Evaluation of reported cases of pertussis: epidemiological study in a large city in Spain

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We retrospectively analysed the incidence rate of reported cases of pertussis in Barcelona during 2009–2012 according to age, sex, type of medical centre and vaccination status. We included 748 confirmed or suspected cases, 613 (82.0 %) of which were confirmed by laboratory testing and the remaining 135 (18.0 %) by epidemiological evidence. The highest reported incidence of pertussis was amongst 1 year olds [96.1 per 100 000 person-years, 95 % confidence interval (CI): 84.3–109.1]. The majority of confirmed and suspected cases were reported in 2011 and 2012, and the total incidence (confirmed or suspected) was 6.3 (95 % CI: 5.6–6.9) and 4.2 (95 % CI: 3.6–4.7) per 100 000 person-years, respectively. Incidence increased significantly \((P<0.001)\) in 2011–2012 compared with 2009. Most confirmed cases occurred in children <1 year old (87.9 %). Cases were confirmed by real-time (RT)-PCR (87.5 %; 95 % CI: 81.3–87.6) and bacterial culture (13.7 %; 95 % CI: 11.0–17.1). We recommend performing RT-PCR in suspected cases with no epidemiological link to a confirmed case.

INTRODUCTION

Pertussis is a highly contagious disease of the upper respiratory tract. It is caused by the coccobacillus Bordetella pertussis. The incubation period is usually 5–10 days, but can be up to 21 days, and clinical manifestations usually last between 4 and 6 weeks. The disease has three stages: catarrhal, paroxysmal and convalescent (Long, 2009).

The incidence of pertussis has increased in recent years in countries with high vaccine coverage, and this re-emergence of pertussis has led to more frequent diagnosis and improved vaccination recommendations, and booster vaccination strategies have been established in children, adolescents and adults (Crespo et al., 2011; Manzanares et al., 2013; Solano et al., 2014).

Pertussis has significant morbidity and relatively high mortality rates, particularly in children <1 year old. In European countries with high vaccination coverage (>95 %) amongst children, the distribution of cases appears to have shifted to adolescents and adults (Díez-Domingo et al., 2004; Gil et al., 2001).

In the region of Catalonia, 90 % of cases of pertussis in <1 year olds had been vaccinated according to the current diphtheria–tetanus–acellular pertussis vaccine (DTaP) schedule for their age group and 87 % of cases aged 5–9 years had been fully vaccinated with five doses of DTaP vaccine. Despite these high levels of vaccination coverage, however, pertussis circulation has not been fully controlled (Sala-Farré et al., 2013). This questions the efficacy of current immunization programmes.

In Spain, pertussis remains an endemic infection that causes a considerable number of cases annually. The clinical diagnosis and laboratory confirmation of reported cases are necessary in order to take preventive and control measures in the event of an outbreak. Prompt treatment of cases, chemoprophylaxis of those with an epidemiological link and vaccination of susceptible populations are also necessary (Comisión de Salud Pública del Consejo Interterritorial del Sistema Nacional de Salud, 2013).

Laboratory diagnostic procedures may be grouped into two categories: direct methods, based on isolating the bacterium by culture or detecting it by direct immunofluorescence or real-time (RT)-PCR, and indirect methods, based on determining the patient-specific immune response (serology). Only direct methods are accepted for surveillance purposes (Sanz Moreno et al., 2002).
Barcelona is the capital city of the autonomous community of Catalonia, Spain, and the second largest city in the country, with a population of 1.6 million within its administrative limits, according to the Spanish National Statistical Institute. It is reported by the United Nations as the sixth most populous urban area in the European Union, after Paris, London, the Rhur, Madrid and Milan. As in other European cities, an increase in the number of reported cases of pertussis has been observed in Barcelona in recent years (Tozzi et al., 2007).

The objective of this study was to compare the incidence rate of suspected and confirmed cases of pertussis in Barcelona during 2009–2012 according to age group, sex, type of medical center and vaccination status, and to compare the frequency of confirmation by RT-PCR and culture tests in the laboratories.

**METHODS**

**Population and case definition.** We conducted a retrospective observational study including all cases reported to the Epidemiology Service of the Barcelona Public Health Agency [Agencia de Salud Pública de Barcelona (ASPB)], Barcelona, Catalonia, Spain, between 2009 and 2012. Suspected cases had to meet the clinical case definition established by the National Epidemiological Surveillance Network of the National Centre for Epidemiology, Carlos III Health Institute, Madrid, Spain, as follows: infectious disease characterized by the appearance of cough for ≥2 weeks, and paroxysmal cough followed by respiratory stridor and post-cough vomiting or apnoea. The diagnosis of pertussis required only the presence of cough accompanied by at least one of the other symptoms, with no other apparent cause. Suspected cases were defined as those meeting the criteria for clinical case definition, but without laboratory or epidemiological evidence. Confirmed cases were defined as those with positive RT-PCR or *B. pertussis* culture, and those who met the clinical case definition and/or were linked epidemiologically (person with compatible pertussis symptoms in contact with a confirmed case).

When a case of pertussis is reported to a public health surveillance unit in Barcelona, a clinical sample must be sent to a reference laboratory for confirmation. The methods of choice are bacterial culture and RT-PCR.

Individuals who did not meet criteria for either confirmed or suspected cases were considered to be non-cases.

**Microbiological techniques**

**Culture media.** First, a nasopharyngeal swab was spread on a Petri dish with Regan–Lowe agar medium with 40 µg cephalexin ml⁻¹, after which it was introduced into a tube with Regan–Lowe semisolid enrichment medium. Both media were incubated aerobically in a humid atmosphere at 37 °C. The culture dishes were incubated for 7 days and growth of typical colonies of *Bordetella* spp. was checked daily from the third day. At 48 h after inoculation, the swabs that had been maintained in semisolid enrichment medium were subcultured on Regan–Lowe agar plates and incubated under the conditions described above.

Colonies suggestive of *B. pertussis* were identified by Gram stain, oxidase production, the absence of urease and agglutination with specific anti-*B. pertussis* antisera (Becton Dickinson Diagnostic Systems) (Begotza et al., 2007).

**RT-PCR.** Genetic material was extracted from 200 µl emulsion in a saline swab using a Tissue Extraction kit (Qiagen). The collected extract was amplified in 50 µl elution buffer using primers BP1 (5’-GATTCATAGTTGTATGATGTT-3’) and BP2 (5’-TTCAGG-CACACAACTTGATGGCCG-3’), and a 181 bp fragment of the insertion sequence IS481 and its isoform IS1002, which is specific to *B. pertussis* (García-Martínez et al., 2006; Poddar, 2004), was detected using a probe-type molecular beacon (5’-FAM-CGGACCTTCTTGA-TGGCCTCGAATTGTTCCG-BHQ-3’).

Amplification resulted in a final volume of 10 ml, to which we added 0.2 mM dNTP, 0.5 µM each primer, 3 µM MgCl₂, 0.3 µM probe, 1 ml Master Mix (Roche Molecular Biochemicals) and 2 µl sample or control. In each amplification mixture, 0.05 U uracil-DNA glycosylase µl⁻¹ (Roche Molecular Biochemicals) was used to avoid contamination. As an internal positive control for the PCR, LightCycler Control kit DNA (Roche Molecular Biochemicals) was used with β-globin, which includes the primers (1 µl) and probe (0.5 µl) for the amplification and detection of a gene fragment. Negative controls (sterile distilled water and samples collected from healthy individuals without *B. pertussis* and a positive control (DNA of *B. pertussis*) were used in each amplification reaction. RT-PCR amplification was carried out using a LightCycler (Roche Molecular Biochemicals), with initial incubation for 10 min at 95 °C followed by 35 cycles of 5 s at 95 °C, 15 s at 58 °C and 15 s at 72 °C. As general measures to prevent contamination of the samples, we used separate work areas pre- and post-PCR steps, biological safety cabinets, and filtered pipette tips.

In samples with a very low load of *B. pertussis* or non-viable residues, RT-PCR could not discriminate between *B. pertussis* and *Bordetella bronchiseptica*. In these cases, a modified technique which highly concentrates the samples was needed to discriminate between species (Koidl et al., 2007).

**Variables studied.** We studied age (categorized into six groups: <1, 1–4, 5–8, 9–12, 13–16 and ≥17 years), sex, reporting year (2009, 2010, 2011 or 2012) and vaccination status (‘properly vaccinated’ cases that had received four anti-pertussis doses).

The reporting centres were categorized as hospitals, primary-care centres and others (schools, individuals or identified by ASPB staff during an epidemiological investigation for another cause).

The clinical manifestations studied were: cough for ≥2 weeks, paroxysmal cough, respiratory stridor, post-cough vomiting, apnoea, fever, pneumonia and other complaints (including seizures and encephalopathy).

DTaP vaccination coverage in the Barcelona population for the study period was determined by reviewing the electronic clinical histories of the population of 6-year-old children assigned to primary-care centres in Barcelona city.

**Statistical analysis.** Pertussis incidence rates and 95% confidence intervals (CIs) of reported cases in the period from 2009 to 2012 were calculated according to age, sex, vaccination status, type of reporting centre and year of onset of symptoms.

The annual population of Barcelona for each year studied was used to determine the incidence rate, according to the Statistical Institute of Catalonia (http://www.idescat.cat/en/poblacio/padro.html).

We performed a bivariate analysis in which we compared confirmed cases and suspected cases (in terms of age group, sex, reporting centre, reporting years and clinical manifestation) and calculated odds ratios (ORs) and their 95% CIs.

In order to obtain an adjusted OR (aOR), we conducted a multivariate logistic regression with confirmation status as the dependent variable.
and variables for which the \( P \) value in the bivariate analysis was <0.1 as the independent variables. We selected the category with the lowest percentage of confirmation as the reference category.

We also studied the subpopulation of subjects for whom laboratory diagnostic testing had been carried out (RT-PCR and/or culture) and calculated the frequency of confirmation by RT-PCR and culture tests.

We used PASW Statistics v.18 and Epidat v.3.1 for the analysis of detection, and OpenEpi v.3.01 to calculate incidence rates and 95% CIs.

**Ethics statement.** Demographic and clinical data were obtained from the epidemiological questionnaire used by the ASPB Epidemiological Service, and the data were treated and analysed anonymously. The analysis was carried out retrospectively and involved data collected during routine epidemiological surveillance. Therefore, no ethical approval or informed consent was required. All data were treated in a strictly confidential manner according to the ethical principles of the Helsinki Declaration of 1964 revised by the World Medical Organization in Edinburgh, 2000, and to Spanish Data Protection Law 15/1999.

**RESULTS**

The incidence of pertussis differed according to age, with the highest incidence in children <1 year old and those aged 1–4 years. In <1 year olds, there was a higher incidence of both confirmed and suspected cases. We also observed high incidences of total cases and confirmed cases amongst 5–8 and 9–12 year olds. Similar incidences were observed in males and females (\( P=0.617 \)). Incidence rates were higher in 2011 and 2012 than in 2009 (\( P=0.001 \)) (Table 1).

We detected 1066 possible cases, of which 318 were discarded for not meeting the clinical case definition. We included 748 confirmed and suspected cases in the study: 71 reported in 2009 (9.5 %), 98 reported in 2010 (13.1 %), 347 reported in 2011 (46.4 %) and 232 reported in 2012 (31.0 %). In total, 613 cases (82.0 %) were confirmed and 135 (18.0 %) were suspected (Table 2).

According to age group, most confirmed cases were observed in <1 (31.0 %), 1–4 (19.3 %) and ≥17 year olds (19.4 %). There was a slight preponderance of females (54.7 %) over males (45.3 %).

According to reporting centre, hospitals reported 72.3 % of cases, primary-care centres reported 27.4 % and other sources reported 0.3 %. We observed a significant increase in the number of cases in 2011 (347 cases) and 2012 (232 cases) compared with 2009 (71 cases) and 2010 (98 cases) \( (P<0.001) \).

In total, 784 RT-PCR tests were performed, of which 536 were positive and 248 were negative.

Amongst the 613 confirmed cases, 541 were laboratory confirmed: 457 (87.5 %, 95 % CI: 81.3–87.6) were confirmed by RT-PCR only, five (13.7 %, 95 % CI: 11.0–17.1) were confirmed by culture only and 79 (14.6 %, 95 % CI: 11.5–17.7) were confirmed by both tests. In the remaining 72 confirmed cases (11.7 %), confirmation was based on an epidemiological link.

The percentage of confirmed cases was higher than that of suspected cases in <1, 5–8 and 9–12 year olds. The confirmation rate was slightly higher in females (83.8 %)

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**Table 1.** Incidence rate of confirmed and suspected cases of pertussis according to age, sex and reporting year: Barcelona, Spain, 2009–2012

Data source: ASPB. Incidence rate determined using the population of Barcelona, according to the Statistical Institute of Catalonia.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Incidence rate per 100 000 person-years (95 % CI)</th>
<th>Confirmed cases</th>
<th>Suspected cases</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;1)</td>
<td>Total (confirmed + suspected) cases</td>
<td>96.1 (84.3–109.1)</td>
<td>84.5 (73.5–96.7)</td>
<td>11.6 (7.8–16.5)</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>1–4</td>
<td></td>
<td>14.5 (12.3–17.1)</td>
<td>9.6 (7.8–11.7)</td>
<td>4.9 (3.7–6.5)</td>
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<tr>
<td>5–8</td>
<td></td>
<td>13.0 (10.8–15.5)</td>
<td>11.2 (9.2–13.6)</td>
<td>1.7 (1.0–2.7)</td>
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<tr>
<td>9–12</td>
<td></td>
<td>8.5 (6.6–10.7)</td>
<td>7.4 (5.7–9.4)</td>
<td>1.1 (0.5–2.0)</td>
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<tr>
<td>13–16</td>
<td></td>
<td>4.8 (3.5–6.6)</td>
<td>3.9 (2.7–5.5)</td>
<td>0.9 (0.4–1.8)</td>
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<tr>
<td>≥17</td>
<td></td>
<td>0.8 (0.7–0.9)</td>
<td>0.6 (0.5–0.8)</td>
<td>0.1 (0.09–0.2)</td>
</tr>
<tr>
<td>All cases</td>
<td></td>
<td>3.3 (3.1–3.6)</td>
<td>2.7 (2.5–3.0)</td>
<td>0.6 (0.5–0.7)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td></td>
<td>3.1 (2.8–3.4)</td>
<td>2.4 (2.2–2.8)</td>
<td>0.6 (0.5–0.8)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>3.6 (3.3–4.0)</td>
<td>3.0 (2.7–3.4)</td>
<td>0.5 (0.4–0.7)</td>
</tr>
<tr>
<td><strong>Reporting year</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2009</td>
<td></td>
<td>1.2 (1.0–1.6)</td>
<td>1.0 (0.7–1.3)</td>
<td>0.2 (0.1–0.4)</td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td>1.8 (1.4–2.1)</td>
<td>1.5 (1.2–1.9)</td>
<td>0.2 (0.1–0.3)</td>
</tr>
<tr>
<td>2011</td>
<td></td>
<td>6.3 (5.6–6.9)</td>
<td>4.9 (4.3–5.5)</td>
<td>1.3 (1.0–1.7)</td>
</tr>
<tr>
<td>2012</td>
<td></td>
<td>4.2 (3.6–4.7)</td>
<td>3.5 (3.0–4.0)</td>
<td>0.6 (0.4–0.9)</td>
</tr>
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</table>
than in males (79.6 %), but the difference was not statistically significant.

We observed a statistically significant difference (P < 0.001) between the confirmation rate in hospitals (80.1 %) and that in primary-care centres (73.2 %; aOR 2.8; 95 % CI: 1.7–4.6). The highest confirmation rate was observed in 2010 (88.8 %; aOR 2.5; 95 % CI: 1.2–5.4) followed by 2012 (84.9 %; aOR 2.1; 95 % CI: 1.2–3.5), considering 2009 as the reference category.

The most common clinical manifestations (Table 3) were cough lasting ≥ 2 weeks (81.1 %), paroxysmal cough (55.2 %; aOR 1.4; 95 % CI: 0.6–3.2) and post-cough vomiting (32.5 %; aOR 1.3; 95 % CI: 0.6–2.8), considering cough ≥ 2 weeks as the reference group.

The rate of hospitalization due to pertussis infection amongst young infants was high, with 3.5 cases per 100 000 person-years (95 % CI: 3.0–3.9). The number of hospitalizations in < 1 year olds was 31 (39.2 % of all hospitalizations) in 2009, 39 (34.8 %) in 2010, 83 (18.9 %) in 2011 and 75 (17.3 %) in 2012. The mean ± SD hospital stay was 6.3 ± 5.1 days.

Four deaths attributable to pertussis infection were reported to the ASPB between 2009 and 2012. The case/fatality ratio was 2.0 per 100 000 person-years in < 1 year olds. All four deaths were confirmed cases, three by RT-PCR (0.5 %), one also (0.2 %) by culture and by epidemiological link. The children who died were < 2 months old and had received no doses of pertussis vaccine. One was born at 36 weeks of gestation (preterm delivery with risk of death due to pertussis). The four infants had atypical clinical manifestations, with apnoea or inspiratory stridor, but no cough ≥ 2 weeks, post-cough vomiting or paroxysmal cough. Mean lymphocyte count was 61.100 ml⁻¹ and all cases had leukocytosis with 30 000 white blood cells ml⁻¹. One infant had a co-infection with respiratory syncytial virus (RSV) and two had bronchiolitis. The clinical parameters and clinical severity scores on admission and during hospitalization were: (right and left) systemic–diastolic biventricular dysfunction, biventricular hypertrophy, mild-moderate pulmonary hypertension, sinus tachycardia, and mild pericardial and pleural effusion. The median length hospital stay was 10.7 days (95 % CI: 10.6–10.7).

Of the confirmed cases, 377 (61.5 %) had been vaccinated. Amongst laboratory confirmed cases, 97 (25.7 %) properly vaccinated children were < 1 year old, 81 (21.5 %) were aged 1–4 years and 94 (24.9 %) were aged 5–8 years. Amongst cases with clinical suspicion, 11 (13.6 %) properly vaccinated children were < 1 year old, 40 (49.4 %) were aged 1–4 years and 12 (14.8 %) were aged 5–8 years.

Infant vaccination coverage with three or more doses of DTaP in the population of 6 year olds was estimated to be
suggest that a substantial number of primary-care physicians do not use pertussis testing and may not be able to recognize the clinical symptoms of this infection in adolescents (Dempsey et al., 2009). This study is based on a passive surveillance system, which is affected by many limitations. The underdetection of pertussis in adolescents, young adults and adults is mainly due to its atypical clinical characteristics, and the lack of laboratory confirmation. The true epidemiological impact of pertussis is not always perceived, and surveillance and further studies are needed to fine-tune pertussis prevention strategies (Gabutti et al., 2012). Pertussis is often overlooked as a cause of chronic cough, especially in adolescents and adults. Several symptoms are classically thought to be suggestive of pertussis, but their diagnostic value is uncertain. The presence or absence of post-cough emesis or inspiratory whoop modestly changes the likelihood of pertussis; therefore, clinicians must use their overall clinical impression to decide on the need for additional testing or empirical treatment (Cornia et al., 2010).

Despite the difference in the reporting of confirmed and suspected cases, the similarity in incidence rates depending on age suggests that the incidence estimates based on reported cases in Barcelona are valid and may better reflect the true incidence of pertussis, which would facilitate implementation of preventive health actions at the community level.

As in previous studies, our results show that, whilst improvements in the vaccination programme have reduced morbidity due to pertussis in childhood, they have not affected the increase in adolescent and adult pertussis. The high circulation rates of B. pertussis in the latter age groups may even limit the effectiveness of paediatric vaccination (de Greeff et al., 2010).

In Europe, 43 482 cases were reported during 2003–2007 (overall incidence: 4.1 per 100 000 inhabitants) (Celentano et al., 2005; Schmidt et al., 2001). These authors concluded that the true rates amongst European adults had doubled in 5 years.
High circulation of B. pertussis amongst adults, which is the combined effect of declining immunity and pathogen adaptation, may support the idea of introducing adolescent and adult booster vaccinations (Forsyth et al., 2005).

The percentage of confirmation was also higher in <1 year olds, as observed by other authors, mainly because very young children are hospitalized more frequently (Gabutti et al., 2012; Vieira et al., 2010).

Nonetheless, a decrease in the rate of hospitalization has been observed in recent years in our study, despite an increase in incidence in this age group, which suggests a possible increase in reporting of clinically milder cases.

As observed by Bisgard et al. (2004) in four states in the USA, we also observed no significant difference between sexes in the percentage of reported cases.

Whilst Catalonia has a reference laboratory for the diagnosis of pertussis, many hospitals and laboratories, some with private activities, follow independent guidelines. There is also no consensus amongst the various regions of Spain regarding recommendations for establishing regular quality controls, as in other countries (Tatti et al., 2013), despite the proposal at the European level that guidelines must include standardization and quality assurance of microbiological diagnostics methods (RT-PCR and culture) (He et al., 2012).

This may be of interest in our study because it influences the interpretation of the RT-PCR and culture tests during the clinical phase of the disease. A recent study reported that the risk of false-positive results remains quite high and recommended establishing uniform quality control procedures to be implemented regularly (Caro et al., 2009). Also, the use of RT-PCR markedly improves the diagnosis of pertussis, which presents new perspectives for epidemiological and vaccine efficacy studies (He et al., 1996).

Bacterial culture was not performed in many Spanish laboratories for a number of reasons (Begoña et al., 2007; Sanz Moreno et al., 2002). Bacteria of the genus Bordetella are extremely labile, slow growing, and need specific transport and incubation conditions. Furthermore, sample collection by laboratory personnel and immediate seeding on plates with appropriate culture media is not always sufficient to ensure growth (Lapaeva et al., 1981).

The highest incidence rate was observed amongst <1 year olds, particularly in 2011 and 2012 (6.3 and 4.2 per 100 000 person-years, respectively), whilst that amongst adults >17 years old was lower at 0.8 (95% CI: 0.7–0.9) per 100 000 person-years. Determining the incidence of diagnosed pertussis (clinically or with laboratory evidence) in adults can be difficult because of non-specific symptoms. Furthermore, routine surveillance data, such as those used for case ascertainment in this study, are known to underestimate the true population incidence (Markov & Crowcroft, 2007; Ward et al., 2006).

Children <1 year old belong to the highest risk group because in most cases they are not old enough to have completed their immunization schedule (Montella et al., 2007) and we observed the highest percentage of confirmed cases in this group. Increased surveillance in schoolchildren, adolescents and adults is an important control activity from the point of view of public health. Adults play an important role as a likely source of infection in the family and the community (Campins et al., 2013; Heininger & Cherry, 2006; Tan et al., 2005), and are also rarely diagnosed with pertussis. Clinical manifestations in adolescents and adults are often atypical and limited to moderate cough, which causes underdiagnosis of pertussis. Therefore, persistent coughing in this age group should always be considered in the differential diagnosis, especially amongst those who could transmit the infection in households with vulnerable members, such as newborns and small children.

Four of the reported cases <2 months old who were included in our study died as a result of the infection. B. pertussis infection is estimated to cause 600 000 deaths worldwide annually, with a disproportionate number appearing in unvaccinated infants. Pertussis is particularly troublesome because it does not necessarily present itself in its commonly known classical stages. Therefore, in very young and non-immunized children, the disease may have a fulminant process characterized by severe leukocytosis, neurological involvement and serious cardiopulmonary failure that can be accompanied by pulmonary hypertension, persistent hypoxia and death (Paksu et al., 2013).

One of the four fatal cases included in our study had a RSV co-infection, which is not uncommon and can even be further complicated by co-infection with Bordetella parapertussis (Walsh et al., 2008).

The mechanisms underlying the specific defence against pertussis infection are still not completely understood (Giammanco et al., 2003). Preliminary evidence suggests that viral–pertussis co-infections are common in non-vaccinated infants. Co-infection with B. pertussis is observed in 8.5% of infants <6 months old that are hospitalized with viral bronchiolitis. To avoid underdiagnosis, pertussis should be considered in all non-vaccinated infants admitted for lower respiratory tract infection (Nuolivirta et al., 2010).

The four deaths included in this study occurred in young infants with atypical clinical manifestations and complicated clinical parameters. Approximately 90% of all deaths due to pertussis in the USA occurred in young infants (Paddock et al., 2008). The pulmonary histopathological examination revealed a descending infection dominated by necrotizing bronchiolitis, intra-alveolar haemorrhage and fibrinous oedema, with marked leukocytosis, and luminal aggregates of abundant leukocytes in small pulmonary arteries, veins and lymphatic vessels. Pertussis should be suspected in any infant death associated with marked leukocytosis, bronchopneumonia or refractory pulmonary hypertension, particularly in children aged ≤4 months. The recognized physiological responses of the infant lung to hypoxia suggest that B. pertussis pneumonia triggers a cascade of events including acute pulmonary vasoconstriction.
and a pertussis toxin-mediated increase in the circulating leukocyte mass. These responses ultimately compromise pulmonary blood flow, exacerbate hypoxaemia and create a vicious cycle of refractory pulmonary hypertension (Paddock et al., 2008).

In 2001, DTaP vaccination coverage amongst schoolchildren aged 6–8 and 9–11 years was 85.5 and 87.6%, respectively (Plans, 2005). However, despite high levels of vaccination coverage, pertussis circulation cannot be fully controlled. The results of these studies question the efficacy of current immunisation programmes (Campins-Martí & Moraga-Llop, 2004; Sala-Farré et al., 2013).

Further epidemiological research evaluating the impact of intervention strategies is crucial for prevention in susceptible populations (Miyashita et al., 2013). Future research should include studies of the impact of vaccination policies and recommendations, such as evaluation of the ‘cocoon’ strategy (Mooi et al., 2014), and assessment of vaccination effectiveness and efficacy. Microbiological research is also needed to assess a potential shift in circulating strains of B. pertussis.

Differences in epidemiological surveillance systems across Europe have highlighted the need for a global surveillance system that can improve the impact of prevention activities in European countries, as their estimated incidence figures are highly variable (Guiso et al., 2011; Koidl et al., 2007).

This study has various strengths and limitations. Its main strength is the fact that it is population-based. A possible limitation is the retrospective nature of the research, as all research data were from the past, although the collection of information was not retrospective. Despite this theoretical limitation, there do not appear to be any biases that might invalidate the study. In particular, no bias was observed in the results of the microbiological tests or in the selection of patients who were considered suspected cases.

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