We report a clinical case of meningoencephalitis with subdural empyema in an immunocompromised farmer caused by toxigenic Clostridium perfringens type A, which was identified by 16S RNA gene analysis of cerebrospinal fluid and subdural empyema. In immunocompromised patients, C. perfringens should be considered a potential pathogen of sepsis.

CASE REPORT

In June 2011, a 74-year-old farmer developed headache, fever, nausea with emesis, and fatigue within 24 h prior to admission to a community hospital. Due to a systemic inflammatory response syndrome (SIRS), he was referred to the intensive care unit (ICU). Empirical intravenous antibiotic treatment with piperacillin-tazobactam and clarithromycin was started based on the clinical presentation of pneumonia with a suspicious infiltrate in the basal lobe. The patient was immunosuppressed secondary to unclassified myelodysplastic syndrome (MDS) and was on continuous steroid treatment for bronchiolitis obliterans organizing pneumonia. Three weeks prior to hospital admission, the farmer had been struck by a cow, but did not seek medical attention. Two hours after admission, the patient developed a generalized seizure with postictal reduced consciousness and left-sided Todd’s paresis. A cerebral computed tomography (CT) showed a hyperdense subdural parieto-occipital lesion on the right side with adjacent brain edema, suggestive of a chronic subdural hematoma (Fig. 1a). Therefore, the patient was referred to a tertiary care hospital for neurosurgical intervention. There, in addition to fever and tachycardia, reduced consciousness, left-sided hemiparesis, and slight neck stiffness were present. The laboratory findings revealed the following: white blood cell count, 1.02 × 10^9/ml (34% neutrophils, 63% lymphocytes, 1% eosinophils, and 2% monocytes); thrombocyte count, 23 × 10^9/ml; prothrombin ratio, 58%; international normalized ratio (INR), 1.5; serum C-reactive protein, 13.3 mg/dl; and HIV and hepatitis B negative. Based on clinical presentation with headache, fever, epileptic seizure, and slight neck stiffness, a central nervous system (CNS) infection was postulated. Antibiotic treatment was changed to intravenous ceftriaxone, amoxicillin, metronidazole, vancomycin, and acyclovir in order to cover microorganisms potentially causing central nervous system (CNS) infection (including Listeria spp., anaerobic bacteria, penicillin-resistant Streptococcus pneumoniae, and herpes simplex virus). Due to impaired coagulation with increased risk of spinal bleeding, a lumbar puncture was initially declined. A cerebral CT scan showed a progression of the subdural lesion to the right frontal lobe, suggestive of an active subdural process, such as empyema (Fig. 1a and 1c). Two days after reconstitution of coagulation, the right frontal part of the lesion was evacuated through a burr hole for microbiological workup. An intraventricular drain (IVD) was inserted on the contralateral side. The intraoperative macroscopic findings supported the radiological diagnosis of subdural empyema. Samples from cerebrospinal fluid (CSF) and empyema were processed for CSF cell count, cytospin and Gram staining, bacterial broad-range 16S RNA gene PCR, fungal broad-range PCR targeting the internal transcribed spacer (ITS) region, and microbiological cultures. Unfortunately, initial analysis of the CSF cell count failed, and CSF drainage was suspended immediately after surgery due to cloting of the catheter. The direct staining of both specimens showed plump, Gram-positive rods with blunt ends suggestive of Clostridium spp. C. perfringens was finally identified in both samples by bacterial broad-range 16S RNA gene PCR followed by sequence analysis of the amplicon (4). A nested PCR as the second amplification step was not required, for which the time to result was substantially reduced. PCR amplicons were sequenced (±500 bp), and homology analysis was performed using IDNS software (SmartGene, Zug, Switzerland). A homology of 99%, with a minimal of 0.5% difference from the second homologous species, allows for identification at the species level. The sequences of the PCR products (465 and 468 bp, respectively) showed 100% identity to the 16S rRNA gene of Clostridium perfringens.

The second homologous species was Clostridium saccharobutylicum, with an identity of 94.7%, enabling an accurate assignment of C. perfringens present in both specimens. The sequence electropherograms gave no indication for a possible polymicrobial infection. A broad-range fungal PCR targeting the internal transcribed spacer (ITS) region (15) was performed using the DNA extracts of the cerebrospinal fluid and the subdural empyema, which was negative for both samples. Subsequently, C. perfringens toxin typing was performed by multiplex PCR (34) followed by sequence analysis for confirmation, which showed the presence of the cpa gene (encoding alpha-toxin) in both CSF and subdural empyema samples. PCR for cpe (β-toxin), cpb2 (B2-toxin), etx (ε-toxin), iap (β-toxin) and cpe (enterotoxin) genes were negative. Anaerobic cultures on brucella agar remained negative even after 10 days of growth.

Received 28 March 2012 Returned for modification 4 May 2012 Accepted 7 August 2012 Published ahead of print 15 August 2012 Address correspondence to Michael Hugelshofer, mhugelshofer@gmx.ch. M. Hugelshofer and Y. Achermann contributed equally to this article. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.00802-12
The most frequent clinical manifestation of *C. perfringens* infection is myonecrosis, or gas gangrene. If only minor amounts of toxins are produced, infections may present as a mild, self-limiting disease. If local infections spread via hematogenous dissemination, every organ might be involved, including the brain. Central nervous system manifestation of *C. perfringens* infection in humans is rare (9). Most commonly, the meninges are affected (1, 3, 6, 7, 10–14, 16–19, 21, 22, 23–25, 29, 30) after clinical manifestations of sepsis (31). Rarely, focal or diffuse encephalitis with or without pneumocoealus has been presented (2, 13, 27, 29), and only a single case of subdural empyema has been reported in the current literature (23). In our patient due to the clinical findings of generalized epileptic seizure, radiological evidence of progressive brain edema, and detection of *C. perfringens* type A by molecular analysis of CSF and subdural empyema, we diagnosed a meningoencephalitis and subdural empyema with *C. perfringens*. We were unable to cultivate *C. perfringens*, probably due to the antibiotic treatment started 3 days before sampling. However, the diagnosis was unambiguous according to (i) direct microscopic detection of plump Gram-positive, rod-shaped bacteria in large quantities in two independent samples of the subdural lesion and (ii) 100% identification of the 16S rRNA gene PCR amplicon with *C. perfringens* in both clinical samples (cerebrospinal fluid and subdural empyema). In addition, the *cpa* gene, which codes for the virulence factor alpha-toxin of *C. perfringens* type A, was amplified and confirmed by sequence analysis.

Usually, infections with *C. perfringens* start from a recent surgical wound, trauma, or intra-abdominal disease, such as infections of the biliary tract or other gastrointestinal infections (26). However, in most of the cases of *C. perfringens* meningitis, the site of infectious origin remains unidentified (6, 11, 13, 14, 18, 21, 23, 24, 28), as in our patient. *C. perfringens* is a common part of the intestinal flora of domestic animals, such as cattle, and the bacteria are spread into the environment by feces (33). In our case, gastrointestinal disease caused by a food-borne infection was ruled out. There was a history of minor trauma caused by a strike of a cow’s hoof 3 weeks before onset of symptoms. Possibly, *C. perfringens* type A was carried on the cow’s hoof and transmitted into the wound. Due to the preexisting coagulopathy and the history of trauma, we assume a chronic subdural hematoma as the site of onset for the CNS manifestation. Although repeated normal aerobic and anaerobic blood cultures remained negative, a transient bacteremia with *C. perfringens* type A is likely to have happened in a chronically exposed and immunocompromised farmer.

Subdural empyema and diffuse encephalitis with *C. perfringens* are reported to be fulminant and have a fatal outcome in 30 to
In conclusion, we report the first case of cerebral infection due to *C. perfringens* type A manifested with subdural empyema and diffuse meningoencephalitis, which was diagnosed by 16S rRNA sequencing and a multiplex PCR approach. The patient was successfully treated with targeted antibiotic therapy and surgical removal of subdural empyema. Even though rarely reported, *C. perfringens* type A should be considered a differential pathogen in a patient with immunosuppression. If no pathogen can be cultivated due to ongoing antibiotic treatment, molecular analysis such as broad-range 16S RNA PCR is a valuable and rapid tool leading to an accurate diagnosis.

**Nucleotide sequence accession number.** The 16S sequence of the patient’s isolate has been submitted to GenBank under accession no. JQ782390.

**REFERENCES**