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The incidence of *Bordetella pertussis* infections estimated in the population from a combination of serological surveys

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Summary

Objectives

Bordetella pertussis circulates even in highly vaccinated populations. There is a considerable amount of infection in adults. For designing more effective vaccination schedules it is important to quantify the age-dependent relation between the number of notified cases and the number of infections.

Methods

We used a statistical relationship between the time since infection and the IgG antibody titers against pertussis toxin, derived from a longitudinal data set, to estimate time since infection for all individuals in a cross-sectional population-based study (1995–1996) based on their titers. Age-specific incidence of infection with *B. pertussis* was calculated and compared with the age-distribution of notified cases of pertussis in 1994–1996.

Results

Estimated incidence of infection was 6.6% per year for 3–79-year olds, annual incidence of notified cases 0.01%. Estimated age-specific incidence of infection was lowest for 3–4-year olds (3.3%) and increased gradually up to the age of 20–24 years (10.8%). The number of notified cases was highest for 3–9-year olds.

Conclusions

In the Dutch population *B. pertussis* infections occur more frequently and in elder age-categories than suggested by notifications. Mathematical modeling could explore what booster vaccination strategies are most effective in reducing severe disease among not (completely) vaccinated infants.

Introduction

Despite widespread vaccination, infection with *Bordetella pertussis* remains a cause of considerable morbidity even in countries with high vaccination coverage.^{1, 2, 3, 4 and 5} The continuing circulation of the pathogen is attributed to waning of vaccine-induced immunity, which leads to the occurrence of (often undiagnosed) pertussis infection among previously vaccinated children, adolescents and adults.^{6, 7, 8, 9, 10, 11, 12 and 13} Like others, we have shown that the definition of reliable criteria for positivity associated with high levels of IgG to pertussis toxin (IgG-PT) in a single serum sample (one-point serology) can greatly enhance the detection of pertussis infections.^{4, 7 and 10}

Inclusion of positive one-point serology as laboratory confirmation for notification in the Netherlands has increased the notification rate considerably, especially among older children and adults.¹ However, the use of surveillance data of notified infections for understanding the epidemiology of pertussis remains limited by the lack of registration or notification discipline as well as age-dependent diagnosis and reporting. Circulation of *B. pertussis* in vaccinated children, adolescents and adults plays an important role in the continuing transmission of the pathogen to infants too young to be vaccinated, in whom disease is most severe and possibly fatal.^{8, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20}

To design better preventive measures, for example by determining the optimal ages for booster vaccinations, insight is needed into the age-specific incidence of all infections with *B. pertussis* as opposed to only those symptomatic infections that are diagnosed and reported. Information about the seroprevalence of IgG-PT antibodies in the general population in combination with knowledge about the rate of decline of IgG-PT antibody levels after infection with *B. pertussis* offers the opportunity to study the incidence of infection in various age groups in the population irrespective of clinical course, diagnosis and reporting frequency. Pertussis toxin is expressed only by *B. pertussis* and cross-reacting antigens have not been described.^{21 and 22}

Furthermore IgG-PT responses occur in most patients with *B. pertussis* infection and high levels persist only temporarily.¹⁰ We estimated the incidence of *B. pertussis* infections in the population using a novel two-stage approach. A statistical description of the decline in antibody levels after infection as derived from a small scale longitudinal study²³ was combined with data of the age-specific distribution of IgG-PT in sera derived from a large scale cross-sectional study of the general population¹⁰ to estimate the age-specific incidences of infection for the age range 3–79 years. These were compared with notification data of reported clinical cases of pertussis. Implications for vaccination-strategy are discussed.

Material and methods

Collection of sera from population and patients

Detailed descriptions of the collection of sera from the general population in the Netherlands ($n = 7756$) and of follow-up sera from patients with diagnosed clinical pertussis ($n = 85$) have been published elsewhere.^{10, 23 and 24} In short, for the cross-sectional study in the general population, 40 municipalities were sampled with probabilities proportional to their population size. An age-stratified sample (classes 0, 1–4, 5–9, ... 75–79 years) of 380 individuals was randomly selected from each municipality. Subjects were asked to give a blood sample. Samples were collected in the period from October 1995 to December 1996 and

stored at -70°C until use. The participation rate was 55%. Sufficient serum for pertussis serology was available for 7756 of 8359 participants.

In a longitudinal study,²³ for 86 episodes 313 follow-up samples from 85 patients clinically diagnosed with pertussis (paroxysmal cough lasting more than 2 weeks) were obtained from one pediatric practice in the period 1989–2000. One patient had a second episode of (clinically confirmed) pertussis during the follow-up period: both episodes have been included in this analysis.²⁵ The follow-up period ranged from 6 months to 11 years and the number of serial sera per patient ranged from 2 to 11. Eleven episodes were from patients less than 6 months of age at the time they contracted pertussis, 69 episodes were from patients between 6 months and 17 years of age, and six patients were between 30 and 41 years old.

Notification data

Pertussis notification data for the period 1994–1996 were obtained from the Dutch Inspectorate of Health. The case definition included clinical symptoms and laboratory confirmation (or close contact with a person with laboratory-confirmed pertussis). The clinical symptoms in the case definition are a serious cough, lasting more than 2 weeks, coughing attacks, or coughing followed by vomiting in combination with at least one of the following symptoms/findings: apnea, cyanosis, characteristic cough with whooping, subconjunctival bleeding, or leucocytosis.

Laboratory confirmation was defined as either a positive culture of *B. pertussis* or *B. parapertussis*, or positive two-point serology. Two-point serology was considered positive if a 4-fold rise of IgG-antibodies against pertussis toxin in paired sera was found to be a concentration of at least 20 U/ml. Only since April 1997, positivity of one-point serology has been formally included in the case definition as being acceptable as laboratory confirmation of pertussis for notification (criterion: IgG-PT concentration in single serum > 100 U/ml). However, already in the years before 1997, a small fraction of the patients with positive one-point serology was reported.¹

Antibody assay

In the longitudinal study, patient sera had been submitted immediately after sampling and were assayed in the routine setting of the serology laboratory of our institute within 4 days after receipt. In the cross-sectional study, sera that had been collected in 1995/1996 were assayed in 1997/1998 in the same routine setting at a rate of approximately 200 sera per week. The IgG-PT was measured by ELISA as previously described.²⁶ The IgG-PT assay has an upper limit (500 U/ml) above which the values are not further differentiated. The lower detection limit of the assay is 5 U/ml. Results are expressed in "local U/ml".

Analysis

In Teunis et al.,²³ a skewed hyperbolic function was used to describe the relationship between the log time since infection with *B. pertussis* and the log IgG-PT antibody titer. This four-parameter function was fitted to the data for each individual patient from the longitudinal study of diagnosed clinical pertussis patients yielding a set of response curves with variation among individuals (Fig. 1). On a linear time scale, the antibody response rises very fast (within a few days) and then declines very slowly over a period of several years. Therefore, the rising part of the response curve may safely be neglected and an inverse function

can be determined on the basis of the long monotone declining part of the response. For every value of the log titer an average time since infection can be calculated (Fig. 1).

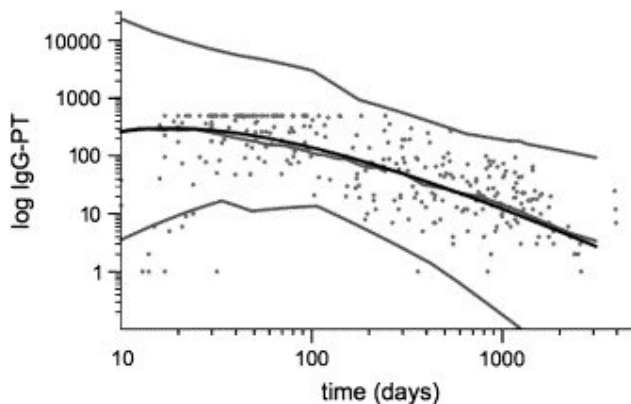


Figure 1. Measured IgG-PT concentration (titer) as a function of the time since last infection (for the study population in Teunis et al.²³). The black line shows the point-wise average of individual estimates, the gray lines show the point wise 2.5%, 50% (median) and 95% percentiles illustrating the magnitude of individual variation in responses. The dots are individual results.

We used those point-wise averages of the inverse response curves to estimate the times since last infection for the cross-sectional study population. Fig. 2 shows the IgG-PT antibody distribution in the participants from the cross-sectional population-based study (adapted from¹⁰).

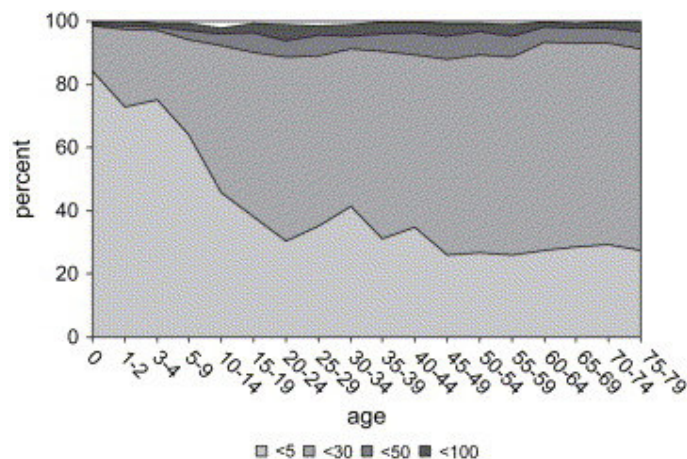


Figure 2. The age-specific IgG antibody levels in the population-based cross-sectional study in the Netherlands. Note that the categories are cumulative, i.e. individuals in the lower categories also belong to all higher categories.

A series of cut-off values was chosen (10, 20, 30, 40, 50, 60, 80, 100, and 150 U/ml) for which we calculated the (age-specific) incidence of infection with *B. pertussis* in the population. The estimated numbers of infected individuals were corrected for the discrepancy between the age-distribution of the cross-sectional study population and the age-distribution of the Dutch population in 1996 by weighting each 1-year age-class with an appropriate correction factor. The estimates of incidence of infection were limited to 3–79-year olds. We did not

estimate the incidence of infection in younger age-classes because IgG-PT in infants can be maternally derived and can be induced by vaccination (at the time of the study given at 3, 4, 5 and 11 months of age). Above 3 years of age, those factors do not influence the IgG-PT, because maternally derived antibodies disappear within 1 year, and IgG-PT induced by vaccination declines rapidly to very low or undetectable levels within 1 year after the last immunization.^{26 and 27}

The average numbers of notified cases per 100,000 by age group in the period 1994–1996 were calculated using the age-distribution of the Dutch population in 1994–1996. The period of 1994–1996 was chosen for reasons of comparability with the period for which the number of infections was estimated. For each age-class, the case-to-infection ratio could then be estimated by dividing the average numbers (per 100,000) of notified cases by the age-specific estimates of incidence of infection.

To assess the influence of individual variation in responses on the uncertainty of the incidence estimates we chose to compute a confidence interval for every cut-off value based on the following assumptions: (a) the longitudinal study population and the cross-sectional study population are the same in their responses to pertussis infection; (b) the variability in times since infection for different cut-off values is the same for the longitudinal and the cross-sectional study population; and (c) the log times since infection are normally distributed with mean and standard deviation calculated from the log times since infection from the longitudinal study.

Results

We describe the results for some of the cut-off values, namely the values 40, 50, 80, and 100 U/ml, because those values are considered to be the most conclusive for identifying recently infected individuals. After infection with *B. pertussis* the average time needed for the high IgG-PT level induced by infection to decline to 100 U/ml is 58.6 (CI [54.2, 63.2]) days, to 80 U/ml it is 102.6 (CI [95.8, 109.8]) days, to 50 U/ml it is 208.9 (CI [195.4, 223.3]) days, and to 40 U/ml it is 297.6 (CI [279.2, 317.2]) days (compare Fig. 1). Using a cut-off level of 100 U/ml the estimated percentage of the population that has been infected in the past 58.6 days was 0.84% (i.e. 0.84% of the population sera contained IgG-PT of at least 100 U/ml) resulting in an estimated incidence in the year before serum sampling of 5.2% (365.25/58.6 times 0.84%). A cut-off value of 80 U/ml yielded an estimated incidence of 6.6% (365.25/102.6 times 1.86%), a cut-off value of 50 U/ml an estimated incidence of 6.6%, and a cut-off value of 40 U/ml an estimated incidence of 7.2% in the year before serum sampling.

In summary, after standardization of the estimated incidences for various cut-off values to the time period of 1 year, one obtains remarkably consistent incidence estimates for a wide range of cut-off values (Fig. 3).

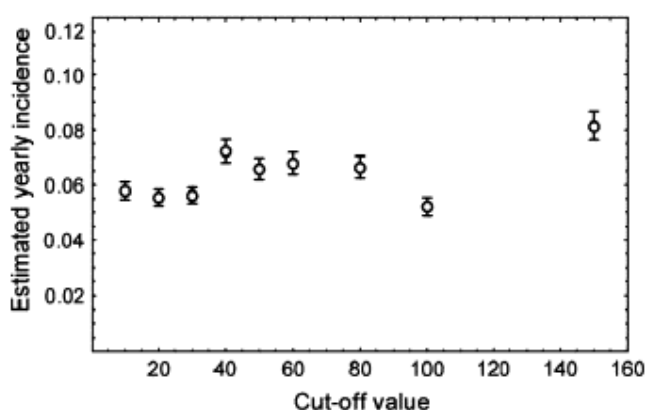


Figure 3. The estimated yearly incidence (as a fraction of the population) for different cut-off values and confidence intervals. It is questionable whether the estimates for cut-off values lower than 50 U/ml can be interpreted as valid estimates for recent incidence, but it is striking that even for those uncertain parameter regions estimates lie in the same range as for higher cut-off values.

The above results also hold for an age-stratified analysis, where the incidence of infection is estimated separately for each age category using the same range of cut-off levels of antibody titers. The consistency of the estimates for the yearly incidence of infection across different cut-off values indicates that the functional relationship between time since last infection and antibody titer as inferred from the longitudinal study in²³ is a useful description of the immune response to IgG-PT. In Fig. 4 the estimated age-specific incidence of infection with *B. pertussis* in the population is shown as calculated for the cut-off of 50 U/ml. The estimated incidence of infection the year before blood sampling (1995–1996) amounted to 6571 per 100,000 (6.6%) on average for 3–79-year olds. This was 685 times higher than the incidence of notified cases in the period 1994–1996 of 9.6 per 100,000 (0.01%) for 3–79-year olds (10.9 per 100,000 for all age groups), meaning that only one of 685 cases of infection is reported.

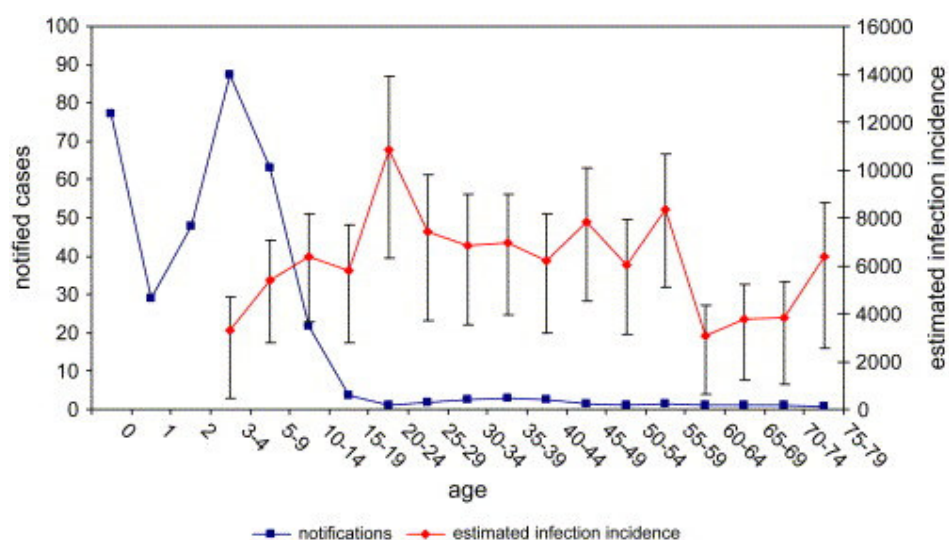


Figure 4. Estimated incidence of infection with *B. pertussis* per age group per 100,000 per year (open circles, right scale) and the annual incidence of notified cases of pertussis per age group per 100,000 averaged over the period 1994–1996 (filled circles, left scale).

As shown in Fig. 4, the estimated incidence of infection is considerably higher for all age groups in comparison to the incidence of notified cases (note the different scales). There is a remarkable difference in the age-distributions of estimated infection rates vs. notified cases. The incidence of notified cases is high among 0-year olds (77.2 per 100,000), 3–4 (87.4 per 100,000) and 5–9-year olds (63.1 per 100,000) and decreases sharply till 20–24 years (1.2 per 100,000). For 30–44-year olds a somewhat higher reported incidence is observed (2.6–2.8 per 100,000). For those aged between 45 and 69 years the incidences fluctuate between 1.2 and 1.6 per 100,000, while the incidence is lowest for the oldest age groups (70 years and above). In contrast, the estimated incidence of infection is lowest for 3–4-year olds (3299 per 100,000). It increases sharply up to the age of 20–24 years (10,831 per 100,000) and decreases again afterwards to a level of around 6500 per 100,000 in the age groups 25–55 years. In the oldest age groups a further decrease to around 4000 per 100,000 can be seen.

Discussion

Incidence and age profile of infections

There is agreement in literature about the fact that pertussis vaccination reduces transmission.^{28, 29 and 30} To estimate the amount of that reduction requires knowledge about the fraction by age of clinical cases among all infections. This fraction is determined by a complex interplay of the age-dependent force of infection, immunity, and reporting behavior. Techniques of the analysis of serologic data have made it possible to identify individuals with recent *B. pertussis* infections and compare seroprevalence data with notifications of symptomatic disease.^{31, 32 and 33}

IgG-PT levels in the Netherlands can be interpreted as markers of recent infection, because firstly the amount of pertussis toxin in the Dutch whole cell vaccine is very low, and second, because vaccine-induced IgG-PT levels are minimal and short-lived.¹⁰ However, also in populations in which pertussis vaccines are used that contain moderate to high amounts of pertussis toxin, vaccine-induced IgG-PT declines to barely detectable levels within 2–4 years.^{34, 35, 36 and 37}

Two major issues clearly emerge from our analysis. Firstly, the estimated incidence of infections with *B. pertussis* is considerable in all age groups and much higher than the reported incidence.¹ We estimated that around 6.6% of the Dutch population had experienced infection with *B. pertussis* in the year before serum sampling, while, in contrast, the incidence of notifications in 1994–1996 amounted to 0.01% per year.¹ Secondly, the age-specific profile of the reported cases diverges remarkably from the estimated age-specific profile of incidence of infection with *B. pertussis* in the population. While the highest incidence of reported symptomatic cases is observed among children aged 3–9 years, the incidence of infection is lowest among 3–4-year olds, increases with age and peaks for 20–24-year olds. Therefore, most cases are notified in those age-categories with the lowest incidence of infection.

Our cross-sectional study was performed in a limited time period (1995–1996). However, we believe that the age-specific profile of incidence of infection with *B. pertussis* is rather stable over time. Repeating the cross-sectional study can only assess whether or not this is correct. However, some support for our hypothesis is found in the similarity of the sero-profile of IgG-antibodies against pertussis toxin in 548 vaccinated children of 1–12 years in 1980, the sero-profile of about 800 individuals of all ages in 1994 and the sero-profile in the present study in

1995–1996.³⁸ Also, the fact that our findings in adolescents and adults are in agreement with the high incidences (3.3–8% per year) found in prospectively followed cohorts of small numbers of adolescents and adults in the USA in other time periods may be seen as supporting our hypothesis.^{6, 39 and 40} The immune response of IgG-antibodies against pertussis toxin after infection with *B. pertussis* shows large variation among individuals.²³ Here, we worked with the point wise (for each titer value) averages of the individual response curves (Fig. 1). We applied this procedure to a large representative population sample, and therefore we assumed that variation on the individual level averages out on the population level. We assumed that the longitudinal study population and the cross-sectional study population are identical in their responses to pertussis infection. With the size of the longitudinal sample as it is available to us at present, we can only say that the responses in different patient categories did appear to be similar.²³ In a more recent study which also included patients of older age groups it was shown that age did not significantly affect the rise, peak and decline of IgG-PT antibody titres after infection.⁴¹

Several factors are responsible for the large discrepancies between reported pertussis cases and the estimated cases of infection in their incidences and age-profiles. The amount of underreporting varies by age, because severity of disease, medical care seeking and diagnostic power are varying with age. Indeed, a high rate of underreporting has been observed mainly in older children, adolescents and adults.^{7, 9, 12, 13 and 42} Recently, Strebel et al. performed active case finding among older children and adults (10–49 years).⁴³ An incidence of symptomatic infection with *B. pertussis* of 0.5% per year was found, which was about 100-fold higher than the incidence of notified cases in that age category. A similar high incidence of symptomatic infections with *B. pertussis* among adults was found in a highly vaccinated region of France.⁴⁴

Another set of factors that influence the age-specific incidence profile of pertussis infections is related to the dynamics of transmission and immunity. The transmission of air-borne infections is strongly determined by the age-dependent patterns of mixing in a population.⁴⁵ The peaks observed in Fig. 4 of age-specific incidence in the population might be related to high contact rates, the lower infection rate for those aged 60 years and older to a lower contact rate in that age group. The gradual increase with age of the incidence of infection to a peak of 10.8% in 20–24-year olds suggests that there is a high variability in the duration of vaccine-induced immunity, which in some may be less than 2 years, in others more than 10 years. However, the incidence of notified cases of pertussis is highest among 3–9-year olds, suggesting a strong reporting bias in that age category, but also showing that susceptibility for symptomatic infection with *B. pertussis* in some may re-emerge shortly after vaccination. The mean incidence of 6.6%, if constant in time, indicates that on average, within a period of 20 years the entire population experiences infection, i.e. that vaccination against pertussis will be followed on average by three episodes of natural infection during life. Indeed, there are strong indications that immunity wanes also after natural infection and that re-infection is possible. German investigators estimated the duration of the protective period following natural infection at 20 years.⁴⁶ Versteegh et al. recently described four individuals in whom re-infection was documented, respectively, 3.5, 5, 6 and 12 years after the first.²⁵

Implications for vaccination

In the Netherlands, the age-specific profile of notifications with the highest incidence in those aged 3–9 years led to the decision of the Dutch Health Council to introduce a booster vaccination with acellular pertussis vaccine at 4 years of

age.⁴⁷ Since siblings play a role in transmission to vulnerable infants,^{14 and 15} one expects the incidence of severe pertussis in infants to decline. However, on the basis of the incidence estimates presented here, we expect that the introduction of the booster vaccination at 4 years on the long run could postpone infection to older age groups. Since these age groups may have more contacts with vulnerable infants, this could imply that on the long run booster vaccination might even lead to an increase of the incidence of severe pertussis in infants.

Conclusion

More insight is needed into the role of adults as compared to siblings in the transmission of *B. pertussis* to young unvaccinated infants. The results of the present study support the findings of others in that adults are an important source of infection.^{8, 12, 13, 14 and 16} Mathematical modeling studies are needed to study the effects of different vaccination strategies on the age-specific incidence of (symptomatic and asymptomatic) pertussis infections. Previous modeling studies^{48 and 49} had to cope with the lack of data concerning the force of infection and the age-dependent fractions of symptomatic and notified cases of infection. While we are still far from having a solid quantitative basis on which to build reliable mathematical models, we think that our study investigated an essential link between the transmission dynamics as described by mathematical models and notification data of pertussis infections. This methodology can be used more generally to estimate infection frequency from seroprevalence data.

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