First Case of Type E Wound Botulism Diagnosed Using Real-Time PCR[▽]

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Wound botulism is a growing problem among injecting drug users. The condition is often difficult to diagnose, with laboratory confirmation in only 50% of the cases. Here we present a real-time PCR-based method for the diagnosis of wound botulism caused by *Clostridium botulinum*. The assay includes an internal amplification control which is amplified simultaneously with the genes encoding neurotoxin types A, B, and E. This method was used to detect the first case of wound botulism in an injecting drug user in Sweden. In addition, to the best of our knowledge, this is the first reported case of wound botulism caused by *C. botulinum* type E.

Wound botulism has been, except in times of war, a very rare form of botulism, occurring mainly in patients with traumatic and surgical wounds that were contaminated with dirt containing *Clostridium botulinum* spores. The spores germinate and botulinum neurotoxin is produced in vivo. The symptoms of botulism are caused by the irreversible binding of the neurotoxin to presynaptic motor-neuron terminals, which blocks acetylcholine transmission across the neuromuscular junction. Botulism typically presents as a progressive, symmetrical, flaccid, descending paralysis without sensory involvement; it creates a pathognomonic clinical picture, but due to the rarity of the condition, the diagnosis of botulism is frequently delayed or even missed. Between 1943 and 1990, only 47 cases were reported to the Centers for Disease Control and Prevention, Atlanta, GA, and in the literature (9). Since then, however, there has been an upsurge of cases (1, 3, 5, 6, 9, 20, 21, 26, 34). The new risk group is injecting drug users (IDUs), especially those that use skin or muscle "popping," in which the drug is injected subcutaneously or intramuscularly (26). The first case of botulism in an IDU was identified in New York in 1982 and involved cocaine (8), but most of the recent cases have involved heroin (1, 3, 5, 20, 21, 25, 26, 28, 30, 34). Soft-tissue infections resulting in abscesses at injection sites or associated with cellulitis can be caused by several different *Clostridium* spp., as well as by *Bacillus cereus* (11, 25). These infections may be due to spore-contaminated injecting paraphernalia or drugs. To make them suitable for injection, drugs are often dissolved in acids, which enhance tissue damage and thus create a favorable milieu for bacterial spores to germinate and initiate a wound infection. So far, all confirmed cases of wound botulism have been caused by *C. botulinum* types A and B (31).

To confirm a clinical diagnosis of botulism, samples should be sent to a specialized reference laboratory. The diagnostic procedure consists of the detection of botulinum neurotoxin by the mouse lethality assay and/or the isolation of *C. botulinum*. If wound botulism is suspected, a serum sample is examined for botulinum toxin and pus or tissue samples are examined for the presence of *C. botulinum* and, if the samples are of sufficient size, for the presence of toxin.

However, microbiological confirmation of wound botulism has been possible in only around 50% of the cases (31). The reason may be incorrectly taken samples, insufficient sample sizes, low levels of circulating toxin, or the start of antibiotic treatment before sampling. If the latter is the cause, dead cells may still be present in the wound, thus permitting PCR detection. Several different PCR methods have been developed for the detection of *C. botulinum* in food and clinical samples (4, 7, 10, 15, 22, 23, 33). However, most of these methods have been developed for conventional PCR and require gel electrophoresis and/or probes to confirm the identity of the target. In addition, they do not usually contain an internal amplification control (IAC), which has been suggested to be mandatory by both the European Committee for Standardization and the International Organization for Standardization (18).

We have previously developed a real-time PCR method for the detection of *C. botulinum* type B (10, 24). In this report, we describe assays for the detection of *C. botulinum* types A and E. The assays include an IAC that is amplified simultaneously with the target genes encoding botulinum neurotoxin types A, B, and E. This real-time PCR approach was used to detect the first case of wound botulism in an IDU in Sweden, and this case is, to our knowledge, the first reported case of wound botulism caused by *C. botulinum* type E.

CASE REPORT

The patient was a 24-year-old homeless woman who had been injecting heroin and amphetamine for over 2 years. Previously, she had been treated for recurrent skin and soft-tissue abscesses following intramuscular and subcutaneous injections. Ten days before hospital admission, an abdominal wall abscess

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TABLE 1. Bacterial strains used in the specificity test of the PCR assays

Species	Strain(s)[a]
C. botulinum	
Type A	ATCC 3502, ATCC 19397, Atlanta 2204-4, Johannesson 263, ATCC 25763, SMRICC 87, SMRICC 89, Madison 109A
Type B	ATCC 7949, ATCC 17841, Eklund 2b, SMRICC 91, SMRICC 92, SMRICC 93, Johannesson 105-66, Atlanta 3025, BL 105/66
Type E	CB-S3E, CB-S4E, CB-S6E, CB-S21E, CB-S25E, CB-K27E, CB-K44E, CB-K58E, CB-K83E, CB-K114E, CB-124E, ATCC 9564, BL E64 S20 St15, BL E823, BL 1177, BL 1890
Type F	ATCC 35415, ATCC 23387, Craig 610B8-6F, Atlanta 2821, Johannesson, ATCC 25765, BL F1
Clostridium butyricum	ATCC 19398
Clostridium perfringens	ATCC 14810, ATCC 3629, ATCC 12925, ATCC 3624, ATCC 3626, ATCC 13124
Clostridium septicum	SVA G2, BL 8/65
Clostridium sporogenes	ATCC 19404, ATCC 11437, K54 SBL, CCUG 15941
Clostridium novyi	BL SVA 89
Clostridium difficile	ATCC 9689
Clostridium ramosum	BL 174
Escherichia coli	ATCC 25922, O157
Staphylococcus aureus	NCTC 7428, ATCC 8181
Streptococcus agalactiae	CCUG 4208, NCTC 8181
Proteus mirabilis	ATCC 43071
Proteus vulgaris	CCUG 6327
Pseudomonas aeruginosa	ATCC 27853
Enterococcus faecalis	ATCC 29212
Serratia marcescens	ATCC 13880
Stenotrophomonas maltophilia	CCUG 5866
Klebsiella pneumoniae	ATCC 10031
Bacteroides thetaiotaomicron	ATCC 29741
Bacteroides fragilis	ATCC 25285
Listeria monocytogenes	NCTC 1194
Campylobacter jejuni	CCUG 10937, CCUG 11284
Yersinia ruckeri	CCUG 21537
Yersinia pseudotuberculosis	CCUG 5855
Yersinia enterocolitica	JH 19, JH 23, Box 27/3
Yersinia frederiksenii	176-36/80
Yersinia kristensenii	Y891
Salmonella enterica serovar Typhimurium	51K61

[a] Strains with designations including the following abbreviations are from the indicated sources: ATCC, American Type Culture Collection; SMRICC, Swedish Meat Research Institute Culture Collection; SVA, Swedish National Veterinary Institute; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

had been incised by a general practitioner, and antibiotic therapy with flucloxacillin had been prescribed. The culture of wound secretions yielded growth of *Staphylococcus aureus*, beta-hemolytic group G streptococci, and unspecified anaerobic bacteria.

Eight days later, the patient started to experience dysphagia and throat pain and sought medical care in an emergency room 1 day after the onset of these symptoms. At that time, she complained of generalized weakness and difficulty in speaking, swallowing, and holding her head upright. She was afebrile and manifested a normal level of consciousness. A psychogenic cause for her symptoms was suspected, and she was referred for psychiatric assessment. Antibiotic therapy was changed to clindamycin due to persistent purulent drainage from multiple gluteal and abdominal wall abscesses.

On the following day, the patient's condition worsened with progressive cranial nerve symptoms and weakness of all extremities. She was admitted for observation in a neurological ward. One day after this admission, she developed respiratory arrest, after which she was intubated and transferred to an intensive care unit (ICU).

Upon ICU admission, the patient was tetraplegic and had bilateral ptosis and facial paralysis but was fully conscious with intact sensorium. Lumbar puncture and brain-imaging studies did not reveal any pathological changes. An electromyogram showed a nonspecific pattern. A clinical diagnosis of wound botulism was made, but antitoxin was not administered due to the extended duration of symptoms before the diagnosis was considered. A tracheostomy was performed 2 days after ICU admission. A chest X ray obtained after tracheal intubation showed right-lower-lobe infiltrates compatible with aspiration pneumonia, and antibiotic therapy with cefuroxime was started.

After 2 months, the patient began to gradually recover muscle power in the extremities, but she still required ventilatory support for 134 days before she could be completely weaned from mechanical ventilation. Subsequently, she made a full recovery of muscular function.

TABLE 2. Sequences and fluorescent dye of primers and hybridization probes used for real-time PCR

Target gene	GenBank accession no.	Primer or probe	Nucleotide positions	Nucleotide sequence (5′→3′)	Source or reference
cntA	M30196	AS-11d	435–455	TG(C/T)AGGACAAATGCAACCAGT	Modified from reference 33
		AS-22	697–717	TCCACCCCAAAATGGTATTCC	33
		ToxA-Fluo	545–570	TGAAACTGGAACTTGTTTTGCTTCTG-FL[a,b]	This study
		ToxA-Red	517–542	LC640-GGTGGATTTAAATCTCCTTCTTCAGG-p[a,c]	This study
cntE	X62683	fToxE	1658–1673	TGAATCAGCACCTGGA	This study
		rToxE	1974–1982	GGTTAGCTTCAGTAGTAAA	This study
		ToxE-Fluo	1913–1936	TGTCAATAAACCTGTGCAAGCAGC-FL[b]	This study
		ToxE-Red	1939–1973	LC640-TATTTGTA(A/G)GCTGGATACAACAAGT (A/G)TTAGTAGAT-p[c]	This study
IAC		LC143-3′Fluo		GCGGTGGGTTTTGTTGTCTTCTCTATT-FL[b]	Modified from reference 27
		LC150-5′Red705		LC705-CTATCCTTGAGCCGTAGGCCACTATCG-p[d]	27

[a] The probe is located on the antisense strand.
[b] The donor probe is labeled with fluorescein (FL) at the 3′ end.
[c] The acceptor probe is labeled with LC Red 640 (LC640) at the 5′ end, and the 3′ hydroxyl is blocked with a phosphate molecule (p).
[d] The acceptor probe is labeled with LC Red 705 (LC705) at the 5′ end, and the 3′ hydroxyl is blocked with a phosphate molecule (p).

MATERIALS AND METHODS

Culture conditions and DNA extraction. All strains used for specificity studies are listed in Table 1. All anaerobic strains were grown anaerobically in tryptone-peptone-glucose-yeast extract broth at 37°C, as described previously (10). All aerobic strains were grown in tryptone broth (Oxoid Ltd., Basingstoke, United Kingdom) at 37°C.

One milliliter of an overnight culture was harvested at $10,000 \times g$ for 5 min. After the discarding of the supernatant, the cells were resuspended in 200 µl of Tris-EDTA buffer. For gram-positive strains, 32 µl of lysosyme (50 mg/ml) and 1.6 µl of mutanolysin (5,000 U/ml; Sigma Chemicals Co., St Louis, MO) were added and the sample was incubated at 37°C for 30 min before extraction using the Easy-DNA kit according to the instructions of the manufacturer (protocol no. 3; Invitrogen BV, Groningen, The Netherlands). The DNA concentration was measured on a TD-700 fluorometer (Turner Designs, Sunnyvale, CA) using the PicoGreen double-stranded DNA quantitation kit (Molecular Probes, Inc., Leiden, The Netherlands).

Primer and probe design. The primers and probes used in this study are listed in Table 2. The assay for the type B neurotoxin gene has previously been described by Lövenklev et al. (24). An alignment of sequences was made using the BioEdit sequence alignment editor (16). Published nucleotide sequences for the neurotoxin genes of *C. botulinum* type E (accession numbers X62683, AB039264, AB040123, AB040125, AB040126, AB040128, AB082519, AB088207, X62088, X62089, EF028403, EF028404, and AB037704) and type A (accession numbers M30196, AF461540, AF488749, EF033126, EF028393, EF028392, EF028391, AY953275, X52066, X52088, and X73423) were collected from the GenBank Sequence Database (http://www.ncbi.nih.gov/Genbank/). Several different primers and probes were tested, some from the literature and some designed using the LightCycler probe design software, version 1.0 (Idaho Technology Inc., Salt Lake City, UT). The specificity was checked against published sequences by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and tested using PCR. Several probes were designed and compared. The hybridization probes consisted of two parts: a donor probe labeled with fluorescein at the 3′ end and an acceptor probe with the 5′ labeled with LC Red 640 and the 3′ hydroxyl blocked with a phosphate molecule. The acceptor probe for the IAC was labeled with LC Red 705.

Construction of the IAC. The method of Stöcher et al. (32) was used to generate multiple internal controls that were amplified by the same primers used for the detection of the neurotoxin genes of *C. botulinum* types A, B, and E. A DNA sequence (IC T69) already used as an internal control for *Salmonella* spp. and containing a binding site for a pair of hybridization probes (27) was used as starting material, and additional primer-specific sequences were added in a conventional preparative PCR with composite primers (Table 3). By using the previous PCR product as a template in the next preparative PCR, after a purification step with the QIAquick purification kit (QIAGEN, Hilden, Germany), the primer-specific sequences were added, one by one, to the original DNA fragment. Preparative PCRs were performed in a GeneAmp 9700 thermal

cycler (Perkin-Elmer Cetus, Norwalk, CT) using a mixture of 0.2 µM (each) deoxynucleoside triphosphates (dNTPs; Roche Diagnostics GmbH, Mannheim, Germany), 1× *Taq* reaction buffer (Roche), 1 U of *Taq* DNA polymerase (Roche), 0.5 µM (each) composite primers (Sigma-Genosys), and PCR-grade water added to reach a final mixture volume of 20 µl, to which 5 µl of the template (1 µg/ml) was added. The following cycle parameters were applied for the first PCR: 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 60°C, and 40 s at 72°C; and 7 min at 72°C. In the following PCRs, the times for each step of the cycles were increased to 40 and 60 s, respectively.

After each preparative PCR, the product was examined by agarose gel electrophoresis. For final purification, DNA was cut out from the gel and purified using the QIAquick gel extraction kit (QIAGEN). The final product was examined in a conventional PCR with the respective *C. botulinum*-specific primers (Table 2). To confirm the expected nucleotide sequences, the generated internal control was subjected to sequence analysis. Competitive real-time PCR was performed with *C. botulinum* DNA, toxin types A and E, reference strains ATCC 3502 and ATCC 9564, and different amounts of the internal control to determine the optimum amount.

PCR conditions. Real-time PCR amplification was carried out in a LightCycler instrument (Roche). For type B, the protocol from Lövenklev et al. (24), which has previously been used to detect a case of infant botulism (M. Lövenklev, personal communication), was followed. The PCR mixture specific for the *cntE* gene contained 1× *Tth* PCR buffer (Roche), 4 mM MgCl$_2$ (Roche), 0.2 mM (each) dNTPs (Roche), 0.3 µM (each) primers (Sigma-Genosys), 50 copies of the IAC, 0.15 µM probes for the IAC, 0.3 µM probes for *cntE* (TIB Molbiol,

TABLE 3. Composite primers used for preparative PCR for the generation of the multiple IAC

| PCR	Primer	Nucleotide sequence (5′→3′)
1	Forward: fBn-P139	AAAGTAGATGATTTACCAATTGTAGTGAAATT ATCGCCACGTTCGGGCAA
	Reverse: rBn-P141	GTTAGGATCTGATATGCAAACTATCATCGCAC CGTCAAAGGAACCGTAA
2	Forward: As11-fBn	TGCAGGACAAATGCAACCAGTAAAGTAGATGA TTTACCAATTGTA
	Reverse: As22-rBn	TCCACCCCAAAATGGTATTCCGTTAGGATCTG ATATGCAAACTA
3	Forward: fToxE-As11	TGAATCAGCACCTGGATGCAGGACAAATGCAA CCAGT
	Reverse: rToxE-As22	GGTTAGCTTCAGTAGTAAATCCACCCCAAAAT GGTATTCC
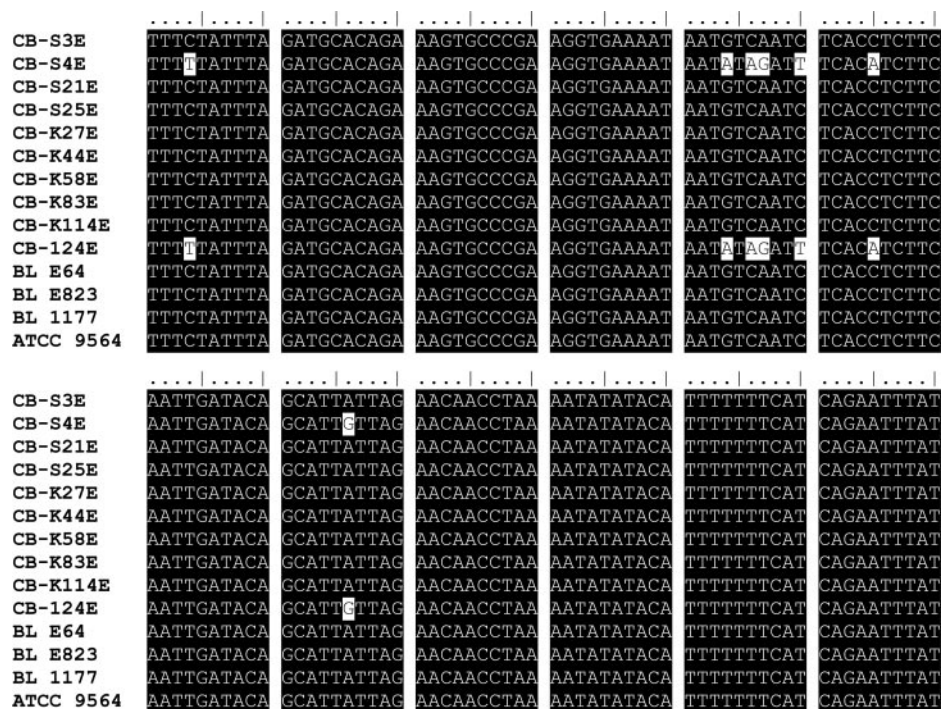
FIG. 1. Alignment of part of the *cntE* nucleotide sequences determined in this study with the corresponding region, positions 1787 to 1906, of the sequence from NCTC 11219 (accession number X62683).

Berlin, Germany), 1.25 U of *Tth* DNA polymerase (Roche), and 4 μl of template solution. The PCR mixture specific for the *cntA* gene contained 1× *Tth* PCR buffer (Roche), 3 mM MgCl₂ (Roche), 0.2 mM (each) dNTPs (Roche), 0.5 μM (each) primers, 50 copies of the IAC, 0.15 μM probes for the IAC, 0.225 μM probes for *cntA* (TIB Molbiol), 1.25 U of *Tth* DNA polymerase (Roche), and 4 μl of template solution. The total volume added to each capillary was 20 μl. The LightCycler amplification protocol used started with an initial denaturation at 95°C for 60 s, followed by 45 cycles of 95°C for 0 s, annealing and fluorescence acquisition at 56°C for 5 s, and elongation at 72°C for 25 s. The specificity was confirmed using a melting-curve analysis. It consisted of 95°C for 0 s and 50°C for 15 s, followed by an increase in temperature of 0.2°C/s up to 90°C. The *cnt* gene was detected in channel F2, and the IAC was detected in channel F3. A color-compensating file was created to avoid spectral overlap of multiple fluorophores.

Sensitivity and amplification efficiency (AE) were tested using series of DNA dilutions. The AE was calculated using the following equation: AE = 10^(−1/s) − 1, where *s* is the slope of the line formed by plotting the crossing-point value against the logarithm of the DNA concentration (22a). For type A, DNA from strain ATCC 3502 was used, and for type E, the DNA was from strain ATCC 9564.

Sequencing. Conventional PCR was used for amplification, and the amplicon was purified using the QIAquick PCR purification kit (QIAGEN). The fragment was sequenced using the BigDye kit (Applied Biosystems) according to the chain termination method (29). The PCR mixture used contained 40 to 100 ng of a PCR fragment, 2 μl of BigDye premix, 0.5 μM primer (Sigma-Genosys), and water added to reach a final mixture volume of 10 μl. The PCR program consisted of 25 cycles of 95°C for 30 s, 50°C for 15 s, and 60°C for 4 min. The DNA was precipitated with a mixture of 1 μl of 3 M sodium acetate (pH 5.2) and 29 μl of 96% ethanol, and after centrifugation, washing with 70% ethanol, and drying, the DNA was sequenced on an ABI PRISM 3100 genetic analyzer (Applied Biosystems) at the BM Unit, Lund University, Lund, Sweden. The sequencing was done three times in each direction, and the results were compared using the BioEdit sequence alignment editor (16).

Laboratory tests. Pus specimens from two abdominal wall abscesses were cultured anaerobically on selective agar (*C. botulinum* isolation) medium and fastidious-anaerobe agar (LabM) and examined for the presence of *C. botulinum* (12). Both samples were also inoculated into enrichment chopped-meat-carbo-hydrate (CMC) medium (17). After incubation for 2 days, the CMC cultures were used for subculturing onto fastidious-anaerobe agar and fresh CMC me-

dium. Samples for DNA extraction and PCR were taken both from the original culture and from plates. Unfortunately, the original sample material was insuf-ficient to allow direct extraction. Cell-free CMC culture supernatants were tested for the presence of botulinum neurotoxins by the mouse bioassay (17). In addi-tion, a serum sample was examined for the presence of botulinum toxin. The pus samples (swab samples) could not be tested for toxin directly, due to the insuf-ficient sample size.

One milliliter of culture was harvested and DNA was extracted as described above. Samples taken from a plate with a sterile loop were dissolved in 200 μl of Tris-EDTA before proceeding as described above. The samples were analyzed as described above using 4 and 8 μl of template, undiluted, diluted 10-fold, or diluted 100-fold. When an increase in fluorescence could be seen in the late cycles, an additional 10 cycles were added to get clearer detection.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in GenBank under accession numbers EF517320 to EF517333.

RESULTS

Oligonucleotides specific for part of the type A and type E neurotoxin genes were constructed (Table 2). During the de-sign of the type E hybridization probes, it was found that two strains, CB-S4E and CB-124E, displayed punctual mutations compared to the other strains sequenced in this study and the sequences reported in GenBank (Fig. 1). The differences are in the same places in both strains. The ToxE-Red probe was therefore redesigned with two degenerated positions. The se-lectivity of both the type A and type E real-time PCR assays was evaluated using 40 strains of *C. botulinum*, 16 strains of other *Clostridium* spp., and 26 non-*Clostridium* strains (Table 1). The PCR products of the primers selected were shown on an agarose gel to be the predicted 335 bp for type E strains, 283 bp for type A strains, and 216 bp for type B strains. None of the other strains gave amplified PCR products when tested with
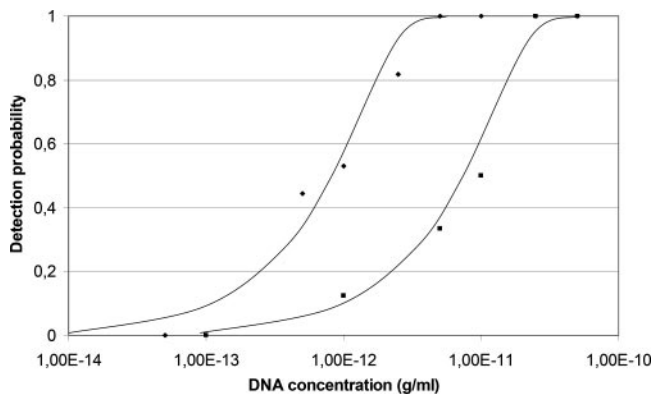
FIG. 2. Detection probability for purified DNA in water. Diamonds represent *C. botulinum* type A, and squares represent *C. botulinum* type E.

conventional PCR or any fluorescent signal when tested with real-time PCR, and there was no cross-reaction between strains. The IAC gave the expected sizes of 0.33 kb with type B primers, 0.37 kb with type A primers, and 0.41 kb with type E primers. The amount of the IAC added did not change the detection limit of the assay (data not shown). The detection limit was at least 20 fg per reaction for type A and 100 fg for type E (Fig. 2). The lowest amount detected was 2 fg for type A, equivalent to 0.5 genomes, and 4 fg for type E, equivalent to 1 genome, assuming an approximate genome size of 3.89 Mbp. The AE with the IAC present was 1.01 for type A and 0.92 for type E, calculated in the linear range between 1 μg/ml and 0.1 ng/ml.

The real-time PCR approach to detect *C. botulinum* types A, B, and E was used on the clinical abscess material from a patient with suspected botulism (see Case Report). Abscess samples were cultivated in CMC medium for 48 h prior to DNA extraction. The PCR results were positive for *C. botulinum* type E. The laboratory confirmation was obtained by the isolation of *C. botulinum* type E and the detection of botulinum toxin type E by the mouse bioassay. A toxin assay of serum failed to demonstrate the presence of botulinum toxin.

DISCUSSION

In this study, we report a sensitive real-time PCR approach to detect *C. botulinum* types A, B, and E. It is proposed to be used to complement the conventional mouse bioassay and to reduce the number of animals needed for the detection and typing of *C. botulinum*. The hope is that eventually the mouse bioassay can be phased out and completely replaced by other techniques, as is recommended by international legislation (Directive 86/609/EEC). Even though there are several published real-time PCR methods for *C. botulinum*, few include an IAC that is amplified by the same primers. In addition, our approach appears to be more sensitive, with a detection limit of 20 fg for type A and 100 fg for type E. In comparison, Yoon et al. (35) report a detection limit of 0.1 ng for type A, Akbulut et al. (2) report a detection limit of 70 fg for type A and 195 fg for type E, and Kimura et al. (22) report a detection limit of 0.5 pg for type E. Fenicia et al. (14), who include an IAC, do not state a detection limit for pure DNA but claim that it is less

than 60 genomic copies for type A, i.e., equivalent to approximately 230 fg. Akbulut et al. (2) also used an IAC; however, it is an exogenous control and uses different primers, which may be affected in different ways by inhibitors (19). In addition, they do not seem to have added it to all samples, since it uses the same dye as one of their probes. Several of our samples were shown to be highly PCR inhibitory (data not shown), which shows the need for an IAC to avoid getting false-negative results.

It appears that delayed diagnosis of wound botulism is not rare (28), and it is even possible that the diagnosis may have been missed in some cases. In the present case, in which antibiotics had been administered before botulism was suspected and samples were taken, it took several days of culturing and repeated subculturing to isolate the bacteria, while PCR could be performed on material from an early stage in the process. The clinical presentation of this patient, i.e., symmetrical, descending, flaccid paralysis in a female IDU, is highly suggestive of wound botulism, but due to the rarity of the disorder, the patient's symptoms and signs were initially misinterpreted. Several other diagnoses, including hysteria, were considered before botulism was considered. Early diagnosis might have allowed for treatment with antitoxin, which is effective in reducing the severity of the symptoms of botulism and the length of hospitalization if administered early in the course of the disease. In a study from California, a lower proportion of patients who had received antitoxin within 12 h of presentation required mechanical ventilation (28) than those who received it later. In addition, the duration of mechanical ventilation was shorter among such patients (median, 45 versus 60 days). Our patient required full ventilatory support for more than 4 months after the disease onset.

Although wound botulism cases in various geographical regions have been described previously, no cases in Sweden have hitherto been reported. Reported risk factors for wound botulism are subcutaneous or intramuscular injection of heroin (skin popping) and female gender (26). We have not been able to identify the source of this patient's infection, but it is likely to be heroin contaminated with spores of *C. botulinum*. In California, black tar heroin is imported from Mexico, but in southern Sweden, most black heroin originates from Afghanistan. We were not able to get access to heroin to test for *C. botulinum*, and the contamination may be restricted to certain batches. The most probable source of contamination is the "cutting" process, when the drug is diluted before sale (26). Since type E spores are the most common in Swedish soil (13), the cutting was probably done locally. It is also possible that the syringe was contaminated.

Outbreaks of wound botulism have occurred in many geographical locations, but so far we are not aware of other cases in IUDs in Sweden. However, this case illustrates that wound botulism is yet another complication of injecting drug use that has to be considered by clinicians and demonstrates the utility of real-time PCR as a complement to traditional methods of diagnosis.

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