who had IgG antibodies level between 1.51-1.99 IU/ml.

Conclusions. The findings of this study concluded that the Yemeni military recruits were not enough immunized to pertussis and with confirmed of low awareness about vaccination and medical history related to pertussis infection particularly among this high-risk of Yemeni community, therefore the routine a cellular booster vaccination of adults is a key way to indirectly protect infants and community.

KEYWORDS : *Bordetella pertussis*, Humoral, Antibodies, ELISA, Yemen

Introduction

Bordetella pertussis one of the main causing whooping cough (pertussis) in humans. Although vaccination against the disease is effective, the bacterium is still circulating among population and can affects all age groups in countries where the immunization rate is low as well as in countries where high levels of immunizations exists and can even cause death. In developing countries, vaccination coverage is increasing and more cost-effective whole-cell pertussis vaccines (wP) are mainly in use¹. Although vaccination is effective, pertussis is still severe to new-borns and unvaccinated infants, even causing mortality in these age groups^{2,3}. However, it has been shown that immunity can dramatically decline during the 12 years after vaccination, causing adolescent and adult susceptibility. Many previous studies show that the immunity level, as induced either by the whole-cell vaccine or after natural infection, wanes over time⁴. High immunity lasts about 3 years, and then gradually declines for 12 years⁵. In spite of the growing recognition of the importance of pertussis in adults, the whole-cell vaccine cannot be combined as a booster with diphtheria and tetanus toxoids because of the relatively high rates of adverse effects. 12–32% of adult's pertussis play an important role in the transmission of this disease to infants, and are considered to be the main reservoir of the organism as mentioned in the reports. Recently, there have been large outbreaks of pertussis e.g. in the USA, the UK and Australia. These outbreaks indicate that pertussis is circulating among people, and it is causing epidemics even in highly vaccinated populations^{6,7,8}.

In Yemen despite vaccination campaign, pertussis is relatively common in the general population therefore, the aim of this study was to investigate the humoral immune response of *B. pertussis* antibodies among Yemeni military recruits.

Materials and Methods

Sera of this study were collected and analyzed between March and October 2013 for research on the prevalence of level of *B. pertussis* IgG antibodies among Yemeni Military recruits at 48 Model Medical Hospital. All subjects had previously been informed about the pertussis study, and had provided written informed consent for use

of their sera in this study. The predesigned questionnaire was on Socio-demographic data as age, sex, marital status, occupation, education, and vaccination status. All information of Military recruits identifiers were kept anonymous in order to strictly protect participants confidentiality, and they were asked to complete a questionnaire.

Laboratory methods

Blood samples were collected from a random sample of Yemeni adult male military recruits were allowed to clot at room temperature for one hour, then cooled at room temperature before being centrifuged. Military recruits ages ranged from 18 to 54 years and sera were stored at -20°C until tested for detection of anti-pertussis toxin (anti-PT) IgG using quantitative analysis using a commercially available Enzyme-linked Immunosorbent Assay (ELISA kit, DRG International, Inc., Germany) and interpreted according to the manufacturer's instructions. Level >100 IU/ml indicated acute pertussis infection or recent vaccination, while 40–100 IU/ml were interpreted as probable past exposure to pertussis. Level of 5–40 IU/ml was interpreted as no evidence of recent acute infection and <5 IU/ml indicated seronegativity.

Statistical analysis

Antitoxin of IgG levels and data according to age group were analyzed using SPSS software (version 20; IBM Inc., Armonk, NY, USA). Quantitative data were expressed as mean values, Standard deviation (SD) median and range when the data was normal distributed or as percentages, chi-square (χ^2) test was used for comparison of two variables to determine the p value and odd ratio (OR) was used with 95 % confidence interval. P values P<0.05 was considered statistically significant.

Results

This study was conducted on 188 Yemeni military recruits at 48 Model Medical Hospital, Sana'a city, Yemen and all studied persons were tested for *B. pertussis* IgG antibodies level. Table 1. Shows five categories of age destruction, the most of participated military recruits were in age group 20-24 years 81 (43.1%), followed by age

group 25-29 years 57 (30.3%), age group <20 years 9 were 4.8% only. While military recruits among age group 30-34 years 14 were (7.4%) and in age group ≥35 years 27 were 14.4%.

Table 1. Age distribution among Yemeni military recruits at 48 Model Medical Hospital

Age / years	Percentage (%)
<20 (n=9)	4.8
20-24 (n=81)	43.1
25-29 (n=57)	30.3
30-34 (n=14)	7.4
≥ 35 (n=27)	14.4

Table 2. Shows the positive protected rate against *B. pertussis* among different age groups. The total crude protective rate was 92.6%. The highest protective rate was observed among age group <20 years it was 100% with associated odds ratio of protection equal to 1.1 and *p*=0.38. The lowest protective rate was observed in age group 25-29 years old in which it was 89.5% with no-associated odds ratio of protection equal to 0.6 and *p*=0.29.

Table 2. Positive protected rate against *B. pertussis* among different age groups

Age groups/years	Positive B.P antibody n=174		OR	CI	X ²	p
	No.	%				
<20 (n=9)	9	100	1.1	1.04-1.13	0.76	0.38
20-24 (n=81)	75	92.6	1.01	0.3-3.5	0.00	0.98
25-29 (n=57)	51	89.5	0.6	0.2-1.95	1.13	0.29
30-34 (n=14)	13	92.9	1.1	0.12-23	0.0	0.96
≥ 35 (n=27)	26	96.3	2.3	0.3-48.7	0.64	0.42
Total (n=188)	174	92.6				

OR Odds ratio >1 at risk, CI Confidence intervals, P probability value <0.05 (significant)

Table 3. Susceptibility rate for *B. pertussis* among different age groups

Age groups/years	Negative B.P antibody n=14		OR	CI	X ²	P
	No.	%				
<20 (n=9)	0	0,0	0	0	0	0
20-24 (n=81)	6	7.4	0.99	0.3-3.3	0.00	0.98
25-29 (n=57)	6	10.5	1.8	0.5-6.13	1.13	0.28
30-34 (n=14)	1	7.1	0.96	0.13-6.8	0.00	0.96
≥ 35 (n=27)	1	3.7	0.44	0.02-3.5	0.64	0.42
Total (n=188)	14	7.4				

OR Odds ratio >1 at risk, CI Confidence intervals, P probability value <0.05 (significant)

Table 3. Shows the susceptibility rate for *B. pertussis* among different age groups. The age group <20 years was zero and the susceptibility rate was 6 (7.4%) in the age group 20–24 years. The highest susceptibility rate was 6 (10.5%) among age group 25–29 years and the susceptibility rate among age group 30–34 years that was 1 (7.1%).

Table 4. The relationship between vaccination history against *B. pertussis*, Number of Doses and immunological status

Characters	Positive B.P antibody n=174		OR	CI	X ²	P
	No.	%				
Vaccinated (n=158)	146	92.4	0.9	0.0-4.5	0.03	0.85
Unvaccinated (n=30)	28	93.3	1.2	0.22-7.9	0.03	0.85
No. of dose :						
One dose (n=31)	28	90.3	0.7	0.2-3.4	0.27	0.6
Two doses (n=67)	63	94	1.4	0.4-5.6	0.33	0.56
Three doses (n=60)	55	91.7	0.83	0.24-3.01	0.1	0.75

OR Odds ratio >1 at risk, CI Confidence intervals, P probability value <0.05 (significant)

Table 4. Shows the relationship between vaccination history against *B. pertussis*, number of doses and immunological status among military recruits, the prevalence of positive *B. pertussis* antibodies among those who received vaccine was 146 (92.4%) and the prevalence was 28 (93.3%) among those who did not receive vaccine. Based on the number of doses, the lowest prevalence of positive *B. pertussis* antibodies was 28 (90.3%) noticed among one dose only of vaccine whereas, the highest prevalence was 63 (94%) among two doses of vaccine. While the prevalence rate among three doses of vaccine was 55 (91.7%).

Table 5. The relationship between clinical signs history of *B. pertussis* infections and positive *B. pertussis* IgG antibodies among different age groups.

Signs	positive B.P antibody n=174		OR	CI	X ²	P
	No.	%				
Respiratory Tract infection (n=71)	69	97.2	3.94	0.8-26.4	3.6	0.05
Cough and fever in the last 3 months (n=129)	123	95.3	3.2	0.95-11.1	4.7	0.03
Cough and fever in the last 6 months (n=17)	14	82.4	0.32	0.1-1.7	2.82	0.09
Cough and fever in the last year (n=21)	19	90.5	0.74	0.14-5.2	0.15	0.7

OR Odds ratio >1 at risk, CI Confidence intervals, P probability value <0.05 (significant)

Table 5. Shows the relationship between clinical signs history of *B. pertussis* infections and positive *B. pertussis* antibodies where the highest prevalence of positive *B. pertussis* antibodies was 69 (97.2%) among those who had respiratory tract infection, the rate among 123 of recruits was (95.3%) for signs of history cough and fever for 3 months, While the lowest rate was 14 (82.4%) among signs of cough and fever for last 6 months. Finally for those recruits whose suffering

from cough and fever in the last year he rate was 19 (90.5%). The statistically significant and a significant risk were noticed among those who had respiratory tract infection and among those who had cough and fever in the last 3 months in which OR= (3.94, 3.2), CI= (0.8-26.4, 0.95-11.1) and $p=0.05, 0.03$) respectively.

Table 6. The level of *B. pertussis* IgG antibodies among different age groups

Level of antibodies	No.	Percentage (%)
<0.65 IU/ml	14	7.4
0.65-1.5 IU/ml	47	25.0
1.51-1.99 IU/ml	73	38.8
>2.0 IU/ml	54	28.7

Table 6. Shows the level of *B. pertussis* among military recruits who had antibodies level <0.65 IU/ml were 14 (7.4%) and 0.65-1.5 IU/ml were 47 (25%). Those who had 1.51-1.99 IU/ml were 73 (38.8%) and >2.0 IU/ml were 54 (28.7%).

Discussion

There has been no information on pertussis in Yemen, which is a major cause of morbidity and mortality in infants and children worldwide in spite of the availability of a good protected vaccine⁹. It is also considered to be endemic bacterial infection, but incidence or prevalence rate among children or adults in Yemen are unknown. Although, Yemen introduced universal immunization against pertussis for infants in early 1970s of the last century, but feedback information in coverage rate of vaccination and its efficacy in the community have been ignored for long period¹⁰. Our study findings showed that 92.6% all military recruits were seropositive, disagreed with other study performed in Iran¹¹. The protective rate of pertussis IgG antibody was higher in age group >20 years (100%), than in 25—29 years (89.5%), similar findings were reported in Korea and Singapore among adults, where higher protective response rate was found among younger adults than older¹². Result can be explained by that pertussis vaccines is administered routinely to children, and immunity wanes over time and adults become more susceptible to disease, or to the different degrees of exposure to natural boosters and nutritional status and socioeconomic factors, race factors, or the type and quality of vaccines used and number of doses which had been taken¹³. The lower rate in older ages make older adults at risk of acquiring pertussis, therefore a safe and effective pertussis booster vaccine for adolescents and adults is highly recommended for routine use. Finding of this study was 28.7% of tested adults showed high level of *B. pertussis* IgG antibodies, these observation indicate that an increase in *B. pertussis* IgG antibodies was the predominant abnormal antibody result pattern among adults suggests that many adults had recent pertussis infections¹⁴. In addition 25% of total adults had low level IgG antibodies which indicate past vaccination, past pertussis infection and or suspected of having a different infections that induces low reactive antibodies, such infections include *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae* infections, both of which have been associated with prolonged cough illness, although the cough duration tends to be shorter than it is with pertussis^{15,16}. *B. parapertussis*, *B. bronchiseptica*, *B. holmesii*, and nonencapsulated *Haemophilus influenzae* also induce low level of -reactive antibodies^{14,17}; there may also be other, as-yet-unknown, microbes that trigger the production of such antibodies. Specifically, in a study of army personnel with prolonged cough illness in Korea, it was found that a number had low level of IgG reactive antibodies¹⁷. Concerning the rest study group, 7.4% developed antibody amounts (<0.649IU/ml) or negative for antibodies, indicating a poor anti-pertussis response after receiving a full course of vaccine it might be guessed of this finding, that these individuals were hypo-responsive to the immunization and that their antibodies may rapidly wane over time or the quality of vaccine used was very bad. Even in these instances, losses of antibody do not necessarily imply loss of protection¹⁸. Considering anti-pertussis may disappear in substantial proportion of vaccine after initially successful vaccination, a booster dose of vaccine, following the administration

of the primary course is recommended by most national bodies. Previous studies showed that protection is still maintained among vaccinated, even in developing countries, despite waning or undetectable anti-pertussis antibodies levels¹⁹.

In addition, among 137 participants whose antibody concentrations were between 0.65-1.99 IU/ml with 72.8 %, more likely represented exposure to pertussis in the past years. This result is comparable with a seroepidemiological study conducted in Denmark²⁰. Using a cut-off point of > 50 IU/ml, the prevalence of pertussis infection in the population was 5.6% (95% CI 4.1-7.8). In Netherlands, one study found that 9.3% of the population low age group had antibody level of more than 62.5 IU/ml, suggestive of pertussis infection in previous years²¹. An estimate of the incidence of pertussis based on serological titers in five European countries found that the seroincidence of infection was approximately 1-6% per annum with a peak in adolescent to young adult age group²². China also reported that approximately 5.2% of adult population 18-50 years of age had pertussis infection using a cut-off point of antibody level > 30 IU/ml²³. However, a recent study emphasized that the incidence estimates of pertussis infection based on antibody measurement alone were higher than the incidence from the annual report²⁴.

Regarding to relationship between clinical signs history of *B. pertussis* infections and positive *B. pertussis* IgG antibodies among Yemeni military recruits, we found the highest rate of our study (97.2%) among respiratory tract infection and even stronger correlations between evidence of vaccination doses and antibody rate. This could be because the humoral immune response to have enhanced binding to *B. pertussis*²⁵, thus increasing pertussis infection risk. However, a seroepidemiology study in Denmark found no significant link between smoking status and recent pertussis infection²⁶.

Regarding gender this study was among military male because female not allowed to join military services, this due to stress of reserves group, but our study agreed with previous studies where higher male seropositivity has also been reported in previous from Mexico ($p=.0007$) Korea ($p=.023$)²⁴, but studies from Spain²⁷, Greece²¹ and the Gambia²² have reported no significant differences in seropositivity between genders even some previous reports mentioned that the woman who does not have protective anti-pertussis antibodies cannot pass the protection to her child during pregnancy, thus the new-born infant is susceptible to the disease²⁸. Overall, it is unclear whether there is a true link between pertussis and gender and, if there is, the cause is unknown.

This study finding needs to be interpreted in the light of certain limitations. Based on sample selection for this study that was conducted in special republican guard hospital and sample was only the Male military recruits, because there are culture capture of female to involve in the military services therefore will not be exactly representative of the general Yemeni population of that age group. However, the study population was selected from donor healthy individuals who were admitted to the Blood Banking Department, and all those who had non fit with the policy of blood donation were excluded. Whilst this study target does not exactly represent all military recruits of community-based study population, due to the selection of healthy donors. Furthermore, selecting a hospital based sample from whom blood is routinely drawn for investigations has helped to avoid any selection bias that may occur by selecting volunteers who would agree to participate in the study due to concerns regarding their immunity status. The study due to financial problem we couldn't use molecular Detection methods we only used commercialize available kit for the determination of antibody levels. However, if there are two or multiple methods of antibody detection were used it would have contributed to increase the validity of the data, compared to using a single test kit.

Conclusion

The protective rate of this study is higher in adults, and decrease the

protection rate in older adults, or in other words, increasing age was showed to be correlated with decreasing protective antibodies. The findings of this study concluded that the Yemeni military recruits were not enough immunized to pertussis and with confirmed of low awareness about vaccination and medical history related to pertussis infection particularly among this high-risk of Yemeni community, therefore the routine a cellular booster vaccination of adults is a key way to indirectly protect infants and community.

Conflict of Interest.

The authors declare that there is no conflict of interests regarding the publication of this paper.

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