

# *Bordetella pertussis* Infection Is Common in Nonvaccinated Infants Admitted for Bronchiolitis

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**Background:** Preliminary evidence suggests that viral-pertussis coinfections are common in nonvaccinated infants.

**Subjects and Methods:** *Bordetella pertussis* infection was studied by polymerase chain reaction in nasopharyngeal aspirates in 142 infants <6 months of age, who were admitted for bronchiolitis. Viral etiology, documented by antigen detection or polymerase chain reaction in nasopharyngeal aspirate, was respiratory syncytial virus (RSV) in 105, rhinovirus in 8, influenza A virus in 8, and other viruses in 10 cases. Only 11 samples were negative.

**Results:** *B. pertussis* infection was found in 12 (8.5%) cases, being coinfection with RSV in 8 (67%) cases (7.6% of all RSV infections). In a retrospective analysis, RSV-pertussis coinfections and sole RSV infections did not differ for the presence of cough. Preliminary evidence was found that a history of coughing spells was associated with *B. pertussis* identification.

**Conclusions:** Coinfection with *B. pertussis* was present in 8.5% of <6-month-old infants, who were hospitalized for viral bronchiolitis. To avoid underdiagnosis, pertussis should be considered in all nonvaccinated infants admitted for lower respiratory tract infection.

**Key Words:** *Bordetella pertussis*, bronchiolitis, infant, mixed infection, respiratory syncytial virus

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The simultaneous occurrence of respiratory syncytial virus (RSV) and *Bordetella pertussis* infection was first reported 20 years ago.<sup>1</sup> Currently, the development of polymerase chain reaction (PCR) tests for RSV and for *B. pertussis*, in particular, has allowed confirmation of this observation.<sup>2–5</sup> *B. pertussis* coinfection has been documented in from <1%<sup>6,7</sup> to 16%<sup>5</sup> of RSV infections in nonvaccinated infants. *B. pertussis* was identified in 23% of <5-month-old infants hospitalized for severe respiratory disorders like apnea and respiratory failure.<sup>2</sup>

Until the early, 2000, children developed pertussis in our country (National Institute for Health and Welfare, Finland) despite approximately 95% coverage with whole cell pertussis vaccine given at 3, 4, 5, and 24 months of age. In 2003, the vaccination program was modified and booster vaccination by acellular pertussis vaccine was given at 6 and 11 to 13 years of age.

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Since 2005, acellular pertussis vaccine has been given at 3, 5, and 12 months and at 4 and 14 to 15 years of age, resulting in a decrease of pertussis incidence in children of all ages.

Between 2001 and 2004, 205 infants aged <6 months were enrolled in a study of the viral etiology of bronchiolitis. Nasopharyngeal aspirates (NPAs) were obtained for antigen detection by immunofluorescence for 7 respiratory viruses and for genome detection by PCR for 9 respiratory viruses; both tests were available for RSV.<sup>8</sup> In 2009, 142 good-quality frozen NPA samples were available for *B. pertussis* PCR. To supplement prospectively collected data (A. Halkosalo et al, unpublished data),<sup>8</sup> data on cough symptoms were collected retrospectively from the patients' records. The aim of the study was to evaluate how often respiratory viral infections, RSV infections in particular, are mixed infections with *B. pertussis* in infants with bronchiolitis.

## MATERIALS AND METHODS

A total of 205 healthy full-term infants younger than 6 months of age, who were hospitalized for bronchiolitis in the Department of Pediatrics, Tampere University Hospital (Finland) between December 1, 2001 and May 31, 2002 and between October 28, 2002 and May 31, 2004, were enrolled in the study. The Ethics Committee of the Tampere University Hospital District approved the study. An informed consent was obtained from parents before enrolling the children.

Bronchiolitis was defined as acute lower respiratory illness characterized by rhinorrhea, cough, and diffuse wheezes or crackles. NPA were obtained from all infants for antigen detection by indirect immunofluorescence for RSV, influenza A and B virus, adenovirus and parainfluenza virus 1, 2, and 3, according to routine practice at the hospital laboratory.<sup>8</sup> In addition, reverse transcription (RT) PCR was used for detection of the same viruses and also of rhinovirus<sup>8</sup> and human metapneumovirus<sup>8</sup> and human bocavirus (A. Halkosalo et al, unpublished data). For the present analysis, combined results of immunofluorescence and RT-PCR were used.

One year after bronchiolitis, 129 children (63%) attended the follow-up visit in 2003 to 2005, at an average of 18 months of age (range: 13–24). The parents had recorded prospectively the illness history of the child in diaries during the follow-up period. At the time of the follow-up visit, a questionnaire was used to register the data on postbronchiolitis episodes of wheezing, ear infections, and inserted tympanostomy tubes, as well as on the use of antibiotics, bronchodilators, and corticosteroids.<sup>9</sup>

In 2009, good-quality frozen NPA samples were available for *B. pertussis* PCR from 142 (69%) children hospitalized for bronchiolitis 5 to 8 years earlier. *B. pertussis* was studied by an in-house PCR at National Institute for Health and Welfare, Turku (Finland), as described earlier in more detail.<sup>10</sup> One of the authors (P.K.) reviewed retrospectively the patient cards of the hospital and classified, being not aware of the viral or pertussis findings, the cough symptoms present on admission into 3 categories: cough with spells, cough without spells, and no cough. In addition, the use of antibiotics, with special focus on macrolides effective for pertussis, was registered. All infants were <6 months old; 96

(67.6%) infants were entirely nonvaccinated for <3-month olds and 46 were 3- to 5-month olds who were vaccinated once or twice. Thus, no 5- to 6-month-old children had completed the primary pertussis vaccination series.

Student *t* test and Fisher exact test were used in the statistical analyze of the data.

## RESULTS

*B. pertussis* involvement was found in 12 of 142 (8.5%) infants hospitalized for bronchiolitis. Their mean age was 10.4 weeks (SD: 5.1) compared with 9.9 weeks (SD: 6.6) in pertussis-negative infants, and 8 of 12 (67%) were girls. Pertussis-positive infants were not more likely to have older siblings than infants with viral bronchiolitis alone. All infants with *B. pertussis* involvement were <20 weeks old (Table 1).

Pertussis was associated with RSV in 6 cases, with RSV and another virus in 2 cases, and with other viruses than RSV in additional 3 cases (Table 1). Therefore, *B. pertussis* was identified in 8 (7.6%) of all 105 RSV infections. Only 1 case was solely associated *B. pertussis* infection.

Overall, 135 (95%) infants presented with cough; 24 had coughing spells and 111 had cough with no spells (Table 2). Coughing spells were present in 5 (41.7%) pertussis-positive infants compared with 19 (14.6%) pertussis-negative infants (*P* = 0.038). The 1 infant with *B. pertussis* infection alone had coughing spells.

There were no differences in the clinical findings for those with only viral infection compared with *B. pertussis* coinfection. The need or duration of supplementary oxygen, feeding support, and presence of fever, need, and duration of inhalation therapy or duration of intensive care or hospital treatment were similar (Table 3). Only 2 pertussis-positive infants (17%) were treated with macrolides and 5 (42%) were treated with other antibiotics (vs. 51% of pertussis-negative ones, *P* = 0.163).

Twelve months after bronchiolitis, 114 of 142 (80%) children attended the control visit, at which time the parents reported doctor-diagnosed postbronchiolitis wheezing in 39 (34%) cases and use of steroids for wheezing in 22 (19%) cases. There was no significant difference between the pertussis-positive and pertussis-negative cases

**TABLE 1.** Age, Gender, and Viral Findings in Infants With Bronchiolitis, in Relation to the Presence or Absence of *Bordetella pertussis* by Polymerase Chain Reaction

Findings	<i>B. pertussis</i> Positive (N = 12)	<i>B. pertussis</i> Negative (N = 130)
Age in weeks, mean (SD)	10.4 (5.1)	9.9 (6.5)
Boys/girls	4/8	67/63
Older siblings	8 (67%)	91 (70%)
Breastfeeding	6 (50%)	91 (70%)
Age <4 weeks	1 (8%)	31 (24%)
Age 5–8 weeks	4 (33%)	33 (25%)
Age 9–12 weeks	1 (8%)	26 (20%)
Age 13–16 weeks	4 (33%)	16 (12%)
Age 17–20 weeks	2 (17%)	11 (9%)
Age 21–24 weeks	0 (0%)	13 (10%)
Viral infection	11 (92%)	120 (92%)
RSV	8 (67%)*	97 (81%)
Rhinovirus	1 (8%)	7 (6%)
Influenza A	1 (8%)	7 (6%)
Other viruses	1 (8%)†	9 (7%)

\*RSV with bocavirus in 1 case and RSV with adenovirus in 1 case.

†One case with parainfluenza 3 virus.

Statistical significance: no significant differences between the groups.

RSV indicates respiratory syncytial virus.

**TABLE 2.** Respiratory Syncytial Virus (RSV) and *Bordetella pertussis* Findings in Infants in Relation to the Presence of Cough

Microbe Findings	Group 1 (Coughing Spells) (N = 24)	Group 2 (Cough, No Spells) (N = 111)	Group 3 (No Cough) (N = 7)
<i>B. pertussis</i> + RSV–	2	2	0
<i>B. pertussis</i> + RSV+	3	5	0
<i>B. pertussis</i> – RSV+	15	77	5
<i>B. pertussis</i> – RSV–	4	27	2
All <i>B. pertussis</i> +	5 (21%)*	7 (6%)†	0 (0%)

\**P* = 0.038 versus group 2; †*P* = 0.644 versus group 3.

**TABLE 3.** Treatment During Hospitalization in the Infants With *Bordetella pertussis* Infection

	<i>B. pertussis</i> Positive (N = 12)	<i>B. pertussis</i> Negative (N = 130)
Supplementary oxygen	4 (29%)	24 (19%)
Duration of oxygen therapy*	4 (1–10)	3.5 (1–14)
Feeding support	5 (36%)	49 (38%)
Duration of feeding support*	7.8 (3–13)	4.5 (1–12)
Duration of hospital stay*	6.8 (3–22)	4.7 (1–15)
Intensive care treatment	1 (8%)	0
Duration of intensive care*	5 (5)	0
Antibiotics	7 (58%)	51 (39%)
Macrolide	2 (29%)	1 (2%)
Amoxicillin	4 (68%)	39 (76%)
Penicillin	1 (14%)	7 (14%)
Cephalosporin	0	3 (6%)

\*Duration in days, mean (range).

Statistical significance: no significant differences between the groups.

**TABLE 4.** Incidence of Pertussis in Children (Cases/100,000) in the Study Years 2001–2004, in the Year 2006 and in the Year 2009 in Finland

Year	Age 0–4 yr	Age 5–9 yr	Age 10–14 yr
2001	7.9	16.2	27.3
2002	24.5	40.7	41.3
2003	53.7	100.2	109.0
2004	82.3	128.5	179.0
2006	10.4	7.9	6.5
2009	9.8	7.0	7.1

Data obtained from the National Infections Register of Finland, maintained by the Institute of Health and Welfare, Helsinki, Finland. Both physicians and laboratories are obligated to report microbiologically verified pertussis cases in the register.

(data not shown). Likewise, there were no significant differences between the groups in the use of antibiotics, ear infections, or inserted tympanostomy tubes after bronchiolitis (data not shown).

During the study years 2001 to 2004, the incidence of pertussis, based on laboratory reports was constantly increasing in Finnish children of all ages (Table 4). The respective incidence rates and their increases in the primary area of our hospital were similar (data not shown). In 2006, 3 years after the first modification of the pertussis vaccination program and 1 year after the final change of the vaccination program, the figures were lower than in 2001 (Table 4).

## DISCUSSION

There are 3 main results in the present study. First, pertussis diagnosed by PCR was surprisingly common, nearly 10%, in

nonvaccinated infants hospitalized for bronchiolitis. Second, two-thirds of the cases had mixed infections with RSV. The findings are similar to another study from Finland,<sup>4</sup> in which 8% of <6-month-old bronchiolitis patients had pertussis by PCR during an RSV epidemic. Similarly, more than 20% of infants requiring intensive care during an RSV epidemic had pertussis in a study from the United Kingdom.<sup>2</sup> Third, our preliminary evidence suggests that the coughing spells were associated with *B. pertussis* coinfection. However, RSV and pertussis cases could not be separated by the presence of cough, in agreement with the 20-year-old observation from the United States<sup>1</sup> and with newer studies from the United Kingdom and Finland.<sup>2,4</sup> Therefore, the typical features of RSV bronchiolitis, or even the detection of RSV etiology, do not exclude pertussis in nonvaccinated infants.

In the 1980s, Nelson et al studied upper respiratory secretions by culture and direct fluorescent antibody assay, for RSV and *B. pertussis* in 180 infants hospitalized for respiratory tract infection.<sup>1</sup> Pertussis was identified in 29 (15%) cases; 15 were mixed infections with RSV and 14 were single *B. pertussis* infections. Pertussis was associated with prematurity, but there were no other significant differences between infants with pertussis alone, RSV infection alone, or mixed pertussis-RSV infection. About 20 years later, Crowcroft et al<sup>2</sup> studied *B. pertussis* culture and PCR in 126 infants aged <6 months requiring intensive care for lower respiratory tract infection; 25 (20%) had pertussis and 9 were mixed infections with RSV. Infants with pertussis suffered from cough, apnea, and whooping cough more often than infants without pertussis.<sup>2</sup> Korppi and Hiltunen<sup>4</sup> studied viral etiology of infection by antigen detection in 117 infants hospitalized for bronchiolitis at <6 months age and *B. pertussis* etiology by PCR in those 88 in whom parents or nurses reported cough. *B. pertussis* was identified in 9 (8%) infants, and 7 were mixed infections with RSV. In a recent study from France, PCR to *B. pertussis* was positive in 16% of <4-month-old children hospitalized for RSV infection.<sup>5</sup> In the present study, pertussis was not clinically suspected in any bronchiolitis case, but nearly 10% of them proved to be *B. pertussis* positive by PCR. Thus, all these studies suggest that pertussis is underdiagnosed in infants with respiratory infection, including infants with presumably viral bronchiolitis and with positive RSV identification.

There are 2 recent studies from the United States with opposite results. Siberry et al found only 1 *B. pertussis* positive case by PCR in 166 infants admitted to hospital during RSV season,<sup>6</sup> and Walsh et al found no *B. pertussis* positive cases by PCR in 204 infants <18 months of age presenting with bronchiolitis.<sup>7</sup> The results from the United States suggest that screening of *B. pertussis* is not useful if the prevalence of pertussis is low, as was the case in those study populations. Our results from 2001 to 2004 represent an era of a higher pertussis incidence than currently the case in Finland. The incidence decreased substantially in children at all ages after the 2 alterations in the national vaccination program in 2003 and 2005. Currently, the last booster vaccination is given at 14 to 15 years of age in our country. The occurrence of pertussis needs continuous monitoring in different age groups, including young adults also with no regular booster vaccinations against pertussis.

The in-house PCR used in the present study has been sensitive and specific in diagnosing pertussis. The test was positive in 57 (48%) of 117 nasopharyngeal swabs obtained from elementary school children during an outbreak, including all 6 culture-positive cases.<sup>10</sup> During another outbreak, PCR was positive in 18 (45%) of 40 NPA samples obtained from children with susceptible pertussis, including all 3 culture-positive cases and 5 (35%) of the 14 seropositive cases.<sup>10</sup> In contrast, the high sensitivity of PCR

carries a risk of positive findings that are not clinically significant. However, this risk is less in infants than in older children. In addition, the percentage increase in laboratory confirmed pertussis cases diagnosed by PCR compared with culture was moderate, ranging from 9% to 26%, in a multicenter 5-year study in <6-month-old infants from the United Kingdom.<sup>11</sup>

Preliminary evidence was found that coughing spells as such are not diagnostic for pertussis. In addition, there were no significant differences between *B. pertussis* positive and negative cases in the severity of the disease, although estimated by many objective measures. In a recent retrospective study from Israel, *B. pertussis* was identified by PCR in 11 (15%) of 74 <12-month-old infants treated in the pediatric intensive care unit.<sup>12</sup> The pertussis-positive and -negative cases differed similar to our results, only for presence of paroxysmal and prolonged cough. Our pertussis-positive group, like the respective groups in many other studies,<sup>4,5,12</sup> was quite small, being underpowered to reveal small albeit real differences between the groups. In the present study, all infants improved despite the fact that antibiotics effective for pertussis were not used. The patients of the present study were collected prospectively, but data on cough history and antibiotic treatment were retrospective. In future, prospective studies powered sufficiently to allow multivariate, and subgroup-specific stratified analyses are needed to answer to these 2 important questions: the clinical value of coughing history and the effectiveness of antibiotics.

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