Journal of Microbiology, Immunology and Infection (2017) 50, 327–332

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Seroepidemiology of pertussis among elementary school children in northern Taiwan

Ching-Chia Kuo [a], Yhu-Chering Huang [a,*], Yu-Chia Hsieh [a], Ya-Ling Huang [b], Yu-Chiau Huang [b], Yung-Tai Hung [c]

[a] *Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[b] *Department of Laboratory Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[c] *Department of Political Science, National Taiwan University, Taipei, Taiwan*

Received 19 March 2015; received in revised form 7 July 2015; accepted 23 July 2015
Available online 31 July 2015

KEYWORDS
children;
pertussis;
seroepidemiology;
Taiwan

Abstract *Background/Purpose:* Pertussis has been considered a vaccine-preventable "childhood disease", but a shift in age distribution has been reported worldwide. We conducted a seroepidemiological study in 2013 in Taiwan to elucidate the seroprevalence of pertussis among elementary school children.
Methods: With a multilevel randomized method, which included 14 variables (4 population variables, 4 socio-educational variables, and 6 medical facilities' variables), the 29 executive districts of New Taipei City, Taiwan, were categorized into five strata. From each stratum, the number of school children as well as the number of elementary schools were proportionally selected. Enzyme immunoassay was applied for pertussis immunoglobulin-G measurement.
Results: A total of 936 children from 14 schools were recruited. Most participants (98.89%) received at least three doses of acellular diphtheria-tetanus-pertussis vaccine. The overall seropositive rate for pertussis was 33.97%. The seropositive rate was highest for students in Grade 1 (49.36%) and then declined with time, except for Grade 6 students. Students from Grade 1 to Grade 4 had a significant higher seropositive rate (37.18% vs. 27.56%, $p = 0.002$) than those from Grade 5 to Grade 6, but a lower geometric mean titer (18.71 NovaTec Unit/mL vs. 20.04 NovaTec Unit/mL, $p = 0.20$). For the class grades, geometric mean titers were positively correlated with seroprevalence ($p < 0.005$).
Conclusion: Currently, almost one-third of elementary school children in Taiwan were seropositive for pertussis, a rate lower than expected. Seroprevalence declined with increasing class

* Corresponding author. Department of Pediatrics, Chang Gung Children's Hospital, Number 5, Fu-Hsin Street, Kweishan 333, Taoyuan, Taiwan.
E-mail address: ychuang@cgmh.org.tw (Y.-C. Huang).

http://dx.doi.org/10.1016/j.jmii.2015.07.006

grades except for Grade 6. The current national immunization program may not provide adequate protection for children against pertussis.

Introduction

Pertussis is a highly contagious acute respiratory tract disease caused by *Bordetella pertussis*, an exclusive human pathogen. Characterized by paroxysmal severe coughing spells, pertussis sometimes causes severe complications in infants and young children including pneumonia, seizure, encephalopathy, and even death.[1] Despite progress in vaccinations, pertussis remains one of the top 10 causes of death worldwide in childhood, accounting for 30,000,000—50,000,000 cases and nearly 300,000 deaths in children every year.[2] So far, breakthrough diseases in cycles were still observed every 3—5 years even in vaccinated populations.[3,4]

In Taiwan, whole-cell diphtheria-tetanus-pertussis vaccines had been offered to children free of charge at 2 months, 4 months, 6 months, and 18 months of age since 1954, based on the national immunization program. The incidence of reported pertussis cases per year has declined significantly thereafter from 77 cases per million in 1955 to less than one case per million in 1970, and this low incidence was retained from 1971 to 1991 before an unexpected outbreak developed in 1992.[5] Two types of acellular diphtheria-tetanus-pertussis (DTaP) vaccines from Kaketsuken (Chemo-Sero-Therapeutic Research Institute), Okubo, Kumamoto, Japan [DPT "KAKETSUKEN" SYRINGE consisting PtxS1B and filamentous hemagglutinin (FHA)] and SmithKline Beecham Biologicals (became GlaxoSmithKline Biologicals since 2000), Rixensart, Belgium (INFANRIX consisting of PtxS1B, FHA, and Prn1 from the Tohama strain) were licensed for use since 1996 and 1998, respectively, at the patient's own expense. The DTaP vaccine was later universally substituted by the diphtheria and tetanus toxoid with acellular pertussis, *Haemophilus influenzae* type b, and inactivated polio vaccine; INFANRIX-IPV + Hib consisting pertussis toxin (PT), FHA, and pertactin from GlaxoSmithKline Biologicals, Rixensart, Belgium, or PEDIACEL consisting PT, FHA, pertactin, and fimbrial agglutinogens 2 + 3 from Sanofi Pasteur, Toronto, Ontario, and included in the expanded program on immunization (EPI), since 2010. In addition, since March, 2009 the reduced dose tetanus, diphtheria, and acellular pertussis vaccine (Tdap; ADACEL consisting PT, FHA, pertactin, fimbrial agglutinogens 2 + 3 from Sanofi Pasteur, Toronto, Ontario) was introduced into EPI as a booster for children from 5 years of age to elementary school Grade 1.

Completion of at least three doses of acellular pertussis vaccine was thought to result in higher efficacy in preventing typical whooping cough and in preventing mild pertussis disease, compared with administration of one to two doses only.[6] However, in children who completed five doses of pertussis vaccine, pertussis was still observed in older children, and the odds increased as the time since last DTap increased, suggesting waning protection despite the five-dose-DTap schedule.[7] An increase in yearly reported pertussis cases of children aged 5—9 years was observed in Taiwan recently, but both of their associated vaccination status and seroepidemiologic information were lacking.[5] The purpose of this study was to establish the seroepidemiologic data of pertussis among children attending elementary schools in New Taipei City, Taiwan, which would be the pilot study of nationwide surveillance, and would be provided as a reference of the national immunization program in Taiwan.

Methods

Ethics statement

The study proposal was reviewed and approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taoyuan, Taiwan in 2012. Informed consent in written form was obtained from all participants, as well as their guardians.

Study populations

A cross-sectional survey of anti-*B. pertussis* immunoglobulin (Ig)-G antibodies in the population of elementary school children in New Taipei City was conducted from September 2012 to June 2013. New Taipei City comprises 29 administrative districts and is the second largest special municipality in northern Taiwan. A total of 225,234 pupils resided in this city, which accounted for 16.5% of all primary school children in Taiwan in 2012. A multistage stratified method with probability proportional to size sampling was employed to obtain samples. The whole 29 administrative districts of New Taipei City were classified into five strata based on 14 variables, which included four demographic variables [population density (persons/km^2), proportion of population older than 65 years, younger than 15 years, and younger than 6 years], four socio-educational variables (number of low-income households per 10,000 people, number of near-poor households per 10,000 people, proportion of agriculture population, proportion of population with college degree or above), and six medical facilities' variables (number of physicians per 10,000 people, number of nursing staffs per 10,000 people, number of nursing staffs in health centers per 10,000 people, number of medical personnel in health centers per 10,000 people, number of staffs in health centers per 10,000 people). Elementary schools in each stratum were selected with selection probability proportional to their size. One class was drawn from each grade in a sampled school, that is, six classes in total were drawn from each sampled school. In each selected school, the number of students in

each class were also selected with probability proportional to their size. Finally, students were randomly selected in each class. Accordingly, 558 students would be selected from six schools in Stratum 1, 130 students from two schools in Stratum 2, 66 students from two schools in Stratum 3, 140 students from two schools in Stratum 4, and seven students from two schools in Stratum 5. In total, at least 901 students should be obtained. Subsequently, we planned to recruit a total of 936 students from the selected schools. Blood was drawn from selected students for the serologic tests of pertussis. All participants were relatively healthy without acute illness while blood samples were drawn between February 2013 and April 2013. Past medical history was gathered via a questionnaire from each participant and the pertussis vaccination status of each participant was provided by the Centers for Diseases Control of Taiwan (CDC-Taiwan) and the student vaccination record in each school.

Determination of pertussis-specific antibodies

Sera were stored at -20°C before measurments. Enzyme immunoassay (NovaLisa Bordetella pertussis IgG/IgA ELISA, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) was applied for the qualitative determination of IgG-class antibodies against *B. pertussis* in the participants' sera. Sera were tested following the directions of the manufacturers. Based on the instructions of NovaLisa, the antibody titers were acquired using NovaTec Units (NTU). A NTU was defined as the mean sample absorbance value $\times$ 10/cut-off value. The results were considered positive for titers $>$11 NTU, negative for titers below 9 NTU, and equivocal between 9 NTU and 11 NTU.

Statistical analysis

Based on timing of blood sampling and age on school enrollment, students in Grade 1 were mostly born between September 2005 and August 2006; students in Grade 2 were born between September 2004 and August 2005, and so on. Nine hundred and twenty-eight students out of 936 (99.15%) students were in the correct age group of their Grade levels during the survey period. As Tdap was included in EPI as a booster for children $>$5 years since 2009 in Taiwan, all the students from Grade 1 to Grade 4 were supposed to have received a further booster vaccine against pertussis. Pertussis-specific antibody titers were presented as geometric mean titers (GMTs). Differences in seropositive percentages between different groups were tested using the Chi-square test. The unpaired *t* test was used to compare the statistic deviances between GMT levels in different Grade. The trend of decreasing GMT was assessed using a linear regression method. Data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, Illinois, United States). A p value $<$ 0.05 was considered statistically significant.

Results

A total of 936 students from 14 elementary schools were recruited in this study for serologic testing of pertussis, of whom 48.29% were boys. Among the 936 children, 318 were positive for anti-*B. pertussis* IgG antibody, with an overall seropositive rate of 33.97%. Seropositive rates were not significantly different between genders. Among schools, seropositive rates varied from 16.67% (Shi-Ding elementary school, Shi-Ding district) to 41.67% (Shuang-Xi elementary school, Shuang-Xi district), both schools in Stratum 5 (p = 0.18). The seropositive rate was below 20% for students in two schools (Jin-Mei elementary school, Jin-Shan district; Shi-Ding elementary school, Shi-Ding district). Stratum-wisely, seropositive rates ranged from 29.17% for the Stratum 3 to 35.59% for Stratum 1. There was no statistically significant difference in seropositive rates either among schools (p = 0.16) or among strata (p = 0.66). Details are shown in Table 1.

Among 936 participants, 34 with unclear vaccine status were thus excluded for further analyses of the association between vaccine doses and seroprevalence. Eight hundred and ninety-two (98.89%) participants received at least three doses of pertussis vaccine, and the seropositive rate ranged from 30.77% to 37.5%. In this population, 576 (64.57%) received a Tdap booster vaccine at the age of $\geq$5 years and had a higher seropositive rate than those who did not (36.63% vs. 31.65%, p = 0.07). Detailed data are shown in Table 2.

Grade-specific pertussis seroprevalence and GMTs are illustrated in Figure 1. The seropositive rate was highest at 49.36% for students in Grade 1, decreased to 39.1% for Grade 2 (p = 0.04), and thereafter decreased to a nadir of 25% for Grade 4 and Grade 5. The seropositivity for students

Table 1 Seroprevalence among 936 elementary school children in five strata.[a]

School* (Administrative district)	Participant No.	Positive cases	
		N (%)	GMT[b]
Stratum 1	576	205 (35.59)	10.55
Zhong-Shan (Ban-Qiao)	96	39 (40.63)	11.22
San-Zhong (San-Zhong)	96	39 (40.63)	12.22
Zhong-Gang (Xin-Zhuang)	96	37 (38.54)	11.11
Lu-Zhou (Lu-Zhou)	96	32 (33.33)	9.77
Ji-Mei (San-Zhong)	96	29 (30.21)	9.57
Ju-Guang (Ban-Qiao)	96	29 (30.21)	9.38
Stratum 2	120	41 (34.17)	9.28
Cheng-Fu (San-Xia)	60	18 (30)	8.95
Xin-Xing (Dan-Shui)	60	23 (38.33)	9.62
Stratum 3	72	21 (29.17)	9.06
Jin-Mei (Jin-Shan)	36	7 (19.44)	7.94
Jin-Long (Xi-Zhi)	36	14 (38.89)	10.17
Stratum 4	144	44 (30.56)	9.64
Gan-Yuan (Shu-Lin)	72	28 (38.89)	11.68
Wu-Gu (Wu-Gu)	72	16 (22.22)	7.6
Stratum 5	24	7 (29.17)	9.21
Shi-Ding (Shi-Ding)	12	2 (16.67)	7.67
Shuang-Xi (Shuang-Xi)	12	5 (41.67)	10.76
Total	936	318 (33.97)	

[a] The five strata were classified according to 14 variables (see text).
[b] GMT: geometric mean titer, in NovaTec unit/mL.
*There was no significant difference among schools (p = 0.16) and strata (p = 0.66).

Table 2 Association between vaccination doses and geometric mean titre[a] in 902[b] children.

	4 doses + 1 booster	4 doses	3 doses + 1 booster	3 doses	1 or 2 doses + 1 booster	1 or 2 doses
Participant No.	544[b]	303[b]	32[b]	13[b]	6	4
Positive						
N (%)	199 (36.58)	96 (31.68)	12 (37.5)	4 (30.77)	1 (16.67)	0
GMT[c]	19.09	19.75	14.41	23.25	12.8	N/A
Equivocal No.	25	11	0	0	0	0
Negative No.	320	196	20	9	5	4

[a] Among 936 participants, 34 with one or two doses of pertussis vaccine but booster status unclear were excluded.
[b] Eight hundred and ninety-two (98.89%) participants received more than three doses of pertussis vaccine, among them 576 with a Tdap booster had a higher seropositive rate (36.63% vs. 31.65%, $p = 0.16$); compared with their nonboostered group, both "4 doses + 1 booster" ($p = 0.17$) and "3 doses + 1 booster" ($p = 0.74$) possessed higher seropositive, insignificant statistic difference between the "4 doses + 1 booster" and "3 doses + 1 booster" groups ($p = 0.92$) was observed.
[c] GMT: geometric mean titer, in NovaTec unit/mL.
N/A = not available.

in Grade 6 was higher than that for Grade 5, but did not reach statistical significance (30.13% vs. 25%, $p = 0.18$). Individuals from Grade 1 to Grade 4 had a significant higher seropositive rate (37.18% vs. 27.56%, $p = 0.002$) than those from Grade 5 to Grade 6, but a lower anti-*B. pertussis* IgG GMT (18.71 NTU/mL vs. 20.04 NTU/mL, $p = 0.20$) for seropositive participants. Similar to seropositivity, anti-*B. pertussis* IgG GMTs of the students decreased significantly from 13.37 NTU/mL for students in Grade 1 to a nadir of 7.76 NTU/mL for Grade 4, and then increased to 9.7 NTU/mL for Grade 6. No statistically significant difference was observed between GMTs for students in Grade 5 and in Grade 6 ($p = 0.12$). Seropositive rates were positively correlated with anti-*B. pertussis* IgG GMTs ($p < 0.005$).

Discussion

To the best of our knowledge, this is the first cross-sectional study applying a multilevel randomized sampling method to establish pertussis seroepidemiology data in Taiwanese elementary school children. This study disclosed a seropositive rate of 33.97% for pertussis among elementary school children in New Taipei City in spring time, 2013. All of the seropositive rates, either stratified by strata, schools, or Grades, were <50%.

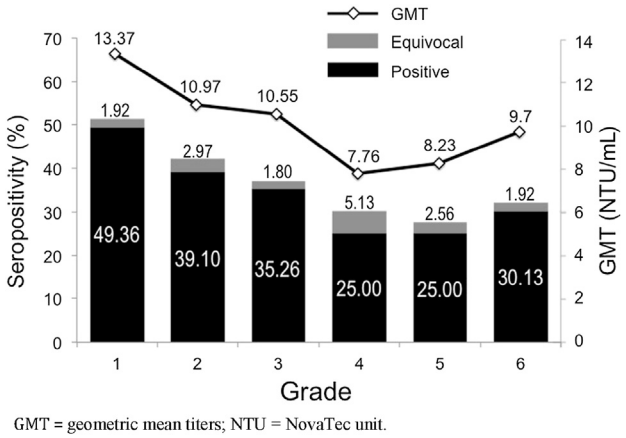

Figure 1. Grade-specific pertussis seropositive rates and change of geometric mean titers, in NovaTec unit/mL. The seropositive rate declined to a nadir at Grade 4 and Grade 5, but then rose at Grade 6. Both the seropositive rate ($p = 0.31$) and the geometric mean titers ($p = 0.03$) were higher at Grade 6 than at Grade 4. The seropositive rate was positively correlated with anti-*Bordetella pertussis* immunoglobulin-G titers ($p < 0.005$). GMT = geometric mean titers; NTU = NovaTec unit.

Because of mild symptoms or atypical manifestations, laboratory confirmation is usually crucial in the diagnosis of pertussis, which includes DNA detection with polymerase chain reaction or measuring *B. pertussis*-specific antibodies by enzyme-linked immunosorbent assay (ELISA).[8,9,10] ELISAs detecting antibodies to *Bordetella* antigens were developed during the acellular vaccine trials, and were employed in seroepidemiological studies.[11] Most of the ELISAs used purified antigens, of which only PT is specific for *B. pertussis*, although use of mixed antigens has been reported. Previous studies have focused mainly on anti-*B. pertussis* IgG antibodies, since the role of IgA antibodies was unclear in the vaccinated population.[8,12] Commercial kits measuring IgA have been reported to have sensitivities ranging between 0.53 and 0.73, with specificities ranging between 0.67 and 0.94, indicating probable limited diagnostic value of IgA measurement in clinical circumstances.[13] Kits using PT as an antigen showed linearity compared with the World Health Organization reference preparation, whereas ELISA kits using mixed antigens showed no linear correlation.[13] In this study, we applied the NovaLisa Bordetella Pertussis IgG/IgA ELISA kit, which used PT and FHA as antigens. NovaLisa ELISA kit was reported to have a sensitivity up to 95% detecting pertussis IgG. Despite that the fact that its specificity was only 54% as demonstrated by Riffelmann et al,[13] NovaLisa Pertussis IgG/IgA ELISA was considered capable of meeting our seroepidemiologic purpose and producing reliable results.

Lai et al[14] demonstrated that in a pediatric population highly immunized with diphtheria and tetanus toxoid and polio vaccines in Singapore, seroprevalence of pertussis waned rapidly over time compared with that of concurrently vaccinated diphtheria and poliovirus.[14] Their study also remarked that the prevalence of pertussis IgG antibody declined with time since the last pertussis vaccine, and remained only at ~50% in those who last received pertussis vaccine ≥4 years ago, which suggested the need of a booster dose at adolescent stage. The rapid waning of protection from vaccination was

not observed only in Singapore. A recent meta-analysis study in the United States suggested that assuming 85% efficacy, DTaP provided an average of only 3 years of protection, and the difference between regimens (3-dose vs. 5-dose schedule) did not significantly affect the annual odds of pertussis, which was actually higher in older age group.[15]

As stated above, Tdap was included in EPI as a booster for children >5 years since 2009 in Taiwan, hence all the students from Grade 1 to Grade 4 in our cross-sectional study, but not for those from Grade 5 to Grade 6, were supposed to have received a further booster vaccine against pertussis. According to the data from CDC-Taiwan, the vaccination rate for the third and fourth dose of DTaP-Hib-IPV reached 96% and 94%, respectively, and the vaccination rate for Tdap was >97% by 2011. Despite the massive administration of pertussis vaccine, the seropositive rate was still <50%, even in those receiving their Tdap booster vaccines within just 1 year prior to this study. Seroprevalence in students from Grade 1 to Grade 4 declined with time, the students in Grade 5 had the same seroprevalence as that for Grade 4, and students in Grade 6 even had a higher seropositive rate ($p = 0.31$) and higher GMTs ($p = 0.03$) than those in Grade 4 (Figure 1). The overall low seroprevalence may be associated with the transition of EPI from whole-cell vaccine to acellular vaccine. Gambhir et al[16] indicated that the priming acellular pertussis vaccine may have lower efficacy and shorter duration of protection than the whole-cell vaccine. Sheridan et al[17] also illustrated the different effectiveness of whole-cell and acellular vaccine. But the serial inter-grade decrease of GMT levels from Grade 1 to Grade 4 with an inverse increase from Grade 5 to Grade 6 could not be explained. The participants in this study might choose to receive the acellular vaccine as the primary series of vaccination at their own expense instead of the whole-cell vaccine included in the EPI, but we did not have enough information to conclude that the high-grade students had a higher rate of vaccination with whole-cell vaccine, and thus developed a higher GMT level. As protection from the vaccine waned over time, older children were prone to develop break-through pertussis infections. In addition to the lack of Tdap booster administration, circulating bacteria evolved away from vaccine antigens might also increase the chance of natural *B. pertussis* infection among high-grade students since their last vaccination against pertussis, resulting in higher antibodies.

Pertussis was thought to be a vaccine-preventable childhood disease.[18,19] After the initiation of massive vaccination in many countries, the disease prevalence decreased significantly but a shift in the age distribution of this disease has been reported in Japan,[20] United States,[21,22] Canada,[23] Australia,[24] China,[25] and Korea.[26] Lin et al[5] showed a significant upward trend in the pertussis incidence in adolescents aged 10–14 years in Taiwan between 1993 and 2004.[5] According to up-to-date surveillance data from CDC-Taiwan, infants aged <1 year ($n = 240$, 44.28%) still accounted for the highest proportion of all reported cases in the past decade (between 2005 and 2014), followed by adolescents aged 10–14 years ($n = 96$, 17.71%), and adults aged 35–39 years ($n = 31$, 5.72%).

The shift in age distribution may be contributed by multiple factors. Firstly, massive vaccination not only protected vaccinated population from pertussis, but also made the vaccinated less exposed to wild-type *B. pertussis* booster. Secondly, vaccine-induced immunity against pertussis was reported to wane 4–12 years after the last vaccination.[27] This study revealed that anti-*B. pertussis* antibodies in Taiwanese elementary school children may have decreased faster than we expected. Based on the model by Lai et al,[14] the seroprevalence threshold should achieve at least 90–95% to establish herd immunity against pertussis. With such universally low seroprevalence among the participants in this study, the current immunization program in Taiwan may be incapable of achieving adequate herd immunity in elementary school children, and outbreaks of pertussis among school children might take place under such a circumstance. Thirdly, the upward trend in adolescents and adults might be the tip of the iceberg,[28] because physicians in Taiwan are unfamiliar with pertussis and thus most pertussis cases were under-diagnosed or undiagnosed.[29] Atypical symptoms, lack of seeking medical evaluation, and misperception that pertussis remained a "childhood disease" all lead to under-diagnosis, and enabled the infected adolescents and adults to transmit pertussis to younger children with possible severe complications,[30] especially in those who had not completed their vaccinations.

For the waning antibodies from vaccination, immunization for adolescents against pertussis was recommended in the United States, Canada, and European regions.[29,31,32] Routine use of acellular pertussis vaccine was protective among adolescents and adults, and might reduce the overall disease burden and transmission to children.[33] As more effective and less reactogenic new vaccines are unlikely to be commercially available in the following decade, it has also been suggested that a universal vaccination in all age groups at frequent intervals is required to develop herd immunity,[34] although the optimal timing and frequency of booster doses are uncertain at the moment.[17] Seroepidemiology status for pertussis among Taiwanese adolescents and adults may be important to determine an endemic disease burden and to optimize an immunization program with the Tdap booster.

In 2013, only one-third of elementary school children in New Taipei City, Taiwan, were seropositive for pertussis, a rate lower than expected. Seroprevalence in students from Grade 1 to Grade 4 declined with time but was still significantly higher than that for Grade 5 and Grade 6. The current national immunization program may not provide adequate protection for children against pertussis, and a booster vaccine administered at regular intervals from adolescent stage may be beneficial to ensure herd effects and decrease the burden of illness.

Conflicts of interest

All authors declare that they have no conflicts of interest associated with the materials discussed in the article.

Acknowledgments

This study was supported in part by a grant from Centers for Disease Control, Taiwan (DOH101-DC-1016). We would like to thank Ms. Kun-Ju Tsai, a member of Public Health Department of New Taipei City Government, for her helpful support.

References

1. Hutchins SS, Cochi SL, Brink EW, Patriarca PA, Wassilak SG, Rovira EZ, et al. Current epidemiology of pertussis in the United States. *Tokai J Exp Clin Med* 1988;**13**(Suppl):103—9.
2. Crowcroft NS, Pebody RG. Recent developments in pertussis. *The Lancet* 2006;**367**:1926—36.
3. Rohani P, Earn DJ, Grenfell BT. Impact of immunisation on pertussis transmission in England and Wales. *Lancet* 2000;**355**: 285—6.
4. Rohani P, Earn DJ, Grenfell BT. Opposite patterns of synchrony in sympatric disease metapopulations. *Science* 1999;**286**:968—71.
5. Lin YC, Yao SM, Yan JJ, Chen YY, Chiang CS, Wu HS, et al. Epidemiological shift in the prevalence of pertussis in Taiwan: implications for pertussis vaccination. *J Med Microbiol* 2007; **56**:533—7.
6. Zhang L, Prietsch SO, Axelsson I, Halperin SA. Acellular vaccines for preventing whooping cough in children. *Cochrane Database Syst Rev* 2014;**9**. CD001478.
7. Misegades LK, Winter K, Harriman K, Talarico J, Messonnier NE, Clark TA, et al. Association of childhood pertussis with receipt of 5 doses of pertussis vaccine by time since last vaccine dose, California, 2010. *JAMA* 2012;**308**:2126—32.
8. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. *Clin Microbiol Rev* 2005;**18**:326—82.
9. Reder S, Riffelmann M, Becker C, Wirsing Von Konig CH. Measuring immunoglobulin g antibodies to tetanus toxin, diphtheria toxin, and pertussis toxin with single-antigen enzyme-linked immunosorbent assays and a bead-based multiplex assay. *Clin Vaccine Immunol* 2008;**15**:744—9.
10. Van Gageldonk PG, Van Schaijk FG, Van Der Klis FR, Berbers GA. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. *J Immunol Methods* 2008;**335**:79—89.
11. Hallander HO, Storsaeter J, Mollby R. Evaluation of serology and nasopharyngeal cultures for diagnosis of pertussis in a vaccine efficacy trial. *J Infect Dis* 1991;**163**:1046—54.
12. Knuf M, Zepp F, Meyer C, Grzegowski E, Wolter J, Riffelmann M, et al. Immunogenicity of a single dose of reduced-antigen acellular pertussis vaccine in a non-vaccinated adolescent population. *Vaccine* 2006;**24**:2043—8.
13. 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.
14. Lai FY, Thoon KC, Ang LW, Tey SH, Heng D, Cutter JL, et al. Comparative seroepidemiology of pertussis, diphtheria and poliovirus antibodies in Singapore: waning pertussis immunity in a highly immunized population and the need for adolescent booster doses. *Vaccine* 2012;**30**:3566—71.
15. Mcgirr A, Fisman DN. Duration of pertussis immunity after DTaP immunization: a meta-analysis. *Pediatrics* 2015;**135**:331—43.
16. Gambhir M, Clark TA, Cauchemez S, Tartof SY, Swerdlow DL, Ferguson NM, et al. A change in vaccine efficacy and duration of protection explains recent rises in pertussis incidence in the United States. *PLoS Comput Biol* 2015;**11**:e1004138.
17. Sheridan SL, Frith K, Snelling TL, Grimwood K, Mcintyre PB, Lambert SB. Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. *Expert Rev Vaccines* 2014;**13**(9):1081—106.
18. Deeks S, De Serres G, Boulianne N, Duval B, Rochette L, Dery P, et al. Failure of physicians to consider the diagnosis of pertussis in children. *Clin Infect Dis* 1999;**28**:840—6.
19. Orenstein WA. Pertussis in adults: epidemiology, signs, symptoms, and implications for vaccination. *Clin Infect Dis* 1999; **28**(Suppl 2):S147—50.
20. Aoyama T, Harashima M, Nishimura K, Saito Y. Outbreak of pertussis in highly immunized adolescents and its secondary spread to their families. *Acta Paediatr Jpn* 1995;**37**:321—4.
21. Brooks DA, Clover R. Pertussis infection in the United States: role for vaccination of adolescents and adults. *J Am Board Fam Med* 2006;**19**:603—11.
22. Winter K, Harriman K, Zipprich J, Schechter R, Talarico J, Watt J, et al. California pertussis epidemic, 2010. *J Pediatr* 2012;**161**:1091—6.
23. Skowronski DM, De Serres G, Macdonald D, Wu W, Shaw C, Macnabb J, et al. The changing age and seasonal profile of pertussis in Canada. *J Infect Dis* 2002;**185**:1448—53.
24. Quinn HE, Mcintyre PB. Pertussis epidemiology in Australia over the decade 1995-2005—trends by region and age group. *Commun Dis Intell Q Rep* 2007;**31**:205—15.
25. Zhang Q, Zheng H, Liu M, Han K, Shu J, Wu C, et al. The seroepidemiology of immunoglobulin G antibodies against pertussis toxin in China: a cross sectional study. *BMC Infect Dis* 2012;**12**:138.
26. Choe YJ, Park YJ, Jung C, Bae GR, Lee DH. National pertussis surveillance in South Korea 1955-2011: epidemiological and clinical trends. *Int J Infect Dis* 2012;**16**:e850—4.
27. Wendelboe AM, 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.
28. Rothstein E, Edwards K. Health burden of pertussis in adolescents and adults. *Pediatr Infect Dis J* 2005;**24**:S44—7.
29. Tan T, Halperin S, Cherry JD, Edwards K, Englund JA, Glezen P, et al. Pertussis immunization in the global pertussis initiative North American region: recommended strategies and implementation considerations. *Pediatr Infect Dis J* 2005;**24**:S83—6.
30. Wendelboe AM, Njamkepo E, Bourillon A, Floret DD, Gaudelus J, Gerber M, et al. Transmission of Bordetella pertussis to young infants. *Pediatr Infect Dis J* 2007;**26**:293—9.
31. Centers for Disease Control and Prevention (CDC). Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:13—5.
32. Wirsing Von Konig CH, Campins-Marti M, Finn A, Guiso N, Mertsola J, Liese J. Pertussis immunization in the global pertussis initiative European region: recommended strategies and implementation considerations. *Pediatr Infect Dis J* 2005; **24**:S87—92.
33. Ward JI, Cherry JD, Chang SJ, Partridge S, Lee H, Treanor J, et al. Efficacy of an acellular pertussis vaccine among adolescents and adults. *N Engl J Med* 2005;**353**:1555—63.
34. Cherry JD. The present and future control of pertussis. *Clin Infect Dis* 2010;**51**:663—7.