Is there a link between infant botulism and sudden infant death?
Bacteriological results obtained in Central Germany

Abstract Despite the fact that botulism was described in Germany for the first time by Kern in 1820, the disease is almost forgotten in this country. Only about 10–20 cases of classical botulism (intoxication) are recorded every year, including 1–2 cases of clinical infant botulism. As we assumed a high incidence of botulism to be connected with cases of sudden infant death (SID), we undertook the research work presented here. From every case of unexpected infant death up to 12 months of age, standardised specimens (blood, liver and intestine) were taken at autopsy. They were tested for the presence of botulinum neurotoxin (BoNT) and/or bacterial forms of Clostridium botulinum with subsequent BoNT neutralisation tests by the international standard mouse bioassay. Age, sex, pathological findings and season were recorded. Over a 5-year period, 75 samples including 57 SID cases were tested. Free toxin was found in nine and bacterial forms were detected in six samples. Toxin neutralisation revealed the definite presence of BoNT/BoNT producing bacteria (mainly type E), whereas another 11 toxin tests were inconclusive. According to international literature, these 15 cases are to be interpreted as infant botulism. Conclusion: the results show a remarkable incidence of infant botulism without any known previous medical history, partly hidden as sudden infant death. We propose to systematically search for botulism in connection with sudden infant death.

Keywords Germany · Infant botulism · Sudden infant death

Abbreviations BoNT botulinum neurotoxin · SID sudden infant death

Introduction

Botulism is caused by the blockage of neural transmission in the cholinergic synapses due to botulinum neurotoxin (BoNT) produced by Clostridium botulinum. However, there may be other clostridial strains which produce BoNT as well [10].

The classical form of botulism in humans, a foodborne intoxication by the direct uptake of BoNT, has been seen less frequently. During the last 25 years, an average number of 24 cases/year has been reported in the United States [31], and in Germany about 10–20. The importance of infant botulism, however, has been growing ever since its first description in 1976 [23] to an average of 71 cases per year in the United States [1]. In Germany, approximately one case is reported per year, but there are no official estimates.

Infant botulism displays a spectrum in its clinical severity from mild paralysis, often diagnosed as “failure to thrive”, to signs of paralysis of all muscles which may appear slowly and progress over 1–3 days to the “floppy infant syndrome”. The fulminant form leads to sudden and unexpected death and may be taken for sudden infant death (SID). By definition, it can be diagnosed post-mortem only [1, 3, 13]. Epidemics are not characteristic for infant botulism [13]; its incidence, however, is certainly underestimated in paediatrics [9, 27, 29, 39], although more than 300 scientific papers have been published [26].

SID is defined as the sudden death of any infant or young child which is unexpected by history, occurring in association with sleep, and lacking explanation after post-mortem investigation. Obviously, there are different suspected or proven reasons and risk factors which could lead to sudden death including or excluding botulism [1, 16, 19, 20, 26, 31, 35]. Both clinical forms of infant botulism are due to resorption of BoNT, which is produced under certain conditions by vegetative forms of C. botulinum in the intestine. These in turn have grown out of spores which had been taken up orally. The physiological conditions in the digestive tract of
children up to the age of 12 months are considered to be very favourable for the colonisation by the ingested spores, whereas older children and adults rarely fall sick (hidden form of botulism) [13, 21]. Arnon et al. [3] extrapolated laboratory findings to show that $2.6 \times 10^8$–$2.6 \times 10^9$ vegetative cells of *C. botulinum* Type A produce enough lethal toxin in the intestine to kill a 7 kg infant.

**Material and methods**

The Institute of Forensic Medicine, Göttingen, is in charge of all forensic autopsies of public (legal) interest within a region of approximately 150 km around the town of Göttingen in the centre of Germany [27]. In this study, no sociological data, e.g., living conditions, social situation, nutritional habits, environment, were considered. They will be published elsewhere.

By German law, an autopsy has to be performed in any case of unexpected and/or uncertain cause of death. From every child with sudden unexpected death younger than 12 months and not being victim of an accident or an obvious disease, samples of intestine, heart blood, and liver were collected under aseptic conditions. During a 5-year period, 75 cases were examined without any clinical signs of botulism.

Samples were transported from pathology to the bacteriological laboratory at ambient temperature by courier. The specimens were examined bacteriologically by standard methods which had been compiled recently by the CDC [12]. Free toxin (BoNT) was directly screened by soaking the samples in gelatine-phosphate-buffer pH 6.2 (1:1 w/v) overnight at 4°C, followed by centrifugation (9000g for 15 min at 10°C). The supernatants were not filter-sterilised. Toxin tests were done in 20–25 g white mice of our institute’s breeding station. Extracts (0.5 ml) were administered by i.p. injection. The animals were observed for 96 h for any clinical signs, e.g., respiratory difficulties, wasp paralysis, or death. In the case of death, toxin neutralisation was carried out by mixing 0.5 ml of the sample preparation with 0.05 ml antitoxin according to the specification of the antitoxin suppliers. After 1 h at room temperature, this mixture was injected into mice accordingly [32].

Together with the neutralisation test, the initial toxicity test was repeated and had to be positive again if neutralisation results were considered as being decisive. Additionally, the injected samples were checked for rod-shaped bacteria under the microscope. To reduce costs and especially to save laboratory animals, we first tested the intestine and only the other samples in negative cases.

Internationally standardised botulinum antitoxins are not produced in Germany. According to availability, we used those from Institut Pasteur (Paris, France), OVI (Ondersteempoort, South Africa), ID-Lelystad (Lelystad, The Netherlands), which were all of certified neutralising capacity. Own antitoxins were standardised to the ID-Lelystad ones. Not all samples were tested with all monovalent antiserum. Different pooled types of antitoxins had to be accepted as screening results according to availability. Types F and G were not tested.

Presence of *C. botulinum* spores or vegetative forms was tested using a 5-day-incubation in Reinforced Clostridial Medium (Merck, Darmstadt, Germany) under anaerobic conditions and 37°C in duplicate (previously heated 60°C/30 min and unheated). Cultures were tested thereafter for BoNT using the bioassay. Finally, in this study only those samples were considered being BoNT positive which were neutralised by antitoxins. The results were compared statistically ($t$-test, $P \leq 0.05$) with reference to age, sex, seasonal influences and proof of BoNT.

**Results**

From January 1995 to December 1999, 75 cases were examined 57 of which were pathologically suspected of being SID. In 15 cases (20%), the presence of BoNT was proven (9 direct toxin tests, 6 with preceding bacteriological enrichment). These included 11 (19.3%) of the suspected SID group. In a further 11 cases, toxin neutralisation was inconclusive with the available antitoxins and recorded as negative. Table 1 shows the typing of BoNT in all 15 positive cases; 80% being type E or the pooled types A, B, E. No monovalent types A, B, or C were neutralised.

The age distribution (in days) of all 75 samples and the 15 positive BoNT tests is shown in Fig. 1. Almost 66% of all samples and positive BoNT tests were in the age group of up to 6 months. Table 2 gives the detailed age values of all sample groups mentioned above. The results of statistical analyses are given as Box Whisker plots concerning the interactions between BoNT negative and positive groups respectively, as well as gender versus time of the year (Fig. 2) or age (Fig. 3). There was a statistical significance only between the sexes and age in the positive BoNT group ($P = 0.016$), and also between males and age in the positive BoNT and SID groups ($P = 0.026$).

The seasonal distribution of all samples and all positive BoNT tests is given in Fig. 4. There was no statistical difference.

**Discussion**

The identification of toxinogenic *C. botulinum* (toxin, vegetative or spore forms) in clinical cases and in necropsy specimens of children of about less than 1 year of age is considered as a confirmation of infant botulism [1, 12, 22, 23]. No *Clostridium* should be found in healthy infants under 12 months of age although rare cases of asymptomatic carriers have been reported [1, 8, 34, 36, 37].

The transport time from necropsy to bacteriological examination could have had a negative influence on the diagnosis. Other bacteria present in the sample may produce proteolytic enzymes which may destroy free toxin or impede growth of *C. botulinum* and/or toxin formation in the test tube [32]. So there could have been even more *C. botulinum* in the original samples. For this discussion, all positive tests for BoNT neutralisation are grouped together; the specific toxin types are not considered separately. In the literature, all the different types of *C. botulinum* are cited as causing infant botulism.

**Table 1** Differentiation of BoNT types of all 15 positive cases. (*NN* no neutralisation possible with available antitoxins)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BoNT neutralisation</th>
<th>Antitoxins</th>
<th>Monospecific</th>
<th>Pooled</th>
<th>NN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin type</td>
<td>Number ($n$)</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Total neutralised ($n$)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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