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The Prevalence of PCR-Confirmed Pertussis Cases in Palestine From Archived Nasopharyngeal Samples

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Abstract

Background: Pertussis caused by *Bordetella pertussis* is a vaccine-preventable disease causing whooping cough in humans of all ages. This study reports infection rate of pertussis in Palestine between the years 2004-2008 from archived nasopharyngeal samples collected from clinically- suspected cases.

Methods: A convenience archived DNA samples collected from 267 clinically-suspected pertussis cases were investigated for *B. pertussis*. Laboratory diagnosis was done by examining all DNA samples using polymerase chain reaction (PCR).

Results: Approximately 49% (130/267) were confirmed by PCR. A pertussis peak was shown to occur in 2008 with 77% (100/130) of PCR-confirmed cases isolated in that year. PCR-confirmed cases existed in all Palestinian districts with highest rate in Ramallah, Bethlehem, Jenin and Al-Khalil. Half of the PCR-confirmed cases (68/130) were less than 2 months old. The positivity rate among who had three doses of vaccine (at 2, 4 and 6 months) was 38%, and became 50% with the fourth dose at 12 months.

Conclusion: The prevalence of pertussis was found to be significantly high among infants less than 2 months old. Active pertussis surveillance using rapid PCR assays is essential, as it is helpful in prompt diagnosis and treatment of patients with pertussis.

Keywords: Acellular pertussis vaccine (aP), *Bordetella pertussis,* Palestine, PCR, Pertussis vaccine, Whole-cell pertussis vaccine (wP)

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Introduction

Pertussis (whooping cough) is an acute illness caused by *Bordetella pertussis*, and less frequently by *Bordetella* spp. It is gram-negative, aerobic, non-motile coccobacilli. Pertussis is a highly communicable and vaccine-preventable respiratory tract disease with a worldwide distribution. Outbreaks usually occur every 3–5 years.[1,2] Pertussis infection affects all age groups, but it is mainly symptomatic in children and more serious in young unvaccinated or partially vaccinated infants. Pertussis can be fatal in newborns, infants, and children under 2 years old.[1,3–5]

Effective vaccines (whole-cell pertussis vaccines) became available in the 1940s, and since that time the rate of infection has been reduced dramatically in countries in which universal vaccination of infants and children is implemented. Multicomponent acellular vaccines (aP) are now in use in many developed countries worldwide.[3] These vaccines do not provide lifelong immunity, however, occasional local epidemics continue to occur.[6–9] In contrast, whole-cell vaccines (wP) are used in low-income countries. The old generation vaccine of wP has shown an efficacy of 80%. However, the undesired components such as endotoxins that cannot be eliminated during production may inevitably be associated with a greater incidence of adverse effects.[9]

In the pre-vaccine era, incidence, case morbidity and fatality rates were very high, and the disease mainly affected children under 5 years of age. The adoption of pertussis vaccine combined with tetanus and diphtheria toxoids in 1974, as part of the WHO's Expanded Program of Immunization, has reduced the number of cases as well as mortality rate.[10] Recently, increasing number of cases among infants, children and adults has been reported in developed countries.[2,3] A possible explanation for this shift in epidemiology could be due to improved diagnostic tests, increased awareness among medical community, genetic changes in the organism and waning immunity. Many countries have therefore introduced a booster dose of vaccine for pre-school children, and others have considered an additional booster dose for adolescences and adults.[3,4,11] The WHO estimate puts the annual number of cases in developing countries at 50 million, with one-third of a million deaths. Case fatality rate among infants is as high as 4%.[12] Furthermore, a surprising increase in incidence rates of pertussis has been reported in several

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developed and developing countries including the United States, Canada, Israel, Brazil, European countries, Iran, Tunisia and Turkey.[13–20]

In Palestine, pertussis is a notifiable disease by law. The Palestinian immunization schedule includes 4 doses of whole-cell vaccine initially at 2, 4, 6 and 12 months, but since 2008 the fourth dose is given at 18 months.[21,22] Definitive diagnosis of pertussis is based on a combination of clinical signs and symptoms, history of exposure to a confirmed case and appropriate laboratory tests.[12,23]

In this pilot study, we report for the first time the epidemiological status and infection rate of pertussis from clinically-suspected cases using polymerase chain reaction (PCR) assay from 2004–2008. In addition, the incidence rate of pertussis in the period between 2000 and 2016 was reported.

Materials and Methods

Specimens

Archived DNA samples were extracted from nasopharyngeal swabs of 267 infants and children with clinically-suspected pertussis who were admitted to hospitals in West Bank, Palestine, between September 2004 and June 2008. The nasopharyngeal specimens were collected using Dacron swabs and were stored at -20°C until tested.

The presumable clinical diagnosis of pertussis was based on the WHO clinical case definition which requires paroxysms of cough lasting at least two weeks with respiratory whooping post-tussive vomiting, without other apparent causes.[12] However, less than 2 weeks of paroxysmal cough was adopted to account for milder infections to prevent missing cases. The infection rate of pertussis was defined as the number of PCR-confirmed cases over the total number of tested cases.

DNA Extraction

DNA was extracted using the boiling method described previously by Lind-Brandberg et al.[24] Nasopharyngeal swabs were briefly inserted in 1.5 mL sterile Eppendorf tubes containing 400 µL sterile nuclease-free distilled water. Swabs were allowed to stand for 3–5 minutes, mixed by vigorous vortex for 15–20 seconds, and incubated at 100°C for 10 minutes on a thermo-block. Afterwards, the tube was left to cool for 2 minutes, and then centrifuged for 15 minutes at 14 000 rpm. The DNA in the supernatant was used immediately for PCR or kept at -20°C until use.

Polymerase Chain Reaction

The *B. pertussis* DNA was amplified by PCR assay described previously using primers PIp1 (forward primer: 5' CCC ATAAGC ATG CCC GAT TGA C 3') and PIp2 (reverse primer: 5' CGC ACA GTCGGC GCG GTG AC 3') targeting the insertion sequence IS481.[24] A Biorad C1000 thermal cycler was used for amplification. Negative control of sterile nuclease-free distilled water and positive control (culture-confirmed isolates) were used in each run. PCR product was run by electrophoresis on agarose gel, and visualized under UV by G: Box-Syngene. The size of the PCR amplicon was 121bp.

Statistical Analysis

The Epi Info™ 7 statistical package (Centers for Disease Control and Prevention, Atlanta, USA) was used for data management and analysis. Fisher exact test and chi-square were used to establish an association between variables. The statistical difference was considered significant when $P < 0.05$.

Results

Of the 267 clinically-suspected cases, 56.5% (151/267) were males. Patients were from 10 Palestinian districts with 28% from Al-Khalil, which is the most populous district (Figure 1). Information regarding the age of the 33 subjects was not available (Table 1). Out of 234 patients, 58.5% (137/234) were infants less than two months old. Fourteen were above 12 months of age with infection rate of 50% (7/14). Of the total nasopharyngeal samples, 130 (49%) were positive for *B. pertussis* DNA (Table 1). From 2000–2016, the mean incidence rate of pertussis was 1.7, ranging from 0.16 to 7.3 cases per 100 000 persons (Figure 1).[25]

A sharp increase in pertussis cases was observed during the first six months of 2008 in comparison to the previous three years, indicating that the year 2008 was a peak year. The number of PCR-confirmed pertussis cases in 2008 was 100 out of 130 (77%). Concomitantly, the Ministry of Health also reported the year 2008 as the peak year of clinically-suspected pertussis cases (Figure 1). Cases in the peak year were mainly from districts of Al-Khalil, Bethlehem, Ramallah and Jenin. A second peak of PCR confirmed cases was also reported in

Table 1. Age Group Versus *Bordetella* pertussis PCR Results

Age Group (mon)	PCR (-)	PCR (+)
0 to 2	68	68
3 to 4	26	37
5 to 6	8	5
7–12	5	3
> 12	7	7
Unknown	23	10
Total	137	130

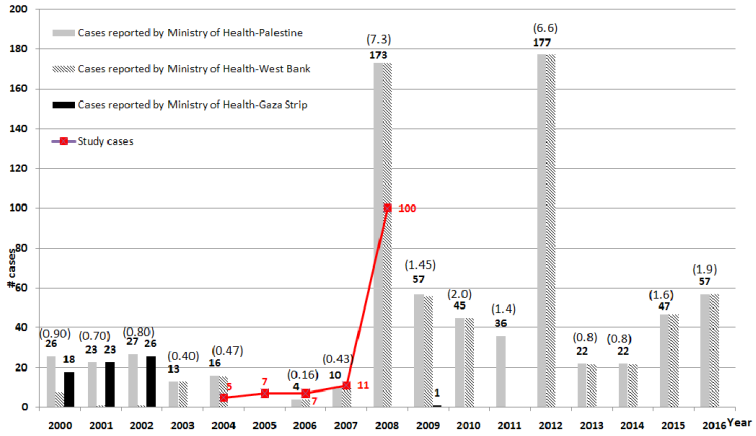

Figure 1. Graph Distribution of *Bordetella pertussis* Cases Over Time From 2000 to 2016. The numbers between brackets represent incidence rate per 100 000 using the Palestine population as the denominator when pertussis cases are reported from the West Bank and Gaza Strip, while, the population of the West Bank was used when zero cases were reported from Gaza Strip. The bold numbers show the number of cases. The absence of cases from Gaza strip is due to lack or absence of laboratory-confirmed cases.

2012. As far as the official figures were based on clinical manifestations from 2000 to 2008, and the PCR assay from 2009 to 2016.

The geographical distribution of pertussis cases in Palestine are shown in Figure 2. The infection rate was highest in Ramallah, Bethlehem, Jenin and Al-Khalil. Pertussis was shown to exist in all Palestinian districts with varying infection rates.

Approximately 52% (68/130) of the PCR-confirmed pertussis cases were less than 2 months old who did not get their first dose of vaccination according to the Palestinian immunization program. The infection rate among infants between 3 and 4 months old who had their first dose of vaccination was 59% (37/63). The infection rate in infants aged 5–6 months who had recently their second dose of vaccination was reduced to 38.4%, which was the same for infants 7–12 months who had their third dose of vaccination, while those who had their fourth dose at 12 months, had an infection rate of 50% (7/14) (Table 1, Figure 3). Among PCR-confirmed cases, no statistically significant difference was shown between age groups above and below 2 months ($P=0.69$, 95% CI: 0.73–1.20). Infants of the age group >12 months were actually above 18 months old, which according to the new Ministry of Health policy adopted in 2008, did not have the fourth booster dose (Table 1). Sex had no significant role in the distribution of pertussis infection ($P=0.22$, 95% CI: 0.92–1.51). The pertussis infection was shown to be seasonal in 75% (96/128) of the PCR-confirmed cases, equally distributed in April, May, and June.

Discussion

In spite of good vaccination coverage that may be more than 90% worldwide and over 98% among Palestinians, pertussis is still an endemic infection in most developed and developing countries including Palestine.[21,25] During the last 2 to 3 decades, an increase in incidence of pertussis, with outbreaks every 3–4 years, had been reported worldwide in young vaccinated individuals and unvaccinated or partially vaccinated children.[2,16,17]

To the best of our knowledge, this was the first study that investigated the infection rate of pertussis using PCR from archived nasopharyngeal swabs of clinically-suspected cases in Palestine. In this study, the infection

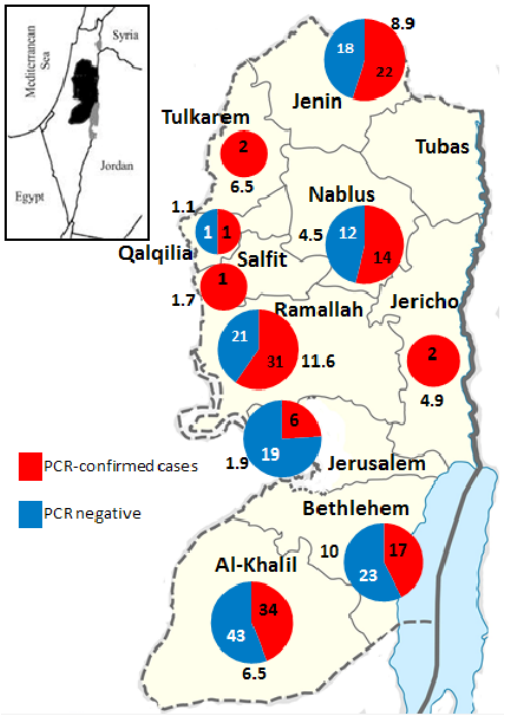

Figure 2. Map Showing Geographical Mapping of the Study Population in the Palestine study. Numbers next to each pie chart represent the incidence rate per 100 000.

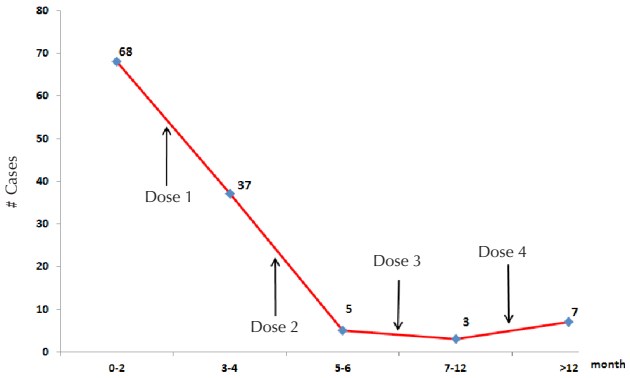

Figure 3. Line Graph Showing the Absolute Numbers of Pertussis Cases Versus Age of Investigated Infants. Arrows show the doses of vaccination on the time line 2, 4, 6 and 12 months according to the schedule adopted by the Palestinian Ministry of Health until 2007. Numbers on the line graph represent the numbers of PCR-confirmed cases.

rate of PCR-confirmed pertussis was 49% (130/267) with a peak in the first half of the year 2008, which is a reflection of the total number of suspected cases reported by the Ministry of Health during the same period (Figure 1). The infection rate of the PCR-confirmed cases in the present study is in agreement with that reported in south-eastern Minnesota, USA during an outbreak in 2012 (60%), and in Peru between 2010–2012 (39.5% among infants less than 1 year, and 73.55% in those less than 3 months).[26,27] On the other hand, lower infection rates have been reported in Tunisia (20%), Iran (10.5%), Turkey (26%), Brazil (35%), and Israel (7%–19%).[15,16,18–20,28] The discrepancy in the infection rate could be due to the sensitivity of PCR method and age of the study samples. Similarly, another peak in the West Bank, Palestine was reported in 2012. This finding is in congruence with other studies in the United States, Sweden, Turkey, Tunisia, Iran, Brazil, Poland and anywhere that the whole-cell vaccine is adopted.[8,16,18–20,29,30]

Although the infection is reported in almost all Palestinian districts, cases are mainly focused in Al-Khalil, Bethlehem, Ramallah and Jenin, which may be due to the outbreak occurred in the aforementioned districts in 2008 and active response of medical personnel in those districts.

The infection rate among the study population had a decreasing trend following the first, second and third vaccine doses at months 2, 4, and 6. This indicates an accumulating effectiveness of the vaccine following each dose, but increased number of cases after the third dose which could be due to small sample size (Figure 3).

Moreover, the vaccine used in Palestine is a whole-cell vaccine (wP) given as a part of the Penta vaccine (Serum Institute of India LTD, Hadapsar, Pune, India) for the first three doses and part of the diphtheria-pertussis-tetanus (DPT) vaccine combination (Biofarma, Bandung, Indonesia) for the fourth booster dose. The effectiveness of high-efficacy whole-cell vaccine ranges from 83% to 96% .[9] The manufacturers of the wP vaccine currently in-use in Palestine, never mentioned the efficacy of their product. Meanwhile, despite high immunization coverage in Palestine (>98%) which is higher than the critical percentage for Pertussis (92%–94%), the infection rate is still significantly high, emphasizing the need of re-evaluating the pertussis vaccine policy

Pertussis infection in Palestine had shown a seasonal pattern with most cases occurring in the months of April, May and June. Although mothers are a significant source of infection for their infants, this could also be due to the picnic season, which starts in late spring/early summer each year. During these seasons, students come into close range with one another during travel, mealtimes and while playing.[31] These students could be infected or more importantly, could take infection home. Crowded kindergartens with infants and young children may also form a site for spreading infection to partially-vaccinated infants. Other studies have also reported seasonality, but in different months.[8,19,20,31]

The main limitation of the present study is the PCR assay that targeted the insertion sequence IS481 of *B. pertussis* which could present other *Bordetella* spp. Under-reporting is another limitation since not all clinically-suspected cases seek medical care. Another limitation is the system of diagnosis in Gaza Strip which is totally based on clinical diagnosis rather than laboratory confirmation; therefore, it dropped from official statistics in annual Palestinian health reports as shown in Figure 1.

This study concluded that PCR-confirmed pertussis infection in Palestine is significantly high among infants under 2 months old who are referred as clinically-suspected cases. Finally, surveillance with rapid PCR method for *B. pertussis* is essential in infants less than 1 year old and is helpful for prompt diagnosis and treatment.

Authors' Contribution

M.

Conflict of Interest Disclosures

None of the authors have any competing interests, whatsoever.

Ethical Statement

The study was approved by the Palestinian Ministry of Health under the reference number 162/2055/2015.

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