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CLINICAL AND EPIDEMIOLOGICAL STUDY

The respiratory pathology in infants with sudden unexpected deaths in whom respiratory specimens were initially PCR-positive or PCR-negative for *Bordetella pertussis*

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Abstract
Background In a previous controlled study, we investigated the relationship between *Bordetella pertussis* infections and sudden unexpected deaths among German infants (sudden infant death syndrome, SIDS). In this present study, we investigated further the respiratory pathology in a subset of infants in the original study.
Methods Originally, there were 234 infants with SIDS and, of these, 12 had either a nasopharyngeal swab (NPS) or a tracheal swab specimen (TS) that was positive for *B. pertussis* by polymerase chain reaction (PCR). Here, tissue specimens from eight infants who were originally PCR-positive were compared with tissue specimens from seven infants in whom the original PCR studies were negative.
Results The histopathologic diagnoses were as follows: 14 of 15 had pulmonary edema and the remaining case had early diffuse alveolar damage. Although 14 of 15 cases had some histologic or clinical evidence suggesting respiratory tract infection, the features were more consistent with a viral etiology, and in none were the findings typical of respiratory disease attributable to *B. pertussis*.
Conclusions The findings in this present investigation do not support a direct role of *B. pertussis* at the site of infection (ciliated epithelium) in the causation of SIDS. The clinical aspects of this study were carried out in the 1990s when pertussis was widespread in Germany. Therefore, the original finding of some PCR-positive cases is not surprising. The possibility that *B. pertussis* infection could still be a factor in some SIDS cases, e.g., by a systemic release of toxins, cannot be definitely ruled out.

Keywords *Bordetella pertussis* · Sudden unexpected death · SIDS · PCR · Histopathology

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Introduction

In the past, it was noted in Sweden, Norway, and England and Wales that infant deaths diagnosed as sudden infant death syndrome (SIDS) increased when pertussis was epidemic in the population [1, 2]. Almost two decades ago, we carried out *Bordetella pertussis* polymerase chain reaction (PCR) studies in Germany on nasopharyngeal swab (NPS) specimens from 51 infants with sudden deaths and nine (18%) were *B. pertussis*-positive [3]. A weakness of this initial investigation was that clinical information on respiratory illnesses prior to death had not been carefully obtained.

Between 1995 and 1997, we then carried out a prospective controlled study to investigate the relationship between *B. pertussis* infections and SIDS among German infants [4]. In that study, NPS or tracheal swab (TS) specimens were obtained for *B. pertussis* PCR from 234 infants and from 441 matched control infants. The PCR results were positive for *B. pertussis* in 12 (5.1%) of the case subjects and from 5.3% of control subjects. In a more

recent study, we studied the pathology of fatal infant pertussis using immunohistochemistry and molecular techniques [5].

Based on this previous experience, we have looked further for a possible causal relationship between *B. pertussis* infection and SIDS in this present investigation. To do this, we evaluated the histopathologic features and employed an immunohistochemical (IHC) stain in the trachea, larynx, bronchi, and lungs in cases of sudden unexpected deaths, from our 1995–1997 study, in whom PCR specimens were positive or negative for *B. pertussis*.

Methods

For this investigation, institutes for legal medicine who, in the original study [4], had contributed at least one *B. pertussis* PCR-positive case subject were contacted and asked for the available lower respiratory tract autopsy specimens from these overall 12 cases. In addition, for each PCR-positive case, lower respiratory tract autopsy specimens were requested from *B. pertussis* PCR-negative subjects from the same study to serve as controls.

Formalin-fixed, paraffin-embedded respiratory tissues from these German infants who died during 1995–1997 from SIDS were evaluated at the Centers for Disease Control and Prevention (CDC). Tissue samples were stained at the CDC by using hematoxylin and eosin and by an immunoalkaline phosphate technique with a monoclonal antibody which reacts to lipooligosaccharide A of *B. pertussis* (IHC staining) [5].

In the original German study, SIDS was described as "sudden infant death which was unexpected by history and in which the autopsy fails to demonstrate an adequate cause of death". The initial study protocol was approved by the ethics committee of the University of Erlangen (Erlangen, Germany).

Results

Respiratory specimens were received from 8 of 12 infants that had NPS or TS specimens taken at the time of autopsy who tested positive and seven specimens were received from deceased infants who had tested negative for *B. pertussis* by PCR [4]. Fourteen of the 15 infant deaths in whom tissue specimens were evaluated at the CDC were initially diagnosed as SIDS by the German pathologists. Presented in Tables 1 and 2 are the histopathologic findings and the pathologic opinions at the CDC and the descriptive findings of the German pathologists and reports by parents when available. In Table 1, the findings in the eight cases who had initial NPS or TS *B. pertussis* PCR-positive samples in Germany are presented and in Table 2, similar findings for the seven cases that were *B. pertussis* PCR-negative in Germany are presented.

In Figs. 1 and 2, selected histopathologic findings in the tissues examined at the CDC from initially PCR-positive

Table 1 Histopathology and pathologic diagnosis in eight infants with sudden death in whom initial nasopharyngeal swab (NPS) or tracheal swab (TS) samples were positive for *Bordetella pertussis* by polymerase chain reaction (PCR)

Case no.	Data from the CDC laboratory		German pathologic diagnosis and clinical data
	Histopathology	Pathologic Dx	
237/95	Trachea: focal, mild submucosal mononuclear inflammation. Lung: diffuse intraalveolar edema	Mild tracheitis and pulmonary edema	SIDS plus respiratory tract infection; bilateral otitis media
148/96	Trachea: no significant histopathology. Larynx: focal mild submucosal inflammation. Lung: patchy intraalveolar edema	Pulmonary edema	SIDS plus respiratory tract infection
374/96	Trachea, larynx, and bronchi: extensive mixed submucosal inflammatory cell infiltrates and focal ulceration. Lung: diffuse intraalveolar edema	Pulmonary edema	SIDS plus laryngotracheitis and bronchitis
174/95	Lung: diffuse intraalveolar edema	Pulmonary edema	SIDS. onset of rhinitis and cough
863/95	Bronchi: focally denuded mucosa without significant inflammation. Lungs: patchy intraalveolar edema	Pulmonary edema	SIDS plus mild signs of respiratory tract infection
1216/95	Trachea: no significant inflammation. Lung: multifocal interstitial pneumonitis and diffuse intraalveolar edema with scattered colonies of large bacterial rods (postmortem?)	Interstitial pneumonitis and pulmonary edema	Brain edema
1221/95	Trachea: no significant inflammation. Lung: diffuse intraalveolar edema	Pulmonary edema	SIDS plus mild signs of respiratory tract infection; cough
128/95	Trachea and bronchi: multifocal submucosal inflammatory cell infiltrates and mucosal denudation. Lung: diffuse intraalveolar edema	Tracheobronchitis and pulmonary edema	SIDS plus severe respiratory tract infection

Table 2 Histopathology and pathologic diagnosis in seven infants with sudden death in whom initial nasopharyngeal swab (NPS) or tracheal swab (TS) were negative for *Bordetella pertussis* by polymerase chain reaction (PCR)

Case no.	Data from the CDC laboratory		German pathologic diagnosis and clinical data
	Histopathology	Pathologic Dx	
342/95	Trachea: multifocally denuded mucosa, with focal mild inflammation. Lung: diffuse intraalveolar edema	Mild tracheitis and pulmonary edema	SIDS plus respiratory tract infection; food aspiration
31/96	Trachea: no significant histopathology. Lung: patchy intraalveolar edema	Pulmonary edema	SIDS plus respiratory tract infection
18/97	Trachea and larynx: multifocal mucosal ulceration with mild focal submucosal inflammation. Lung: patchy intraalveolar edema	Mild tracheitis and pulmonary edema	SIDS plus respiratory tract infection
268/95	Bronchi: extensively denuded mucosa without significant inflammation. Lungs: intraalveolar hemorrhage and macrophages, suggestive of early diffuse alveolar damage	Early diffuse alveolar damage	SIDS plus mild respiratory tract infection
1024/95	Trachea: no significant histopathology. Lung: patchy intraalveolar edema	Pulmonary edema	SIDS plus mild respiratory tract infection
1888/95	Bronchi: multifocal mucosal ulceration with mild focal submucosal inflammation. Lung: patchy intraalveolar edema	Bronchitis and pulmonary edema	SIDS plus interstitial pneumonia; hepatitis
1940/95	Lung: diffuse intraalveolar edema	Pulmonary edema	SIDS

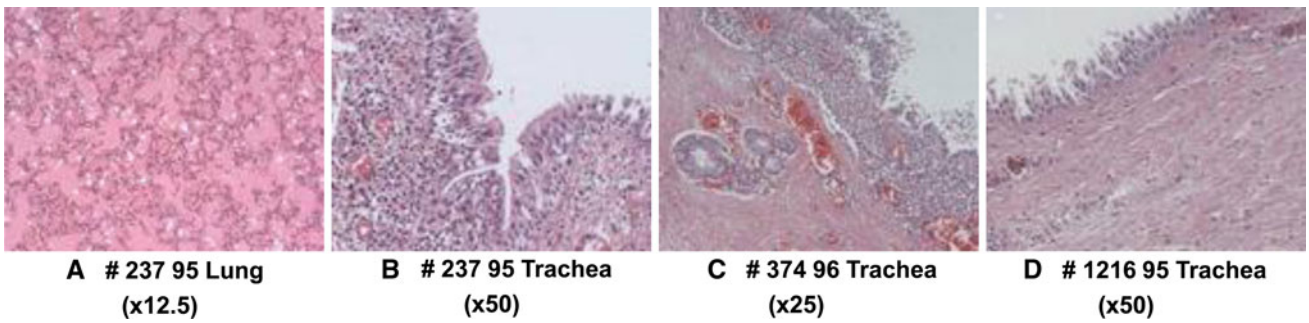

Fig. 1 Histopathologic findings of selected infants from Table 1. **a** Extensive intraalveolar edema. **b** Focal, mild, predominantly mononuclear inflammatory cell infiltrates in the lamina propria of the trachea. **c** Abundant lymphoplasmacytic inflammatory cell infiltrates in the lamina propria of the trachea with prominent submucosal congestion. **d** Trachea with no significant inflammatory cell infiltrates

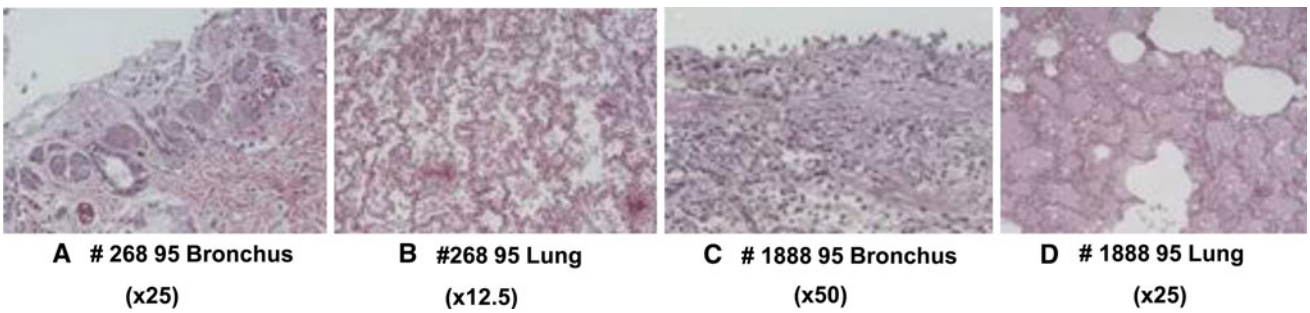

Fig. 2 Histopathologic findings of selected subjects from Table 2. **a** Bronchus with extensively denuded and necrotic mucosal epithelium without conspicuous inflammatory cell infiltrates. **b** Lung with early diffuse alveolar damage characterized by intraalveolar macrophages and fibrin, and interstitial and intraalveolar edema. **c** Mild, predominantly mononuclear inflammatory cell infiltrates in the submucosa of the bronchus, with patchy loss of epithelium. **d** Extensive intraalveolar edema

and PCR-negative infants are presented. No remarkable differences were noted between the pathologic findings of the eight cases with initially *B. pertussis* PCR-positive findings and those of the seven cases in whom the initial PCR studies were negative. In all but one case pulmonary edema was the major finding. Interestingly, the pathologic

findings in three cases (three in the PCR-positive group and three in the PCR-negative group) had findings suggestive of a respiratory viral infection. Of the eight cases who were initially PCR-positive, in addition to pulmonary edema, two had tracheobronchitis, one had mild tracheitis, and one had interstitial pneumonitis. Of the seven cases who were initially PCR-negative, two had mild tracheitis, one had bronchitis, and one had early diffuse alveolar damage.

Of note, IHC staining for *B. pertussis* was negative in all specimens. In none of the 15 cases were there typical findings of fatal pertussis (pulmonary hemorrhage, septal edema, necrotizing bronchopneumonia, and leukocyte aggregates in septal and pleural veins and lymphatics) [5].

Discussion

The clinical aspects of this study were carried out when pertussis was widespread in Germany, so that finding some PCR-positive cases is not surprising, as asymptomatic or mild infections with *B. pertussis* are common [4, 6, 7]. Importantly, there were no apparent differences in the histopathologic findings between cases that were initially either PCR-positive or PCR-negative.

Although all but one of the 15 cases had some evidence of respiratory tract infection, in none was it typical of fatal *B. pertussis* infection, i.e., in none of the infants was there evidence of a descending lower respiratory tract infection dominated by necrotizing bronchiolitis, fibrinous edema, or prominent angiolymphatic collections of leukocytes [5]. Rather, the histopathological findings were most consistent with a mild viral infection.

In light of these histopathologic findings, the data from this study does not support a direct role of *B. pertussis* at the site of infection (ciliated epithelium) in the causation of SIDS. However, since it has been noted that reported SIDS increases during epidemic pertussis [1, 2] and since *B. pertussis* DNA has been found in respiratory specimens in infants with sudden unexplained deaths [3, 4], it is possible that the *B. pertussis* infection could still be a factor in SIDS through the systemic release of toxin or toxins. In this regard, pertussis toxin (PT) would seem to be a possibility, since it can cause hypoglycemia [8], but if PT were the cause, one might expect to see signs of the associated leukocytosis with lymphocytosis in the histological specimens from the respiratory tract, which was not the case in our investigations. Although the cause of the paroxysmal cough in pertussis is not known, it is presumed to be one or more toxins that mediate cough through a central nervous system site [8]. SIDS could be an outcome from apnea (a characteristic complication in infants with pertussis) resulting from toxin effects in *B. pertussis*-infected infants.

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Conflict of interest None.

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