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Seroprevalence of IgG antibodies to pertussis toxin in children and adolescents in Estonia

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ABSTRACT

Background and aims: Despite high immunisation coverage and frequent booster doses, the national notification rates of pertussis in Estonia have been increasing. The peak of 97/100,000 was reached in 2010 which is the highest incidence rate since 1962 (210/100,000).

We aimed to measure the prevalence of pertussis toxin (PT) IgG type antibodies in subjects of <18 years and to estimate the pertussis infection activity in a recently non-immunised cohort.

Methods: In a cross-sectional serosurvey, all consecutive leftover sera were collected in the Tartu University Hospital during April–August 2012. Anti-PT IgG concentration was measured by commercial ELISA and analysed in yearly cohorts. The antibody concentrations ≥62.5 IU/mL was considered suggestive to pertussis in the last year among 9- to 14-year-olds.

Results: The GMC of the anti-PT-IgG was 7.4 IU/mL (95% CI 6.9–8.0). In the total of 1053 serum samples, the highest proportion of sera with high antibody titres ≥125 IU/mL and ≥62.5 IU/mL were at the ages when pertussis vaccine boosters were given: 7 years 10.9% (95% CI 4.1–22.3) and 2 years 36.9% (95% CI 25.3–49.8), respectively. Approximately half of all sera had undetectable anti-PT IgG levels. The estimated incidence of *Bordetella pertussis* infection among 9- to 14-year-olds in the year before serum sampling was 6.3% (95% CI 3.3–10.8), which is at least 60 times higher than the officially reported incidence of pertussis disease in respective years.

Conclusions: The serologic method is not suitable for diagnosing pertussis in instances when the last pertussis immunisation was less than one year ago. The relatively high proportion of subjects with undetectable anti-PT IgG levels and the relatively low rate of officially reported pertussis cases suggest that low antibody levels do not necessarily indicate the absence of protection. The estimated incidence rate of pertussis is much higher than officially reported figures, which suggests that asymptomatic/mild *B. pertussis* infection remains unrecognised and unreported.

1. Introduction

Pertussis is the most frequent vaccine preventable disease in childhood, despite the high immunisation coverage in medium to high income countries [1]. Today, pertussis with classical symptoms is far less common than mild disease or pertussis infection that includes in addition asymptomatic carriage of *Bordetella pertussis*; asymptomatic carriers are likely the source of transmission [2].

In Estonia, pertussis is a notifiable disease [3]. Until 2012 the vast majority of notifications were based on positive serology using qualitative ELISA tests, whereas culture and PCR were rarely used. In 2012, most laboratories in Estonia switched to more specific quantitative ELISA kits that measure only pertussis toxin (PT) antibodies, as is recommended by the EU Pertstrain group [4].

According to the data of the Health Board of Estonia, incidence of pertussis has risen during the last decade, similar to many other

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countries that have switched from whole cell vaccines to acellular vaccines. However, the notification that solely relies on the reporting by general practitioners and hospitals is far from ideal and rather reflects incidence of pertussis disease but underestimates *B. pertussis* infection [5,6]. On the other hand, pertussis in Estonia may also be over-reported due to relying on qualitative serological tests that are known to have high sensitivity but low specificity, particularly in recently and frequently immunised populations [7]. The findings that 22.8% of pertussis cases were reported under the age of five (data in file of the Health Board of Estonia), in spite of four doses of vaccine and high immunisation coverage (about 95%) is an indirect proof of over-reporting. The incidence of pertussis in infants who are too young to be vaccinated can be considered as an indicator of pertussis activity [8], in Estonia it was 27 cases per 100,000 in 2012 (data in file of the Health Board of Estonia).

Population-based seroepidemiological studies are used to estimate the incidence rate of pertussis infection as they capture both pertussis disease and infection and enable between-country comparisons [9–15]. A quantitative measurement of PT IgG type antibodies which are specific to PT and do not cross-react with other antigens is most commonly used [16]. The anti-PT cut-off values vary, but the following levels are often employed: ≥125 IU/mL indicating recent or active pertussis infection and ≥62.5 IU/mL suggesting pertussis infection in the previous year [10,11,17,18]. Various anti-PT IgG levels, ranging from <2 IU/mL [19] to <30 IU/mL [13], have been used to described negative sera in different studies, but most authors define sera with <5.0 IU/mL as negative [10–12,14,17].

In this cross-sectional serosurvey, we first aimed to measure the prevalence of PT IgG type antibodies in the Estonian population of people aged <18 years. Secondly we aimed to estimate the prevalence of the *B. pertussis* infection in 2012 in a subset of not recently immunised children.

2. Methods

2.1. Study population and design

The samples were collected in the Children's Clinic of Tartu University Hospital which is one of the two paediatric hospitals in Estonia and has a catchment area of 87,000 subjects <18 years. According to the hospital and outpatient databases, there were no clinical or laboratory confirmed pertussis cases diagnosed during the time of sampling from April to August 2012. All consecutive left-over sera (200 μl to 2 mL) of hospitalised and ambulatory patients <18 years with any diagnosis were stored until predefined sample size requirements were fulfilled for each yearly cohort. Samples were stored first at +2 to +8 °C for a maximum period of one week and then at −80 °C until analysed. No information other than the age was collected.

Data on the annual notified incidence of pertussis and immunisation coverage were derived from the reports of the Health Board of Estonia [http://www.terviseamet.ee/en/cdc/estonian-communicable-disease-bulletins-2005-2013.html, http://www.terviseamet.ee/nakkushaigused/vaktsineerimine/riiklik-immuniseerimiskava-ja-selle-taitmine.html].

2.2. Measurement of IgG antibodies

The concentration of anti-PT IgG was measured by a commercial ELISA kit (Euroimmun, Lübeck, Germany) in accordance to the manufacturers' protocol in Quattromed HTI Laborid OÜ. The sensitivity and specificity of the assay is reported of 100% and 95.5%, respectively. The anti-*B. pertussis* toxin IgG ELISA calibration curve was linear in the ranges between 5 and 174 IU/mL. To calculate GMC for antibody levels below and above the cut-off value of the assay the value of 2.5 IU/mL and 175 IU/mL, respectively, were given arbitrarily.

2.3. Statistics

The required sample size was calculated based on the estimated level of seroprevalence of 6–9% of pertussis in the Netherlands [9,20]. Accordingly, 55 ± 6 of subjects were required in each yearly cohort.

The anti-PT IgG antibodies were presented as geometric mean concentrations (GMCs) with 95% confidence intervals (CIs). The antibody levels were further divided into four categories as follows: ≥125 IU/mL – very high, suggesting pertussis infection/immunisation in last 6 months; 62.5 to <125 IU/mL – high, suggesting pertussis infection/immunisation in last 7–12 months; 5 to <62.5 IU/mL – mid-range, exposure to pertussis infection/immunisation >12 months previously; <5 IU/mL – undetectable [10,11,17,18].

If original data were in different units, it was converted as suggested by Cagney et al., Giammanco et al., Guiso et al., Pebody et al., and Riffelmann et al. [4,7,11,18,21].

Pertussis incidence was calculated only for a recently non-immunised cohort aged 9–14 years. The cut-off level of 62.5 IU/mL was chosen [11] and the average time for the high anti-PT IgG after infection to decrease to 62.5 IU/mL was expected to be 208.9 days (95% CI 195.4–223.3) [9]. The estimated yearly incidence was calculated as described by de Melker et al. [9] – 365.25/208.9 × the percentage of the population sera contain anti-PT IgG of at least 62.5 IU/mL.

Analyses were performed using Microsoft Excel 2013 and Stata 12.1 (last accessed 10.05.2014).

2.4. Ethics

The study was approved by the Ethics Committee of Tartu University. Informed consent was not required as all sera was collected anonymously.

3. Results

3.1. Immunisation against pertussis in Estonia

Since 2012, a total of six doses of acellular pertussis vaccine are given as part of a government financed immunisation programme at 3 month, 4.5 month and 6 month for primary-vaccination (Infanrix-IPV + Hib® or Pentaxim®) and at 2 years (Infanrix-IPV + Hib® or Pentaxim®), 6–7 years (Infanrix Polio® or Tetraxim®) and 15–17 years (Boostrix® or Adacel®) as boosters [22]. Both Infanrix®, a three component vaccine, and Pentaxim®, a two component vaccine contain 25 μg pertussis toxoid (Ptx) per dose. Before the year 2008 different DTPw (DTP Pasteur Merieux®, Tetract-Hib®, Pentact-Hib®, Tetracoq®) were used (personal communication with the Head of Department of Marketing Authorisations of State Agency of Medicines).

The immunisation times and received doses are presented in Fig. 1.

Immunisation rates have consistently been around 95% in most age cohorts except 6- to 7-year olds (88% in 2012), because the immunisation in this age group started in 2008.

3.2. Concentrations of anti-PT IgG

A total of 1053 blood sera samples, equally distributed across each yearly cohort (mean number of samples 58.5 (SD 4.2)), were analysed.