Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A cross sectional survey measuring sero-incidence of pertussis infection among Japanese junior and senior high school students in 2013 and 2014

Hideyuki Ikematsu [a,*], Naoki Kawai [a,b], Shigehiro Yajima [b]

[a] Japan Physicians Association, Japan
[b] Gifu Prefecture Medical Association, Japan

ARTICLE INFO

Article history:
Received 16 January 2017
Received in revised form 3 April 2017
Accepted 26 May 2017
Available online xxxx

Keywords:
Pertussis
Toxin
Antibody
Sero-epidemiology
Adolescent

ABSTRACT

Pertussis in adolescents has been increasingly documented in recent years, but diagnosis from the clinical symptoms is difficult. Serological diagnosis with IgG antibody to pertussis toxin (IgG PT) is useful for detecting pertussis cases in this population. However, no serological criterion for recent infection has been fully validated and large-scale, longitudinal serological data among Japanese junior and senior high school students are lacking.

Paired serum samples of 3243 junior and senior high school students, collected in 2013 and 2014, were analyzed for IgG PT and its relationship to possible risk factors.

Regression analysis showed an average decrease of 35% in IgG PT between 2013 and 2014. In 2013, 4.4% of the students showed IgG PT levels ≥100 EU/mL, as did 3.7% in 2014. The seroincidence, defined as [IgG PT] change from <100 in 2013 to ≥100 EU/mL in 2014, was 10.3 cases per 1000 person-years. A 4-fold rise in IgG PT was seen in 2.1% of the students, with significant differences between schools and significant correlations to two risk factors, "over 2 weeks coughing" and "exposure to a person with over 2 weeks coughing". A substantial number of students had IgG PT ≥100 EU/mL despite the observed 35% yearly decrease in IgG PT level. The local foci of ≥4-fold IgG PT increase in specific schools suggests the persistent circulation of *B. pertussis* in Japanese adolescents. The results also support a "≥4-fold rise in IgG PT" as a useful component of the sero-epidemiological surveillance for pertussis.

1. Introduction

The endemic and cyclic nature of pertussis epidemiology suggests that large portions of the population, of all ages, are regularly exposed to recurrent Bordetella pertussis (*B. pertussis*). As a result, certain levels of antibodies to the antigens, including pertussis toxin (PT), are maintained in the population. Pertussis sufferers often experience insignificant titer increase, creating a problem defining recent contact with *B. pertussis* [1,2]. Recent studies have suggested that acellular pertussis vaccines may not protect as well as whole-cell vaccines or previous infection against infection and transmission [3]. The impact of asymptomatic and undiagnosed pertussis infection on the dynamics of *B. pertussis* infection and disease are important.

In the context of recent sizeable pertussis outbreaks in several countries, numerous studies have been published on the effectiveness of pertussis vaccines and their duration, showing that the protective immunity elicited by vaccination wanes over time [4–6]. However, the complexity of the factors involved leaves the precise speed and extent of this waning still under debate [7]. On the other hand, a recent multicenter, test-negative case-control study conducted in Japan indicates that acellular pertussis vaccine in a routine immunization program administering four doses from age 0 to 2 years has a long-term preventive effect against pertussis, probably for more than 9 years [8].

To assess the impact of a vaccination program against pertussis infection, reliable surveillance tools are necessary. However, it is well recognized that there are limitations to the sensitivity and specificity of the methods currently used to diagnose pertussis infection. A serological test that measures the concentration of immunoglobulins against *B. pertussis*-specific Pertussis Toxin (PT), IgG PT, is considered useful for diagnosis through provision of a sensitive, specific indicator of recent infection with *B. pertussis* [9–11]. However, several limitations exist for serological diagnosis by the antibody level, which must include diagnosis based on both natural infection and the immunization status. Furthermore, the antibody titer does not necessarily correlate with clinical symptoms, as sub-clinical infections commonly occur with aging.

* Corresponding author at: 2-18-30 Hakataekihigashi, Hakata-ku, Fukuoka 812-0013, Japan.
E-mail address: ikematsu@gray.plala.or.jp (H. Ikematsu).

http://dx.doi.org/10.1016/j.vaccine.2017.05.082
0264-410X/

Numerous different thresholds have been used in various studies around the world [12]. The variety of ELISA and other assays used, along with differing thresholds in different populations with varied immunization contexts and history make comparisons across studies treacherous and questionable. Nonetheless, the one thing that is commonly demonstrated in all seropeidemiology studies of pertussis is the endemicity of pertussis infection, which affects susceptible individuals before they are vaccinated or several years after their last dose of vaccine, when the vaccine-induced immunity has waned.

Very little large-scale, sero-epidemiological data concerning pertussis in Japan are available. Our study targeted a large cohort of junior and senior high school students in Gifu Prefecture, Japan. Blood sampling in consecutive years was intended to yield reliable data for exploring the incidence of pertussis infection and to give insight into the reliability of single time point IgG PT quantification. Questionnaires were used to investigate a range of risk factors possibly associated with pertussis infection. The distribution of IgG PT levels and the changes over an observation period of one year were analyzed, and the relation to risk factors was also investigated. The threshold of 100 EU/mL was selected for use in the present study as this is the official diagnostic threshold recommended in Japan. It is consistent with positivity values used in other sero-epidemiology studies of pertussis [10].

2. Subjects and methods

2.1. Subjects

Students of eight junior high (JHSs) and two senior high schools (HSs) in Gifu Prefecture were recruited. These schools are located in an urban area in adjacent cities, Gifu and Kagamihara. All JHS1 students were 12 or 13 years old: They begin the school year (April in Japan) at 12 years-of-age and become 13 on their birthday in the study year. Subsequent grades follow the same pattern, plus one year. Japanese children receive three doses by one year-of-age and one additional dose at two years. Over ten years had passed since the last vaccination for the students studied. The study was approved by the Ethics Committee of the Gifu Prefecture Medical Association. Paired serum samples were collected at the same time of year in the 2013 and 2014 school years. Two questionnaires were distributed, one for vaccination status and past history of pertussis infection and the other for exposure to possible risk factors for pertussis infection. The school grade at study entry was used for age classification of the participants.

2.2. Measurement of IgG antibody to pertussis toxin (IgG PT)

IgG PT was measured using a commercially available enzyme immunoassay kit (Denka Seiken, Japan) [13] by a commercial laboratory test center (BML, Japan). In brief, a flat-bottom microplate is coated with FHA or PT. anti-FHA IgG antibodies or anti-PT IgG antibodies and peroxidase-labeled goat anti-human IgG polyclonal antibodies are allowed to react. anti-FHA IgG antibodies or anti-PT IgG antibodies in samples are detected by measuring the enzyme activity. The lowest detection limit of the tested sera is 10 EU/mL. The antibody level is quantified by comparison to a reference sample, JNIH-10 (IgG PT: 250 EU/vial). The EU value with JNIH-10 displays 70–80% of the IU value by the international standard of 06/140.

2.3. Questionnaires

The first questionnaire, distributed in 2013, included past history of vaccination against pertussis infection. The second, concerning possible risk factors, was conducted in 2014 and queried the presence of clinical signs consistent with pertussis disease, socio-demographic factors, co-morbidity, and healthcare and medication history, along with possible risk factors for exposure to *B. pertussis*, such as persistent cough among close contacts.

2.4. Statistical analysis

Three criterion were used for estimation of the seroincidence of infection by *B. pertussis*; IgG PT of 100 EU/mL, change of IgG PT <100 EU/mL in 2013 to ≥100 EU/mL in 2014, and four fold-increase of IgG PT from 2013 to 2014.

Differences in the geometric mean (GM) of IgG PT among the schools and grades were tested by one-way ANOVA. IgG PT in 2013 and 2014 was compared by paired *t*-test. Tendency of IgG PT change among school grades was tested by Cochran-Armitage test. The incidence of *B. pertussis* infection, using the criteria for IgG PT change, was compared by Fisher's *t*-test. The association between seroincidence or IgG PT change and the various measured co-factors, such as clinical signs of pertussis and risk factors for exposure, was tested using Fisher's exact test. All analyses were performed using SAS® version 9.3 (SAS Institute, Inc., Cary, NC, USA) software. Statistical significance was determined at $p < 0.05$.

3. Results

The data on serum IgG PT in 2013 and 2014 and the information from the completed questionnaires of 3243 students were analyzed. Immunization status was verified from the questionnaire and showed that 3125 (96.4%) students had been vaccinated. The completion of four doses by age two was confirmed for 2915 (89.9%) students. "Unvaccinated" was reported by 89 (2.7%) students and "unknown" was reported by 19 (0.6%).

The distributions of the log-transformed IgG PT for 2013 and 2014 are depicted in Figs. 1 and 2. The distribution pattern was quite similar for both years. The mode of the tested samples was IgG PT 10 EU/mL, which is due to the log-transformation. The distribution was not even. A small group was found with IgG PT ≥100 EU/mL in both 2013 and 2014.

The relation between the IgG PT levels of 2013 and 2014 are depicted as a scatter plot of the log-transformed IgG PT levels (Supplementary Fig. 1). The regression equation for IgG PT in 2014 compared to 2013 was (IgG PT in 2014) = 9.7 + 0.65 × (IgG PT in 2013), with a correlation coefficient of 0.7115.

The IgG PT GMs and medians for 2013 and 2014 by sex and school grade are listed in Table 1. The IgG PT was similar for the male and female students in both years. The IgG PT GMs in 2013 and 2014 ranged from 13.28 to 17.74 EU/mL for the JHS1, JHS2, and JHS3 students and from 19.02 to 20.7 EU/mL for the HS1 and HS2 students. The IgG PT GM was significantly higher in HS1 students than in JHS1, JHS2, and JHS3 students in 2013 ($p = 0.0084$, 0.0008, and 0.0009, respectively, by ANOVA) and was significantly higher in HS1 and HS2 students than in JHS1 and JHS2 students ($p = 0.0009$, 0.0029, 0.0008, and 0.0027, respectively, by ANOVA).

The estimated number of *B. pertussis* recent infection according to the putative criterion is listed in Table 2. The number of students with IgG PT ≥100 EU/mL was 143 (4.4%) in 2013 and 121 (3.7%) in 2014. Among the 143 students who had IgG PT ≥100 EU/mL in 2013, 89 (62.2%) continued to have IgG PT ≥100 EU/mL in 2014. The number of male and female students with IgG PT ≥100 EU/mL was almost equal. The percentage of students with IgG PT ≥100 EU/mL in 2014 by school grade ranged from 2.7% to 6.0%, increasing significantly from JHS1 to JHS3 ($p = 0.0323$). There was no significant difference between HS1 and HS2 ($p = 0.6364$).

Please cite this article in press as: Ikematsu H et al. A cross sectional survey measuring sero-incidence of pertussis infection among Japanese junior and senior high school students in 2013 and 2014. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.05.082