

Severe anthrax outbreaks in Italy in 2004: considerations on factors involved in the spread of infection

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SUMMARY

Anthrax is a disease of humans and animals caused by the encapsulated, spore-forming *Bacillus anthracis*. In Italy, anthrax is normally a sporadic disease. During the summer 2004, anthrax broke out in the Basilicata, in southern Italy, a region with a low prevalence of anthrax in which vaccination had been suspended since 1998. The disease involved several animals in few weeks and in a large area. Over 41 days, 81 cattle died, as well as 15 sheep, 9 goats, 11 horses and 8 deer. The Multiple-locus Variable-Number Tandem Repeats Analysis (MLVA) showed that all the 53 isolates belonged to the Cluster A1a, genotype 1. The results of the Single Nucleotide Repeats (SNRs) Analysis showed that 48/53 *B.anthracis* strains belonged to a single clonal lineage, the sub-genotype *sgt - eB*. Two sporadic mutants, *sgt - eB,m1* and *sgt - eB,m2*, were isolated, only one managing to infect other herds. Factors that could have contributed to the spread of infection, such as the transmission of spores by insect vectors and the favourable weather conditions were evaluated.

KEY WORDS: *Bacillus anthracis*, Animals, Epidemic

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Anthrax is a disease of human beings and animals caused by the encapsulated, spore-forming, large gram positive rod *Bacillus anthracis*. The bacteria grow vegetatively within the body tissues of the host, sporulation occurs when vegetative organisms are exposed to the atmosphere (Turbull *et al.*, 1990). In Italy, anthrax is a sporadic disease. Every year, a few outbreaks occur in the known risk areas of central-southern Italy and in the major islands. The disease involves unvaccinated herbivores on pasture and is rare in

northern Italy. The Multiple Locus Variable-Number Tandem Repeats Analysis (MLVA) with 8 Variable Number Tandem Repeat (VNTRs) of Italian isolates proved the presence of seven genotypes belonging to cluster A1a. Because of the similar genomic pattern, we presume that they evolved from a common ancestral strain (Fasanella *et al.*, 2005; Di Marco *et al.*, 2004; Kreidl *et al.*, 2006). In Basilicata, a region of south Italy, anthrax is historically an endemic disease with low prevalence. In this region, in the period preceding the epidemic (1975-2003), 45 anthrax outbreaks were reported involving sheep, goats, cattle and red deer. The analysis of isolates from the outbreaks showed that genotype 1 was predominant (Palazzo *et al.*, 2004). Because of the low prevalence of the disease, anthrax vaccination in Basilicata was suspended in 1998.

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Herewith we describe repeated anthrax outbreaks that occurred in Basilicata in a scattered way and affected a variety of animal species from different herds over a large area. Because of the unusual characteristics of this epidemic and the large number of animals involved in few weeks in a large area, some factors favourable for anthrax were evaluated, including spread by insect vectors and the presence of weather conditions increasing anthrax spores concentration in soil. The characteristics and evolution of the anthrax epidemic are presented in Figure 1. The first outbreak was reported on July 28th 2004, it occurred in the centre of Basilicata and killed only one bovine. A second outbreak occurred on August 25th in the south of Basilicata, at a distance of 23

km from the previous one. During the subsequent 40 days, from August 25th to October 3rd, 41 farms were involved in an area of 900 km², including 13 towns, in which there were about 7,000 cattle and 33,000 sheep and goats. The epidemic killed 124 animals of different species: 81 cattle, 15 sheep, 9 goats and 11 horses. In addition, in the Pollino National Park, located in the same area, 8 deer died (Fasanella *et al.*, 2007). The anthrax infection evolved in three different phases (Figure 1). The first, from the 6th day post initial identification (*dpii*) to the 22nd *dpii*, was the most severe, 26 outbreaks occurred and 96 animals died. The highest number of deaths was recorded in the 15th *dpii*. In the second phase, from the 26th *dpii* to the 29th *dpii*, 7 outbreaks and 17 dead animals

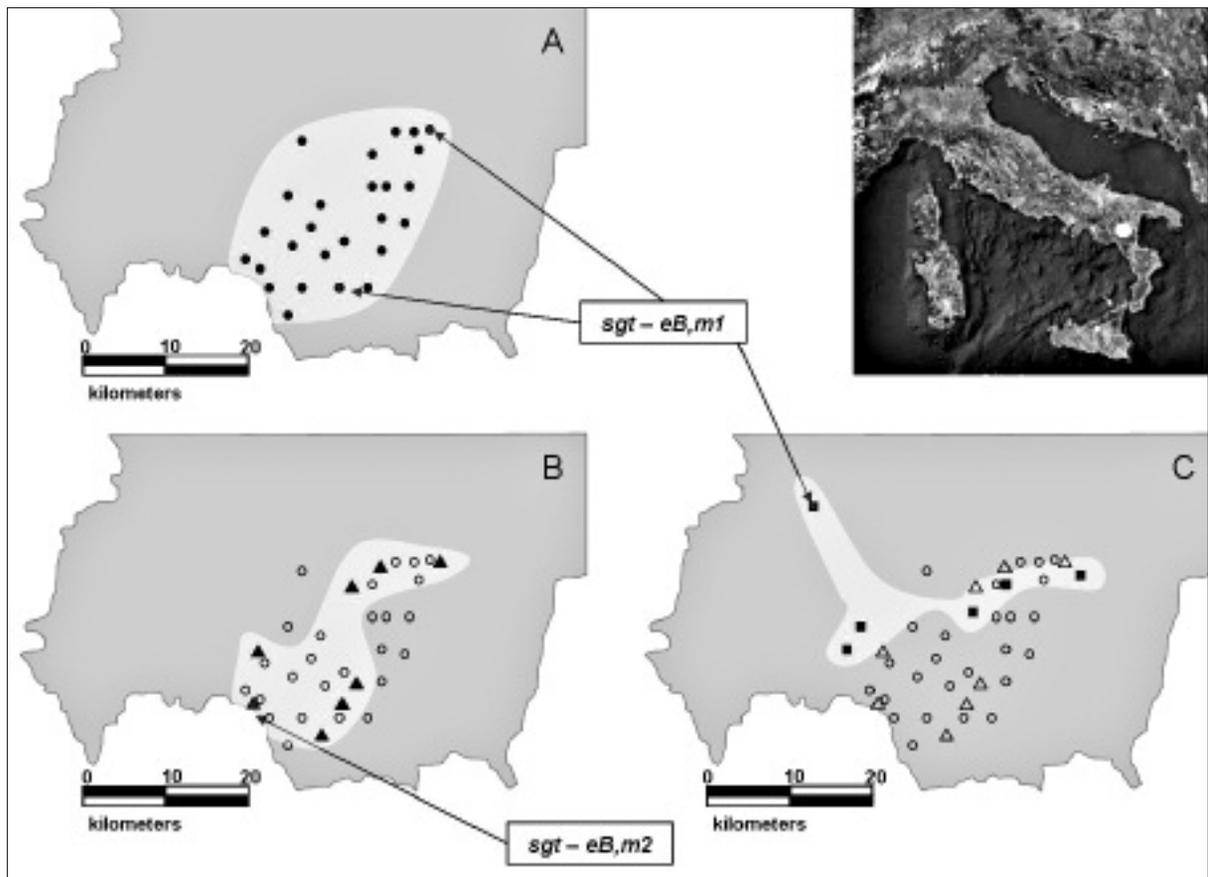


FIGURE 1 - The map shows the evolution of the anthrax epidemic in Basilicata. The epidemic included 13 towns in an area of about 900 km² involving forty-one farms. Three different phases were detected: A) first phase, with 26 anthrax outbreaks (▲) in which 96 animals died. The mutant strain of *B. anthracis* sgt - eB,m1 was isolated twice from different outbreaks; B) second phase, with 7 outbreaks (○) and 17 dead animals. The mutant sgt - eB,m2 was isolated from only one outbreak; C) last phase, with 5 outbreaks (■) and 8 dead animals. The mutant sgt - eB,m1 was isolated from only one outbreak.

were reported. In the last phase, from the 34th *dpii* to the 36th *dpii*, 5 outbreaks occurred and 8 animals died.

All *B.anthraxis* strains isolated from the epidemic were tested by the MLVA with 8 VNTRs and with 25 VNTRs, as previously indicated (Fasanella *et al.*, 2005; Lista *et al.*, 2006). The results indicated that all 53 isolates belonged to the Cluster A1a, genotype.

In this study, all isolates were additionally tested by the Single-Nucleotide Repeats (SNRs) analysis, that allows the differentiation of strains with extremely low levels of genetic diversity, according to previous protocols (Stratilo *et al.*, 2007; Kenefic *et al.*, 2008).

The anthrax outbreaks occurred in Basilicata when the weather conditions were particularly favourable for anthrax. The 2004 was characterized by a rainy spring followed by a hot dry summer. The SNR results indicated that the strain isolated from July 28th outbreak had a SNR pattern different from the others and thus, this earlier outbreak can be excluded from the following epidemic. All the epidemic isolates belonged to a single clonal lineage, the sub-genotype epidemic of Basilicata *sgt - eB*. From August to October, from different outbreaks, a mutant was isolated showing a single mutation in the CL33 fragment (*sgt - eB,m1*); this mutant was isolated from horse, sheep and cattle. In addition, in an outbreak that occurred on the 26th *dpii*, a different mutant was detected carrying a single mutation in the CL12 fragment (*sgt - eB,m2*) (Figure 1).

The origin of these mutants was unknown: after several passages *in vivo* and *in vitro*, we cannot demonstrate, that they derived directly from the *sgt - eB* Basilicata sub-genotype. We think that *sgt - eB,m1* and *sgt - eB,m2* were just two more sporadic events, only one (*sgt - eB,m1*) involved in the infection of other herds.

The cyclical nature of anthrax outbreaks may be determined by climatic factors: extreme weather changes, such as a drought followed by heavy rains, could generate a high soil concentration of anthrax spores causing disease in grazing animals, producing the occasional outbreaks followed by long disease-free intervals. The disease incidence usually increases during a hot dry summer after a very rainy spring. Usually, anthrax outbreaks are limited both geographically and temporally, tending to appear as few cases in re-

stricted areas. Sometimes, however, anthrax shows the characteristics of an epidemic. In the 1997 an epidemic of anthrax occurred in the western part of the Edwards Plateau in Texas involving both livestock and wildlife, especially white-tailed deer (*Odocoileus virginianus*). This climatic potential for an epidemic has been known for many decades in western Texas and its association with tabanid flies, which are known locally as *Charbon* flies (Hugh Jones, 1999; Hugh Jones, De Vos, 2002).

The anthrax outbreaks in Basilicata, could be favoured by a rainy spring followed by a hot dry summer. Also in this case, however, we cannot exclude the role of seasonal mechanical vectors, whether necrophilic or haematophagic, in spreading anthrax infection.

The first cases of epidemic involved grazing cattle and the owners discovered dead animals after a long delay, from two to over four days. For many days the carcasses were exposed to wild carnivores, wild boars and insects. We assume that flies had an important role in the spread of spores because during the first phase the number of dead animals was high and many animals were not grazing but were kept in barns. Many insects had a prolonged access to the infected carcasses, particularly in proximity to the blood-effusions. Over the following days, the veterinary services and the farmers removed the carcasses promptly thus reducing the level of insect contamination and consequently the number of dead animals (Figure 1). Many studies have hypothesized insect vectorial transmission. The transmission could occur as result of insect bites or deposition of anthrax spores present on the body parts or in the feces of the vectors (Turel - Knudson, 1987).

Anthrax may be transmitted to goats through the blowfly (*Calliphora erythrocephala*) and the housefly (*Musca domestica*) (Sen - Minett, 1944). A few sources report the anthrax syndrome in cattle and they link it to fly bites (Davies, 1983; Krishna - Mohiyuden, 1958). The percentage of anthrax transmission obtained in these studies was very low. However, in every study were employed anthrax spores but, theoretically, insects can transmit either spores or vegetative forms and no studies provided data on the inoculum amount necessary to induce infection with the vegetative forms of anthrax. Moreover, the virulence of the

inoculum depends on the anthrax strain employed.

In conclusion, following the anthrax vaccination suspension in 1998, two sporadic outbreaks occurred in 2004: one in July, which did not spread, and a second in August which sparked the epidemic. The epidemic was severe and afflicted a large area. Probably, the insect vectors had a role in spreading the infection.

This episode would make it clear that the background sporadic cases are very important in the epidemiology of anthrax disease. While epidemics catch everyone's attention, routine vaccination is successful by preventing these sporadic cases and thus cost effectively preventing ground recontamination.

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