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Pertussis-associated persistent cough in previously vaccinated children

Nicola Principi,[1] David Litt,[2] Leonardo Terranova,[1] Marina Picca,[3] Concetta Malvaso,[4] Cettina Vitale,[5] Norman K. Fry,[2] Susanna Esposito[6,*] for the Italian Pertussis Group for Persistent Cough in Children

Abstract

To evaluate the role of *Bordetella pertussis* infection, 96 otherwise healthy 7- to 17-year-old subjects who were suffering from a cough lasting from 2 to 8 weeks were prospectively recruited. At enrolment, a nasopharyngeal swab and an oral fluid sample were obtained to search for pertussis infection by the detection of *B. pertussis* DNA and/or an elevated titre of anti-pertussis toxin IgG. Evidence of pertussis infection was found in 18 (18.7 %; 95 % confidence interval, 11.5–28.0) cases. In 15 cases, the disease occurred despite booster administration. In two cases, pertussis was diagnosed less than 2 years after the booster injection, whereas in the other cases it was diagnosed between 2 and 9 years after the booster dose. This study used non-invasive testing to show that pertussis is one of the most important causes of long-lasting cough in school-age subjects. Moreover, the protection offered by acellular pertussis vaccines currently wanes more rapidly than previously thought.

Persistent cough, mainly secondary to infection, is a relatively common clinical problem in paediatrics [1]. It was previously shown that *Bordetella pertussis* is an important cause of this condition [2] and that school-age children and adolescents have the highest incidence of pertussis-associated persistent cough [3]. In these children, the cough is frequently different from that which characterizes typical pertussis cases and is only rarely accompanied by recurrent vomiting and exhaustion. Consequently, the diagnosis is delayed or never made, leading to dissemination of the infection and an incorrect evaluation of the true epidemiology of pertussis [4].

In recent years, it has been demonstrated that the protection offered by pertussis vaccination in the first year of life only lasts for 4–12 years, and this short period of protection is one of the main reasons for the resurgence of pertussis [5]. Consequently, many European countries have recommended the administration of a booster dose of pertussis vaccine at pre-school age and during adolescence [6]. However, the efficacy of this schedule may be debated because cases of pertussis among children who have received booster doses of vaccine have been reported [7, 8]. The main aim of the present study was to evaluate the role of *B. pertussis* infection in the determination of persistent cough in school-age children and adolescents, and to analyse whether children who have received booster doses of vaccine were protected from pertussis-associated cough.

This study was coordinated by the Pediatric Highly Intensive Care Unit of the University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and was carried out between April 2015 and January 2017. It was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy. Written informed consent was obtained from either the parent(s) or legal guardian(s) of each study participant, and the children aged >8 years signed to confirm their consent.

Otherwise healthy 7- to-17-year-old children who were suffering from a cough lasting from 2 to 8 weeks were prospectively recruited from 10 primary care paediatricians working in Italy. The duration of cough for inclusion was chosen to meet the definition of persistent pertussis according to the British Thoracic Society (i.e. a cough lasting 3 to 8 weeks) [9] and the criteria used by the World Health Organization (WHO) for the clinical case definition of pertussis (i.e. cough of at least 2 weeks) [10]. Children suffering from a disease frequently associated with chronic cough (i.e. cystic fibrosis, bronchiectasis, chronic heart failure and neurological diseases), those with known

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Author affiliations: [1]Pediatric Highly Intensive Care Unit, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [2]Respiratory and Vaccine Preventable Bacteria Reference Unit, Public Health England, London, UK; [3]Primary Care Paediatrician, Milan, Italy; [4]Primary Care Paediatrician, Latina, Italy; [5]Primary Care Paediatrician, Palermo, Italy; [6]Department of Surgical and Biomedical Sciences, Pediatric Clinic, Università degli Studi di Perugia, Perugia, Italy.

***Correspondence:** Susanna Esposito, susanna.esposito@unimi.it

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immunodeficiency and those with an underlying chronic disease were excluded. Moreover, to avoid the risk of considering pertussis cases in children with recent pertussis vaccination, patients who had been given the pre-school booster (PSB) vaccine against pertussis less than 1 year before the onset of cough were also excluded. Their pertussis vaccination status was established by consulting the official vaccination chart issued by the Regional Vaccination Service.

At enrolment, a nasopharyngeal swab and an oral fluid sample were obtained from each child by the primary care paediatricians with the intention of detecting *B. pertussis* genomic DNA in the nasopharynx and to determine the anti-pertussis toxin (anti-PT) IgG titre as an indicator of recent *B. pertussis* infection. The swabs were obtained using an ESwab kit and a polypropylene screw-cap tube with an internal conical shape filled with 1 ml liquid Amies medium (Copan Italia SpA, Brescia, Italy). Oral fluid was collected using an Oracol saliva collection kit (Malvern Medical Development Ltd, Worcester, UK). Both samples were immediately sent by mail to the central laboratory centre in Milan, where they always arrived within 48 h of collection. Nasopharyngeal samples were processed within 24 h for the detection of *B. pertussis* by means of a real-time PCR assay as described by Tatti KM *et al.* [11]. The oral fluid was eluted from the Oracol swab using transport medium [12] and then stored at −80 °C until shipment to Public Health England, Colindale, London, UK for testing. Once the sample arrived, it was analysed using an IgG antigen-capture enzyme-linked immunosorbent assay (GACELISA) for the detection of anti-pertussis toxin IgG. This GACELISA acts as a surrogate for anti-pertussis toxin IgG serology, and an oral fluid titre of 70 aU has been shown to be equivalent to a serology cutoff of 70 IU ml^{-1} [12]. Using these cutoffs, it has been demonstrated that the oral fluid assay can detect seropositive subjects with a sensitivity of 92.9 % and a specificity of 93.8 % [12]. Negative oral fluid titres ($\leq$70 aU) were only accepted as valid if the sample contained a total concentration of IgG $\geq$1 μg ml^{-1} [12].

The criteria for laboratory confirmation of pertussis infection were defined as the detection of *B. pertussis* genomic DNA extracted from the nasopharyngeal swab and/or the determination of an anti-pertussis toxin IgG titre >70 arbitrary units (aU) in the patient's oral fluid.

Initially, 100 children were enrolled. However, in four cases, the collection of oral fluid failed because an adequate volume of liquid was not obtained for analysis. In total, 96 patients (43 males and 53 females; mean age±SD, 9.6 ±3.1 years) were included in the study. All of them had received three doses of a three-component acellular pertussis vaccine when 3, 5 and 12 months old according to the immunization schedule recommended by the Italian Ministry of Health [13]. However, three children had not been given the recommended PSB at 5–6 years, and none of those $\geq$13 years old had received the adolescent booster dose.

Laboratory evidence of recent pertussis infection was found in 18 (18.7 %; 95 % confidence interval, 11.5–28.0) patients (Table 1). In two cases, the findings were based solely on the detection of *B. pertussis* DNA in the nasopharyngeal swab. In 13 cases, evidence of pertussis infection was supported by high (>70 aU) anti-pertussis IgG titres in the oral fluid. In

Table 1. Time elapsed since the pre-school pertussis booster vaccination in children with persistent cough and laboratory-confirmed pertussis

Age at pertussis diagnosis	Duration of cough (weeks)	*Bordetella pertussis* in nasopharyngeal swab	IgG-PT (aU)	Time since PBS
7 years and 3 months	5	No	92	19 months
6 years and 8 months	4	No	268	16 months
7 years and 6 months	5	No	202	2 years and 2 months
8 years and 6 months	6	No	78	3 years and 1 month
9 years and 5 months	2	Yes	0	3 years and 4 months
9 years and 2 months	6	No	130	3 years and 6 months
10 years and 1 month	5	No	147	4 years and 3 months
10 years and 2 months	6	No	>1000	4 years and 3 months
10 years and 3 months	6	No	98	4 years and 4 months
10 years and 3 months	3	Yes	491	4 years and 6 months
11 years and 6 months	6	No	134	5 years and 9 months
11 years and 6 months	3	Yes	391	6 years and 1 month
12 years and 1 month	3	Yes	358	6 years and 1 month
12 years and 4 months	6	No	738	6 years and 2 months
8 years and 6 months	6	No	414	7 years and 5 months (no PBS)
15 years and 2 months	2	Yes	4	9 years and 1 month
12 years and 1 month	7	No	308	11 years (no PBS)
14 years	6	No	243	13 years (no PBS)

IgG-PT, oral fluid anti-pertussis toxin IgG titre; PBS, pre-school pertussis booster vaccination.

three cases, the patients were positive for both of the studied indicators. *Bordetella pertussis* DNA was detected in children with a cough lasting only 2–3 weeks, whereas the patients with high oral fluid titres had coughs lasting 4–7 weeks. Pertussis infection was confirmed in the three children who had not received the PSB vaccination. In 15 cases, the disease occurred despite booster administration. In two cases, pertussis was diagnosed less than 2 years after the booster injection, whereas in the other cases the disease developed between 2 and 9 years after the booster dose (in eight children <5 years after vaccination).

The data collected during this study using noninvasive tests showed that pertussis is one of the common causes of long-lasting cough in school-age children and they are in agreement with the findings previously reported by studies performed in England, Wales, the Netherlands and New Zealand [7, 14, 15]. In this study, laboratory evidence of pertussis infection was found in approximately 18 % of enrolled children, even though all of them had received full primary vaccination against pertussis, and most had been given a booster pertussis vaccine dose immediately before entering school.

The majority of pertussis cases occurred in children who had received the last vaccine dose 4–6 years before cough onset. This is earlier than in the previous UK study [6], and indicates that the protection offered by the acellular pertussis vaccines currently wanes more rapidly than had previously been thought. Although the Italian and UK schedules are not identical, this suggests that administration of the booster doses according to the currently recommended schedule may be inadequate to provide long-term protection for all of the vaccinated subjects [7, 16]. Supporting this finding, a recent study reported that the efficacy of a booster dose at age 13 years was 68.8 % during the first year after vaccination and decreased to 8.9 % by $\geq$4 years after vaccination [8]. The limited duration of protection is further highlighted by the evidence that pertussis was diagnosed in three of the patients enrolled in our study only 18–24 months after vaccination. Although there is a possibility that residual antibodies from vaccination could potentially have affected the results of the oral fluid testing [12], leading to an improper diagnosis, it is highly likely that the infections diagnosed in these patients were true cases of pertussis. The individual titres of IgG antibodies against PT in oral fluid were substantially above the cut-off, with a high positive predictive value having previously been reported for this cut-off [12]. Further studies are needed to evaluate the true epidemiology of pertussis and the protection provided by the available vaccines. An easy-to-perform and noninvasive diagnostic measure, such as oral fluid testing, could facilitate the achievement of these objectives, improving the development of new vaccines and pertussis vaccination strategies.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy. Written informed consent was obtained from either the parent(s) or legal guardian(s) of each study participant, and children who were aged >8 years signed to confirm their consent.

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