Investigations of 2 Cases of Diphtheria-Like Illness Due to Toxigenic *Corynebacterium ulcerans*

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**Background.** We present 2 case reports in the United States and investigations of diphtheria-like illness caused by toxigenic *Corynebacterium ulcerans*. A fatal case occurred in a 75-year-old male Washington resident who was treated with clindamycin but did not receive equine diphtheria antitoxin. A second, nonfatal case occurred in a 66-year-old female Tennessee resident who received erythromycin and diphtheria antitoxin.

**Methods.** Both case patients and close human and animal contacts were investigated by their respective state health departments.

**Results.** *C. ulcerans* isolated from the patient who died was resistant to erythromycin and clindamycin. For both isolates, conventional polymerase chain reaction results were positive for A and B subunits of diphtheria toxin gene tox, and modified Elek tests confirmed toxin production. The source of infection remained undetermined for both cases. Neither patient was up-to-date with diphtheria toxoid vaccination.

**Conclusion.** These case reports highlight the importance of early treatment with diphtheria antitoxin, the selection of effective antimicrobial agents, and prevention through up-to-date vaccination.

Toxigenic *Corynebacterium ulcerans* was first isolated from the throat of a patient with respiratory diphtheria-like illness in 1926 [1]. Toxigenic strains of *C. ulcerans* produce a diphtheria toxin that is similar to that produced by toxigenic strains of *Corynebacterium diphtheriae* [2, 3]. Diphtheria toxin contributes to the formation of a pseudomembrane that characterizes respiratory diphtheria and may cause cardiac and neurologic sequelae. *C. ulcerans* also produces a dermonecrotic toxin that is similar to that produced by *Corynebacterium pseudotuberculosis* [2].

*C. ulcerans* is a commensal in animals and has been isolated from a wide host of domestic and wild animals (table 1) [4–18]. These animals may serve as reservoirs for human infection. *C. ulcerans* causes mastitis in cattle and goats. Handling of infected dairy animals and consumption of contaminated milk have been associated with respiratory diphtheria–like disease caused by *C. ulcerans* [19]. *C. ulcerans* is also reported to cause cutaneous lesions in humans [20, 21].

The epidemiology of human infections caused by *C. ulcerans* is not well known. As shown in table 2, respiratory diphtheria–like illnesses caused by toxigenic strains of *C. ulcerans* are increasingly reported from developed countries [3–5, 22–41]. *C. ulcerans* accounted for 21 (58%) of 36 human toxin-producing isolates of *Corynebacterium* species in the United Kingdom from 1997 through 2002 [42], 3 (33%) of 9 isolates in Canada from 1999 through 2003 [43], and 1 (33%) of 3 isolates in Italy from 1990 through 2001 [44].

In the United States, 5 confirmed or probable cases of respiratory diphtheria caused by *C. diphtheriae* (including 1 imported fatal case) were reported during the period 1999–2005. During the same period, the Centers for Disease Control and Prevention (CDC) received reports of 2 cases (1 fatal) of diphtheria-like illness caused by *C. ulcerans*; prior to 1999, the last reported case of diphtheria-like illness caused by *C. ulcerans* in the United States occurred in 1996 [31]. We report the
epidemiologic investigations of these 2 cases of respiratory diphtheria–like illnesses caused by *C. ulcerans* in Washington and Tennessee.

**METHODS**

The Washington State Department of Health and the Tennessee Department of Health conducted epidemiologic investigations of patients A and B. The investigations included reviewing both patients’ medical records, identifying close household contacts, obtaining nasopharyngeal swab specimens from contacts, offering postexposure prophylaxis with penicillin or erythromycin, and administering an age-appropriate diphtheria toxoid vaccine to those who were not current with vaccination. Personnel from the Minnesota Department of Health interviewed family members and investigated cattle at the Minnesota dairy farm visited by patient A. Oral and rectal swab specimens were obtained from animal contacts of both patients.

**RESULTS**

**Patient A (Washington).** In 1999, a 75-year-old white non-Hispanic man presented to an emergency department with a 3-day history of sore throat with difficulty in swallowing and fever. The result of a rapid streptococcal test of a throat swab specimen was negative. The patient was prescribed erythromycin and sent home; the patient reported allergies to cephalosporins and penicillin. He returned to his primary care physician the next day because of persisting sore throat and worsening difficulty swallowing and breathing, and he was hospitalized. On examination, the patient’s oral temperature was 38.3°C, and he had copious nasopharyngeal secretions. Direct laryngoscopic examination revealed ulceration, with yellowish exudate on the posterior pharynx. His initial WBC count was 1500 cells/mm³, with 66% polymorphonuclear cells, 5% band cells, and 23% lymphocytes. During the first 3 days of hospitalization, the patient received intravenous clindamycin. On the fourth day after hospital admission, the drug was replaced with metronidazole, ciprofloxacin, and vancomycin, after the local laboratory reported that culture of throat specimens grew 4+ diphtheroids that did not show typical *C. diphtheriae* morphology by methylene blue staining and after diphtheria was excluded as a likely diagnosis. Although the patient had signs of a diphtheria-like illness, diphtheria antitoxin was not recovered. The patient developed dyspnea and underwent intubation after an episode of aspiration. His condition rapidly deteriorated, with development of pulmonary edema and evidence of cardiogenic shock. Despite nasotracheal intubation and mechanical ventilation, the patient died on the following day.

The patient had a significant history of Felty syndrome and had been receiving daily 10-mg doses of oral methylprednisolone for the preceding 11 years. He had no travel history outside of the United States, but he had spent 2–3 days visiting friends on a farm in Minnesota 10 days prior to the onset of his illness. The patient had no history of interacting with anyone who had recently traveled abroad or any person with skin ulcers. He had reportedly received a booster tetanus and diphtheria vaccination ≥12 years prior to this illness.

Postmortem examination demonstrated a thick, gray membrane that extended from the throat into the bronchial tree and into the esophagus. The membrane had sloughed in some areas, and there was narrowing of the airways due to edema. No significant inflammatory changes were noted in the

<table>
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<th>Reference</th>
<th>Year</th>
<th>Country</th>
<th>Animal source</th>
<th>Clinical presentation</th>
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<td>United Kingdom</td>
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<td>Dromedary camel</td>
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<tr>
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<td>1967</td>
<td>United Kingdom</td>
<td>Dairy herds</td>
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myocardium or in peripheral nerves. Postmortem specimens from the larynx, trachea, and lungs were obtained for culture and conventional PCR testing [45].

Culture of throat specimens obtained during the laryngoscopic examination revealed heavy growth of diphtheroids (4+) on day 3 of hospitalization. The isolates were forwarded to the Washington State Department of Health Public Health Laboratory (Shoreline, WA), where the agent was identified as *C. ulcerans* 4 days after the patient died. The agent was resistant to clindamycin and erythromycin but susceptible to penicillin, sulfamethoxazole, ciprofloxacin, vancomycin, and cephalosporins. The CDC confirmed these results and demonstrated the presence of toxigenic *C. ulcerans* by the modified Elek test [46] and by positive conventional PCR [45] for subunit A and subunit B of the diphtheria toxin gene (*tox*). In addition, the conventional PCR results were positive for the *tox* gene in postmortem formalin-fixed tissue specimens from the larynx and trachea but were negative in lung tissue specimens. Real-time PCR [47] performed on this isolate revealed atypical amplification of subunit A and no amplification of subunit B of the *tox* gene.

**Patient B (Tennessee).** In 2005, a 66-year-old white, non-Hispanic woman complained of fatigue, sore throat, and difficulty in swallowing. Her symptoms worsened, and on the following afternoon, she presented to the emergency department of a local hospital. She was afebrile (oral temperature, 37.1°C) and had sinus tachycardia, with a heart rate of 105 beats per min. A throat examination revealed an erythematous palate and edematous pharynx and uvula, with a covering exudate that extended into the nasopharynx. CT of the neck confirmed marked edema of the uvula and soft palate and demonstrated near obliteration of the nasopharynx but did not suggest epiglottitis or a retropharyngeal abscess. The result of a rapid streptococcal test was negative.
The patient was hospitalized and initially received methylprednisolone, ceftriaxone, and clindamycin and, later, erythromycin and ampicillin/sulbactam. At hospital admission, her WBC count was 12,600 cells/mm³, with 86% granulocytes and 7% lymphocytes. During the night, the patient developed respiratory difficulty, with oxygen desaturation to 68%. There was no clinical evidence of neurological abnormality or cardiac abnormality by electrocardiogram. By the following morning, the palatine erythema and edema had worsened, her voice became more muffled, and her breathing became noisy. The patient underwent emergency intubation and taken to the operating room, where tracheostomy was performed. During tracheostomy, an extensive, thick, yellowish, fibrinous, sloughing membrane was noted to cover the lower half of the uvula, extensively coating the posterior aspect of the soft palate and the entire nasopharynx and extending into the trachea. The membrane measured about 5 × 3 cm. It was gradually removed by peeling and suctioning, leaving behind an intact mucosa with mild, punctate bleeding. Direct laryngoscopic examination demonstrated a normal hypopharynx, larynx, and epiglottis. There was marked swelling of the soft palate and pharynx. The attending physician suspected diphtheria and treated the patient with 60,000 U of diphtheria antitoxin (DAT) obtained on the same day from the CDC. Throat swab specimens were obtained for *C. diphtheriae* testing. The patient had an uneventful recovery.

At the Tennessee State Health Department laboratory (Nashville, TN), cultures of throat specimens grew black colonies on tellurite media that were suggestive of *Corynebacterium* species. The isolate and membrane specimen were identified as *C. ulcerans* at the CDC. The organisms were susceptible to penicillin, erythromycin, clindamycin, ceftriaxone, and ciprofloxacin. Results of conventional PCR were positive for both subunit A and subunit B of the *tox* gene. However, real-time PCR revealed atypical amplification of subunit A and no amplification of subunit B. Toxin production in the isolate was demonstrated by a positive modified Elek test result. A commercial laboratory reported the serum antibody level to diphtheria toxin to be 0.036 IU/mL (protective level, ≥0.1 IU/mL) in this patient.

The patient had not received vaccination against diphtheria during the previous 30 years. She lived in a farmhouse with her husband and had not traveled during the 2-week period before onset of illness. She seldom had direct contact with the animals, although she owned 4 horses and 2 dogs; she did not own dairy animals. Her close and frequent contacts included her children and their families. One daughter had frequent close contacts with immigrants from Central America. Members of her church had recently returned from a 1-week cruise to the Caribbean that included island stops.

**Contact investigation.** As shown in table 3, no specimen from human and animal contacts of either case patient grew *C. ulcerans*.

**DISCUSSION**

Several published case reports have linked human *C. ulcerans* infections to consuming unpasteurized milk from infected cows or having close contact with infected dairy animals [13, 18, 19]. However, in the majority of reports, patients neither consumed raw milk nor had contact with dairy animals (table 2). In 2 recent reports, human infection followed contact with dogs with chronic labial ulceration, rhinorrhea, and sneezing [4, 5];...
in 1 report, the same *C. ulcerans* strain was isolated from both the patient and her dog [5]. Although a human case resulted from possible exposure to an infected stray cat with rhinorrhea [25], in another report, close household contacts of 2 infected cats with rhinorrhea did not acquire infection [8]. In our reports, nasal and rectal specimens from dairy animals, horses, and dogs were culture negative for *C. ulcerans*, suggesting that there might be other reservoirs and/or novel ways of transmission.

The clinical features of diphtheria-like illness caused by *C. ulcerans* are similar to those of clinical respiratory diphtheria, and laboratory investigations, treatment, public health response, and preventive interventions are identical for the 2 types of infection. Infection with *C. ulcerans* or *C. diphtheriae* should be considered in the differential diagnosis of membranous pharyngitis. Culture of throat specimens is the gold standard for diagnosis of diphtheria-like illness caused by *C. ulcerans*. Although no special culture medium is required for growth, media such as Tinsdale or tellurite agar are useful for rapidly identifying potentially pathogenic *Corynebacterium* species. Growth of diphtheroids not showing typical morphology for *C. diphtheriae* by methylene blue staining does not rule out clinical diphtheria or exclude *C. ulcerans*. A modified Elek test is used to confirm toxin production. A positive result of conventional PCR of isolates or tissue specimens detects the presence of subunits A and B of the tox gene but does not confirm whether the strain is producing toxin. PCR may be particularly useful to detect the presence of a strain containing the tox gene when antimicrobial therapy is initiated prior to specimen collection. A newly developed but not commercially available real-time PCR test [47] is used at the CDC to provide more rapid results than conventional PCR for *C. diphtheriae*. However, in this report, real-time PCR analysis of *C. ulcerans* isolates from both case patients produced atypical amplification of subunit A and no amplification of subunit B. Although conventional PCR to detect the presence subunits A and B of the tox gene may be useful for screening for *C. diphtheriae* and *C. ulcerans*, these findings indicate that additional studies are required to determine the role of real-time PCR testing of toxigenic *C. ulcerans* isolates.

*C. ulcerans* causes severe diphtheria-like illness, and patients often require hospitalization (table 2). As for clinical diphtheria, the mainstay of treatment is equine DAT. Providers should promptly administer DAT to a patient with respiratory diphtheria–like illness, after testing for sensitivity to DAT and without awaiting laboratory confirmation. Failure or delay in administering DAT can lead to a fatal outcome. The dose of DAT varies from 40,000 IU to 100,000 IU and depends on the site and extent of the membrane and the duration of symptoms. In the United States, DAT is available from the CDC under a US Food and Drug Administration–approved Investigational New Drug protocol and can be obtained by contacting the Director’s Emergency Operations Center at the CDC [48].

Although antimicrobial agents are not a substitute for DAT, they eliminate *C. ulcerans* from the respiratory tract and, thereby, limit toxin production, reduce disease severity if administered early, and halt potential transmission. In vitro studies have demonstrated that *C. ulcerans* is susceptible to a wide range of antimicrobial agents [49]. The recommended antimicrobial agents and their dosage for treatment and postexposure prophylaxis are the same as those for treatment of clinical diphtheria. Generally, erythromycin and penicillin are effective and are the preferred antimicrobial agents for treatment of respiratory diphtheria–like illness caused by *C. ulcerans*. To our knowledge, this is the first report of respiratory diphtheria–like illness caused by a *C. ulcerans* strain that was resistant to erythromycin and clindamycin but susceptible to penicillin, vancomycin, ciprofloxacin, sulfamethoxazole, and cephalosporins. The finding of an erythromycin-resistant strain of toxigenic *C. ulcerans* highlights the importance of testing strains of this organism for susceptibility to antimicrobials used for treatment and/or postexposure prophylaxis.

Because of the rare possibility of transmission to close contacts from patients with diphtheria-like illness caused by *C. ulcerans* [50], contact investigations can be limited to household members and other persons who have intimate physical contact or direct exposure to respiratory secretions of a case patient or to animals infected with *C. ulcerans*. In this report, *C. ulcerans* was not isolated from any close contacts (human or animal), and this finding is consistent with other case reports [3–5, 22–41]. Nevertheless, close face-to-face contacts of patients should receive postexposure chemoprophylaxis after nasopharyngeal and throat swab specimens are obtained for culture and should be placed under surveillance for a week for evidence of disease [51].

Up-to-date immunization with a diphtheria toxoid vaccine will prevent diphtheria and diphtheria-like illness caused by *C. ulcerans* by maintaining adequate levels of antibodies to diphtheria toxin. In this report, both case patients were older adults who were not up-to-date with booster immunization against diphtheria. Because diphtheria or diphtheria-like illness caused by *C. ulcerans* may not provide an adequate protective immune response, case patients should receive an age-appropriate diphtheria toxoid vaccine during the convalescent period. Close contacts of case patients should also receive an age-appropriate diphtheria toxoid vaccine according to the childhood, adolescent, and adult immunization schedules, as recommended by the Advisory Committee on Immunization Practices. The Advisory Committee on Immunization Practices recommends that all children receive a routine series of a pediatric diphtheria toxoid vaccine (diphtheria and tetanus toxoids and acellular pertussis antigen vaccine and diphtheria and tetanus toxoids)
at ages 2, 4, 6, and 12 months and between 4 and 6 years of age. Adolescents should receive a booster dose with tetanus toxoid and reduced diphtheria toxoid and acellular pertussis vaccine, preferably between 11 and 12 years of age, and then tetanus and diphtheria vaccine every 10 years thereafter during adulthood [52, 53]. For added protection against pertussis, adults who have not previously received a dose of tetanus toxoid and reduced diphtheria toxoid and acellular pertussis vaccine should receive a single dose of such vaccine to replace the next dose of tetanus and diphtheria vaccine [54].

In summary, heightened clinical awareness of respiratory diphtheria–like illness caused by C. ulcerans is critical for early recognition and prompt administration of treatment with DAT and appropriate antimicrobial agents. Finding of a strain resistant to first-line antimicrobials highlights the need for alternative antimicrobial susceptibility testing of C. ulcerans. Using real-time PCR to rapidly confirm the presence of the tox gene in C. ulcerans may lead to false-negative results, and this test requires further evaluation for toxigenic C. ulcerans isolates. Lastly, age-appropriate vaccination with diphtheria toxoid vaccines and timely decennial boosters should be encouraged to prevent disease.

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