Seroprevalence of Pertussis among Danish Patients with Cough of Unknown Etiology

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The common perception that pertussis is only a childhood disease is not correct. Vaccination or infection with Bordetella pertussis provides only short-lived protection against pertussis—and the majority of the population is consequently at risk of contracting pertussis. We evaluated the seroprevalence of pertussis antibodies (IgG against pertussis toxin) in serum samples from 265 Danish patients, aged 8 years and older, with coughs of unknown etiology. Depending on the cutoff chosen, we found that 2.6% to 10.9% of these patients were seropositive for pertussis. Of 178 patients with a reported duration of cough between 2 weeks and 3 months, 3.4% to 12.4% were seropositive for pertussis, indicating recent infection. Our study indicates that B. pertussis infection may be underdiagnosed among older children and adults with coughs in Denmark.

Pertussis is a highly contagious (3) bacterial infection of the respiratory tract, caused by the Gram-negative bacterium Bordetella pertussis (45), that can be fatal for unvaccinated infants. In the early 19th century, mortality due to pertussis in Denmark was very high: approximately 10% of all infants below the age of 1 year died of pertussis (32). During an epidemic in the early 1950s, the incidence among infants was approximately 11,000 per 105 (32). Since the introduction in 1961 of pertussis vaccination in the Danish childhood immunization program (see the Statens Serum Institut website [www.ssi.dk]), the incidence of pertussis has decreased dramatically. The incidence in Denmark in 2008 was 149 per 105 for infants and 9 per 105 for the whole population (12), and the latest case of infant death from pertussis in Denmark was reported in 2005 (35). The low incidence and mortality of pertussis in Denmark have probably contributed to low awareness of pertussis in the population, as well as among general practitioners.

However, pertussis can affect all age groups in the population, since neither vaccination nor previous pertussis infection provides lifelong protection; rather, they provide protection lasting 4 to 20 years (48). A recent study among general practitioners in France showed that only 6% of all cases of prolonged coughing among adults were considered for laboratory investigation for pertussis (10), and the situation in Denmark could very well be the same. Although adult pertussis is rarely fatal, confirmation of adult pertussis is nevertheless important, since adults with pertussis have been shown to be a major source of infection for infants (5, 11, 47). Adults with pertussis can present with mild and atypical symptoms (51), which can make clinical diagnosis difficult.

Pertussis can be confirmed in the laboratory by culture, PCR, or serology. Culture and PCR are useful only at an early stage of the disease, whereas serology can be used for a longer period (43). Serological diagnosis by means of measuring IgG antibodies against pertussis toxin (anti-PT IgG) displays high sensitivity and high specificity (4, 6, 17). In addition to its diagnostic purpose, anti-PT IgG serology is frequently used for seroprevalence studies among the general population (6, 19, 34, 36, 38) and among individuals with coughs (1, 7, 18, 20, 22, 23, 25, 27, 31, 37, 39, 40, 42, 46, 49). In line with this, recent studies from the Netherlands (33) and Sweden (19) have shown that 2.5% and 3% of the adult populations, respectively, had elevated anti-PT IgG titers, indicative of recent pertussis. These studies all point to the fact that the true incidence of pertussis is substantially higher than that reported for the respective populations.

The objective of this study was to determine the seroprevalence of anti-PT IgG among Danish individuals with coughs of unknown etiology.

MATERIALS AND METHODS

Sera. Sera were collected from Danish patients with coughs of unknown etiology during the period from October 2006 to June 2008. Patients were identified through a laboratory registry at the Statens Serum Institut and were eligible when a sputum and/or blood sample submitted for the examination of atypical bacterial pneumonia (Chlamydia pneumoniae, Chlamydia psittaci, Legionella pneumophila, or Mycoplasma pneumoniae [CLM] tests) yielded negative results. A letter requesting a blood sample was sent to patients who had negative results in one of the following diagnostic tests: (i) a combined serologic analysis for the detection of serum antibodies to Chlamydia, Legionella, and Mycoplasma pneumoniae antibodies (CLM serology), (ii) a combined PCR analysis from a throat swab for the detection of Chlamydia pneumoniae, Chlamydia psittaci, Legionella spp., and Mycoplasma pneumoniae DNA (CLM PCR), or (iii) a combined PCR analysis from a throat swab for the detection of Chlamydia pneumoniae and Mycoplasma pneumoniae DNA (CM PCR). Only patients more than 7 years old were contacted. All patients involved were provided with a letter of information and a short questionnaire regarding the start of the cough and its severity on a scale from 0 to 10, where 0 corresponded to “No particular effect on well-being.” 5 corresponded to “Very uncomfortable, major reduction of work ability and sleep,” and 10 corresponded to “Unbearable, very painful, constant fear of suffocation.” In addition, a written consent form, to be signed at enrollment, was included in the letter.

Collection of serum samples was performed by the patient’s general practitioners and was approved by the Danish College of General Practitioners (MPU 4-2007) and the Danish Data Protection Agency (2007-54-0061). The study was, moreover, approved by the Scientific Ethical Committee for Copenhagen and
TABLE 1. Distribution of anti-PT IgG seropositivity for 271 healthy blood donors, 68 patients with confirmed pertussis, and 265 patients with coughs of unknown etiology

| Group                        | % Acceptance<sup>a</sup> (no. accepting/total) | % (no.) seropositive according to the following cutoff: |
|------------------------------|-----------------------------------------------|--------------------------------------------------------|
|                              |                                               | 95% (44 IU/ml) | GMT + 2SD (62 IU/ml) | 99% (79 IU/ml) | 100 IU/ml | GMT + 3SD (159 IU/ml) | 99.99% (186 IU/ml) |
| Healthy donors               |                                               | 5.2 (14)       | 1.8 (5)                | 1.1 (3)         | 0.7 (2)   | 0.4 (1)               | 0.4 (1)               |
| Patients with confirmed pertussis |                                               | 86.8 (59)     | 82.4 (56)              | 75.0 (51)       | 69.1 (47) | 63.2 (43)             | 54.4 (37)             |
| Patients with coughs         |                                               | 10.9 (29)      | 9.8 (26)               | 6.0 (16)        | 5.3 (14)  | 3.4 (9)               | 2.6 (7)               |

Duration of cough (days)

| No. of | % Acceptance <sup>a</sup> (no. accepting/total) | % (no.) seropositive according to the following cutoff: |
|--------|-----------------------------------------------|--------------------------------------------------------|
| Age (yr) |                                               | 95% (44 IU/ml) | GMT + 2SD (62 IU/ml) | 99% (79 IU/ml) | 100 IU/ml | GMT + 3SD (159 IU/ml) | 99.99% (186 IU/ml) |
| 8–14   |                                               | 80.0 (4)       | 80.0 (4)               | 80.0 (4)       | 60.0 (3)  | 60.0 (3)               | 60.0 (3)               |
| 15–19  |                                               | 12.5 (1)       | 12.5 (1)               | 12.5 (1)       | 12.5 (1)  | 12.5 (1)               | 12.5 (1)               |
| 20–29  |                                               | 8.3 (1)        | 8.3 (1)                | 0              | 0         | 0                     | 0                     |
| 30–39  |                                               | 7.9 (3)        | 5.3 (2)                | 2.6 (1)        | 2.6 (1)   | 2.6 (1)               | 0                     |
| 40–49  |                                               | 9.6 (5)        | 9.6 (5)                | 3.8 (2)        | 3.8 (2)   | 0                     | 0                     |
| 50–59  |                                               | 7.8 (5)        | 4.7 (3)                | 1.6 (1)        | 1.6 (1)   | 0                     | 0                     |
| 60–69  |                                               | 9.8 (5)        | 9.8 (5)                | 7.8 (4)        | 5.9 (3)   | 3.9 (2)               | 2.0 (1)               |
| 70+    |                                               | 14.3 (5)       | 14.3 (5)               | 8.6 (3)        | 8.6 (3)   | 5.7 (2)               | 5.7 (2)               |

Duration of cough (days)

| No. of | % Acceptance <sup>a</sup> (no. accepting/total) | % (no.) seropositive according to the following cutoff: |
|--------|-----------------------------------------------|--------------------------------------------------------|
| 1–13   |                                               | 12.4 (22)       | 11.2 (20)               | 7.3 (13)       | 6.7 (12)  | 54.5 (8)             | 3.4 (6)               |
| 14–90  |                                               | 7.1 (3)         | 4.8 (2)                | 2.4 (1)        | 0         | 0                     | 0                     |
| 91+    |                                               | 42              |                        |               |           |                       |                       |

<sup>a</sup> Of request to participate in the study.

Frederiksberg, Denmark (KF 01 318860). At the time of collection, serologic diagnosis of pertussis was not available in Denmark.

Sera from 271 anonymous Danish blood donors were also available for the study. These sera had been collected in 2004 (<i>n</i>, 121) and 2006 (<i>n</i>, 150) and were from healthy individuals 17 years old and older.

In addition, sera from 68 Danish patients aged 8 to 65 with laboratory-confirmed <i>Bordetella pertussis</i> infection (PCR or culture) were included in the study. These sera were obtained from a previous study (15), in which originally 69 sera were collected, but one serum sample was omitted due to the patient’s reported use of immunosuppressive drugs. In the present study, only the first sample from each patient was included.

For this study, only patients aged 8 years and older were included, in order to avoid potential interference by antibodies remaining from the preschool booster, which is scheduled at the age of 5 years.

Detection of anti-PT IgG antibodies by ELISA. IgG antibodies to pertussis toxin were measured by an indirect enzyme-linked immunosorbent assay (ELISA) according to a method described previously, which shows highly reproducible results; median coefficients of variation (CVs) for duplicate samples were shown to be 3.3% upon analysis of 1,615 samples (16). Results were expressed as international units (IU) per milliliter according to the WHO International Standard Pertussis Antiserum (code 06/140; National Institute for Biological Standards and Control, Potters Bar, United Kingdom). The IU/ml unit of measurement corresponds to the previously used unit of measurement, ELISA units (EU) per milliliter (50). IU/ml was assigned by comparing the results from a dilution series of the in-house standard to a dilution series of the International Standard. The ELISA is now validated for use as a routine diagnostic test, available for all general practitioners and hospitals in Denmark. The Danish cutoff value for diagnostic purposes is set at 75 IU/ml, as calculated from the anti-PT IgG results from the 271 blood donor sera (mean plus 2 standard deviations [SD] from log<sub>10</sub>-transformed data plus an extra 20% margin as a gray zone).

Statistical analysis. Statistical analyses were performed using SAS statistical software (version 9.1.3; SAS Institute Inc., Cary, NC).

RESULTS

Patients. Throughout the study period, 807 patients with coughs meeting the inclusion criteria were contacted. Of these, 520 were CLM serology negative, 15 were CLM PCR negative, 268 were CM PCR negative, 2 were negative both by CLM serology and by CLM PCR, and 2 were negative both by CLM serology and by CM PCR. A total of 265 patients (32.8%) agreed to participate in the study, had a blood sample taken, and signed a written consent form (patients). Of these, 187 were CLM serology negative, 6 were CLM PCR negative, 71 were CM PCR negative, and 1 was negative both by CLM serology and by CM PCR. Thirteen of the patients had had simultaneous laboratory analyses done by either PCR or culture for the detection of pertussis at the time of the CLM tests, but all 13 of these analyses were negative (10 by PCR, 2 by culture, and 1 by both PCR and culture).

The patients were 8 to 87 years old (median age, 53 years; interquartile range [IQR], 40 to 62 years), and the rate of acceptance was correlated with age (<i>P</i> of <0.001 by Spearman correlation analysis) (Table 1). According to the questionnaire, the length of the cough at the initial CLM test was reported by 225 patients and ranged from −24 days to 1,674 days (median, 27 days; IQR, 14 to 53 days) (results below zero probably reflect problems recalling the exact date for the onset of the cough). The corresponding length of the cough at the date of the study blood sample was 1 to 1,688 days (median, 49 days; IQR, 34 to 81 days). The severity of the cough was reported by 227 patients; it ranged from 1 to 227 (median, 5; IQR, 3.5 to 6.5) and was not correlated either with age (<i>P</i> of 0.17 by Spearman correlation analysis) or with the duration of the cough at the time of the study blood sample (<i>P</i> of 0.11 by Spearman correlation analysis).

None of the participants (neither patients nor blood donors) had been eligible for the pertussis preschool booster, which was introduced in 2003 (2). There are currently no recommendations for adolescent or adult booster vaccinations for pertussis in Denmark. Thus, the most recent pertussis vaccination for all participants was given at the age of 1 year. The anti-PT
IgG antibodies measured for all participants are therefore true postinfection rather than postvaccination antibodies.

Serology. Anti-PT IgG antibodies were measured in sera from 271 healthy blood donors, and the results ranged from 1 IU/ml to 187 IU/ml. The anti-PT IgG levels from the two groups of sera obtained in 2004 and 2006, respectively, were distributed similarly (P of 0.30 by a two-sample t test).

From these blood donor sera, different models for cutoff calculations were used. Using the geometric mean titer plus 2 standard deviations (GMT H11001 2SD), the cutoff was determined to 62 IU/ml. By using the geometric mean titer plus 3 standard deviations (GMT H11001 3SD), the cutoff was determined to 159 IU/ml. By using percentiles, the 95th percentile was 44 IU/ml, the 99th percentile was 79 IU/ml, and the 99.99th percentile was 186 IU/ml. The corresponding assay specificities for each cutoff can be calculated as 1 minus the percentage of seropositive blood donors (assumed to be healthy) and ranged from 94.8% to 99.6%.

When these cutoff values were applied to the collection of sera from 68 Danish patients with confirmed B. pertussis infections, sensitivities ranging from 54.4% to 98.5% were achieved (Table 1).

When the sera from 265 patients with coughs of unknown etiology were analyzed, the anti-PT IgG results ranged from 1 IU/ml to 530 IU/ml. Using the cutoff values mentioned above, the rates of positive results ranged from 2.6% to 10.9% (Table 1). With the often-used cutoff of 100 IU/ml, the rate of positive results was 5.3% (Table 1). The highest seroprevalence was found for children 8 to 14 years old; either 3 or 4 of the 5 samples collected were positive, depending on the cutoff chosen (Table 1). The positive cases were distributed evenly throughout the collection period.

When only the data from patients reporting coughs lasting 14 days to 3 months at the time of donation of the blood sample (178 patients) were used, the rates of positive samples ranged from 3.4% to 12.4% (Table 1). For data from patients with coughs lasting 1 day to 13 days (5 patients), there were no positive results with any of the cutoffs, and for data from patients with coughs lasting 91 days or longer (42 patients), the rates of positive results ranged from 0 to 7.1% (Table 1). The results for the 14- to 90-day-cough group were thus more often positive than those for the rest of the samples—although the difference was not statistically significant (P values ranged from 0.08 to 0.35 by Fisher’s exact test depending on the chosen cutoff). Forty patients did not report the onset of the cough.

The distributions of anti-PT IgG antibody levels in sera from patients and blood donors are shown in Fig. 1 as well as in Table 1. Significantly higher anti-PT IgG levels were found in patient sera than in blood donor sera (P of <0.05 with all cutoffs by Fisher’s exact test). The anti-PT IgG levels of the patients in correlation to patient age, days since the onset of the cough, or the severity of the cough are shown in Fig. 2 to 4. These three figures show that very high anti-PT IgG levels were most frequently found among teenagers and elderly, that high anti-PT IgG levels were seen mostly for coughs lasting between 14 and 90 days, and that there was no correlation between anti-PT IgG results and the severity of the cough (P of 0.35 by Spearman correlation analysis).

Of 13 patients for whom the general practitioner had already considered pertussis and for whom either culture or PCR tests were negative, 3 had elevated anti-PT IgG levels of 69, 88, and 180 IU/ml, respectively.

DISCUSSION

Among 265 Danish patients aged 8 to 87 years with coughs of unknown etiology, we found 2.6% to 10.9% positive for elevated anti-PT IgG antibodies depending on the choice of cutoff (Table 1). For 178 patients with a reported duration of cough between 2 weeks and 3 months at the donation of the
blood sample, we found a higher prevalence, 3.4% to 12.4%, of anti-PT IgG antibodies (Table 1), although the difference was not statistically significant. This corresponds to the period where serology is useful for pertussis diagnosis: the level of anti-PT IgG antibodies will typically begin to increase after 2 weeks of coughing, and the symptomatic period for classic pertussis is approximately 3 months (reviewed in references 26 and 28).

The highest seroprevalence was found for children between the ages of 8 and 14 years. This finding corresponds to the high incidence seen among this age group in Denmark in the years of the study, as confirmed by either culture or PCR (12–14). As mentioned above, none of these children had received the preschool booster vaccine, and the anti-PT IgG antibodies measured are therefore not related to vaccination.

Agreement to participate in this study was correlated with age; older people were more willing to participate—probably because of a more flexible calendar—than students and parents of younger children. This skewed age distribution probably has only minor effects on the validity of this study, since previous research has shown that the magnitude and kinetics of the anti-PT IgG response at pertussis are not correlated with age (15). Accordingly, the probable bias caused by different distributions of age between blood donors and patients is estimated to be low.

Thirteen of the participating patients had been tested previously for pertussis by either PCR or culture, and all 13 were found negative for the presence of 

B. pertussis

. Three of these patients, however, were found to have elevated anti-PT IgG titers. This finding underlines the importance of serology as a diagnostic method for pertussis, since the sensitivities of both PCR and culture decrease with the progression of the disease (42, 44). Furthermore, such false-negative test results could be attributed to the general difficulties of performing a correct

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**FIG. 2.** Anti-PT IgG results from 265 patients with coughs of unknown etiology. Results are plotted against the ages of the patients.

**FIG. 3.** Anti-PT IgG results from 219 patients with coughs of unknown etiology who reported a date for the onset of cough less than 200 days prior to the donation of the blood sample. Results are plotted against the days of cough.
nasopharyngeal swab, to the difficulties in distinguishing pertussis symptoms from the symptoms of other respiratory diseases (18), and to the fact that the bacterial load in the nasopharynx is quite low in adolescents and adults (29). Previous studies confirm the superiority of serology to PCR and culture for the diagnosis of pertussis in adults (reviewed in reference 43).

Anti-PT IgG is widely agreed to be the most sensitive and the most specific test for the serodiagnosis of pertussis (4, 6, 17), and it is therefore also the best choice for seroprevalence studies (34).

Numerous anti-PT IgG seroprevalence studies for pertussis among individuals with coughs have been performed previously (1, 7, 18, 20, 22, 23, 25, 27, 31, 37, 39, 40, 42, 46, 49) (Table 2). However, the cutoffs chosen for these studies differ greatly, making the findings on the prevalence of positive anti-PT IgG serology very difficult to compare. For example, in a Greek study on coughing, a kit-defined cutoff of 11 EU/ml was used, leading to the conclusion that 71% of the individuals were seropositive for *B. pertussis* infection (25). At the other end of the spectrum, in a cough study from Minnesota, an age-specific cutoff of 999 EU/ml was found by a GMT/H11001_3SD approach (42).

Not only the cutoff value but also the choice of antigen is an important issue, as illustrated by a German study of coughing patients in which four different antigens were used and the levels of both IgA and IgG antibodies were determined: seropositivity rates ranging from 10% (antifimbrial IgG) to 54% (anti-pertactin IgA) were reported (39). For serodiagnostic studies, both single-point serology and paired samples are used, and Table 2 clearly shows that the rates of positivity obtained by paired serology can be very different from those obtained by single-point serology. In one study, paired serology yielded more seropositive results than single-point serology (39) (with a very high single-point cutoff at 312 EU/ml), while in five other studies, the opposite was true (7, 27, 37, 42, 49). Judging from these data, it would seem that single-point serology is the better choice for seroprevalence studies using anti-PT IgG analyses.

The seropositivity rates for individuals with coughs, determined by using anti-PT IgG measurements, from 15 studies from different countries in the period 1991 to 2008 are shown in Table 2. Several different approaches to the cutoff value were used, and the seropositivities range from 1.6% to 71%. As noted above, data from studies using different protocols and different cutoffs are difficult, if not impossible, to compare. However, by a rough estimate from Table 2, it would seem that 5 to 15% of individuals with prolonged coughs are perhaps truly seropositive for anti-pertussis antibodies (Table 2).

During the study period, 2006 to 2008, a low incidence of laboratory-confirmed pertussis was reported in Denmark (detected by PCR or culture). A total of 331 laboratory-confirmed pertussis cases were registered in 2006, 455 in 2007, and 513 in 2008—corresponding to population incidences of 6, 8, and 9 per 100,000, respectively (12–14). In comparison, the incidence during the most recent epidemic in Denmark, in 2002, was 36 per 100,000, with a total number of 1,946 cases (24). The number of laboratory-confirmed cases of pertussis for individuals in the same age group as this study (8 years old and older) in Denmark during the study period, from October 2006 to June 2008, was 549 (Statens Serum Institut, registered laboratory data, 2010), and in our small study of coughing patients, we found 7 to 29 additional patients with positive anti-PT IgG titers (Table 1). During the study period, a total of 13,772 samples from patients 8 years old and older were sent to the Statens Serum Institut for laboratory diagnostics of bacterial diseases categorized as atypical pneumonia (6,584 for CLM serology, 3,309 for CLM PCR, and 3,879 for CM PCR) (Statens Serum Institut, registered laboratory data, 2010). If 2.6% to 10.9% of all these samples were positive for anti-PT IgG, these proportions would correspond to 358 to 1,501 pertussis cases. Thus, the actual number of pertussis cases among coughing individuals 8 years old and older in Denmark during the study period is estimated to have been considerably larger than the 549 cases detected.

Several countries have now introduced adolescent and/or adult

![FIG. 4. Anti-PT IgG results from 227 patients with coughs of unknown etiology who reported the severity of cough on a scale from 0 to 10. Results are plotted against severity of cough, with 0 corresponding to “No particular effect on well-being,” 5 corresponding to “Very uncomfortable, major reduction of work ability and sleep,” and 10 corresponding to “Unbearable, very painful, constant fear of suffocation.”](image-url)
pertussis booster vaccines (8, 9, 21, 30, 41), and in the light of the results from the present study, an adolescent and/or adult pertussis booster should perhaps be considered in Denmark.

When the state of Massachusetts introduced serology as a diagnostic method for pertussis in 1987 and improved pertussis surveillance as well, a large increase in the number of laboratory-confirmed adult pertussis cases was seen. Thus, 92% of all pertussis cases in Massachusetts in 1998 were detected in adolescents and adults, while in the rest of the United States, the proportion of confirmed pertussis cases detected in adults was only 47% (51). Serological diagnosis of pertussis has recently been introduced in Denmark in order to improve the diagnosis of coughs of unknown etiology.

The common notion that pertussis is only a childhood disease is not correct, and the awareness among Danish practitioners of the occurrence of pertussis among older children and adults is probably comparable to the low awareness seen in a French study from 2001 (10). Detection of pertussis in adults is particularly important in order to prevent the transmission of the disease to vulnerable infants (5, 11, 47). Our study suggests

| Prevalence of anti-PT IgG | Cutoff method, single-point | Cutoff for seropositivity | Duration of cough | Age group (yr) | Location | Reference |
|--------------------------|-----------------------------|---------------------------|-------------------|-------------|----------|-----------|
| 3.8 (5/130), paired      | >GMT + 3SD                  | Paired: >4-fold difference| ≥6 days           | Students     | California| Mink et al., 1992 (27) |
| 9.2 (12/130), total     |                             | Single: 242.2 EU/ml       |                   | (median, 23)|          |           |
| 12.5 (7/56), paired     | 99th percentile             | Paired: >3.43-fold increase| >14 days          | 18–79       | Germany  | Schmitt-Grobé et al., 1995 (39) |
| 7.6 (11/145), single    | GMT + 2SD                   | Paired: >4-fold increase  | 14–90 days        | >17         | Tennessee| Wright et al., 1995 (49) |
| 2.6 (2/75), paired      |                             | Single: >60 EU/ml         |                   |             |          |           |
| 13 (10/75), single      |                             | >7 days                   | 18–29             |             |          |           |
| 16 (12/75), total       |                             | 2–12 wk                  | 16–77             |             |          |           |
| 2.8 (1/35), paired      | 1/100 of a reference serum  | Paired: >2-fold increase  | 2–130 days        | 13–81       | Illinois | Rosenthal et al., 1995 (37) |
| 12 (19/153), single     | GMT + 2SD                   | Single: >84 in-house units| 2–14 wk           | 24–78       | California| Nennig et al., 1996 (31) |
| 0.8 (1/120), paired     | None                        | Paired: >4-fold increase  | >7 days           | 18–29       | California| Jansen et al., 1997 (23) |
| None (0), paired        | GMT + 2SD                   | Single: 123 EU/ml         | 2–12 wk           | 16–77       | Denmark  | Birkebeck et al., 1999 (7) |
| 6.1 (13/212), paired    | GMT + 3SD (age specific)    | Paired: >2-fold increase  | 7–34 days         | 10–49       | Minnesota| Strebel et al., 2001 (42) |
| 8.5 (18/212), single    |                             | Single: >20 EU/ml         | 14 days           | 5–16        | United Kingdom | Hartden et al., 2006 (20) |
| 7.5 (33/442), paired,   | 99.99th percentile          | Paired: >4-fold increase  | 7–56 days         | 12–90       | Canada   | Senzilet et al., 2001 (40) |
| including PCR and culture| GMT + 3 SD                 | Single: ?                 |                   |             |          |           |
| 22 (40/183), paired     | ≥4 times MLDc               | Paired: >2-fold increase or decrease| 7–69 days         | 18–88       | France   | Gilberg et al., 2002 (18) |
| 11 (15/136), single and paired | From literature | Paired: >4-fold increase and >20 EU/ml | 1–6 wk | <18 | Netherlands | Verstoegh et al., 2005 (46) |
| 37 (64/172), single and paired | From literature | Paired: >4-fold change and >20 EU/ml | ≥14 days         | 5–16        | United Kingdom | Hartden et al., 2006 (20) |
| 15 (5/33), single       | Commercial kit              | 100 EU/ml                | 1 wk to 2 yr      | 1–75        | Taiwan   | Hu et al., 2006 (22) |
| 1.6 (5/307), paired     | From literature             | Paired: >4 fold increase and >20 EU/ml | 2–5 wk | 6–14 | Turkey | Aksakal et al., 2007 (1) |
| 15 (49/307), single     | Commercial kit              | Single: 100 EU/ml        |                   |             |          |           |
| 17 (51/307), total     |                             | ≥3 wk                    | Adults             |             | Greece   | Kapaskelis et al., 2008 (25) |
| 71 (311/441), single    | Commercial kit              | Single: 11 EU/ml         |                   |             |          |           |

* a Given as a percentage (number seropositive/total number), type of serology. Abbreviations: paired, paired samples; single, single-point serology; acute, acute-phase; conv., convalescent-phase.
* b EU/ml correlates 1:1 to IU/ml.
* c MLD, minimal level of detection.
that pertussis should be considered among Danish schoolchildren and adults with coughs and that as many as 10.9% of such individuals could in fact have pertussis.

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