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# Assessment of antibody level and avidity against *Bordetella pertussis* in a cohort of Egyptian individuals aged 1–18 years

**Nihal M. Ibrahim [a,*], Ebtsam M. El-kady [b], Somiaa A. Eissa [c], Ahmed F. Wahby [a]**

[a] *Department of Molecular Biology, National Research Center, Dokki, Cairo, Egypt*
[b] *Department of Microbial Biotechnology, National Research Center, Dokki, Cairo, Egypt*
[c] *Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt*

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ABSTRACT

Pertussis specific antibodies were studied with respect to quality and quantity in a cohort of apparently healthy Egyptian children and adolescents, with their age range between 1 and 18 years, in an attempt to get a close and clear insight into the current humoral immunization status in this specified group and to try find a relation between the antibody levels and their avidities in eradication of this devastating infectious disease. Our results showed that avidity increase was most marked in young school children (6–8 years) where it seemed to reach a plateau in older children and adolescents. Antibody titer was highest in toddlers (1–2 years) and young school children (6–8 years) groups, most probably following vaccination and/or booster doses. Among children aged 1–5 years, 28% had highly avid and 50% had high titer antibodies, whereas in adolescents aged 13–18 years, 70% had highly avid antibodies and only 30% had high titer antibodies. The results clearly demonstrated that while levels of anti-*Bordetella pertussis* (*B. pertussis*) antibodies wane with growing age, the avidity seems to increase, to a plateau, irrespective of further antigen exposure in a pattern showing complete independence of avidity on concentration. The present study draws attention to the importance of avidity measurements, together with conventional ELISAs, for evaluating immunity against pertussis. Being based on a limited sample size, it could open doors for larger-scale surveys to be possible indicators for the need and timing of booster vaccination doses among Egyptians.


* Corresponding author. Tel.: +20 222668203, mobile: +20 1226413601, +20 1282808675.
E-mail address: n-hal@hotmail.com (N.M. Ibrahim).
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## Introduction

Pertussis (whooping cough) is an acute disease of the upper respiratory tract caused by the gram negative bacillus *Bordetella pertussis* (*B. pertussis*). Bacterial pneumonia or respiratory distress is the usual cause of death [1]. Worldwide, pertussis remains one of the top ten causes of

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vaccine-preventable deaths in children under 1 year with an estimated 30–50 million cases and as many as 300,000 pertussis related deaths annually, 90% in developing countries and mostly in infants [2]. In 2000, disability-adjusted life years from pertussis (12.7 million) exceeded those of lung cancer (11.4 million) and meningitis (5.8 million) [3].

Despite high childhood vaccination coverage, since the universal implantation of the whole cell vaccine in the 1940s, pertussis has reemerged as a public health problem worldwide in the past 2–3 decades [4–7]. Waning immunity following infant vaccination and reduced opportunity of pertussis for boosting immunity due to reduced circulation of *B. pertussis* contribute to increased susceptibility to pertussis infection and disease in adolescents who are the main source of infection to vulnerable infants too young to be vaccinated [8–10]. Estimates of the duration of protection following whole cell pertussis vaccination range from 4 to 12 years and following acellular vaccination is approximately 5–6 years [11].

Antibody decay rates and mathematical modeling [12–14] suggest that repeated doses of pertussis vaccines will be needed to maintain protection against pertussis. Moreover, many developed as well as developing countries including the Eastern Mediterranean region [15] have recommended adult vaccination against pertussis. In Egypt, the whole cell vaccine combined with diphtheria and tetanus toxoids (DPT) has been introduced in the schedule of compulsory vaccination for Egyptian children at 2, 4, and 6 months followed by a booster dose in the second year of life [16]. Comparison of pertussis incidence between countries is problematic due to differences in case definition, access to diagnostic tests, clinician awareness and differences in immunization strategies [10]. In many developing countries, identification of pertussis is still limited by patient and physician awareness and the limited sensitivity of diagnostic tests although the WHO estimates demonstrated that these countries have the highest disease burden [17].

The humoral immune response to a specific antigen comprises the magnitude of antibody as well as the affinity of an antibody to its antigen [18]. Clinically, serum antibody level is a useful parameter that could detect the presence of infection and the magnitude of protective antibodies against a certain pathogen following natural infection or vaccination. Qualitative parameters, affinity and avidity, mainly measure the binding strength of a pathogen to specific antibodies thereby determine the efficiency of the circulating antibodies and their ability to induce protection against a disease [19].

Antibody avidity characterizes the functional affinity of multivalent antibody binding with multivalent antigens [18]. Antibody avidity represents the functional measure of affinity maturation of antibodies and is correlated with long term immunity [20,21] and possibly protection against pertussis disease [22]. Antibody levels increase shortly after antigen contact while increase in avidity is much slower [23,24] and appears to be an age dependent process observed from six months onwards [25] and is present at significant levels at 4 years old in children [26,27].

As the antibody quantity and quality are good serological markers of vaccine efficacy, the present study was designed to assess the antibody titer levels and avidities in a cohort of Egyptian children and adolescents (1–18 years) which could be an attempt to find a relation between these two criteria and show how much both parameters would contribute to define the immunization status, with respect to age, against pertussis in the individuals under test.

## Subjects and methods

### *Serum samples*

Serum samples were randomly collected from children and adolescents aged 1–18 years who admitted KIDS hospital, Al-Mohandeseen, Cairo, Egypt, in the period between June 2012 and December 2012 ($n = 59$) and in January 2015 ($n = 33$). Two samples collected from infants 5 and 6 days old were used as control. Most individuals participating in the study suffered minor to moderate health problems although no one had been diagnosed with underlying pertussis disease or other respiratory infections. All participants had been immunized according to the Egyptian national immunization program at 2, 4, 6 months followed by a booster at 18 months. The inclusion criteria include apparently healthy immunized members and those who had suffered transient weakness or instability but with no underlying acute or chronic disease, while the exclusion criteria include individuals who suffer chronic diseases, acute illness or those who require long therapy especially individuals treated with steroids, chemotherapeutics, immunoglobulins or other immunosuppressive drugs, in addition to those who recorded antibody titers $< 50$ or avidity values $< 0.5$.

*The study was completely a random clinical trial and was approved by the research ethics board at KIDS hospital, Al-Mohandeseen, Cairo, Egypt. An oral informed consent was obtained from the parents before starting the protocol.*

### *Antibody titer determination by ELISA*

Serum anti-pertussis antibody titer was measured with the standardized ELISA [28] with minor modifications. Briefly, 96 wells-microtiter plates (Dynatech) were coated overnight at 4 °C with 100 µl of pertussis antigens (dil 1:1000) in the coating buffer (0.05 M carbonate buffer pH 9.6). The pertussis antigens contained equal volumes of the whole cell *B. pertussis* strains 134, 509, and 165 that were kindly provided by the VACSERA authorities. The plates were washed three times with PBS-T buffer (100 mM PBS pH 7.5 containing 0.05% Tween 20) and incubated overnight with 150 µL/well of the blocking buffer (100 mM PBS pH 7.5 containing 0.5% gelatin). Serial dilutions of the tested human sera (100 µL) in the PBS-T were dispensed into duplicate wells and incubated for 2 h at room temperature then overnight at 4 °C. After wash, anti-human alkaline phosphatase conjugate diluted in PBS (1/2500) was added (100 µl/well) and incubated for 2 h at 37 °C. The plates were washed thoroughly for 3–5 times with PBS-T buffer before allowing them to react with 100 µl/well of the substrate solution (4 mµ P-nitro phenyl phosphate, P-NPP, 1 mµ $MgCl_2$ in 1 µ diethanolamine, pH 10). The reaction was allowed to proceed for 30 min at room temperature in the dark before the addition of 1 N NaOH (50 µL). The developed ODs were measured at 490 nm in a Micro ELISA Reader Photometer. A reference serum was used to correct from plate-to-plate errors and the antibody titer was calculated as the antibody dilution that gives an OD of 0.5 absorbency.

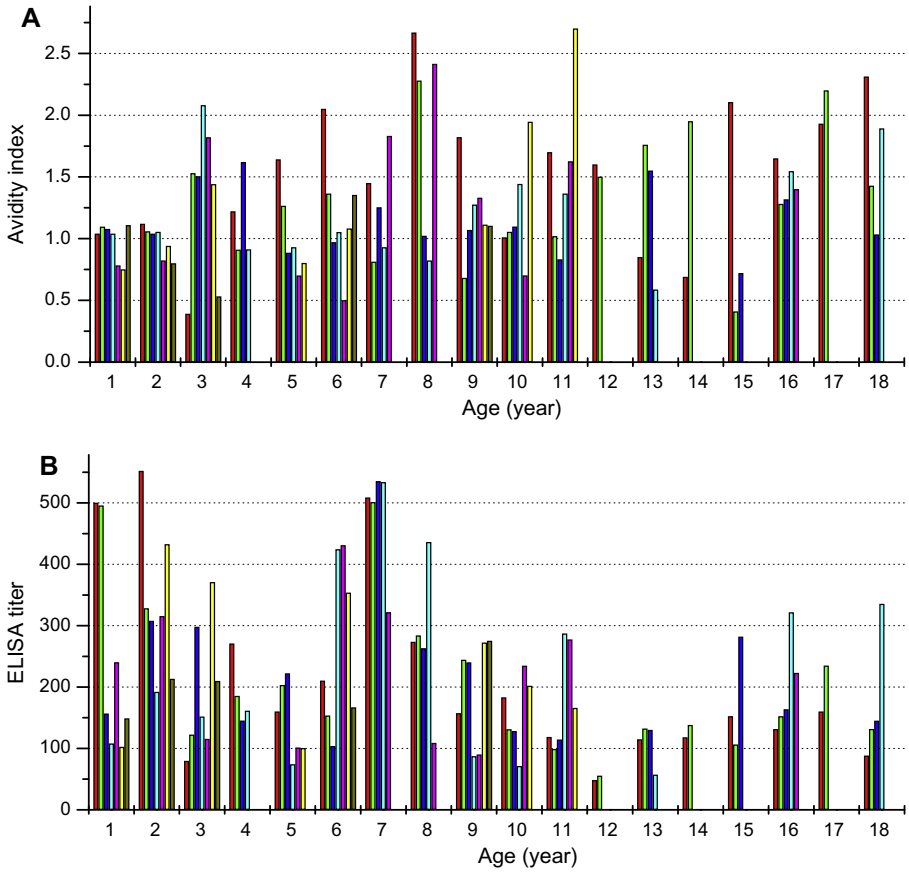


**Fig. 1** Avidity indices (**A**) and ELISA titer levels (**B**) of antibodies specific to pertussis as measured in sera of the study participants.

*Antibody-avidity tests*

Antibody avidity was determined according to [29] with little modifications. Briefly, ELISA plates were coated with pertussis antigens and blocked with gelatin as described under the previous section. The different human sera (dilution 1:30) were allowed to bind the pertussis toxin (1:1000) in the ELISA plates. The formed antigen–antibody complexes in twelve wells were used to determine the avidity index of one serum. Following washing of the plates (3 times) with PBS-T buffer, 100 μL of $NH_4SCN$ in PBS pH 6 were added at five different concentrations (0.25 M, 0.5 M, 1 M, 2 M and 3 M) leaving two wells filled with the dilution buffer alone, without $NH_4SCN$, as a control. After 15 min, the plates were washed with PBS-T and processed as previously described under the standard ELISA in the previous section. A plot of the percentage binding ([OD in presence of $NH_4SCN$/OD in absence of $NH_4SCN$] × 100) versus the $NH_4SCN$ concentration was established and used for interpretation of the avidity index. The $NH_4SCN$ concentration that develops 50% of the binding in the $NH_4SCN$-free sample was considered as the relative avidity index for the tested serum.

## Results

*The humoral response against **B. pertussis** in Egyptian children and adolescents 1–18 years*

Fig. 1 shows the avidity and antibody titer concentrations of 92 sera samples obtained from randomly selected cohort of Egyptian children and adolescents 1–18 years old. The high variation coefficient (CV) values reflect the great variations in antibody response with respect to magnitude and/or avidity among individuals of the same age (Table 1).

*Relationships between antibody avidity, titer and age*

Both antibody titers and avidity associated weakly with age. While antibody avidities to whole cell pertussis antigens correlated positively ($R = 0.368$, Fig. 2A), antibody levels were inversely associated ($R = -0.621$, Fig. 2B) with age. Very poor, almost no, correlation could be detected between avidity and titer of pertussis antibodies ($R = -0.068$, Fig 2C).

*Behavior of avidity and titer of anti-pertussis antibodies over the different age groups*

The behavior of avidity and titer over five different age groups were examined as shown in Fig. 3. It is evident that the avidity tends to smoothly increase by growing age until a plateau was reached in school children and adolescents. On the contrary, ELISA titer showed two maxima: the first in toddlers (1–2 years), and the second in young school children (6–8 years). Assuming that high avid antibodies are those registering avidity indices $\geqslant 1.2$ and high level antibodies are those with ELISA titers $\geqslant 200$, the results showed that among children aged 1–5 years, ~28% had highly avid and 50% had high titer antibodies, whereas in adolescents aged 13–18 years, 70% had highly avid antibodies and only 30% had high titer antibodies

**Table 1** Mean avidity indices and titers of antibodies specific to pertussis in the different age participants.

| Age | | Number (participants) | Avidity index (Mean ± [a]SE) | [b]CV% | ELISA titer (Mean ± [a]SE) | [b]CV% |
|---|---|---|---|---|---|---|
| Group | Years | | | | | |
| Toddlers | 1 | 8 | 0.951 ± 0.059 | 6.27 | 273.99 ± 62.012 | 22.6 |
| | 2 | 7 | 0.976 ± 0.047 | 4.81 | 334.47 ± 47.096 | 14.1 |
| Pre-school children | 3 | 7 | 1.328 ± 0.239 | 17.99 | 192.46 ± 40.419 | 21 |
| | 4 | 4 | 1.165 ± 0.168 | 14.42 | 190.67 ± 27.942 | 14.6 |
| | 5 | 6 | 1.038 ± 0.143 | 13.78 | 143.53 ± 24.824 | 17.3 |
| Young school children | 6 | 7 | 1.195 ± 0.179 | 14.97 | 263.28 ± 51.614 | 19.6 |
| | 7 | 5 | 1.255 ± 0.183 | 14.59 | 480.26 ± 40.147 | 8.4 |
| | 8 | 5 | 1.841 ± 0.381 | 20.7 | 273.12 ± 51.875 | 19 |
| Old school children | 9 | 9 | 1.437 ± 0.187 | 13.01 | 189.32 ± 24.774 | 13.1 |
| | 10 | 6 | 1.208 ± 0.176 | 14.56 | 158.35 ± 24.198 | 15.2 |
| | 11 | 6 | 1.54 ± 0.269 | 17.46 | 176.97 ± 34.521 | 19.5 |
| | 12 | 2 | 1.55 ± 0.05 | 3.22 | 51.76 ± 3.513 | 6.8 |
| Adolescents | 13 | 4 | 1.186 ± 0.278 | 23.4 | 108.46 ± 17.604 | 16.2 |
| | 14 | 2 | 1.32 ± 0.63 | 47.7 | 128.07 ± 10.075 | 7.8 |
| | 15 | 3 | 1.078 ± 0.521 | 48.3 | 180.14 ± 52.62 | 29.2 |
| | 16 | 5 | 1.439 ± 0.069 | 4.8 | 198.38 ± 34.363 | 17.3 |
| | 17 | 2 | 2.065 ± 0.135 | 6.5 | 197.42 ± 37.32 | 18.9 |
| | 18 | 4 | 1.666 ± 0.277 | 16.6 | 175.02 ± 54.784 | 31.3 |

[a] SE, the standard error among the stated number of participants.
[b] CV%, the coefficient of variation = SE/mean × 100.

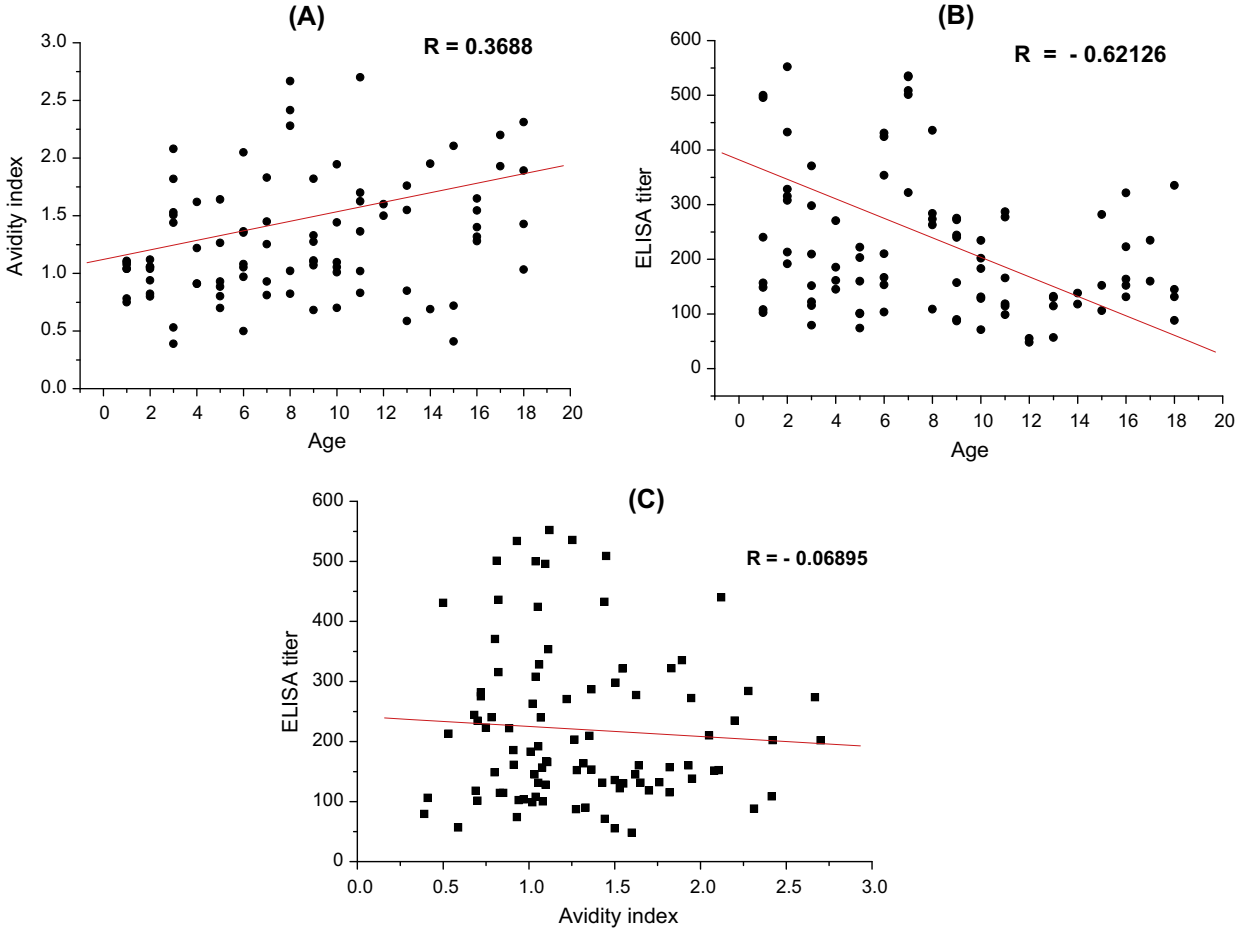


**Fig. 2** Correlation between avidity indexes and serum titer levels of antibodies to whole cell pertussis antigens in relation to age (A and B, respectively) and in relation to each other (C). Linear regression lines are shown as the associated *R* values.

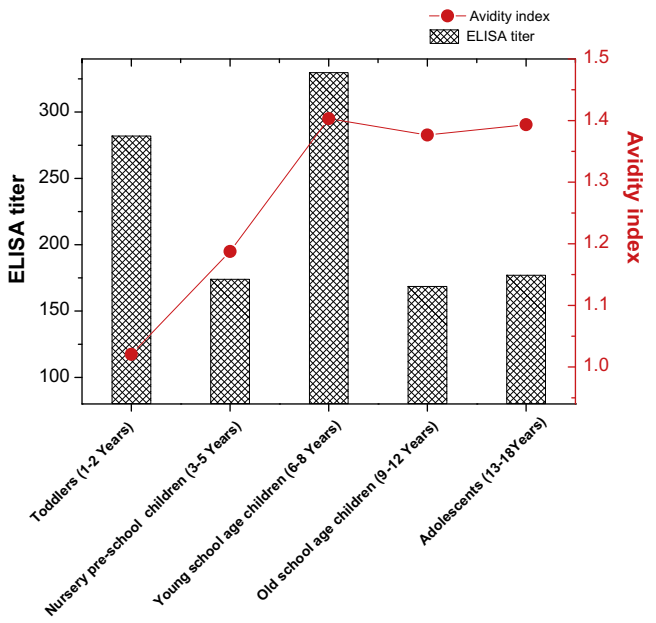


**Fig. 3** Behavior of avidity indices and ELISA titers over five age groups. Each point and column represents the mean value of avidities and titer levels, respectively, for the participants in each age group under test.

(Table 2). On the other hand, it seemed that school children (6–12 years) had an acceptable amount of antibodies, with respect to quantity and quality, as 55% had high avid antibodies and 52.5% had high titer antibodies. However, it is not necessary that the same individuals with high titer antibodies are those carrying high avid antibodies.

## Discussion

The present study was designed to elucidate the humoral immunity, with respect to antibody quality and quantity, in a randomly chosen cohort of Egyptian children and adolescents in an attempt to find a relation between these two criteria and how much both would contribute to define the immunization status, with respect to age, against pertussis in the individuals under test.

According to our results, both antibody levels and avidities associated (albeit weakly) with age; while avidity correlated positively, antibody levels wane with growing age. The lowest antibody levels were recorded in adolescents (13–18 years) in whom the highest avid antibodies were most prominent. The percentage of individuals having high avid antibodies increased from 28% in children (1–5 years) to about 70% in adolescents (13–18 years), whereas the proportion of individuals with high titer antibodies decreased from 50% to 30% along the same previous age groups.

Nowadays, the immunization schedules vary from country to country [10]. The Egyptian national program for obligatory vaccination includes immunization at 2, 4, 6 and 18 months [30]. As it is well known that antibody levels substantially increase following vaccination and fast decrease after then [31–33], accordingly it was completely accepted that the highest antibody titers would be registered in toddlers (1–2 years), but it was surprising to detect such high levels of anti-pertussis antibodies, comparable to those recorded in toddlers, in young school children (6–8 years) as well. This observation could reflect the culture and traditions of Egyptian parents and their inherent fears of infectious diseases that enhance them to optionally revaccinate their children at school entry even against diseases not included in the national immunization program. Otherwise, the possibility that the measured anti-pertussis antibodies were due to silent infection cannot be excluded, especially when we know that antibody concentrations are higher after infection than following vaccination [22].

Although almost no correlation was observed between antibody level and avidity, very low avid antibodies ($<0.5$ M $NH_4SCN$) were mostly of low titer. This might be attributed to that lower proportions of low avid antibodies are bound to the coating antigen which means that the actual quantification of low avid antibodies could be missed. Likewise, very low titer antibodies could readily be eluted by low $NH_4SCN$ concentrations that made the detection and analysis of high avid antibodies preferentially favoured [34]. The degradation of antibodies during storage cannot, however, be excluded.

The values of avidity indices could hardly exceed 2 M $NH_4SCN$ which is too low compared to other antigens tested in our laboratory under the same conditions. This was most likely attributed to the reduction of antibody binding due to susceptibility of the whole cell pertussis antigens to high $NH_4SCN$ concentrations with subsequent denaturation and elution. Moreover, the coating antigen could be different from that found in physiological conditions [35], even though we used three strains of *B. pertussis* as coating antigens. Furthermore, ELISA tests that measure antibody level to the whole cell antigens are generally of low sensitivity and specificity compared to that measured against individual pertussis antigens [36]. Nevertheless, avidity index is not an absolute value but rather a relative measure and strongly varies as a function of the assay conditions [18].

The present study was completely a randomized and unsystematic study, so that the results have to be interpreted with

**Table 2** Distribution of high avid and high titer antibodies specific to whole cell pertussis antigens over the tested groups.

| Age group | | [a]High avid antibodies | | [b]High titer antibodies | |
|---|---|---|---|---|---|
| Years | Number | Number | % | Number | % |
| 1–5 | 32 | 9 | 28.125 | 16 | 50 |
| 6–12 | 40 | 22 | 55 | 21 | 52.5 |
| 13–18 | 20 | 14 | 70 | 6 | 30 |

[a] Antibodies with avidity indices $\geqslant 1.2$.
[b] Antibodies with ELISA titers $\geqslant 200$.

caution because the number of samples is limited, the samples were collected haphazardly from apparently healthy participants, no past history of revaccination or natural exposure to pertussis antigens at any age was available. In addition the time of sampling is different in all age groups that could explain the great variation among participants of the same age. Time of sampling is a very important factor in determining antibody levels and/or avidities; as the antibody levels increase shortly after immunization then rapidly wane, while avidity maturation is a much slower process taking several months but persists longer after exposure to antigens [23,24]. Consequently, highest antibody titer might have been reached before sampling and thus could be missed by late sampling, whereas highest avidity might possibly be missed due to early sampling.

Our results showed somewhat prolonged avidity maturation period of anti-pertussis antibodies. The increase in avidity was most marked in young school children. Later on, a plateau was reached in older school children and adolescents. The low average avidity observed in toddlers might reflect the immaturity of the immune system or a slow rate of avidity maturation along years. Nevertheless, natural and/or booster exposure to pertussis antigens seem to enhance both antibody levels and avidities.

According to Sallam [37] pertussis has almost disappeared since introducing the whole cell Pertussis–Diphtheria–Tetanus vaccine in the Egyptian national program of immunization 1968 [16]. No epidemics or outbreaks have been reported in Egypt as has been described in other well developed countries [38]. A possible reason could be the use of the whole cell pertussis vaccine in priming immunization which is associated with a lower risk of subsequent pertussis disease than the acellular pertussis vaccine [39]. Moreover, immunity after priming doses of an acellular vaccine waned more rapidly than after the priming doses of a whole cell vaccine [40]. The improved diagnostic techniques, the increased physician awareness and reporting in well developed countries have also made a major contribution to high notification rates for pertussis [41].

Identification of pertussis is still difficult. The lack of access to diagnostic methods, misdiagnosis, under-reporting, lack of classic symptoms in adults and older children and low physician awareness all made the true incidence of pertussis in a developing country like Egypt is poorly defined. The true incidence of pertussis is generally considered to be substantially higher than reported by either notifications or hospitalizations in both developed and developing countries[1] [10].

In summary, while levels of anti-*Bordetella pertussis* (*B. pertussis*) antibodies wane with growing age, the avidity seems to increase irrespective of further antigen exposure in a pattern showing complete independency of avidity of concentration. Highly avid antibodies are not necessarily of high titer. Inversely, higher titer antibodies do not always imply (entail) higher quality of antibodies. So that we can come to a conclusion that the decrease in antibody levels in adolescence is compensated by selection of high avid antibodies that might confer some sort of naturally acquired protection against the disease in this age group. Accordingly, we can cautiously say that Egyptians have acquired high immunity in childhood due to active immunization and in teenager hood due to avidity maturation. This, however, does not rule out the presence of participants of low titer and avidity. Because serologic levels of protection have not yet been established [7,42], we cannot decide whether these participants are compromised by the reduction in antibody titer and/or avidity. Further studies have to be conducted on well-known history persons to show how well these immunity parameters correlate with clinical protection.

[1] It may be worth mentioning that the patients of the present manuscript had suffered prolonged cough and high temperature over more than 2–3 weeks. Symptoms exaggerate by using low effective antibiotics against common cold before an intelligent physician truly diagnosed the disease as being whooping cough. Then it took long before complete recovery was assessed. Thanks to God, the patients were old enough to withstand and survive. This gives an idea that pertussis antigens still circulate in the community and complete eradication of whooping cough in Egypt has not yet been accomplished.

## Conclusions

The present report opens doors for further studies on immunity against pertussis, and may be other pathogens, in Egypt and highlights the importance of avidity measurements, together with conventional ELISAs, for evaluating immunity against diseases to enrich the information given about antibodies. However, a wider survey had to be done including larger number of participants from different social levels and different environments before we are able to recommend any boosters in the national immunization program.

## Conflict of interest

*The authors declare that there is no conflict of interest.*

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## References

[1] Edwards KM, Decke MD. Pertussis vaccines. In: Stanley A, Plotkin MD, Walter Orenstein MD, Paul A, Offit MD, editors. Vaccines. Elsevier; 2013. p. 447–92.
[2] Centers for Disease Control and Prevention (CDC). Pertussis: Pertussis in Other Countries; 2012. <http://www.cdc.gov/pertussis/countries.html> [cited 2012 April 7].
[3] Crowcraft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? Lancet Infect Dis 2003;3:413–8.
[4] Watanabe M, Nagai M. Acellular pertussis vaccines in Japan: past, present and future. Expert Rev Vaccines 2005;4:173–84.
[5] He Q, Mertsola J. Factors contributing to pertussis resurgence. Future Microbiol 2008;3:329–39.
[6] Heininger U. Update on pertussis in children. Expert Rev Anti Infect Ther 2010;8:163–73.
[7] Chiappini E, Stival A, Galli L, de Martino M. Pertussis re-emergence in the post-vaccination era. BMC Infect Dis 2013;13:151.
[8] Cherry J. The epidemiology of pertussis: a comparison of the epidemiology of the disease pertussis with the epidemiology of *Bordetella pertussis* infection. Pediatr 2003;115:1422–7.

[9] Águas R, Gonçalves G, Gomes MG. Pertussis: increasing disease as a consequence of reducing transmission. Lancet Infect Dis 2006;6:112–7.
[10] Wood N, McIntyre P. Pertussis: review of epidemiology, diagnosis, management and prevention. Paediatr Respir Rev 2008;9:201–12.
[11] Wendelboe A, Van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J 2005;24:S58–61.
[12] Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study. Br Med J 1988;296:612–4.
[13] Hallander HO, Gustafsson L, Ljungman M, Storsaeter J. Pertussis antitoxin decay after vaccination with DTPa. Response to a first booster dose 3 ½ - 6 ½ years after the third vaccine dose. Vaccine 2005;23:5359–64.
[14] Bailleux F, Coudeville L, Kolenc-Saban A, Bevilacqua J, Barreto L, André P. Predicted long-term persistence of pertussis antibodies in adolescents after an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine, based on mathematical modeling and 5-year observed data. Vaccine 2008;26:3903–8.
[15] World Health Organization (WHO). Regional Consultation to standardize Expanded Program Immunization (EPI) schedule in the Eastern Mediterranean Region of the World Health Organization (WHO EMRO). Cairo, Egypt: VPI/EMRO; 2006, 23p. <http://www.who.int/.../3_EMRO_1_EPI_Schedule_Report.pdf> [cited 2006 October 16-18].
[16] Saad A, Safi-El-Dine A, El-Shamy K. The trend of mandatory vaccination among children in Egypt. Open Vaccine J 2009;2:77–84.
[17] World Health Organization (WHO). Global burden of disease estimates; 2002. <http://www.who.int/healthinfo/bodgbd2002revised/en/index.html>.
[18] Almanzar G, Ottensmeier B, Liese J, Prelog M. Assessment of IgG avidity against pertussis toxin and filamentous hemagglutinin via an adapted enzyme-linked immunosorbent assay (ELISA) using ammonium thiocyanate. J Immunol Methods 2013;387:36–42.
[19] Denoël P, Goldblatt D, de Vleesschauwer I, Jacquet J-M, Pichichero M, Poolman J. Quality of the *Haemophilus influenza* type b (Hib) antibody response induced by Diphtheria-Tetanus-Acellular pertussis/Hib combination vaccines. Clin Vaccine Immunol 2007;14:1362–9.
[20] Ahmed R, Gray D. Immunological memory and protective immunity: understanding their relation. Science 1996;272:54–60.
[21] Goodnow CC, Vinuesa CG, Randall KL, Mackay F, Brink R. Control systems and decision making for antibody production. Nat Immunol 2010;11:681–8.
[22] Barkoff AM, Gröndahl-Yli-Hannuksela K, Vuononvirta J, Mertsola J, Kallonen T, He Q. Differences in avidity of IgG antibodies to pertussis toxin after acellular pertussis booster vaccination and natural infection. Vaccine 2012;30:6897–902.
[23] Goldblatt D, Vaz AR, Miller E. Antibody avidity as a surrogate marker of successful priming by *Haemophilus influenza* type b conjugate vaccines following infant immunization. J Infect Dis 1998;177:1112–5.
[24] Prelog M, Almanzar G, Rieber N, Ottensmeier B, Zlamy M, Liese J. Differences of IgG antibody avidity after an acellular pertussis (aP) booster in adolescents after a whole cell (wcP) or aP primary vaccination. Vaccine 2013;31:387–93.
[25] Klein U, Kuppers R, Rajewsky K. Variable region gene analysis of B-cell subsets derived from a 4-year-old child: somatically mutated memory B cells accumulate in the peripheral blood already at young age. J Exp Med 1994;180:1383–93.
[26] Ridings J, Nicholson IC, Golgsworthy W, Haslam R, Roberton DM, Zola H. Somatic hypermutation of immunoglobulin genes in human neonates. Clin Exp Immunol 1997;2:366–74.
[27] Ridings J, Dinan L, Williams R, Roberton D, Zola H. Somatic mutation of immunoglobulin V (H) 6 genes in human infants. Clin Exp Immunol 1998;114:33–9.
[28] Riffelmann M, Thiel K, Schmetz J, Wirsing von Koenig CH. Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. J Clin Microbiol 2010;48:4459–63.
[29] Pullen GR, Fitzgerald MG, Hosking CS. Antibody avidity determination by ELISA using thiocyanate elution. J Immunol Methods 1986;86:83–7.
[30] Ministry of Health and Population (MOHP). Ministry of health and population/child survival project: the expanding programme of immunization in Egypt; 1984–1994. MOHP/EPI: Egypt; 1995.
[31] Hallander HO, Ljungman M, Storsaeter J, Gustafsson L. Kinetics and sensitivity of ELISA IgG pertussis antitoxin after infection and vaccination with *Bordetella pertussis* in young children. APMIS 2009;117:797–807.
[32] Mooi FR. *Bordetella pertussis* and vaccination: the persistence of a genetically monomorphic pathogen. Infect Genet Evol 2010;10:36–49.
[33] Dalby T, Petersen JW, Harboe Z, Krogfelt K. Antibody responses to pertussis toxin display different kinetics after clinical Bordetella pertussis infection than after vaccination with an acellular pertussis vaccine. J Med Microbiol 2010;59:1029–36.
[34] Butler JE. Solid supports in Enzyme-linked immunosorbent assay and other solid-phase immunoassays. Methods 2000;22:4–23.
[35] Goldblatt D. Simple solid phase assays of avidity. In: Turner MW, Johnstone AP, editors. Immunochemistry 2: a practical approach. Oxford University Press; 1997. p. 31–51.
[36] Heininger U, Cherry JD. Pertussis immunisation in adolescents and adults-*Bordetella pertussis* epidemiology should guide vaccination recommendations. Expert Opin Biol Ther 2006;67:685–97.
[37] Sallam I. Health care in Egypt. Lancet 1998;352:1632.
[38] CDC Centers for Disease Control and Prevention. Pertussis: Pertussis Outbreak Trends; 2013. <www.cdc.gov/pertussis/outbreaks/trends.html>.
[39] Sheridan SL, Ware RS, Grimwood K, Lambert SB. Number and order of whole cell pertussis vaccines in infancy and disease protection. JAMA 2012;308:454–6.
[40] Simondon F, Preziosi MP, Yam A, Kane CT, Chabirand L, Iteman I, et al. A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal. Vaccine 1997;15:1606–12.
[41] Bechini A, Tiscione E, Boccalini S, Levi M, Bonanni P. Acellular pertussis vaccine use in risk groups (adolescents, pregnant women, newborns and health care workers): a review of evidences and recommendations. Vaccine 2012;30:5179–90.
[42] Tan T, Trindale E, Skowronski D. Epidemiology of pertussis. Pediatr Infect Dis J 2005;24:S10–8.