

A serologic study of organisms possibly associated with pertussis-like coughing

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Objective. To assess the frequency of serologic evidence for an infection with microorganisms other than *Bordetella pertussis* in children with pertussis-like coughs.

Methods. The study was performed within a protective efficacy trial of an acellular pertussis vaccine. Children who coughed for >7 days and had no laboratory evidence of recent infection with *B. pertussis* were eligible for the present study. Antibodies to *Mycoplasma*, *Chlamydia*, respiratory syncytial virus and influenza viruses A and B were measured by complement fixation, and antibodies to adenovirus and parainfluenza viruses 1, 2 and 3 were measured by enzyme-linked immunosorbent assay (ELISA) in acute and convalescent serum samples. Significant titer rises (4-fold titer rise in complement fixation, 100% increase of units in ELISA) and concentrations of antibodies beyond age-specific reference values were regarded as indicative of recent infection. In some children IgM antibodies to Epstein-Barr virus and to cytomegalovirus were also measured by ELISA.

Results. A total of 149 of 1179 (12.6%) children had no laboratory evidence of *B. pertussis* infection. Serologic evidence for other infections were found in 56% (83 of 149). Adenovirus (33), parainfluenza viruses 1, 2 and 3 (18), *Mycoplasma pneumoniae* (15) and respiratory syncytial virus (14) were most common. Of this group 48% had been vaccinated against pertussis.

Conclusion. We present data that a proportion of pertussis-like coughs in children may be caused by adenovirus, parainfluenza viruses, re-

spiratory syncytial virus and *Mycoplasma*. The differential diagnosis of pertussis-like coughs by laboratory methods should include these infections, especially in vaccinated children.

INTRODUCTION

Paroxysmal coughing is the primary symptom in the clinical diagnosis of pertussis and the mainstay of the WHO definition of the disease.¹ Nevertheless, it has been suggested previously that pertussis-like coughing can also be observed during infections with other microorganisms, such as *Bordetella parapertussis*, adenoviruses^{2, 2a, 3} and *Mycoplasma*.⁴ Whereas data on the role of *B. parapertussis* are available,⁵ the role of other microorganisms in causing paroxysmal pertussis-like coughs of longer duration has not been elucidated.

We used a field trial of the efficacy of an acellular pertussis vaccine⁶ to evaluate the differential diagnosis of pertussis-like coughs serologically in acute and convalescent sera of children who presented with prolonged coughing (≥ 7 days) and without laboratory evidence of *Bordetella* infection. We measured antibodies to adenovirus, parainfluenza virus (HPIV) types 1, 2 and 3, influenza viruses A and B, respiratory syncytial virus (RSV), *Mycoplasma pneumoniae* and *Chlamydia* spp. A titer increase and/or antibody concentrations beyond the 95% confidence interval were regarded as indicative of a recent infection with the microorganisms tested.

MATERIAL AND METHODS

Setting. The protective efficacy trial⁶ was performed in six areas of Germany. It was designed as a prospective household contact study. Pertussis diagnostics were offered free of charge to everyone in the study areas, who presented with prolonged (≥ 7 days) coughs.

Diagnostic procedures. For laboratory confirmation of pertussis, the physicians took nasopharyngeal swabs and drew an acute phase blood sample. A convalescent blood sample was taken from all patients with a negative culture for *Bordetella pertussis*. Families with household contacts were monitored by regular standardized telephone interviews for a period of 56 days after the onset of cough in the last family member.

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Key words: Pertussis-like, adenovirus, parainfluenza virus, *Mycoplasma*, serology.

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If anyone of the household developed respiratory symptoms, pertussis diagnostics were initiated. In all contacts with cough, the duration of cough, of paroxysmal cough and of other symptoms such as whooping and vomiting were also recorded.

Serology. *Bordetella* serology was performed with a standardized enzyme-linked immunosorbent assay (ELISA), measuring antibodies of isotypes IgG and IgA to pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (69 kDa) as described elsewhere.⁷ The primary pertussis case definition, focusing on a high specificity, required an increase of antibodies to PT and/or FHA of 100% to at least 8 units/ml. For this study secondary definitions of positive *Bordetella* serology were used focusing on the sensitivity of definitions (see below), the results of which are shown in Table 1. The application of these definitions resulted in an increase of *Bordetella*-positive serum pairs from 697 to 821, a total of 69.6% of all cases having a positive *Bordetella* serology.

Sera negative for *Bordetella* infection (see below) were tested for antibodies to *Mycoplasma*, *Chlamydia*, RSV and influenza viruses A and B by standardized microtiter complement fixation (Behringwerke AG, Marburg, Germany), and antibodies to adenovirus and parainfluenza viruses 1, 2 and 3 were measured by ELISA (BAG, Lich, Germany). Acute and convalescent samples from every patient were analyzed in parallel in the same run.

Study population. Subjects ages 0 to 18 years were selected from the original efficacy study population according to the following criteria: A, availability of paired sera and convalescent sample taken 4 to 14 weeks after the acute sample; B, negative culture for *B. pertussis* and *B. parapertussis*; C, negative *Bordetella* serology, which meant no titer increase (<20%) or titer decrease (<20%) of antibodies to PT, FHA and pertactin. The goal was to exclude all subjects who showed a variation in antibody concentration to *Bordetella* antigens that could not be explained by methodologic variance (intraassay coefficient of variation, 7 to 14% depending on methods); D, concentration of antibodies

to *Bordetella* antigens was in the 2 SD range (log normal distribution) of an age-related group with no signs of *Bordetella* infections.

A total of 149 (12.6% of all) subjects fulfilled the above criteria, and their sera were used in this study.

Another 209 subjects (17.8%) showed an increase or a decrease of *Bordetella*-specific antibodies in the range between $\pm 20\%$ and $\pm 50\%$. From this group 50 serum pairs were chosen at random and tested for antibodies to adenovirus, HPIV1, HPIV2 and HPIV3 to verify the specificity of results in the group with negative *Bordetella* serology.

In the sera with a negative *Bordetella* serology, significant titer rises (4-fold titer rise in complement fixation, 100% increase of units in ELISA) were regarded as indicative of recent infection. A recent contact to the infectious antigen was also assumed when the concentration of antibodies in both sera exceeded an age-specific reference range. This range was defined as >2 SD (log normal distribution) for all ELISA methods and as the 95% confidence interval for antibodies measured by complement fixation. For complement fixation antigens, antibodies titers of >1:160 for *M. pneumoniae* and >1:80 for RSV, influenza A and influenza B were regarded as indicative. Samples that showed a seroconversion to more than one microbial antigen were also tested for IgM antibodies to Epstein-Barr viral capsid antigen (Fresenius, Bad Homburg, Germany) and IgM antibodies to cytomegalovirus antigen (Medac, Hamburg, Germany) by ELISA to exclude a possible polyclonal stimulation.

Statistical evaluation. Differences between groups were tested for significance by Fisher's exact test.

RESULTS

Table 2 shows serologic data from 149 children who had pertussis-like coughs with a negative pertussis serology. Increase of antibodies or high concentrations of antibodies to adenoid antigen ($n = 39$), to HPIV antigens ($n = 29$), to *Mycoplasma pneumoniae* antigen ($n = 15$) and to RSV ($n = 14$) were most frequently detected. In 5 of 8 patients who showed a simultaneous response to adenovirus and to HPIV 1, 2 and/or 3, serologic evidence for adenovirus infection was also found in family

TABLE 1. Breakdown of children in vaccine efficacy trial with paired serum samples

	n	%
Total	1179	100
Positive serology for <i>Bordetella pertussis</i>		
Primary case definition	697	59.1
Secondary definitions		
Plus >50% increase of IgG-PT	20	1.7
Plus >50% increase of IgG-FHA	35	3.0
Plus antibodies to pertactin	18	1.5
Plus >50% decrease of IgG-PT	46	3.9
Plus >50% decrease of IgG-FHA	5	0.4
Total positive <i>Bordetella</i> serology	821	69.6
Total indeterminate* <i>Bordetella</i> serology	209	17.8
Total negative <i>Bordetella</i> serology	149	12.6

* For definition see text.

TABLE 2. Serologic findings in 149 patients with negative pertussis serology

Serologic Evidence for Infection with	n	%
Adenovirus	33	22
Parainfluenzavirus 1, 2, 3	18	12
<i>Mycoplasma pneumoniae</i>	11	7
RS virus	10	7
Epstein-Barr virus	4	3
Influenza B	4	3
Influenza A	3	2
Negative for all agents tested	66	44

contacts, and these cases were regarded as adenovirus infections. In serum samples from 4 children antibodies to *Mycoplasma* antigens were detected in high titers ($>1:320$) already in the acute serum, and these children also showed a response to adenovirus ($n = 2$) or HPIV 1, 2 or 3 ($n = 2$) but were regarded as *Mycoplasma* infections. In 4 children responses to multiple antigens were assumed to reflect a polyclonal stimulation caused by a recent Epstein-Barr virus (EBV) infection, which was substantiated by the detection of IgM-anti-EBV capsid antigen. Thus a total of 33 patients showed evidence for recent contact with adenovirus (24 seroconversion, 9 high titer), 18 for HPIV 1, 2, 3 (13 seroconversion, 5 high titer), 11 for *M. pneumoniae* (3 seroconversion, 8 high titer), 10 for RSV (9 seroconversion, 2 high titer) and 11 for other agents. No single serum pair showed an increase or a high concentration of antibodies to *Chlamydia* antigens and no IgM antibodies to cytomegalovirus could be found. In 66 paired sera (44%) no titer increase and/or high antibody titer against any of the antigens tested was found. Overall 31 children (21%) had a household contact with a confirmed pertussis case. Of these contact children 12 showed no titer increase and/or high antibody concentration to any of the antigens tested and 18 children showed responses to 1 or more of the antigens (6 adenovirus, 5 HPIV, 3 *M. pneumoniae*, 2 RSV, 1 influenza A, 1 influenza B) ($P > 0.1$ between groups).

To verify the specificity of the serologic results in the group with negative pertussis serology, 50 randomly selected serum pairs from the group with indeterminate pertussis serology were also tested for antibodies to adenoid and HPIV 1, 2 and 3. Among these one pair had high titers of antibodies to adenoid (2%, $P < 0.001$ as compared with the pertussis negative group), and two pairs (4%, $P < 0.001$ as compared with the pertussis negative group) fulfilled our criteria for a recent contact to HPIV.

The age distribution of children who were seropositive for adenovirus or seropositive for other antigens as compared with those seropositive for *Bordetella* is shown in Figure 1. Adenovirus-infected children displayed an age distribution similar to that of pertussis cases, although children ages 6 to 24 months were relatively more often infected with adenovirus. The age distribution of children positive for other agents in comparison to children with laboratory evidence for pertussis showed a distribution similar to that of pertussis cases.

The gender distribution of adenovirus (45% female) was similar to that of pertussis (52% female), but it differed in those children positive for other agents (64% female) and in the group with no laboratory evidence of recent antigenic contact (36% female). With the excep-

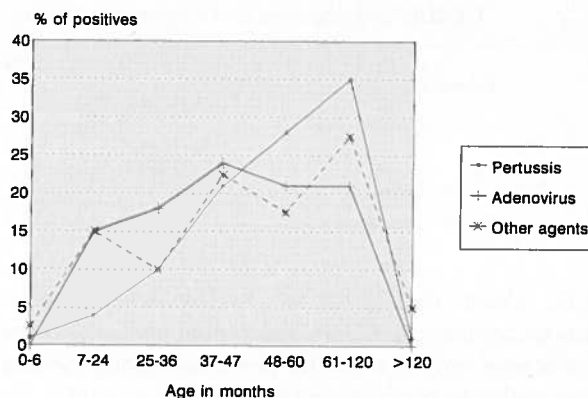


FIG. 1. Age distribution of children with laboratory evidence for pertussis and for adenovirus infection.

tion of RSV infections, which were found only during the winter months, no seasonal pattern could be observed.

Figure 2 shows the duration of paroxysmal coughs in children seropositive for adenovirus as compared with that in children with laboratory evidence for *B. pertussis* infection. Although adenovirus cases had significantly ($P < 0.05$) shorter paroxysms than did pertussis cases, they coughed for a mean of 18 days (pertussis, 35.1 days), and 50% of the children with adenovirus infection would have satisfied the clinical criteria (>21 days of paroxysmal coughing) for pertussis.

Table 3 shows the frequency of whooping and vomiting as well as the pertussis vaccination status of children seropositive for adenovirus, and those seropositive for other agents compared with children with laboratory evidence for pertussis and with the seronegative group. Whereas whoops were significantly ($P < 0.01$) more frequent in pertussis cases, vomiting was a frequent symptom of adenoviral infections ($P > 0.05$) as well in the other infections tested ($P > 0.05$).

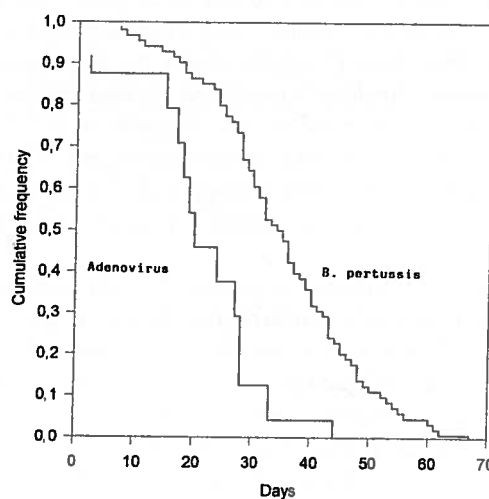


FIG. 2. Duration of paroxysmal cough in children with laboratory evidence for pertussis and for adenovirus infection.

TABLE 3. Symptoms and vaccination status of children seropositive for pertussis and other agents

Symptom	Serology Positive (%) for			Serology Negative (%)
	Pertussis	Adenovirus	Other agents	
Whoops	79 (76/96)*	20 (3/15)	15 (2/13)	17 (4/24)
Vomiting	76 (73/96)	53 (8/15)	31 (4/13)	33 (8/24)
Pertussis vaccination	6 (7/112)	47 (7/15)	46 (6/13)	54 (13/24)

* Numbers in parentheses, number per total.

Finally, about one-half of all children with negative pertussis serology had been vaccinated against pertussis, whereas only 6% in the pertussis group had received acellular pertussis vaccination ($P < 0.001$).

DISCUSSION

This study was restricted to those children in whom a recent contact to *Bordetella* could be excluded by laboratory methods. The requirements for laboratory confirmation of *Bordetella* infection in the efficacy trial were developed to assure the highest possible specificity, whereas this study needed a definition with the highest possible sensitivity. Therefore in addition to the trial case definition we considered all children with titer increases of $>50\%$,^{8,9} those with titer decreases of $>50\%$ ¹⁰ and those with atypically high concentrations of *Bordetella*-specific antibodies⁸ as positive for *Bordetella* serology. This resulted in an increase of putative pertussis cases of 15.1% (697 to 821).

Another cohort of 209 children had an indeterminate (see Materials and Methods) *Bordetella* serology. Testing 50 randomly selected serum pairs from this group showed that a serologic evidence for adenovirus or HPIV infections was significantly ($P < 0.001$) less frequent in this group than in the group with a negative *Bordetella* serology. Thus we assume that even small titer changes to *Bordetella* antigens can sometimes be indicative of recent infection.

The remaining cohort with negative *Bordetella* serology comprised a total of 149 children, which made up 12.6% of the overall cohort. The overall proportion of cases in this cohort, which could be diagnosed by serologic tests for other agents (53%), was comparable with data published previously. Bassett et al.¹¹ found an agent in 31.5% of lower respiratory tract infections by serologic methods, and Korppi et al.¹² established a diagnosis in 43% of 183 coughing Finnish children by serology.

As expected from previous data^{2, 2a, 3} adenovirus was the most frequently encountered agent in pertussis-negative children with pertussis-like coughs, which are thought to be mostly caused by adenovirus type 9.³ *M. pneumoniae* infections were shown to be a possible cause of pertussis-like coughs by Davis et al.⁴ HPIV and RSV, however, have previously not been associated with pertussis-like coughs. Concerning the diagnostic procedure, infections with adenovirus, HPIV and *M.*

pneumoniae can be diagnosed by serologic methods.^{3, 12} RSV infections, however, are best diagnosed by antigen or virus detection in nasopharyngeal specimens,^{2, 2a, 3, 12} and serologic methods lack sensitivity. In our study we had no opportunity to test adequate specimens for direct detection, and thus we may have systematically underestimated the frequency of RSV infections. In four children antibody increases to various antigens could be explained by a possible polyclonal stimulation during a primary infection with EBV. An association of EBV with lower respiratory tract infections in children had been assumed by Ray et al.,¹³ but these authors were also unable to clarify the exact role of EBV in these infections.

The demography of adenovirus, HPIV, RSV and *Mycoplasma* infections did not show significant differences from that seen in the pertussis group. A seasonal pattern could not be observed, apart from RSV infections, which occurred only during winter months. The cohort would have been too small to detect the seasonal fluctuations in HPIV infections described by Knott et al.¹⁴

Although children with adenovirus coughed for a significantly shorter time than "pertussis children," the clinical severity of adenovirus infections is shown by the fact that $>50\%$ of this group would have fulfilled the WHO criteria for pertussis¹ and $>80\%$ would have fulfilled the CDC's clinical criteria (14 days or more of paroxysmal cough).^{15, 16} Furthermore it was astonishing that 20% of children without serologic evidence of "whooping cough" had whoops. Vomiting after a paroxysmal attack occurred as expected in four-fifths of *B. pertussis* infections but also in $>50\%$ of the adenovirus cases. Comparing the vaccination status of the children showed that both the group that was seropositive for other agents and the group seronegative for other agents were significantly ($P < 0.01$) more often vaccinated against pertussis than the pertussis group (6%).

Account should be made for the fact that these data were generated at a time when pertussis was still endemic in Germany with an estimated incidence rate of $>100/100\ 000$ population per year.¹⁷ In countries with a low endemicity of pertussis such as the US,¹⁸ it can be assumed from our data that an estimated 70% of pertussis-like coughs could be attributed by testing for antibodies to adenovirus, HPIV, RSV and *Mycoplasma*. In conclusion our data show that a significant propor-

tion of pertussis-like coughs in children are associated with adenovirus, parainfluenza viruses, respiratory syncytial virus and *Mycoplasma*. These infections should be considered as a differential diagnosis for *B. pertussis* infections, especially in vaccinated children.

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The first of these is the fact that the majority of the cases of this disease are reported from the United States and Europe. It is interesting to note that the disease is not reported from any of the tropical or subtropical regions. This fact is of interest because the disease is thought to be caused by a parasite which is widely distributed in the tropics. The fact that the disease is not reported from these regions may be due to the fact that the parasite is not found in these regions, or it may be due to the fact that the disease is not recognized in these regions. The fact that the disease is not reported from these regions is of interest because the disease is thought to be caused by a parasite which is widely distributed in the tropics. The fact that the disease is not reported from these regions may be due to the fact that the parasite is not found in these regions, or it may be due to the fact that the disease is not recognized in these regions.

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