Pertussis is a highly contagious respiratory disease that can occur in all age groups. Over the past 15 years, an increase in the incidence of reported cases has been observed globally. Local surveillance data have also shown an increase in the number of laboratory-confirmed cases. This raises concerns about waning immunity in adolescents and adults. The aim of this study was to screen for seropositivity against pertussis infection by measuring immunoglobulin G (IgG) antibody titers of *Bordetella pertussis* in an adolescent population in the Western Cape, South Africa. Serum samples were collected from 182 adolescents aged 15-18 years from different racial groups. The SERION ELISA® classic *B. pertussis* IgG assay was used to detect human antibodies in serum. Twenty-seven subjects (15%) were seronegative and 135 (74%) seropositive for IgG antibodies. Racial breakdown showed that 84% of the black, 74% of the coloured, and 69% of the white subjects were seropositive, while the largest percentage (24%) of sero-negative individuals was in the white population group. This study demonstrated a high percentage of individuals in the adolescent age group with sero-positive antibody levels. However, it is unknown if these antibody levels are functionally protective. A more rigorous surveillance system would assist in defining and understanding the epidemiology of pertussis in South Africa, and could provide evidence of the need for booster vaccinations in adolescence and adulthood.

**Introduction**

Pertussis or whooping cough is a highly contagious respiratory disease caused by the Gram-negative coccobacillus, *Bordetella pertussis*. It affects all age groups and has a higher incidence rate in females than in males. Although the highest reported rate of pertussis infection is still in infants, up to 44% of infections now occur in adolescents and adults, even in countries with high vaccine coverage and low mortality. However, many cases are undiagnosed and underreported as a prolonged cough may often be the only manifestation of pertussis infection.

Despite high vaccination rates and effective vaccines worldwide, pertussis is still estimated to cause 295 000-390 000 deaths annually in lower socio-economic countries. The case fatality rate in infants may be as high as 4%. Females are more likely to die in infancy from pertussis than males. South Africa is classified by the World Bank as an upper- to middle-income country, and although only 15% of the population has medical insurance, basic primary health care, including childhood vaccinations, have been provided free of cost to children under the age of six years since 1994. However, there are substantial differences in the quality and outcomes of healthcare services, vaccine timeliness and coverage in the geographical areas in South Africa. For instance, a much higher rate of unimmunised children was found in a poor rural area in KwaZulu-Natal than that in a peri-urban area in the Western Cape.

The whole cell pertussis vaccine, in combination with toxins against diphtheria and tetanus (DTwP), was very effective when it was introduced in the 1940s, but it was commonly associated with local, and less common, more serious, systemic adverse events. These safety concerns prompted the development of the diphtheria, tetanus and acellular pertussis (DTaP) vaccine.

Extensive vaccination studies in the USA revealed that the DTwP vaccine provided longer-lasting protection, while DTaP only provides adequate protection levels for between four and 15 years, following complete vaccination. This results in a susceptible population in mid-adolescence.

When the DTwP vaccine was introduced in South Africa in 1950, it was immediately followed by a marked decline in reported morbidity and mortality as a result of pertussis infection. However, isolated cases were still reported, which
mainly affected the very young. The National Expanded Programme for Immunisation (EPI) was established in South Africa in 1995, and included the DTwP vaccine to be administered at 6, 10 and 14 weeks, with a booster at 18 months of age. Because of the side-effect profile of the whole cell vaccine, DTaP, in combination with Haemophilus influenzae B and the inactivated polio vaccine (Pentaxim™), has been included in the South African EPI from 2009.

Well documented surveillance activities in the USA and Europe show an increasing incidence of pertussis in the adolescent and adult populations, despite a decreasing incidence in childhood. South Africa has experienced a steadily increasing number of laboratory-confirmed pertussis cases in recent years, according to the National Institute for Communicable Diseases (NICD). Although acellular pertussis vaccines appear to induce longer-lasting cellular, than humoral, immune responses, this could be a result of waning immunity. However, other more important factors should be considered, including improved diagnosis and reporting, as well as changes in the $B. pertussis$ population which result in vaccine strain mismatches.

In 2010, more than six-million children in sub-Saharan Africa did not complete their primary vaccination schedule by the age of one year, jeopardising vaccine efficacy. Different methods are used by the Department of Health and the World Health Organization in South Africa to determine vaccine coverage. This could result in huge discrepancies in the results (96% and 64%, respectively reported for 2011).

South Africa shows great diversity in terms of socio-economic development and infrastructure, and these factors greatly impact on the timeliness of vaccination. Poorer communities classically have lower vaccine coverage rates and reduced timeliness. However, despite vaccine coverage of 81-93% in areas of the Western Cape in the late 1980s, a pertussis outbreak was reported in Cape Town in 1988 and 1989. Although no outbreaks have been reported since, data from the NICD indicated an increasing number of laboratory-confirmed pertussis cases in South Africa between 2008 and 2011, in line with international trends. Similar trends worldwide and statistics from the USA indicate an ever-increasing number of pertussis cases and outbreaks, especially in the age group 10-19 years.

The aim of this study was to screen for seropositivity against pertussis infection by measuring the IgG antibodies against $B. pertussis$ in an adolescent population in communities of the Western Cape. The last vaccination against pertussis takes place in South Africa at 18 months of age. The results of this study will contribute to the literature when assessing the need for booster immunisation in the adolescent age group.

Method

Study population and design

Serum samples were selected from a large cohort study of clinically healthy adolescents which focused on childhood obesity and metabolic syndrome in schools in the Western Cape. For this study, the serum samples from 182 individuals were randomly selected from the sample bank, which represented different racial groups (black, coloured and white) and socio-economic backgrounds, based on the area in which the participants lived.

Informed consent was obtained from the learners and parents for participation in the large cohort study. No information on vaccination status was available since the main questionnaire and data capture form were not designed to be used for antibody determination. Additional ethical approval for this substudy was obtained from the Human Research Ethics Committee of the University of Stellenbosch to perform anonymous pertussis antibody determination on the stored serum samples.

Laboratory tests

The SERION ELISA® classic $B. pertussis$ IgG assay (Serion Immunagnostica & Institut Virion-Serion GmbH, Würzburg, Germany) was used to measure human IgG antibody levels against $B. pertussis$ in serum. The solid phase of the IgG enzyme-linked immunosorbent assay (ELISA) is coated with a mixture of pertussis toxin (PT) and filamentous haemagglutinin (FHA). Quantitative IgG, IgM and IgA tests are routinely used for the detection of acute and recent infections of $B. pertussis$ and for epidemiological studies. IgM, IgA and IgG antibodies are produced in the event of primary infection, while an immune response after vaccination consists of IgM and IgG antibodies only. Analysis was performed on the Bio-Rad PhD™ system (Bio-Rad Laboratories, Johannesburg, South Africa) which was validated for $B. pertussis$ antibody levels by using an adjusted extended incubation time. Obtained results were classified as seropositive, indeterminate and seronegative, according to the manufacturer’s assay-specific reference ranges. No separate reference ranges are available for different countries, and age or racial groups. The same cut-off values were applied to all of the subjects.

Data analysis

Data were analysed on GraphPad® Prism v 5 (GraphPad Software, San Diego, USA). Two-tailed Spearman rank correlation coefficients were performed to establish a potential relationship between the parameters. A two-tailed Mann-Whitney U test was performed when comparing pertussis levels between the males and females. A one-way Kruskal-Wallis analysis of variance (ANOVA), followed by the Bonferroni-Dunn post-test, was performed when comparing the results between the different race groups, as well as between the races matched for sex. Significance was set at $p$-value $< 0.05$.

Results

The study group consisted of 37 black (20%), 87 coloured
(48%), and 58 white (32%) individuals. There were 119 females (65%) and 63 males (35%). The mean age was 15.8 years (a range of 15–18 years). No other clinical or relevant laboratory data were available.

IgG antibody levels ranged between 5.5 and 811.9 US Food and Drug Administration (FDA) U/ml. Since no separate reference ranges were available for the African region, the manufacturer’s reference ranges were used (seropositive > 30 FDA U/ml, indeterminate 20–30 FDA U/ml, and seronegative < 20 FDA U/ml). One hundred and thirty-five serum samples (74%) were seropositive, 27 (15%) seronegative, and the remaining 20 (11%) reflected indeterminate results. The gender and racial distribution, as well as different antibody groups, are presented in Table I.

Table I: Gender and racial distribution and antibody classification

<table>
<thead>
<tr>
<th>Gender and racial distribution</th>
<th>Seropositive &gt; 30 FDA U/ml</th>
<th>Indeterminate 20–30 FDA U/ml</th>
<th>Seronegative &lt; 20 FDA U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (n = 119)</td>
<td>88 (74%)</td>
<td>12 (10%)</td>
<td>19 (16%)</td>
</tr>
<tr>
<td>Males (n = 63)</td>
<td>47 (74%)</td>
<td>8 (13%)</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Black (n = 37)</td>
<td>31 (84%)</td>
<td>3 (8%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Coloured (n = 87)</td>
<td>64 (74%)</td>
<td>13 (15%)</td>
<td>10 (11%)</td>
</tr>
<tr>
<td>White (n = 58)</td>
<td>40 (69%)</td>
<td>4 (7%)</td>
<td>14 (24%)</td>
</tr>
<tr>
<td>Total</td>
<td>135 (74%)</td>
<td>20 (11%)</td>
<td>27 (15%)</td>
</tr>
</tbody>
</table>

No statistical difference was found when comparing males and females (p-value 0.629). Similar results were found when comparing black and white, coloured and white, and coloured and black, subjects (p-value 0.494, 0.758 and 0.851, respectively). Insignificant differences were found between the sero-positive and intermediate groups, sero-positive and sero-negative, and intermediate and sero-negative, groups (p-value 0.390, 0.672 and 0.335, respectively).

Discussion

Pertussis is not just a childhood disease, but an infection that can affect all age groups. This has been highlighted by the worldwide increase in observed pertussis cases in the adolescent and adult groups. Only a few countries have introduced booster vaccines for adolescents and adults. Data from studies on several thousand adolescents and adults have confirmed the immunogenicity and safety of the DTaP booster vaccine. No clear serological correlates of protection for pertussis have yet been defined, and the only way of assessing immunity is to screen for seropositivity. Although an adolescent or adult vaccine is available on request in the private sector in South Africa, only a very small proportion of the South African population benefits from this. The last routine booster dose is given in South Africa at 18 months of age.

Booster vaccines induce longer-term protection in individuals, and can also reduce transmission within the general population. This is especially important for vulnerable and susceptible individuals when the immune system is compromised, such as the elderly, individuals of all ages infected with the HIV virus and HIV-exposed, uninfected infants and children.

Pertussis is a notifiable disease in South Africa. However, diagnosis and reporting are suboptimal and the surveillance data are of poor quality. Despite fairly high vaccine coverage at the time, the last pertussis outbreak was reported in South Africa in 1988 and 1989, but increasing numbers of pertussis cases were confirmed by laboratory diagnosis between 2008 and 2011. Most of the locally reported cases were infants and small children, who were either incompletely vaccinated or not protected at all. Although the source of the infection was not recorded in these cases, the presence and effect of adolescents and adults with waning or no immunity against pertussis is likely to have played a role.

Sampling was carried out in adolescents who received their vaccinations 10–15 years ago in this study, before the introduction of the acellular vaccine in South Africa, and in urban areas where high childhood vaccine coverage existed at the time. Therefore, the data should reflect the long-term effect of immunity induced by the whole cell vaccine, rather than incomplete childhood vaccination schedules, or the effect of the acellular vaccine. Although 74% of subjects in this study were seropositive for IgG antibodies against B. pertussis, the remaining 26% had indeterminate or sero-negative results. This is a major concern because these individuals could become infected and might also transmit the disease to others. The literature suggests a female predominance for pertussis seropositivity, but we found a similar proportion of females and males to be seropositive. This was probably owing to the small sample size in this study. Interestingly, the black subjects in this study showed a higher percentage (84%) of seropositivity than the coloured (74%) and white (69%), subjects, while the latter were more likely to be seronegative than their black and coloured counterparts. However, the sample size in this study was too small to enable conclusions to be drawn from these figures. Therefore, the results should be interpreted with caution. No trends could be identified either, owing to the single sampling.

Laboratory diagnosis is often not helpful in isolation, so several other factors should be considered when interpreting the IgG results for B. pertussis. The SERION ELISA® assay for FHA IgG antibodies is known to cross-react with other pertussis species, e.g. B. parapertussis. FHA is regarded as the dominant adhesin for, and is produced and secreted in high concentrations by B. pertussis, but can also be secreted by B. parapertussis and B. bronchiseptica. By measuring FHA on its own or in combination with PT, it is not possible to distinguish between seropositivity resulting from natural immunity due to infection by any of these species, or that resulting from vaccination. ELISA kits that contain the PT antigen only should be used to obtain a diagnosis of B. pertussis infection in patients with clinical symptoms that are compatible with pertussis, or when cross-reactions are suspected. However,
raised anti-PT IgG could reflect exposure or infection, rather than clinical disease. Interpretation may be difficult in recently vaccinated individuals. Therefore, the anti-PT IgG test is used most often for surveillance to evaluate the effectiveness of the pertussis vaccine in vaccinated populations.²⁵

Immunity to both natural infection and vaccination wanes over time and the re-emergence of pertussis infection is typically seen in adolescence and during early adulthood. Individuals can become seronegative within a few years of completing the vaccination.¹²⁴⁶ These individuals are then susceptible to infection. Although this might not be accompanied by clinical disease, transmission to other susceptible or vulnerable individuals is sometimes possible. The susceptibility is a direct result of a change in the level of immunity in the population, owing to waning immunity and re-exposure to the pathogen. Re-exposure can further provide a natural boosting effect without clinical disease developing.²⁶

Environmental, demographic and socio-economic factors contribute to different degrees of exposure to B. pertussis infection. The black subjects in this study lived in lower socio-economic conditions than the coloured and white subjects, and were exposed more often and readily to a wider variety of pathogens because of factors such as overcrowding, and poor sanitation and housing. Despite the fact that only a small number of black subjects were included in this study, the highest percentage of seropositivity (84%) was found in this group.

Since this study used convenience sampling, it had several limitations. Individual vaccination records were not available for any of the subjects. The participants in this study were born between 1990 and 1995, when overall immunisation coverage for this period in the Western Cape was 64.2%.²⁷ The HIV status of the participants was also not known. HIV-infected individuals are likely to be more susceptible to other infections, so HIV status should be considered when interpreting immunological data. The introduction of antiretroviral treatment and better access to health care resulted in increased survival rates in all age groups, but it also led to an increased number of HIV-exposed, uninfected infants. Specific alterations in the immune responses of these infants during the first two years of life had been described. The question remains as to whether or not subsequent seropositivity against pertussis or any other pathogen equates to actual protection against the disease.²⁸⁻⁳⁰

The assay that was used in this study was unable to distinguish between IgG antibodies produced as a result of primary infection or vaccination. Furthermore, while PT is exclusively secreted by B. pertussis, FHA is also secreted by B. parapertussis and B. bronchiseptica. Therefore, it was not possible to distinguish between antibodies against PT, FHA, or both. Cross-reactivity between pertussis and parapertussis had also been described, and could have affected the results in the absence of clinical data or specific microbiological laboratory investigations, such as cultures.²⁵,²³,³¹

Despite high vaccine coverage worldwide, pertussis infection still regularly re-emerges in several areas, and follows a cyclic pattern. This suggests waning of immunity as a result of natural infection and vaccination. Mass immunisation could lead to herd immunity in communities. This is only possible for diseases where humans are the only reservoir, and transmission occurs from person to person, such as pertussis infection. However, this practice limits the opportunity for natural boosting due to re-exposure.²⁶

Acellular booster vaccines, formulated specifically for adolescents and adults, are available worldwide.⁴ Longitudinal studies should be carried out in South Africa on vulnerable groups to assess the need for booster vaccination programmes in adolescence or later, to establish longer-lasting immunity in the community.

Larger studies from different geographical areas, with detailed vaccinations, clinical history and laboratory investigations, including IgA and PT, should be undertaken to validate the results obtained in this small cross-sectional descriptive study. Longitudinal follow-up sampling would also enable the identification of increasing or decreasing trends of seropositivity with increasing age.

**Conclusion**

In South Africa, no major outbreaks of pertussis have been reported recently, despite an increase in the number of laboratory-confirmed cases.²⁸ This could be attributed to natural immunity and constant re-exposure to the antigen, or the result of inadequate reporting and surveillance systems in South Africa. This small study demonstrated a high percentage of individuals in the adolescent age group who were seropositive for pertussis antibodies, but it could not determine if these antibodies were protective or not. In the absence of well defined protection correlates, it is not possible to assume that seropositivity implies protection against disease. This emphasises the need for well structured and documented surveillance programmes in order to evaluate the need for revaccination or booster vaccination for pertussis in adolescent and adult populations.

**Declaration**

A reagent support grant (K-Fund) was obtained from the NHLS to perform the ELISA antibody tests.

**References**

Original Research: The seroprevalence of _Bordetella pertussis_ antibodies in adolescents in the Western Cape