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Short Communication

Anthrax in animals of the Eastern Mediterranean region of Turkey

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In this study, we aim to perform a microbiological evaluation on the anthrax cases encountered in small and large ruminants as well as in equidae in the Eastern Mediterranean region of Turkey between March 2005 and July 2010. Blood specimens from 44 animals raised in the Mediterranean region of Turkey and suspected of having anthrax in light of the clinical examination results between 2005 and 2010, were subjected to bacteriological analysis. Conventional methods such as Giemsa and Gram staining as well as culture and motility tests were carried out. Smears prepared from blood specimens were treated with Giemsa and *Bacillus anthracis* pathogens with typical capsules were investigated. During culture test, specimens were cultivated in nutrient broth, blood agar, and MacConkey agar after which they were incubated at 37°C for 24 - 48 h in an aerobic environment. Colony morphologies and microscopic appearances of the bacteria that grew in the nutrient broth and blood agar were evaluated. Thus, bacteria displaying an appearance of typical hair-like shape and a configuration of long chains similar to bamboo sticks, with nonmotile and R-form like colony view, were diagnosed as *B. anthracis.* The animals with suspected anthrax in this study were comprised of 19 cattle, 14 goats, 10 sheeps and 1 mule. In 12 (27.3%) of 44 specimens, *B. anthracis* was isolated and identified. *B. anthracis* was isolated from the animals with suspected anthrax. Anthrax, recognized as a dangerous zoonosis across the entire world, is still of endemic status in our country and preventive measures should include prevention of illegal animal slaughter by performing efficient supervision, applying routine vaccination programs and raising awareness among the livestock owners.

Key words: Anthrax, animals, *Bacillus anthracis*, pathogens.

INTRODUCTION

Anthrax, also known as charbon, is a contagious and zoonotic infection that is seen in many mammals and humans. In animals, this infection is characterized by high mortality, 42°C fever, splenomegaly, vascular disorders and sepsis, whereas in humans, it has cutaneous, pulmonary and gastrointestinal forms (Bienz et al., 2005; Quinn et al., 2002). Most sensitive species among mammals are cattle, sheep and goat, whereas members of the equidae family demonstrate a moderate sensitivity (Aydin et al., 2006). Although, anthrax is observed across the entire world, it is encountered more commonly in countries of Asia, Africa, Southern Europe, Middle East and Southern America. In our country, while prevalence of both human and animal anthrax cases show a decrease, it is still seen as an endemic event in the eastern and southern regions (Esendal, 2007; Doganay, 2007). *Bacillus anthracis*, the causative agent of the disease, is a gram-positive, aerobic, spore-forming, capsulated, immotile, large rod-shaped bacteria which contains toxins that play a very important role in the development of the disease. The transmission may take place via close physical contact with the skin, carcass, or internal organs of the infected animals as well as consumption of the contaminated animal foods (Aydin et

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al., 2006; Mock and Fouet, 2001). *B. anthracis* is a microorganism that has been occuring as a zoonotic infection in animals and humans since centuries ago and currently it has been classified among the Group A bioterrorism pathogens by Centers for Disease Control and Prevention (CDC). Group A is the most important category in biological warfare which includes pathogens with high mortality such as *B.anthracis* that may cause serious devastations in the community (CDC, 2000; Hawley and Eitzen, 2001).

In this study, we aim to perform a microbiological evaluation on anthrax cases observed in the large ruminants, small ruminants and equidae of the Middle and Eastern Mediterranean regions of Turkey between March 2005 and July 2010.

MATERIALS AND METHODS

Specimen collection

In total, 44 animals (19 cattle, 14 goats, 10 sheeps, and 1 mule) fed in the provinces (Adana, Mersin, Kahramanmaras, Osmaniye, Hatay and Adiyaman) of Middle and Eastern Mediterranean regions and suspected of having anthrax, were included in the current study. Specimens were collected from the blood and internal organs (spleen, liver, lung) of the 44 animals which demonstrated signs that appear to be consistent with anthrax such as high fever, respiratory difficulty, cardiovascular disorders, and sepsis in the clinical examinations; those specimens were dispatched to the Microbiology Laboratory of the Adana Veterinary Control and Research Institute within cold chain containers.

Microbiological analysis

Giemsa and Gram staining methods along with culture and motility tests were conducted on blood and internal organ specimens for isolation and identification of *B. anthracis*. In order to achieve this analysis, smears prepared from blood specimens were dyed with Giemsa and typical capsulated *B. anthracis* pathogens were investigated. Culture tests were carried out with nutrient broth, blood agar and MacConkey agar at 37°C for 24 - 48 h in an aerobic environment. The culture growth characteristics, colony morphologies and microscopic appearances of bacteria grown in the cultures, were evaluated. Thus, non-motile bacteria demonstrating typical hair-shape and bamboo-stick configuration with a rough colony, were evaluated as *B. anthracis* (Koneman et al., 2006; Otlu et al., 2002).

RESULTS

Animals suspected of anthrax were comprised of 19 cattle, 14 goats, 10 sheeps and 1 mule. Clinical examination of the animals exhibited findings such as high fever, hematologic and circulatory disorders, respiratory difficulty, sepsis and hemorrhage from natural orifices. In 12 (27.3%) animals out of 44, *B. anthracis* was isolated and identified. Isolated strains were belonging to specimens of 6 goats, 3 cattle, 2 sheeps and 1 mule Table 1.

Table 1. The distribution of anthrax cases in Eastern Mediterranean.

Provinces	Suspected cases (positive cases)		
	2005 - 2006	2007 - 2008	2009 - 2010
Adana	2 (2)	4 (1)	6 (0)
Mersin	9 (2)	2 (2)	0
K. Maras	3 (3)	2 (0)	5 (1)
Osmaniye	1 (0)	1 (0)	3 (0)
Adiyaman	0	2 (0)	2 (1)
Hatay	1 (0)	1 (0)	0
Total	16(7)	12(3)	16(2)

DISCUSSION

Anthrax is an animal disease which frequently causes cutaneous anthrax and occasionally leads to other forms in humans due to transmission from animals to humans by close contact (Lucey, 2005). Gastrointestinal form of anthrax in humans may develop due to consumption of large amounts of meat and similar products contaminated with *B. anthracis* (Murray et al., 1998). Since *B. anthracis* spores are very resistant against physical conditions, they may stay alive for years in environments contaminated by infected animals. According to the reports of CDC; one of the important reasons why *B. anthracis* causes fear and panic among humans, is its usage in bioterrorism attacks. In such acts, spore form of the pathogen is employed and by inhalation of those spores, sepsis and peracute deaths are encountered (CDC, 2000; Yenen and Doganay, 2008; Blancou and Person, 2003). Regarding the disease caused by this pathogen among animals, as shown by the current study, our country is still recognized as having an endemic status for anthrax. Principal source of transmission via animals is contaminated environment and infected animals. The disease can be seen in acute or peracute forms in animals and the symptoms developing after ingestion of spores through digestive tract include high fever, tremors, respiratory depression, sepsis and emergence of nonclotting dark blood from natural orifices (Quinn et al., 2002; Aydin et al., 2006).

In our country, notifying related governing bodies is obligatory for anthrax and performing autopsy on infected animals is forbidden due to risks concerning transmission and contamination of the environment. However, in cases where the disease cannot be diagnosed by clinical examination and blood analysis, autopsy can be performed after establishing a good level of decontamination. Typical signs of anthrax in the autopsy are known to be subcutaneous hemorrhages, incomplete rigor mortis and enlargement of the spleen along with disruption of tissue integrity (Bienz et al., 2005; Quinn et al., 2002; Aydin et al., 2006).

In Eastern and South Eastern regions of our country where livestock husbandry is widespread, anthrax is prevalent (Anonim, 2006; Anonim, 2009). In a previous study performed between 2001 - 2007, *B. anthracis* was isolated from 34.2% of the animals suspected of having anthrax (Aytekin and Ozkan, 2010). In studies conducted between 2001 - 2007 in Kars and Ardahan which are known to be notable provinces for animal husbandry, *B. anthracis* was determined in 65.4% of 113 animals comprised of 97 cattle and 16 sheep (Otlu et al., 2007). In Turkey, a small-scale epidemic was informed in Kocaeli province where human and animal anthrax cases are known to be uncommon and the subsequent investigation on the subject revealed cutaneous lesions in 12 people due to illegal slaughtering and consumption of cattle meat contaminated with anthrax (Meric and Wilke, 2008).

In our country, prevalence of human anthrax is similar to the prevalence of animal anthrax in regions where anthrax is endemic. In the registry of Ministry of Health of Turkey, there are 2,210 reported human anthrax cases between 2000 - 2005. Cutaneous form is the most common form of human anthrax in Turkey as it is across the world and it is noted to be secondary to having close contact with the infected animals or contaminated products by people working in animal husbandry or their relatives (Esendal, 2007; Doganay, 2007). In our country where illegal animal slaughtering is a fact despite all the efforts towards prevention, anthrax prevalence displays a parallel increase. Particularly people working in animal husbandry are under serious risk for anthrax and the proper control and protection programs should be effectively performed in order to reduce the economical loss to minimal levels and decrease the prevalence of the disease.

In conclusion, *B. anthracis* was isolated from the specimens acquired from animals suspected of having anthrax and raised in the Eastern Mediterranean region of our country. The fight against anthrax, which is recognized as a dangerous zoonotic disease across the world, should include prevention of illegal animal slaughtering by performing adequate supervision, conducting vaccination programs on the animals, and raising awareness among the breeders and raisers of livestock.

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