A Controlled Study of the Relationship Between *Bordetella pertussis* Infections and Sudden Unexpected Deaths Among German Infants

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ABSTRACT. **Objective.** This was a prospective, controlled, multicenter study to investigate the relationship between *Bordetella pertussis* infections and sudden unexpected deaths among German infants.

**Design.** Between 1995 and 1997, all infants who died at 7 to 365 days of age and for whom autopsies were performed in 1 of 8 participating institutes of legal medicine were enrolled. During a standardized autopsy, nasopharyngeal specimens (NPSs) and tracheal specimens were obtained for polymerase chain reaction (PCR) assays to detect *B pertussis*. The oligonucleotide primers FPt1 and FPt2, which specifically amplify a 191-base pair DNA fragment of the pertussis toxin operon of *B pertussis*, were used. Two control subjects (matched according to residence, age, gender, and nationality) were enrolled for each case subject, via a network of pediatricians in private practice, and NPSs were obtained from those infants. Parents of case subjects and control subjects were asked to provide specific information on respiratory illnesses of the child, contact with a known case of pertussis, or close contact with a person with a cough illness during the 4 weeks before death or enrollment, as well as the child’s pertussis immunization status. The pathologists performing the autopsies were unaware of the PCR results.

**Results.** Enrolled were 254 infants (66% male) with sudden unexpected deaths and 441 matched control subjects. Autopsies according to protocol were performed for 234 of the case subjects (92%); a diagnosis of sudden infant death syndrome (SIDS) was made for 76%. For the remaining subjects, causes of death were respiratory or other infections (14%), congenital anomalies or organ failures (4%), aspiration (2%), or accidents or traumatic events (4%). PCR results were positive for *B pertussis* for 12 case subjects (5.1%) (all with SIDS or respiratory infections) and 5.3% of control subjects. Of the 12 case subjects with positive PCR results, 10 (83%) were male. Questionnaires had been returned by the parents of 5 of the 12 infants. Three had experienced a respiratory illness (all with cough), beginning 7, 14, and 19 days before death. None had a known contact with a case of pertussis. Four of 15 control infants (27%) with positive PCR findings for *B pertussis* had a cough illness, including possible pertussis, and 2 of those 4 developed typical symptoms (whooping). Background information was received from 116 parents (46%) of case subjects and from parents of all control subjects. Upper respiratory tract infections within 4 weeks before death were reported for 53% of case subjects and 38% of control subjects. Also, fewer case subjects (33%) than control subjects (68%) had received age-adequate numbers of pertussis vaccine doses.

**Conclusions.** The concept of infection as a factor in SIDS is supported by a number of observations, including the seasonal distribution of the occurrence of SIDS; the high incidence of concurrent upper respiratory tract infections among infants dying as a result of SIDS; the peak age at 3 to 4 months; nicotine use in a child’s household, which predisposes children to respiratory infections such as otitis media; and the protective role of breastfeeding. A prominent role might be suspected for *B pertussis*, for several reasons. 1) *B pertussis* infections in infancy are frequently associated with apneic spells, which are occasionally life-threatening and, if leading to death, might be reported as SIDS. 2) Epidemiologic evidence from the United Kingdom, Sweden, and Norway indicates that SIDS is associated with *B pertussis* infection. 3) In a previously published study, we detected *B pertussis* DNA in the nasopharynx of 9 of 51 consecutive infants (18%) with sudden unexpected deaths. This is the first prospective, controlled study to investigate the possible etiologic role of *B pertussis* in SIDS. Clinically unrecognized *B pertussis* infections were relatively frequent (5.3%) among control infants during the course of our study. The rate of infection was similar or perhaps greater for control subjects, compared with case subjects (1.7%), when only NPS results were compared. This may seem surprising but is supported by other studies, in which asymptomatic infections or mild respiratory illnesses were observed among infants exposed to *B pertussis*. Careful autopsies, including histologic evaluations of organ specimens and use of PCR to detect *B pertussis* in NPSs and tracheal specimens, represented a strength of this study. Our general findings were as expected. The majority of cases were classified as SIDS. The second largest group included infants for whom...
respiratory infections were found. The findings of various other diagnoses, which in several instances would have been undiscovered otherwise, emphasize the need for autopsies after unexpected infant deaths. What is the significance of the identified *B pertussis* infections in 12 cases? Several pieces of evidence support the plausibility of a cause-and-effect relationship. Eight of the 12 case subjects died before 6 months of age, the typical age for death attributable to pertussis. In autopsies, 9 of the subjects were found to have signs of respiratory infections; for 2 infants, the autopsies suggested that death was attributable to a respiratory infection. One additional infant (data not shown) had brain edema (which could have been attributable to hypoxemia during pertussis). Lower rates of completed primary series or age-appropriate numbers of pertussis vaccine doses among case subjects than among control subjects may indicate that immunization against pertussis protects children from death attributable to unrecognized *B pertussis* infection. Moreover, a recent study indicated that immunization with diphtheria-tetanus-pertussis vaccine induces antibodies that cross-react with pyrogenic staphylococcal toxins, which have been implicated in several cases of SIDS. Other microorganisms may be involved in the sudden death of infants, as suggested in this study by the higher rate of a history of concurrent upper respiratory tract infections among case subjects, compared with control subjects. Similarly, in a Scandinavian study, 48% of 244 SIDS case subjects, compared with 31% of 869 control subjects, exhibited symptoms of upper airway infection during the last week before death or interview, respectively. Because SIDS is a diagnosis of exclusion, every attempt should be made to identify a cause of death during autopsy. This should include the search for pathogenic microorganisms in the respiratory tract with the use of PCR and other sensitive tests. In conclusion, *B pertussis* infection was found for 12 of 234 infants (5.1%) with unexpected deaths, and the infections might have contributed to the deaths. *Pediatrics* 2004;114:49–e15.

URL: http://www.pediatrics.org/cgi/content/full/114/1/e9; Bordetella pertussis, SIDS, sudden infant death, polymerase chain reaction.

**ABBREVIATIONS.** PCR, polymerase chain reaction; SIDS, sudden infant death syndrome; ILM, institute of legal medicine; CSC, central study coordinator; NPS, nasopharyngeal specimen; TS, tracheal specimen.

Sudden infant death syndrome (SIDS) is a frequent diagnosis for deaths among infants. The incidence of SIDS in most areas of Europe is ~0.5 to 2 cases per 1000 live births,1 with notable decreases in the past decade, probably because of campaigns to reduce the rates of deaths associated with risky sleeping conditions.2–7 Although a number of biologic and epidemiologic risk factors, such as prone position, hyperthermia, nicotine abuse during pregnancy, and covering of the head, have been identified,8–10 their interactions and the detailed pathophysiologic mechanisms are still not well understood.11,12

We13 and others14,15 previously noted an association between epidemic pertussis and sudden unexpected deaths among infants. This is not surprising, because apnea is a common complication of pertussis among young infants.16,17 In an attempt to assess how frequently SIDS is caused by *Bordetella pertussis* infections, we have been obtaining postmortem nasopharyngeal specimens (NPSs) since December 1990, in collaboration with several German institutes of legal medicine (ILMs). In our initial studies, we failed to culture *B pertussis* from infants with sudden unexpected deaths. Subsequently, however, using polymerase chain reaction (PCR), we found evidence of *B pertussis* infection in 9 of 51 consecutive sudden infant deaths (18%).18 To validate these preliminary findings, in 1995 we initiated a prospective, blinded, multicenter, matched-control study, which is the subject of this report.

**METHODS**

**Participants and Study Design**

ILMs of 8 German universities took part in this study. Every deceased infant 7 to 365 days of age who was transferred to a participating ILM between January 1995 and January 1997 was enrolled as a case subject, irrespective of the reason for autopsy. During a standardized autopsy, NPSs and tracheal specimens (TSs) were obtained with Dacron swabs (MAST, Hamburg, Germany) and shaken vigorously in 0.4 mL of sterile 0.9% saline solution, in Eppendorf vials (Eppendorf Laboratories, Eppendorf, Germany). All specimens were sent via regular mail to the central study coordinator (CSC) at the University Hospital for Children and Adolescents (Erlangen, Germany), where they were maintained at −80°C, separately from other clinical materials, until additional processing (see below). The autopsy findings, including the cause of death, were kept blinded at the ILM until the end of the study.

On the day of the autopsy, the ILM personnel reported the date of birth, gender, nationality, and zip code of the residence of the case subjects to the CSC via telephone or fax. A short questionnaire and an accompanying letter explaining the purpose of the study were forwarded to the parents of the case subjects during counseling, and informed consent was obtained. The following data considered to be relevant for the purpose of the study were collected: birth weight and gestational age of the child, respiratory (with and without cough) illnesses of the child within 4 weeks before death, number of immunizations against pertussis for the child, contact with a known case of pertussis within 4 weeks before death, and close contact with a person with a cough illness within 4 weeks before death.

**Identification of Matched Control Subjects**

Before the start of the study, a network of 83 pediatric offices in the areas of the 8 ILMs had been established. After a case subject was enrolled, the CSC identified the pediatric office closest to the residence of the case subject, for enrollment of 2 control infants. Personnel in the pediatric office identified the first 2 infants who appeared for a regular developmental examination (well-infant visit) whose date of birth was within 1 month of the date of the birth of the case subject and whose gender and nationality were identical to those of the case subject. After informed consent had been obtained from the parent of the control child, a NPS was collected with a Dacron swab and the same data as gathered for the case subjects were collected as described above and sent to the CSC via regular mail. If the office failed to enroll 2 control subjects within 1 week, then the task was delegated to a different pediatric office. If the PCR assay of the NPS was positive for *B pertussis*, then a clinical follow-up visit was requested 4 to 6 weeks later.

**PCR Method**

All NPSs and TSs arriving at the CSC were coded and maintained at −80°C. After inclusion of 10% blinded, negative-control vials (0.9% saline solution only), the frozen NPSs were shipped to the University Children’s Hospital (Basel, Switzerland) in weekly batches, for analysis by PCR, as previously described in detail.19–21 Oligonucleotide primers PTP1 and PTP2, which specifically amplify a 191-base pair DNA fragment of the pertussis toxin operon of *B pertussis*, were used.
Autopsy Procedures and Histologic Methods

Standardized autopsies (details available from W.J.K. on request) were performed at the individual ILMs, and the histologic features of the organs were evaluated centrally by 2 pathologists blinded to the patients’ histories and PCR results. SIDS was defined as “sudden infant death which was unexpected by history and in which the autopsy fails to demonstrate an adequate cause of death.”

Toxicologic Analyses

All participating ILMs except the ILM of the University of Munich performed toxicologic analyses on a routine basis according to internal protocols, using standardized methods. Tests performed included drug screening of urine and blood samples, determination of blood alcohol levels, and hemoglobin-carbon monoxide determination.

Statistical Analyses

For comparison of means, the Wilcoxon rank sum test and Student’s t test were used. Percent comparisons were performed with the χ² test, and odds ratios were calculated as appropriate. P values of <.05 were considered significant. The study protocol was approved by the ethics committee of the University of Erlangen (Erlangen, Germany).

RESULTS

General Results

Between January 1995 and January 1997, a total of 254 infants (66.1% male) with sudden unexpected deaths and 441 matched control subjects were enrolled in the study. For 207 case subjects (81.5%), 2 control infants were recruited. For 1 case subject, 3 instead of 2 control subjects were recruited by mistake. For 21 case subjects (8.3%) no control infant and for 26 case subjects (10.2%) only 1 control infant was identified. The nationality of 251 case subjects was known, and 216 (86%) were German.

For the initial 254 case subjects, basic characteristics such as date of birth, date of death, and gender were known in all instances. However, questionnaires were returned by the parents for only 116 (46%), and answers were complete in only 70 instances (28%). Questionnaires were received for all 441 matched control subjects. The mean age of case subjects was 139 days (range: 7–378 days; median: 112 days) in this subcohort, compared with 137 days (range: 5–378 days; median: 112 days) in the 234 case subjects with complete autopsy findings and 221 case subjects (95%), respectively.

The following analyses were restricted to the 234 case subjects with complete autopsy findings and their 407 respective control subjects. The mean ages of the case subjects (138 days; range: 7–362 days; median: 118 days) and control subjects (137 days; range: 5–378 days; median: 112 days) in this subcohort were almost identical to those for the whole study population. A total of 156 of the case subjects (66.7%) were male, as were 277 of the control subjects (68.1%) (P = .73).

TABLE 1. Final Diagnosis of Cause of Death for 234 Infants for Whom Complete Autopsies Were Performed

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIDS</td>
<td>177 (76)</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>25 (11)</td>
</tr>
<tr>
<td>Other infections</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Malformation/organ failure</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Accidents/trauma</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Aspiration</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>234 (100)</td>
</tr>
</tbody>
</table>

PCR Results

NPSs and TSs were obtained from 234 case subjects (100%) and 221 case subjects (95%), respectively. For the 407 control subjects, NPSs were obtained an average of 10.6 days after the death of the respective case subject. Results for 4 NPSs were unavailable because of false-positive control readings during the PCR assay, and 4 samples were missing.

Twelve of 234 case subjects (5.1%) demonstrated PCR evidence of *B pertussis* infection. Of those 12 subjects, 4 had positive NPS findings (1.7%), 7 had positive TS findings (3.0%), and 1 had positive NPS and TS findings. Twenty-one of 399 control subjects (5.3%) had NPSs that were positive for *B pertussis*. Of the 12 case subjects with positive PCR findings, 10 had a final diagnosis of SIDS and 2 had respiratory infections.

Analysis of Questionnaires

Questionnaires were received from the parents of 122 of 234 case subjects (52%) and all 407 of the control subjects. However, because not all questions were answered in all questionnaires, denominators vary in the following analyses.

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e11
Downloaded from by guest on May 2, 2017
A comparison of characteristics for all case and control subjects for whom information was available is presented in Table 2. There were more preterm infants among the case subjects than among the control subjects. Upper respiratory tract infections within 4 weeks before death were more frequently reported for case subjects and their household members, compared with control subjects. Significantly fewer case subjects than control subjects had received age-appropriate numbers of pertussis vaccine doses. Known epidemiologic links to individuals with pertussis were rare for case subjects (2.2%) and control subjects (1.5%).

Of the 12 case subjects with positive PCR samples, 10 (83%) were male. Unfortunately, questionnaires had been returned by the parents of only 5 of the 12 infants. Three had experienced a respiratory illness (all with cough), beginning 7, 14, or 19 days before death. None had known contact with a person with pertussis.

Characteristics of control patients, stratified according to PCR results (negative versus positive for *B. pertussis*), are shown in Table 3. Control subjects with positive PCR findings for *B. pertussis* were not different from those with negative PCR results with respect to any of the characteristics.

Follow-up information was obtained for 15 of the 21 control subjects with NPSs that were positive for *B. pertussis* in PCR assays. Of the 15 subjects, 1 had rhinitis at the time the NPS was obtained, but that infant did not develop another respiratory illness. Four of the control subjects developed cough illnesses within 4 weeks after the NPSs had been obtained. One 7-month-old infant had a cough illness of 4-week duration, beginning a few days after the NPS had been obtained.

One infant, who had been coughing for 5 days when the NPS was collected, developed typical pertussis, with >4 weeks of whooping. Another infant, who had received 3 doses of pertussis vaccine, developed a cough with whooping of 1-week duration. One unvaccinated infant had an atypical cough illness for 1 week. Overall, 4 of 15 control infants (27%) with positive PCR findings for *B. pertussis* had a...

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**TABLE 2.** Overall Comparison of Characteristics Determined by Questionnaire for 122 Case Subjects and 407 Control Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value No.</td>
<td>Value No.</td>
<td></td>
</tr>
<tr>
<td>Birth weight, g, mean (±SD)</td>
<td>3179 (±737) 91</td>
<td>3410 (±590) 406</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gestational age, wk (±SD)</td>
<td>38.2 (±3.3) 111</td>
<td>39.4 (±1.6) 404</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Denominators are the number of responses for a specific question on the questionnaire. OR, odds ratio; CI, confidence interval; URI, upper respiratory tract infection.

**TABLE 3.** Characteristics for 21 Control Subjects With PCR Results Positive for *B. pertussis*, Compared With 378 Control Subjects With Negative PCR Results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PCR Positive</th>
<th>PCR Negative</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value No.</td>
<td>Value No.</td>
<td></td>
</tr>
<tr>
<td>Birth weight, g, mean (±SD)</td>
<td>3650 (±792) 21</td>
<td>3402 (±580) 377</td>
<td>0.17</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>39.7 (±9.7) 21</td>
<td>39.4 (±4.9) 375</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Denominators are the number of responses for a specific question. OR, odds ratio; CI, confidence interval; URI, upper respiratory tract infection.

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* Within 4 weeks before death (case subjects) or enrollment (control subjects).

† For ≥120 to 149 days of age: ≥1 dose; ≥150 to 189 days of age: ≥2 doses; ≥190 days of age: ≥3 doses.
cough illness suggesting possible pertussis, and 2 of those 4 developed typical symptoms (whooping).

**DISCUSSION**

The concept of infections as a factor in SIDS is supported by a number of observations, including the seasonal distribution of the occurrence of SIDS, with peaks during the cold season; the high incidence of concurrent upper respiratory tract infections among infants dying as a result of SIDS; the peak age at 3 to 4 months, when immunoglobulin G levels among infants reach a nadir, caused by decreases in the levels of passively acquired maternal antibodies and still-insufficient production of their own antibodies; the association with nicotine use in a child’s household, which predisposes children to respiratory infections such as otitis media; and the protective role of breastfeeding. Not surprisingly, a number of different viruses and bacteria have been suggested as causative agents of SIDS, as summarized by Blackwell and Weir. Among microorganisms, a prominent role for *B. pertussis* might be suspected for several reasons, as follows. 1) *B. pertussis* infections in infancy are frequently associated with apneic spells, which are occasionally life-threatening and if leading to death may be reported as SIDS. 2) Epidemiologic evidence indicates that SIDS is associated with *B. pertussis* infection. Nicoll and Gardner examined postnatal infant deaths resulting from respiratory causes and SIDS in England and Wales between 1968 and 1984. During an outbreak of pertussis between 1977 and 1980, they estimated an excess mortality rate for undiagnosed pertussis of 460 to 700 deaths. Similarly, Cherry calculated 362 excess infant deaths caused by pertussis that had been classified as being caused by “other respiratory infectious illnesses” during the same epidemic. Recent data from Sweden and Norway indicate a direct correlation between the incidence of pertussis and the occurrence of SIDS. The relationship between the SIDS mortality rate and the prevalence of *B. pertussis* infections between 1983 and 1988 was investigated. The reported SIDS rate significantly followed the monthly prevalence of *B. pertussis* infections in Sweden as a whole and in the city of Stockholm. In Norway, where (in contrast to Sweden) infants were immunized against pertussis on a routine basis during the study period, there was a significant correlation only during an outbreak of pertussis and not during the whole study period. 3) Our previous findings, in which *B. pertussis* DNA was detected in the nasopharynx of 9 of 51 consecutive infants (18%) with sudden unexpected deaths, support a potential role for this organism in SIDS.

Here we present the first prospective, controlled study investigating the possible etiologic role of *B. pertussis* in SIDS. Careful autopsies, including histologic evaluation of organ specimens, and the use of PCR to detect *B. pertussis* in NPSs and TSs were the strengths of this study. Our general findings (eg, gender and age distribution) were as expected. The majority of cases were classified as SIDS, ie, no plausible cause of death had been found with a careful history, investigation of the death scene, and an autopsy. The second largest group included infants for whom respiratory infections were found. The finding of various other diagnoses, which in several instances would have been undiscovered otherwise, emphasizes the need for autopsies after unexpected infant deaths. Interestingly, all cases of *B. pertussis* infection occurred among infants with a diagnosis of SIDS or death attributable to respiratory infection. Not unexpectedly, the comparison of data solicited with standardized questionnaires from case subjects and matched control subjects revealed a greater proportion of preterm infants, lower mean birth weights, and higher rates of upper respiratory tract infections in the 4-week period before death among deceased infants, compared with control subjects. Interestingly, lower rates of completed primary series or age-adequate numbers of pertussis vaccine doses were found among case subjects, compared with control subjects. The negative association between diphtheria-tetanus-pertussis vaccine immunization and SIDS was observed previously. Although this may indicate that immunization against *B. pertussis* protects children from death attributable to unrecognized *B. pertussis* infection, a recent study suggested that immunization with diphtheria-tetanus-pertussis vaccine also induces antibodies that cross-react with pyrogenic staphylococcal toxins, which have been implicated in several cases of SIDS.

As an initial surprise to us, the rates of positive PCR findings were similar for case subjects and control subjects and the rate was actually higher for control subjects (5.3%) than case subjects (1.7%) when NPS results were compared. A significant decrease in positivity rates from the first year to the second year of the study (6.7% vs 2.9%) was observed (data not shown). This suggests a continuous decrease, compared with the rate of 18% found in our original uncontrolled study, which was performed in 1993–1994. Of note, this decrease parallels the reintroduction of routine childhood immunization against pertussis in Germany in 1991, followed by the introduction of acellular pertussis vaccines for infant immunization in 1995, which led to a dramatic increase in immunization rates among German infants, from an estimated 15% in 1990 to a current coverage of ~90%.

What is the significance of the identified *B. pertussis* infections in 12 cases? Several pieces of evidence support the plausibility of a cause-and-effect relationship between infections and deaths. Eight of the 12 case subjects died before 6 months of age, the typical age of death attributable to pertussis. In autopsies, 9 of the subjects were found to have signs of respiratory infections; for 2 infants, the autopsies suggested that death was attributable to a respiratory infection. One other infant (data not shown) had brain edema (which could have been attributable to hypoxemia during pertussis).

The 5.3% rate of positive PCR results among the control subjects may seem surprisingly high but is supported by other studies.

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Deen et al, in a household contact study, noted asymptomatic infections for 52 of 114 exposed subjects (46%) and infec-
tions for 23 of 53 subjects (43%) with mild respiratory illnesses. Long et al. also noted the widespread silent transmission of *B. pertussis* among families. More recently, Crowcroft et al. noted asymptomatic infections for 10 children who were contacts of case subjects. Of 15 control subjects with positive PCR findings and adequate follow-up information, 1 had typical clinical pertussis and 4 others had nonspecific cough illnesses. The remaining 10 individuals had no respiratory illnesses in association with the positive PCR findings. Some of the positive PCR findings for these control subjects could be false-positive results, but this seems unlikely. In general, false-positive PCR results and precautions for avoiding them are well known, and our laboratory complied fully with standard recommendations. Furthermore, during the course of the study, we sent a total of 332 blinded, negative-control specimens to the PCR laboratory, along with study and clinical specimens, on a routine basis; only 4 (1.2%) yielded positive PCR results, indicating laboratory contamination. Finally, we made to identify a cause of death during autopsy. This should include a search for pathogenic microorganisms in the respiratory tract with the use of PCR assays and other sensitive tests.

### ACKNOWLEDGMENTS

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