Journal of Microbiology and Infectious Diseases / JMID
2012; 2 (1): 9-13
doi: 10.5799/ahinjs.02.2012.01.0034

ORIGINAL ARTICLE

A small cutaneous Anthrax epidemic in Eastern Turkey

Umut Gülaçtı[1], Cemal Üstün[2], Mehmet Özgür Erdoğan[3]

[1] *Elazığ Harput General Hospital, Department of Emergency Medicine, Elazığ, Turkey*
[2] *Elazığ Harput General Hospital, Department of Infectious Diseases and Clinical Microbiology, Elazığ-Turkey*
[3] *Ministry of Health Haydarpasa Numune Training and Research Hospital, Depart. of Emergency Medicine, İstanbul-Turkey*

ABSTRACT

Objectives: This study aims to investigate an epidemic of cutaneous anthrax in Tunceli Province, Eastern Turkey.

Materials and methods: Seven cases with cutaneous anthrax, admitted to emergency room, were diagnosed and followed at Elazig Harput State Hospital in August 2011. The possible sources of epidemic and clinical characteristics of the patients were evaluated.

Results: The mean age of seven cases with cutaneous anthrax was 34.1±8 years, of whom four were male and three were female. All patients were from the same village of Cemisgezek, Tunceli, and all of them had contacted with a dead animal. Amoxicillin/clavulanic acid-resistant *Bacillus anthracis* was recovered from a patient' wound. This resistance may be related with the inappropriate use of antibiotics in animal and animal husbandry practices.

Conclusion: Most of natural originated anthrax cases are cutaneous. Although the incidence of anthrax has decreased in Turkey, the disease is still endemic in the eastern part of the country. *J Microbiol Infect Dis 2012; 2(1): 9-13*

Key words: Anthrax, epidemic, amoxicillin/clavulanic acid, antibiotic resistance

Türkiye'nin Doğusunda Bir Cilt Şarbonu Epidemisi

ÖZET

Amaç: Bu çalışmada Türkiye'nin doğusunda Tunceli vilayetinde meydana gelen bir cilt şarbonu epidemisi incelendi.

Gereç ve yöntem: Elazığ Harput Devlet Hastanesi Acil Servisi'ne Ağustos 2011'de başvuran yedi cilt şarbonu olgusu takip edildi. Muhtemel salgın kaynağı ve hastaların klinik özellikleri irdelendi.

Bulgular: Hastane Acil Servisine başvuran yedi hastaya cilt şarbonu teşhisi konuldu. Hastaların yaş ortalamaları 34,1 ± 8 yıl olup dördü erkek, üçü kadın idi. Hastaların tamamı, Tunceli'nin Çemişgezek ilçesine bağlı bir köyden idiler ve hepsi de köyde ölü bir hayvana temas etmişlerdi. Bir olgunun yara kültüründen amoksisilin/klavulanata dirençli *Bacillus anthracis* izole edildi. Hayvanlarda uygunsuz antibiyotik kullanımının bu tür dirençli bakterilerin gelişimine neden olabileceği düşünüldü.

Sonuç: Doğadan kazanılan insan şarbonu olgularının çoğu cilt şarbonu şeklindedir. Türkiye'de şarbon insidansında düşüş görülmesine rağmen ülkenin doğusunda hastalık halen endemiktir.

Anahtar kelimeler: Şarbon, epidemi, amoksisilin/klavulanat, antibiyotik direnci

INTRODUCTION

Anthrax is a zoonosis caused by *Bacillus anthracis*, a Gram-positive, spore-forming bacterium that naturally infects herbivorous animals. Humans, as incidental hosts, are infected by direct or indirect contact with animals and contaminated animal products. Butchers, veterinarians, and farmers who deal with cattle are at risk for anthrax infection.[1] The major disease forms are cutaneous, respiratory, and gastrointestinal. Approximately 95% of naturally acquired infections are cutaneous in humans. The lesions are mainly distributed on the upper limbs, head, and neck.[2]

The incidence of anthrax is low in developed countries but remains a global concern. *B. anthracis* spores can be used as a biological weapon, as exemplified by the bio-terrorist attacks in 2001 in USA. Anthrax is found globally, with an

Correspondence: Dr. Cemal Üstün, Elazığ Harput General Hospital Department of Infectious Diseases and Clinical Microbiology, Elazığ-Turkey Email: drcustun@gmail.com
Received: 18 January 2012, Accepted: 03 February 2012

incidence of up to 100,000 cases annually.[1,3] In Turkey, 6.730 human anthrax cases were reported between 1990 and 2006. In recent years, the anthrax incidence in Turkey has decreased remarkably. In 2009, 148 cases were reported, while in 2010, only 93 cases were reported. The incidence of anthrax was highest between 1995 and 2005 in Kars Province, where 309 human and 123 animal cases were reported. In Tunceli Province, 25 cases were reported between 1995 and 2005; however, no cases have been reported since 2009.[4,5]

Recently, antibacterial-resistant *B. anthracis* has become an important concern, and amoxicillin/clavulanic acid resistance among *B. anthracis* strains has been reported.[6] However, in Turkey, amoxicillin/clavulanic acid-resistant *B. anthracis* has not been reported.[7,8] In this study, we investigated an epidemic of seven cutaneous anthrax cases in Eastern Turkey.

Patients and Methods

Seven cases with cutaneous anthrax, admitted to emergency room and Infectious Diseases Policlinic, were diagnosed and prospectively followed at Elazig Harput State Hospital in August 2011. Three patients were hospitalized in the Infectious Diseases Clinic, and the others were followed as outpatients. A detailed history, including patients' socio-demographic characteristic and history of contacting with dead animals,, was obtained from the cases during the admission to the hospital. The possible sources of epidemic and clinical characteristics of the patients were evaluated.

Culture material of the vesicular lesion was obtained by needle aspiration. Specimens were cultured on sheep blood agar and incubated under aerobic conditions at 37°C for 48 h. Bacteriologic isolation was performed on the culture material. Resistance to penicillin, ampicillin, amoxicillin/clavulanic acid, and trimethoprim/sulfamethoxazole was evaluated according to the antibiogram (Oxoid-disc diffusion test).

RESULTS

All patients with cutaneous anthrax, from Örenceler Village, Çemişgezek, Tunceli, were diagnosed in August 2011. The patients were admitted to Emergency Room with necrotic, crusted, edematous, and erythematous wound infection on their upper extremities. Three patients (Case 1, Case 2 and Case 3) were initially admitted to emergency room and hospitalized in the Infectious Diseases Clinic. Two days later, the other four patients were admitted to emergency room and, then to Infectious Diseases Policlinic. These patients had been treated in a primary health care center. These patients were followed as outpatients. Three out of seven patients were from the same family, and the others were their neighbors. A total of four cattle had died in the village, and all patients had contacted with these animal.

Case 1

A 33-year-old male farmer presented to the emergency department (ED) with a 3x3 cm crusty, hemorrhagic wound with major erythema and edema on the left arm (Figure 1). He reported that he had carried four dead cows 20 days ago. He complained of fever and had a wound on his left arm. A physical examination revealed a body temperature of 38.7°C (axillary), arterial blood pressure of 120/70 mm/Hg, and a pulse rate of 85 beats/min. The patient was hospitalized in the Clinic of Infectious Diseases. Laboratory examinations revealed leukocytosis (12.800/mm^3), elevated C-reactive protein (CRP) concentration (69 mg/dl), and an erythrocyte sedimentation rate (ESR) of 19 mm/h. The patient was diagnosed with cutaneous anthrax and treated empirically with intravenous ampicillin/sulbactam four times 1 gram and oral ciprofloxacin (two times 500 mg). After incubation, flat, non-hemolytic white colonies with irregular edges were observed. Gram-stained culture smears demonstrated Gram-positive, endospore-forming bamboo-type rods (Figure 2). Identification was established based on catalase positivity. Blood culture samples were found negative. The antibiogram revealed that the isolate was sensitive to ceftazidime, ciprofloxacin, imipenem, and gentamicin. Ampicillin/sulbactam treatment was ended and only ciprofloxacin was continued. On the seventh day, the patient was discharged in a satisfactory clinical condition with the recommendation of outpatient controls and continuous antibiotic treatment for ten days. Progress was satisfactory regarding the outpatient controls.

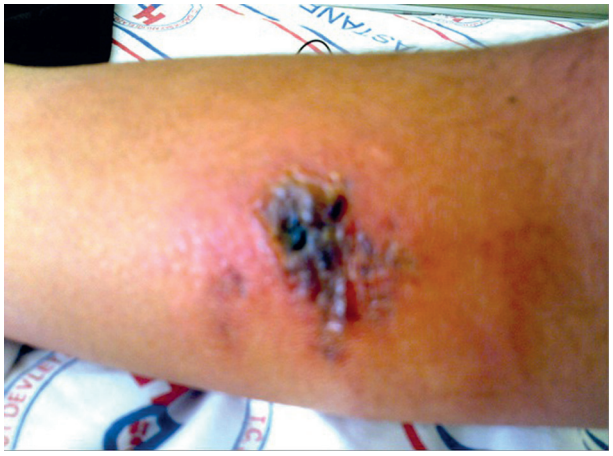

Figure 1. Erythematous and mid-necrotic crusted lesion with extensive edema on the left arm

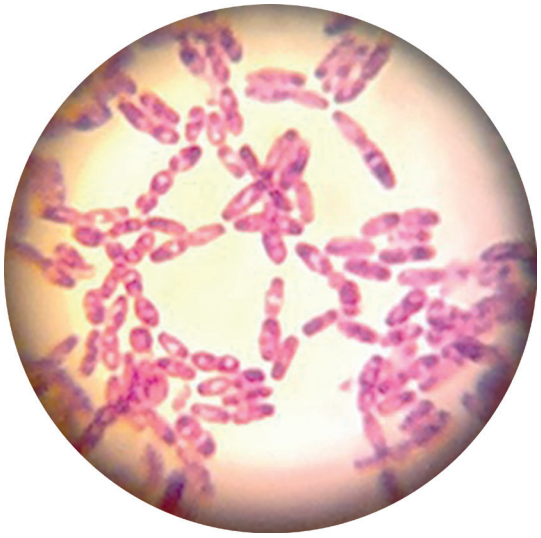

Figure 2. Characteristic central ellipsoidal endospore and squared-end *B. anthracis* as visualized by Gram-staining

Case 2

A 33-year-old male farmer presented to the ED with a 2x2 cm necrotic ulceration with major erythema and edema involving the left forearm (Figure 3). The patient reported the same story as in case 1 (dead cow transport). Physical examination revealed a body temperature of 36.7°C (axillary), arterial blood pressure of 110/70 mm/Hg, and a pulse rate of 80 beats/min. Laboratory examinations showed leukocytosis (9,800 /mm^3), a CRP concentration of 85.8 mg/dl, and an ESR of 33 mm/h. The patient was hospitalized for cutaneous anthrax and treated empirically with oral ciprofloxacin (2x500 mg). The culture of vesicular lesion material did not identify any pathogen, and blood culture revealed no bacterial growth. The patient was discharged in satisfactory clinical condition with recommended outpatient controls and continuous antibiotic treatment for ten days.

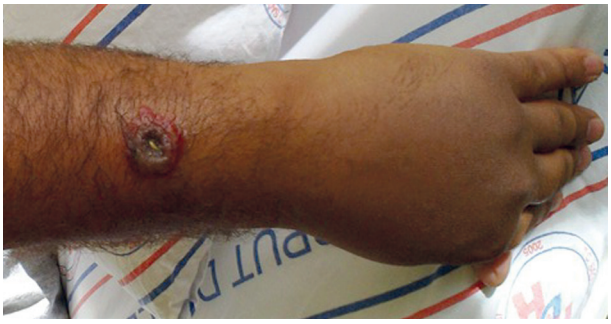

Figure 3. Erythematous, mid-necrotic lesion with edema on the left forearm

Case 3

A 46-year-old male farmer presented to the ED with two necrotic ulcerations on the right forearm (Figure 4). The patient reported the same story of dead cow transport. A physical examination revealed a body temperature of 37.2°C (axillary), arterial blood pressure of 110/80 mm/Hg, and a pulse rate of 75 beats/min. A CBC revealed leukocytosis (11,700 /mm^3), a CRP concentration of 70.8 mg/dl, and an ESR of 29 mm/h. The patient was hospitalized for cutaneous anthrax and treated empirically with oral ciprofloxacin (2x500 mg). On the seventh day, the patient was discharged in satisfactory clinical condition.

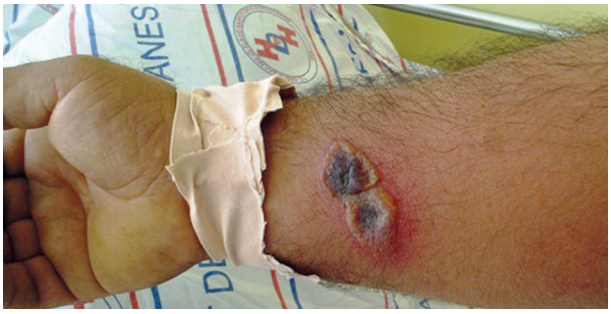

Figure 4. Two erythematous and bullous mid-necrotic ulcerous lesions on the right forearm

Other Cases

The other four cases with cutaneous anthrax, of whom three were female and one was male

(aged 23, 27, 35, and 42 years, respectively) had wide necrotic lesions smaller than 0.5x0.5 cm without accompanying edema on the fingertips. The lesions were self-limiting and recovering. All patients reported carrying dead animals. Antibiotic treatment had been prescribed in a primary health care center one week earlier. Bacteriologic cultures failed in these patients, probably because of the preliminary antibiotic use.

DISCUSSION

Although the incidence of anthrax in Turkey has decreased, the disease is still an endemic zoonosis in the eastern part of the country. Animal husbandry is common in eastern Turkey,[9] and direct contact with infected animals or contaminated animal products is the main source of cutaneous anthrax transmission to humans. Anthrax commonly enters through skin lesions during the slaughtering of infected animals. Papules develop 1-7 days after exposure, and vesicles surrounding the papules appear 48-72 h after papule formation. Anthrax lesions progress through papular, vesicular, and pustular stages until an ulcer forms with a blackened necrotic eschar surrounded by a characteristic zone of brawny edema. Small vesicles surrounding the original lesion form dry eschars.[10] Similar skin lesions developed in our three inpatients and four outpatients.

A diagnosis of anthrax depends on clinical suspicion. Death is often caused by septicemia when antibacterial treatment for cutaneous anthrax is inadequate.[11] Thus, the early diagnosis and treatment of anthrax is critical. A diagnosis of cutaneous anthrax may be confirmed by obtaining bullous-vesicular material. Gram-stained smears of the specimen may reveal Gram-positive bamboo-type rods typical of *B. anthracis*. Also, vesicular culture material may allow for bacteriologic isolation. Cutaneous anthrax can easily be diagnosed based on animal contact and the existence of classical necrotic ulcers in an endemic area. All of our cases were diagnosed based on clinical suspicion. In addition, the bullous-vesicular material observed in our first case had bacterial growth. Surgical intervention is not recommended during the acute phase as it may lead to septicemia and worsening of the infection.[1,11,12] Culture of the bullous-vesicular lesion was not successful (excluding one case) and was not repeated because of the risks of surgical intervention. Wound culture was not successful since two cases presented after the bullous-vesicular phase and the other four cases underwent preliminary antibiotic use. However, in these patients, the disease was easily diagnosed based on the history of animal contact and existence of classical necrotic ulcers.

Penicillin, doxycycline, and quinolones are the primary treatments for anthrax. It is important to change the treatment according to the antibiogram.[7] Natural resistance of *B. anthracis* to penicillin has been reported, and β-lactams are preferred for anthrax treatment.[13,14] The resistance of the bacterium to β-lactams is due to the presence of β-lactamase genes.[15] Cavallo et al.[6] reported 11.5% amoxicillin/clavulanic acid resistance. Some studies have reported the *in vitro* resistance of *B. anthracis* to ciprofloxacin and doxycycline.[16,17] However, natural resistance of *B. anthracis* to these antibiotics has not been reported. Our study documented penicillin and amoxicillin-clavulanic acid resistance in the vesicular material culture of case 1. Penicillin-resistant *B. anthracis* strains have been documented in Turkey. Gültekin reported 5% resistance to penicillin, tetracycline, and cefazoline; 14% resistance to clindamycin; 90% resistance to chloramphenicol; and 100% resistance to cefotaxime.18 Doganay did not observe resistance to amoxicillin-clavulanic acid, cefazoline, or cefoperazone.19 To our knowledge, the resistance of *B. anthracis* to amoxicillin-clavulanic acid has not been reported in Turkey until now. Amoxicillin/clavulanic acid resistant *B. anthracis* was recovered from the vesicular fluid of our first case; however, this resistant strain could not be confirmed by reference laboratory of zoonotic diseases of Refik Saydam National Health Agency in Ankara owing to limited possibility of our hospital. In addition, MIC value of the strain by E-test was not determined because of the same limitation mentioned above. The increasing number of resistant *B. anthracis* strains may be associated with inappropriate antibiotic use in animals. More strict control of animal husbandry may prevent anthrax epidemics and bacterial resistance. Anthrax in animals is lethal and causes a loss of economic resources, 20 but it may be avoidable through vaccination. The control of anthrax in animals can reduce the prevalence in humans.

In conclusion, anthrax is still a common infectious disease in Turkey that may cause epidemics. We argue that education of the at-risk

population and animal vaccination may reduce anthrax prevalence. The detection of antibiotic resistance among *B. anthracis* strains indicates that animal husbandry should be more carefully controlled. Also, improving *B. anthracis* vaccination programs could protect both humans and animals from anthrax.

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