Cutaneous infection caused by *Bacillus anthracis* in Larissa, Thessaly, Central Greece, July 2012

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In July 2012, a confirmed case of cutaneous anthrax infection in a stockbreeder in the prefecture of Larissa, Thessaly, Central Greece was reported. The investigation revealed five related deaths in animals (two dogs and three sheep). Control measures have been taken immediately in order to prevent further spread in humans and animals.

On 7 July 2012, a stockbreeder in his early 60s was admitted to the Department of Medicine, University Hospital of Larissa, Greece with high fever up to 39.5 °C accompanied by rigors, malaise and generalised weakness that had been present for the previous six hours. The patient reported the appearance of three pruritic papular lesions on the left forearm five days earlier. He further reported that he had slaughtered and flayed a sheep six days before admission to hospital.

Case description

Upon hospital admission, the patient was febrile, his vital signs were normal, and during the physical examination three painless ulcers on the left forearm with surrounding vesicles and oedema, covered by black eschars were observed (Figure). The left axillary lymph nodes were significantly swollen. No other signs or symptoms were found during the physical examination.

Laboratory results on the day of hospital admission revealed elevation of acute phase response markers (white blood cells: 17,100/μL (range: 4,000-10,000/μL), neutrophils: 14,600/μL (range: 2,400-6,000/μL), C-reactive protein: 3.5 mg/dL (range: 0.5 mg/dL)). A working diagnosis of cutaneous anthrax was established on the basis of the patient's place of residence and typical clinical presentation. Therefore, intravenous treatment with penicillin (24 million units per day) was started immediately [1]. After 10 days of hospitalisation, he was discharged in good health with clinical and laboratory results indicating complete recovery. Although the possibility of inhalation exposure in this case was very unlikely, the precise conditions of the direct contact that took place during flaying are not

known. Therefore, upon discharge from hospital, the patient received amoxicillin (oral dose of 1,500 mg per day) for an additional 45 days as a post-exposure prophylaxis against the potential development of anthrax pneumonitis.

Laboratory investigation

On 8 July, one day after hospitalisation of the patient, biological samples (smears from pustules) were sent to the Department of Microbiology at the Medical School of the University of Thessaly. Microscopic examination of the smears showed the presence of Gram-positive rods, typical for *Bacillus anthracis*. However, bacterial cultures remained negative; this finding could be explained by the fact that, at the time the samples were taken, the patient was already under penicillin treatment at a high dose.

Blood samples were obtained by the local veterinarian from two more sheep that have died in the same herd after 7 July. These two were also inspected and microscopic examination revealed the presence of

FIGURE

Skin lesions due to cutaneous anthrax infection, Larissa, Thessaly, Central Greece, July 2012

Gram-positive rods and bacterial cultures grown for 24 h on 5% blood agar produced grey-white colonies. Preliminary identification was performed using conventional methodology. Briefly, haemolysis detection and motility testing was performed as described previously, using 5% horse blood and trypticase soy broth (Bioprep, BioPa Kerateas, Greece) [2]. Capsular testing was performed using nutrient agar plates supplemented with 0.7% NaHCO₃ (Bioprep), incubated in 5% CO₂ for 24 h, followed by McFadyen methylene blue staining. Genus and species confirmation, as well as detection of the two *B. anthracis* plasmids, pXO1 and pXO2, responsible for the species' pathogenicity, was performed using SYBR Green real-time PCR and the primer pairs BA813F/R, PAG67/68 and CAP57/58, as well as the Genesig *Bacillus anthracis* Real Time PCR kit (PrimerDesign Ltd, Southampton, UK), which is based on TaqMan chemistry [3].

The microorganism isolated from the sheep was identified as *B. anthracis* and carried the two pathogenic plasmids pXO1 and pXO2; the pXO1 plasmid contains the *lef*, *cya* and *pag* genes, which encode the lethal factor, oedema factor and protective antigen, respectively, while the pXO2 plasmid contains the *cap* gene, which encodes the capsule [3].

Epidemiological investigation

The stockbreeder was contaminated after having handled the slaughtered sheep due to direct contact with the infected animal. He had flayed the animal together with his wife and then fed two dogs with the contaminated meat. These dogs died during the next day. After 36 hours, the specific anthrax cutaneous lesions appeared on the exposed area of the stockbreeder's skin. Since 7 July, two more sheep have died in the same herd. No other death occurred in this or other herd in the same village (Tsabournia).

It can be assumed that the stockbreeder's wife was also exposed to the spores of the infected animal. However, she did not present any signs or symptoms of infection and is now under post-exposure prophylactic treatment.

Control measures

The stockbreeder's wife hasn't developed any symptoms during the maximum incubation period of 15 days, but is currently receiving post-exposure prophylaxis. The residents of the village (Tsabournia) have been informed about this case in order to recognise early clinical symptoms of anthrax and they were advised to seek medical treatment immediately if anthrax was suspected. The local health centre and general practitioners are aware of this need for careful monitoring. Special directions have been given to the stockbreeders of Tsabournia regarding the use of protective equipment. The local Veterinary Authority has taken measures for the correct disposal of animal carcasses, including disinfection of contaminated material and

decontamination of the environment. Mass vaccination of 7,000 animals is currently in progress.

Background information

Anthrax is an acute infectious disease caused by a large, spore-forming, toxin-producing bacterium *B. anthracis* [4]. It is the oldest known zoonosis with worldwide distribution and has been known to man for hundreds of years, mostly as an animal disease, typically in agricultural areas [4,5]. The disease is endemic in many countries of the world, particularly in tropical and sub-tropical areas, such as southern Europe, Asia, Africa, North and South America, and Australia [6,7]. It commonly occurs in well defined endemic areas where environmental conditions are particularly favourable for the survival of the spores. In Europe, there is a definite declining trend: The number of reported human cases remained at around 25 cases per year during a ten-year period (1995–2004), and has since decreased even more (2005: 10 cases, 2006: 16 cases, 2007: five cases, 2008: three cases, 2009: 14 cases) [8–12]. In the last four years, several reports of anthrax infections in heroin drug users have been reported in European countries [13–15].

Until 1979, Greece, particularly the northern part of the country, was considered as an enzootic zone for anthrax [6]. Although the number of animal outbreaks between 1970 and 1979 had declined to almost a quarter of that of the previous decade (1960–1969), there were 300 outbreaks a year, mostly involving sheep. During this period, there were 8,475 sheep and 1,675 bovine losses in 3,669 separate outbreaks. During the same period, 482 human anthrax cases occurred in the country and all patients were from rural areas [6]. The highest incidences were observed in the prefectures of Aetoloakarnania, Evros, Ioannina, Larissa, Rodopy and Thessaloniki [6]. Since then, strict control measures have eliminated the disease and only sporadic cases in animals and humans have been reported. According to the epidemiological reports from the European Centre for Disease Prevention and Control (ECDC), only 38 confirmed human cases of anthrax were reported between 1994 and 2010 [8–12]. However, it should be stated that although anthrax is included in the notifiable diseases and every suspected case should be reported to the Hellenic Center for Disease Control and Prevention (HCDCP), there is some degree of underreporting and the low number of reported cases does not allow general conclusions regarding the accurate incidence trend.

Thessaly is a rural region located in Central Greece and includes four prefectures (Karditsa, Larissa, Magnesia, Trikala). The estimated number of goats and sheep in this region is above 2 million. The large majority of them (more than 1 million goats and sheep) are farmed in Larissa prefecture. According to the records of the local Veterinary Authority of Larissa, three outbreaks of anthrax have been reported in Larissa in the past 35 years (in 1978, in 2000, and in 2006) (unpublished

data). All of them occurred in herds kept in two villages (Livadi and Tsaournia) situated at a distance of 35 km from each other in the area of Elassona, Larissa prefecture. Approximately 90 animals were affected in total, and the outbreaks were contained after correct disposal of animal carcasses and vaccination of exposed animals. According to the epidemiological data of the Veterinary Authority, no case of anthrax in animals or humans has ever been declared in the other three prefectures of Thessaly.

In 1978, anthrax infection had been confirmed in animals of three different herds in Tsaournia. However, no human infection has been reported. Vaccination and appropriate control measures have been taken; since then until the incident described here no other anthrax case in animals or in humans has been reported.

Conclusions

From a public health point of view, anthrax is important for Europe as well as for other regions. Infections still occur in Greece and clinicians should be aware of the disease and of the need for immediate management and reporting to the HCDCP [16].

In the management of the case described above, the level of post-prophylactic treatment may be seen as unusual according to the World Health Organization (WHO) recommendations (no post-prophylactic treatment required in a patient previously treated by intravenous penicillin) [1]. Here, post-exposure prophylaxis was nevertheless recommended after hospital discharge because the precise conditions of direct contact which took place during playing were not clearly known [17].

Early recognition of this suspected human case and reporting to the local authorities without delay have led to the prevention of further spread of the disease both in humans and animals.

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