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ARTICLE

Molecular epidemiology of the *Bacillus anthracis* isolates collected throughout Turkey from 1983 to 2011

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Abstract The main perspective of this study was to determine cross-transmissions amongst anthrax cases and provide detailed information regarding the genotypes of *Bacillus anthracis* isolates circulating in Turkey. A total of 251 *B. anthracis* isolates were obtained from human (93 isolates), animal (155 isolates), and environmental (three isolates) samples in various provinces of Turkey. All isolates were susceptible to quinolones, vancomycin, tigecycline, and linezolid, but not to ceftriaxone. Excluding human isolates, one of the animal isolates was found to be resistant to penicillin, erythromycin, and doxycycline. Multiple-locus variable-number tandem repeats analysis including 8 loci (MLVA8) revealed 12 genotypes, in which genotype 43 was observed at the highest frequency (41.8 %), followed

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by genotype 35 (25.5 %) and genotype 27 (10.4 %). Major subtype A3.a was the predominant cluster, including 86.8 % of the isolates. The MLVA25 analysis for the 251 isolates yielded 62 different genotypes, 33 of which had only one isolate, while the remaining 29 genotypes had 2 to 43 isolates, with a total of 218 isolates (86.9 %). These findings indicate very high cross-transmission rates within anthrax cases in Turkey. The genotypes diagnosed in Turkey are populated in the A major cluster. Penicillin prescribed as the first-choice antibiotic for the treatment of anthrax is still effective.

Introduction

Anthrax is a zoonotic disease caused by *Bacillus anthracis*, which is a Gram-positive, capsule-, and spore-forming rod-like bacterium. The spores of this bacterium are very resistant to external conditions and can survive for decades based on the pH of soil, chemicals, irradiation, humidity, and seasonal features [1, 2]. Soil is the natural reservoir of *B. anthracis*. Herbivores become infected by *B. anthracis* by contacting with spores while feeding on grass. Infection in animals results in gastro-intestinal anthrax and sepsis, ultimately leading to death [3]. Humans acquire anthrax via direct or indirect contact with the infected animals' meat, wool, bone, hair, hide, and so on. Anthrax in humans can be of industrial, agricultural, or laboratory origin [4]. Depending on the method of entry, three clinical forms can be seen as cutaneous anthrax, which is the most common form including more than 95 % of all cases, gastro-intestinal anthrax, and respiratory tract anthrax [5].

Although anthrax decreased to a very low level in developed countries, it has been seen widely in several Asian and African countries where vaccination programs are inadequate [2]. It is hyperendemic in Guinea, Niger, Chad, Zambia, and Zimbabwe in Africa, and Myanmar and Tajikistan in Asia [1, 2, 4]. According to record of the Turkish Ministry of Health, a total of 6,873 human anthrax cases were reported between 1995 and 2005 in Turkey, during which the mortality rate varied from 0.38 to 0.77 per 100,000 people [6]. Looking at the distribution of human anthrax cases on a regional basis in Turkey, the Eastern Anatolia region possesses the highest rate of human anthrax cases, followed by Central Anatolia, which has three times fewer cases than Eastern Anatolia [7–9]. By increasing the rate of animal vaccination and awareness adapted to the control programs in Turkey, the number of human anthrax cases decreased from 396 in 2000 to 148 in 2009, and finally dropped to 93 cases in 2010 [10]. More than 95 % of the human cases in Turkey have been seen as diagnosed with cutaneous anthrax [5]. In terms of animal anthrax cases, a total of 1,304 cattle were influenced by anthrax between 1996 and 2004 in Turkey, and 882 of those died, while the number of influenced and death sheep/goats was 556 and 518, respectively [11].

Molecular tools have been widely used to determine cross-transmissions in *B. anthracis* isolates between or within different sources, such as soil, animals, and humans. Moreover, they provide useful data regarding the introduction and distribution of predominant genotyping patterns in a population, region, and country, or all over the world. The studies used for the molecular typing of anthrax bacilli are commonly based on variable-number tandem repeats located in multiple loci in chromosome and pXO1 and pXO2 plasmids [12–14]. The application of multiple-locus variable-number of tandem repeats analysis (MLVA) for typing *B. anthracis* isolates from different countries revealed that MLVA genotypes possess a geographically specific distribution [15–17]. There has been only two reports in literature the so far [12, 18] on the MLVA types of *B. anthracis*, which covers only a limited number of isolates in Turkey. In order to gain further information regarding cross-transmission rates and dynamics, as well as major genotypes and clones in human, animal, and environmental isolates, the MLVA25 analysis was conducted for typing the 251 *B. anthracis* isolates collected throughout Turkey over a 28-year period. In addition, it was aimed in this study to determine the antimicrobial resistance of the isolates and to address whether there is any relationship between drug resistance and MLVA genotypes.

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Materials and methods

Bacillus anthracis isolates

A total of 251 *B. anthracis* isolates collected both prospectively and retrospectively were analyzed. The isolation and identification of *B. anthracis* were done following the protocol described by Turnbull et al. [2]. The isolates which were nonmotile, sensitive to penicillin and gamma phage, and have positive McFadyean stain were identified as *B. anthracis*. All available clinical, demographic, and epidemiological information of the study population were recorded. Of the 251 isolates, 93 (37.05 %) were cultured from humans, 155 (61.75 %) were from animals, and 3 (1.2 %) were from environmental samples. There was no information in regards to the type of clinical samples for the 21 human isolates. Sixty-nine isolates (95.8 %) out of the remaining 72 human isolates were obtained from the cutaneous anthrax, one isolate (1.4 %) from the blood, and one (1.4 %) from the cerebrospinal fluid. Of the 155 animal isolates, 129 isolates (83.2 %) were acquired from cattle, 23 (14.8 %) were from sheep, and three (1.9 %) were from goats. Detailed information regarding the distribution of the isolates according to the geographic regions, provinces, isolation source, and period is given in Table 1. *B. anthracis* Sterne strain was obtained from the Refik Saydam National Public Health Agency, Ankara, Turkey.

Drug susceptibility testing

All isolates were tested for susceptibility to penicillin G, ceftriaxone, ciprofloxacin, levofloxacin, doxycycline, tigecycline, linezolid, erythromycin, gentamicin, and vancomycin. The susceptibility tests were performed by the agar dilution method on Mueller–Hinton agar medium [19]. Briefly, a bacterial suspension of 2×10^6 CFU/ml was prepared for each isolate and 5 μl of each suspension was inoculated onto agar plates including appropriate concentrations of each antibiotic. The inoculated plates were incubated at 35 °C for 18 h. The minimum inhibitory concentration (MIC) was regarded as the lowest concentration of an antimicrobial agent at which complete inhibition of bacterial growth was observed.

Molecular typing

Multiple-locus variable-number of tandem repeats analysis including 25 loci (MLVA25) was used for the molecular typing of *B. anthracis* isolates following the protocol by Lista et al. [14]. Briefly, 2–3 colonies on a blood agar plate were suspended in 100 μl of TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 7.6). The bacterial suspension was heated at 100 °C for 15 min and then centrifuged at 10,000 × g for 1 min. The supernatant was used as the DNA source. Four multiplex PCR reactions were prepared to amplify 25 loci. The amplification mixture included 2 μl of 10 × PCR buffers, 0.2 mM of dNTP mix, primers with concentrations indicated by Lista et al. [14], 0.75 U of *Taq* DNA polymerase, and 5 μl of DNA suspension. The amplification conditions were as follows: an initial denaturation at 96 °C for 3 min, followed by 36 cycles at 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 120 s. The amplification product was electrophoresed through 2 % agarose gel and amplification was controlled. Then, the PCR products were diluted by 1 to 5 times using distilled water. A quantity of 5 μl of the diluted products were mixed with 40 μl of sample loading solution (Beckman Coulter, Inc., Boston, MA, USA) and 0.5 μl of WellRED labeled size standard (MapMaker 1200; BioVentures, Inc., Murfreesboro, TN, USA). Then, capillary electrophoresis was implemented on a Beckman Coulter 8000 instrument (Beckman Coulter, Inc., Boston, MA, USA) using the following conditions: denaturation at 90 °C for 120 s, injection at 2.0 kV/20 s, and separation at 3.0 kV/3 h. The fragment size of each locus was estimated using the size standard, which included fragments from 75 to 1,200 bp.

Data analysis

The BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) version 6.1 software program was used to analyze the genetic relationship amongst the isolates analyzed. Dendrograms of the MLVA25 and MLVA8 typing results were generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm. MLVA types were compared to epidemiological data.

Results

Drug susceptibility of the isolates

All of the 251 isolates analyzed were found to be susceptible to vancomycin, quinolones, tigecycline, and linezolid, whereas they were resistant to ceftriaxone. It was determined that only a limited number of animal isolates were resistant to penicillin G (one isolate; 0.63 %), doxycycline (one isolate; 0.63 %), erythromycin (one isolate; 0.63 %), and gentamicin (two isolates; 1.3 %). Fifty-eight isolates (23.1 %) were intermediately susceptible to erythromycin. The percentage rate of the isolates susceptible to erythromycin at intermediate levels was 29 % (27 isolates) for those collected from humans and 19 % (31 isolates) for those collected from animal and environmental samples.

Table 1 Distribution of the 251 isolates according to geographic regions, provinces, isolation source, and period

Geographic regions	Provinces	Human (*n*)	Animal (*n*)	Environment (*n*)	Total, *n*(%)	Isolation period
Black Sea (*n*=10)	Amasya	–	1	–	1 (0.4)	2006
	Bolu	–	2	–	2 (0.8)	2004, 2006
	Bartin	–	1	–	1 (0.4)	2005
	Bayburt	–	1	–	1 (0.4)	2006
	Zonguldak	–	1	–	1 (0.4)	2005
	Samsun	–	4	–	4 (1.6)	2006
Central Anatolia (*n*=94)	Ankara	–	13	–	13 (5.2)	2004–2006
	Eskisehir	–	1	–	1 (0.4)	2005
	Kayseri	20	1	1	22 (8.8)	1995–2008
	Kirsehir	3	–	–	3 (1.2)	2010
	Konya	–	8	–	8 (3.2)	2006
	Nevsehir	1	–	–	1 (0.4)	1992
	Sivas	34	4	–	38 (15.1)	1983–2006
	Yozgat	3	4	–	7 (2.7)	1991–2004
	Karaman		1		1 (0.4)	2006
East Anatolia (*n*=143)	Ardahan	1	1	–	2 (0.8)	1997, 2003
	Elazig	3	–	–	3 (1.2)	1996
	Erzurum	–	10	–	10 (4.0)	2005–2006
	Malatya	9	–	–	9 (3.6)	2005–2007
	Kars	1	96	1	98 (39)	1997–2011
	Van	15	2	1	18 (7.2)	2009–2011
	Igdir	–	3	–	3 (1.2)	2006–2008
Southeast Anatolia (*n*=1)	Siirt	1	–	–	1 (0.4)	2009
Mediterranean Region (*n*=2)	Icel	1	1	–	2 (0.8)	1995, 2006
Marmara Region (*n*=1)	Kocaeli	1	–	–	1 (0.4)	2009
Total, *n* (%)		93 (37.05)	155 (61.75)	3 (1.2)	251	1983–2011

MLVA typing results

MLVA analysis yielded complete typing data for the isolates tested. All isolates possessed the pX01 and pX02 plasmid markers. The MLVA25 profile of the 'Sterne' strain for CG3, *bams*44, *bams*3, *vrr*B2, *bams*5, *bams*15, *bams*1, *vrr*C1, *bams*13, *vrr*B1, *bams*28, *vrr*C2, *bams*53, *bams*31, *vrr*A, *bams*25, *bams*21, *bams*34, *bams*24, *bams*51, *bams*22, *bams*23, *bams*30, pXO1-aat, and pXO2-at was 158, 417, 579, 162, 385, 616, 485, 583, 886, 229, 493, 532, 236, 781, 313, 391, 676, 425, 595, 493, 735, 651, 691, 129, and 135 base pairs, respectively.

MLVA8 results

A total of 12 MLVA8 genotypes were determined amongst the 251 *B. anthracis* isolates. All 12 MLVA8 genotypes were found to correspond to 89 G_K genotypes previously determined by Keim et al. [12]. The G_K 43 was the most frequently encountered genotype, which comprised 105

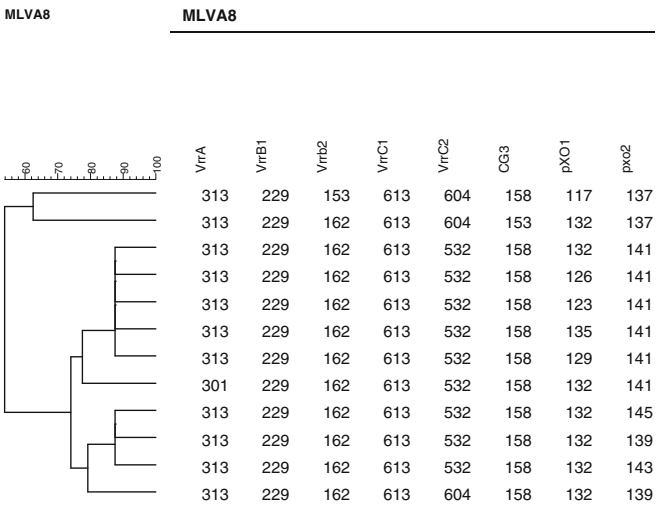
isolates (41.8 %), followed by the G_K 35 genotype with 64 isolates (25.5 %), the G_K 27 genotype with 26 isolates (10.4 %), and the G_K 44 genotype with 16 isolates (6.4 %). The remaining 40 isolates correspond to eight different G_K genotypes of the least occurrence (Fig. 1). The G_K 43 genotype was detected in both animal and human isolates obtained from provinces in the Eastern Anatolia, Central Anatolia, and Marmara regions. The other two dominant genotypes G_K 35 and G_K 27 were detected in isolates obtained from the provinces located in the Eastern Anatolia, Central Anatolia, and Black Sea regions.

All 12 MLVA8 genotypes were in the A major cluster, in which the A3.a cluster comprised 86.8 % of the isolates, the A1.b cluster included 11.6 % of the isolates, and the remaining isolates (1.6 %) corresponded to the A1.a cluster. No isolate/genotype was found in the B major cluster. Except for two provinces, the subtype A3.a cluster was found to be in almost all provinces from where *B. anthracis* isolates were collected in this study. The rare subtype A1.b was found in nine provinces, whereas the second rare subtype A1.a was detected in three provinces (Fig. 2). The clusters A3.a and A1.b have existed in Turkey from 1983 to 2011 and from 1990 to 2011, respectively, while the cluster A1.a existed between 1994 and 2006.

MLVA25 results

Sixty-two different genotypes (from GT1 to GT62) were determined amongst the 251 *B. anthracis* isolates. Thirty-three genotypes comprised only one isolate (one isolate/one genotype). Each of the remaining 29 genotypes comprised 2 to 43 isolates (see the additional file in the Supplementary Material), giving a total of 218 clonally related *B. anthracis* isolates (86.9 % of 251) in the study population. Amongst the 93 human isolates, 37 different MLVA25 genotypes were detected, 19 of the genotypes possessed one isolate each, while the remaining 18 genotypes included a total of 74 (79.6 %) clonally related isolates. Thirty-eight MLVA25 genotypes were determined amongst 158 isolates from animal and environmental samples. A total of 136 isolates (86.1 %) scattered amongst 16 genotypes were found to be clonally related. One environmental isolate acquired from the Central Anatolia region possessed the same genotype as the isolates acquired from the animals in the Eastern Anatolia region, while the other two environmental isolates exhibited unique genotypes.

No correlation was found between the epidemiological data and MLVA25 typing results obtained in this study. The isolates within the same MLVA25 genotypes did not have strict epidemiological correlations with their isolation period, geographic localization, or host distribution. For instance, the most common MLVA25 genotype (GT42) included 43 isolates, in which 27 isolates were obtained from cattle, 11 isolates from humans, and five isolates from sheep. This genotype existed between 1983 and 2011 in Turkey, which was discovered in four different provinces located in two geographic regions in Turkey. The second most prevailing genotype (GT13) included 24 isolates collected between 1993 and 2011, in which five isolates were obtained from humans, 15 isolates from cattle, three isolates from sheep, and one isolate from a goat. This genotype was seen in six different provinces located in three geographic regions (see the additional file in the Supplementary Material).



VrrA	VrrB1	Vrrb2	VrrC1	VrrC2	CG3	pXO1	pxo2	G	N	Geo Region	Cluster
313	229	153	613	604	158	117	137	28	3	TURK	A1.b
313	229	162	613	604	153	132	137	13	4	China TURK	A1.a
313	229	162	613	532	158	132	141	35	64	TURK NAM UK USA	A3.a
313	229	162	613	532	158	126	141	44	16	TURK NAM	A3.a
313	229	162	613	532	158	123	141	33	7	TURK	A3.a
313	229	162	613	532	158	135	141	43	105	TURK	A3.a
313	229	162	613	532	158	129	141	45	2	TURK SAF ARG USA (V770)	A3.a
301	229	162	613	532	158	132	141	41	7	TURK	A3.a
313	229	162	613	532	158	132	145	36	7	TURK	A3.a
313	229	162	613	532	158	132	139	40	4	NAM MOZ ZIM	A3.a
313	229	162	613	532	158	132	143	37	6	TURK	A3.a
313	229	162	613	604	158	132	139	27	26	NOR	A1.b

Fig. 1 A dendrogram of the 12 multiple-locus variable-number tandem repeats analysis including 8 loci (MLVA8) genotypes found among the 251 *Bacillus anthracis* isolates which were typed. Three major clusters were detected; A3.a included 218 isolates (86.9 %), A1.b included 29 isolates (11.6 %), and A1.a included four isolates (1.6 %). *G*: genotype, *n*: no. of isolates, *Geo region*: geographic region: *TURK*: Turkey, *NAM*: North America, *UK*: United Kingdom, *USA*: United State of America, *SAF*: South Africa, *ARG*: Argentina, *MOZ*: Mozambique, *ZIM*: Zimbabwe, *NOR*: Norway

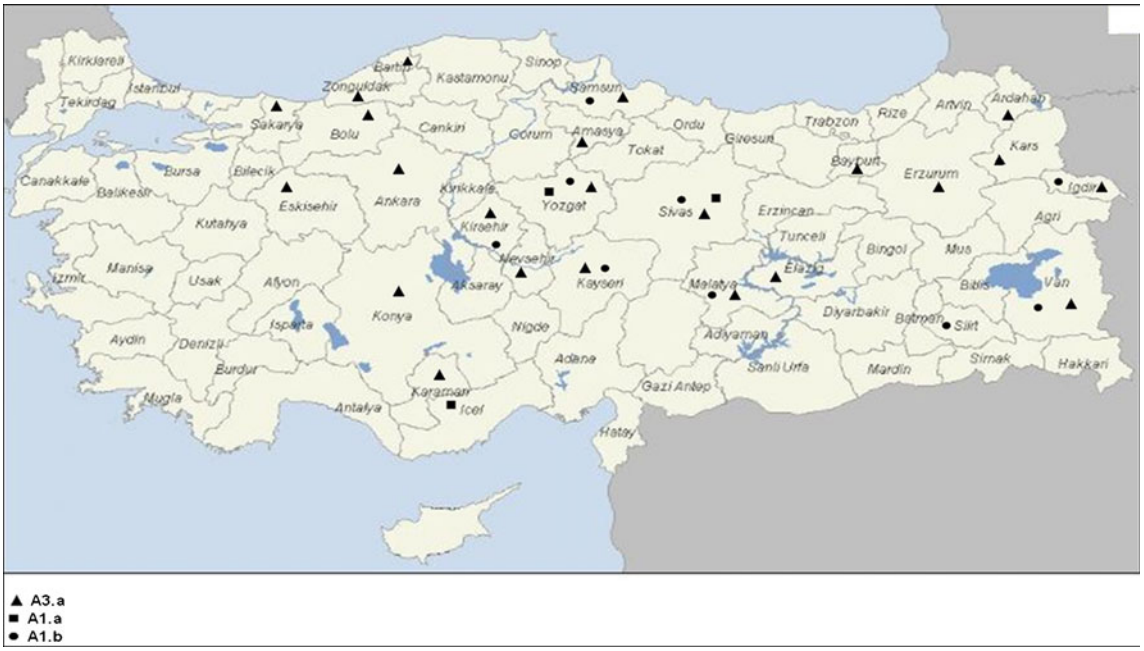


Fig. 2 Distribution of MLVA8 clusters in provinces of Turkey. The predominant A3.a subtype was spread throughout the nation. The subtype A1.b was found to be in nine provinces; Igdir, Van, Malatya, Siirt, Sivas, Kayseri, Yozgat, Kirsehir, and Samsun, whereas the second rare subtype A1.a was detected in only three provinces; Yozgat, Icel, and Sivas

Since most of the isolates showed a homogenous drug susceptibility pattern, a correlation between the drug resistance and genotyping results could not be drawn.

Discussion

The genetic diversity and drug susceptibility of *B. anthracis* isolates circulating in Turkey were investigated from of a total of 251 *B. anthracis* isolates collected from animal (155 isolates), human (93 isolates), and environmental samples over a 28-year period. To the best of our knowledge, this is the first multicentral genotyping study conducted on a large number of *B. anthracis* isolates in Turkey. A great majority of the isolates (62 %) tested in this study correspond to those obtained from animals, which is possibly responsible for a particularly significant number of human anthrax cases in the North-Eastern Anatolia region of Turkey. Nowadays, anthrax is controlled by the vaccination of healthy animals, while infected animals are culled, sacrificed, and burned in compliance with health rules [2]. The presence of *B. anthracis* in three environmental samples collected in three different provinces is a proof of failure in the decontamination procedure applied to died animals in those provinces.

Penicillin is still the first-choice antibiotic applied in the treatment of anthrax. Because *B. anthracis* is highly resistant to second- and third-generation cephalosporins, these antibiotics are not suggested in the treatment of anthrax. Nevertheless, erythromycin, tetracyclines, chloramphenicol, quinolones, and first-generation cephalosporins can be chosen as alternative antibiotics in patients who are allergic to penicillin. In severe infections, doxycycline or ciprofloxacin can be used intravenously [20]. Either no [21, 22] or very low resistance (2.8–11.5 %) to penicillin, especially in animal and environmental isolates, is reported in the literature [23, 24]. In addition, high resistance to second- and third-generation cephalosporins was reported [25]. In agreement with the literature findings, in the current study, all of the *B. anthracis* isolates were found to be susceptible to quinolones, vancomycin, tigecycline, and linezolid, but resistant to ceftriaxone. Although low rates of resistance to penicillin (0.6 %), doxycycline (0.6 %), and gentamicin (1.3 %) were detected amongst the isolates obtained from animals, no resistance was observed amongst the isolates obtained from humans. As indicated in the literature [19, 22], a considerable number of the isolates tested (58 isolates; 23 %) were found to be intermediately resistant to erythromycin; this ratio was 29 % (27 isolates) for those obtained from humans and 19 % (31 isolates) for those from animal samples.

MLVA8 distinguished 12 different G_K genotypes and a considerable number of the isolates (105 isolates) was identified as the G_K 43 genotype. The two other common types were the G_K 35 genotype with 64 isolates and the G_K 27 genotype with 26 isolates. The genotypes G_K 43 and G_K 35 were also observed in two previous studies in Turkey [12, 18]. The G_K genotypes were reported in different countries were classified in branch A and in some studies also in branch B [12, 16, 26]. All of the 12 G_K genotypes found in the current study were in the A major cluster, which is quite common throughout the

world. In the A major cluster, A1.a was determined as being dominant in the North-West part of Bulgaria [27], France [14], Italy [28], and Kazakhstan [26]; A3 in Mongolia [29]; A3.a in Georgia [30], Turkey [12], Iran, and South and East parts of Bulgaria [27]; A4 in Switzerland [31] and Korea [16]. In our study, 86.8 % of the strains were in cluster A3.a, indicating that the *B. anthracis* isolates circulating in Turkey exhibit the 'South Caucasian-Turkish' regional strain pattern, which was also detected in the eastern (Iran) and western (Bulgaria) neighboring countries of Turkey. Using these data, it is proposed that *B. anthracis* isolates which circulated in Turkey descend from a common ancestor, and that minor mutations within few genomic loci lead to different MLVA genotypes amongst the isolates of the common ancestor. While A1.a and A1.b were detected in only a few provinces in Turkey, the predominant A3.a cluster is spread throughout the nation, indicating that the cluster A3.a has existed for a long time. Indeed, it was determined in our laboratory that the clusters A3.a, A1.a, and A1.b have been present in Turkey since 1983, 1990, and 1994, respectively. Furthermore, cluster B was not seen in any of the isolates studied in this laboratory, which seems to be consistent with other laboratory findings [12, 26] that reflected a narrow distribution for cluster B.

MLVA25 molecular typing provides very useful data regarding the origin and magnitude of cross-transmission within and between animals and human anthrax cases in a study population [14, 32]. MLVA25 yielded only one genotype and confirmed cross-transmission amongst 53 *B. anthracis* isolates cultured during a 41-day epidemic time interval in Italy [32]. In another study carried out on 160 *B. anthracis* strains isolated mainly from Italy and France, 67 different MLVA25 genotypes had been identified and the isolates within the same genotype were found to be epidemiologically related [14]. Here, in this study, 62 different MLVA25 genotypes were identified in 251 *B. anthracis* isolates and 86.9 % of the isolates tested were grouped in 29 genotypes, in which each group included 2 to 43 isolates. These findings indicate that the largest number of anthrax cases in Turkey exhibited cross-transmission. A detailed analysis of the grouped MLVA25 types showed that there was no strict epidemiological correlation with the isolates in the same genotype; cross-transmission was not only observed among the isolates cultured from only humans or animals in a specific province in a limited period, but it was also very common between animals and humans and among provinces of different geographic regions in Turkey. The most numerous MLVA25 type (GT42), which includes 43 isolates, has been existing in Turkey for at least 28 years, confirming ongoing cross-transmission amongst the anthrax cases in Turkey.

The main findings in this study are as follows: (1) more than 95 % of the human isolates originated from cutaneous anthrax, while about 83 % of the animal isolates originated from cattle; (2) except for ceftriaxone, there were no or rare rates of resistance to the antibiotics tested; (3) MLVA25 yielded 62 different genotypes in 251 isolates; the percentage rate of the isolates showing a related MLVA25 profile amongst the isolates obtained from humans and animals were 79.6 and 86.1 %, respectively; (4) MLVA8 resulted in 12 G_K genotypes, in which the genotype G_K 43 exhibited the highest percentage population, being 41.8 % out of 251 isolates; (5) the MLVA genotype A3.a was found to be the predominant cluster, including 86.8 % of the isolates, followed by clusters A1.b (11.6 %) and A1.a (1.6 %); (6) no isolate was identified in the B cluster; (7) a strict epidemiological correlation amongst the isolates within the same MLVA genotypes was not observed.

In conclusion, although almost half of the anthrax cases were observed in the Eastern Anatolia region of Turkey, these cases can still be seen all over the country. The high cross-contamination rate (86.9 %) among different hosts (human, animal, environment), provinces, and years (1983–2011) is most probably due to the relocation of animals throughout Turkey. The predominant cluster A3.a suggests that *B. anthracis* isolates circulating in Turkey have a common ancestor including the neighboring countries. The results of the antibiotic susceptibility testing confirmed the current effectiveness of penicillin in the treatment of anthrax cases. All strains tested in this study were delivered to the Bacteriology Laboratory of Refik Saydam National Public Health Agency, Ankara, Turkey, for future studies.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Schmid G, Kaufmann A (2002) Anthrax in Europe: its epidemiology, clinical characteristics, and role in bioterrorism. Clin Microbiol Infect 8:479–488
2. Turnbull PCB, Böhm R, Cosivi O, Doganay M, Hugh-Jones ME, Joshi DD, Lalitha MK, de Vos V (1998) Guidelines for the surveillance and control of anthrax in humans and animals, 3rd edn. World Health Organization (WHO), Geneva
3. Centers for Disease Control and Prevention (CDC) Q & A: anthrax and animal hide drums. Available online at: http://www.bt.cdc.gov/agent/anthrax/faq/pelt.asp
4. Sweeney DA, Hicks CW, Cui X, Li Y, Eichacker PQ (2011) Anthrax infection. Am J Respir Crit Care Med 184(12):1333–1341
5. Doganay M, Metan G (2009) Human anthrax in Turkey from 1990 to 2007. Vector Borne Zoonotic Dis 9(2):131–140
6. http://www.saglik.gov.tr/istatistikler/temel2005/tablo-31.htm. Accessed 30 Apr 2012
7. Turkish Ministry of Health, Basic Health Services General Directorate, Department of Zoonotic Diseases. Available online at:

http://www.saglik.gov.tr/TR/dosya/1-71842/h/zoonotikhastaliklarkatilimci_kitabi. Accessed 1 May 2012
8. Kaya A, Tasyaran MA, Erol S, Ozkurt Z, Ozkan B (2002) Anthrax in adults and children: a review of 132 cases in Turkey. Eur J Clin Microbiol Infect Dis 21:258–261
9. Ozkurt Z, Parlak M, Tastan R, Dinler U, Saglam YS, Ozyurek SF (2005) Anthrax in eastern Turkey, 1992–2004. Emerg Infect Dis 11(12):1939–1941
10. Ertek M (2011) Current situation of anthrax in Turkey. ANKEM Derg 25(Suppl 2):88–91
11. World Organisation for Animal Health (OIE). Annual animal disease status. Available online at: http://web.oie.int/hs2/report.asp?lang=en
12. Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, Jackson PJ, Hugh-Jones ME (2000) Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. J Bacteriol 182:2928–2936
13. Keim P, Van Ert MN, Pearson T, Vogler AJ, Huynh LY, Wagner DM (2004) Anthrax molecular epidemiology and forensics: using the appropriate marker for different evolutionary scales. Infect Genet Evol 4:205–213
14. Lista F, Faggioni G, Valjevac S, Ciammaruconi A, Vaissaire J, le Doujet C, Gorgé O, De Santis R, Carattoli A, Ciervo A, Fasanella A, Orsini F, D'Amelio R, Pourcel C, Cassone A, Vergnaud G (2006) Genotyping of *Bacillus anthracis* strains based on automated capillary 25-loci multiple locus variable-number tandem repeats analysis. BMC Microbiol 6:33. doi:10.1186/1471-2180-6-33
15. Merabishvili M, Natidze M, Rigvava S, Brusetti L, Raddadi N, Borin S, Chanishvili N, Tediashvili M, Sharp R, Barbeschi M, Visca P, Daffonchio D (2006) Diversity of *Bacillus anthracis* strains in Georgia and of vaccine strains from the Former Soviet Union. Appl Environ Microbiol 72(8):5631–5536
16. Ryu C, Lee K, Hawng HJ, Yoo CK, Seong WK, Oh HB (2005) Molecular characterization of Korean *Bacillus anthracis* isolates by amplified fragment length polymorphism analysis and multilocus variable-number tandem repeat analysis. Appl Environ Microbiol 71:4664–4671
17. Simonson TS, Okinaka RT, Wang B, Easterday WR, Huynh L, U'Ren JM, Dukerich M, Zanecki SR, Kenefic LJ, Beaudry J, Schupp JM, Pearson T, Wagner DM, Hoffmaster A, Ravel J, Keim P (2009) *Bacillus anthracis* in China and its relationship to worldwide lineages. BMC Microbiol 9:71. doi:10.1186/1471-2180-9-71
18. Ortatatli M, Karagoz A, Percin D, Kenar L, Kilic S, Durmaz R (2012) Antimicrobial susceptibility and molecular subtyping of 55 Turkish *Bacillus anthracis* strains using 25-loci multiple-locus VNTR analysis. Comp Immunol Microbiol Infect Dis 2012 Mar 22. [Epub ahead of print]
19. Mohammed MJ, Marston CK, Popovic T, Weyant RS, Tenover FC (2002) Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. J Clin Microbiol 40:1902–1907
20. Doganay M (2003) Anthrax. In: Cohen J, Powderly WG (eds) Infectious diseases, vol 2, 2nd edn. American Society for Microbiology Press, Washington, pp 1257–1261
21. Esel D, Doganay M, Sumerkan B (2003) Antimicrobial susceptibilities of 40 isolates of *Bacillus anthracis* isolated in Turkey. Int J Antimicrob Agents 22(1):70–72
22. Luna VA, King DS, Gulledge J, Cannons AC, Amuso PT, Cattani J (2007) Susceptibility of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus pseudomycoides* and *Bacillus thuringiensis* to 24 antimicrobials using Sensititre automated microbroth dilution and Etest agar gradient diffusion methods. J Antimicrob Chemother 60(3):555–567
23. Cavallo JD, Ramisse F, Girardet M, Vaissaire J, Mock M, Hernandez E (2002) Antibiotic susceptibilities of 96 isolates of *Bacillus anthracis* isolated in France between 1994 and 2000. Antimicrob Agents Chemother 46(7):2307–2309
24. Odendaal MW, Pieterson PM, de Vos V, Botha AD (1991) The antibiotic sensitivity patterns of *Bacillus anthracis* isolated from the Kruger National Park. Onderstepoort J Vet Res 58(1):17–19
25. Doganay M, Aydın N (1991) Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis 23(3):333–335
26. Aikembayev AM, Lukhnova L, Temiraliyeva G, Meka-Mechenko T, Pazylov Y, Zakaryan S, Denissov G, Easterday WR, Van Ert MN, Keim P, Francesconi SC, Blackburn JK, Hugh-Jones M, Hadfield T (2010) Historical distribution and molecular diversity of *Bacillus anthracis*, Kazakhstan. Emerg Infect Dis 16 (5):789–796
27. Antwerpen M, Ilin D, Georgieva E, Meyer H, Savov E, Frangoulidis D (2011) MLVA and SNP analysis identified a unique genetic cluster in Bulgarian *Bacillus anthracis* strains. Eur J Clin Microbiol Infect Dis 30(7):923–930
28. Fasanella A, Van Ert M, Altamura SA, Garofolo G, Buonavoglia C, Leori G, Huynh L, Zanecki S, Keim P (2005) Molecular diversity of *Bacillus anthracis* in Italy. J Clin Microbiol 43(7):3398–3401
29. Okutani A, Tungalag H, Boldbaatar B, Yamada A, Tserennorov D, Otgonchimeg I, Erdenebat A, Otgonbaatar D, Inoue S (2011) Molecular epidemiological study of *Bacillus anthracis* isolated in Mongolia by multiple-locus variable-number tandem-repeat analysis for 8 loci (MLVA-8). Jpn J Infect Dis 64(4):345–348
30. Merabishvili M, Natidze M, Rigvava S, Brusetti L, Raddadi N, Borin S, Chanishvili N, Tediashvili M, Sharp R, Barbeschi M, Visca P, Daffonchio D (2006) Diversity of *Bacillus anthracis* strains in Georgia and of vaccine strains from the former Soviet Union. Appl Environ Microbiol 72(8):5631–5636
31. Pilo P, Perreten V, Frey J (2008) Molecular epidemiology of *Bacillus anthracis*: determining the correct origin. Appl Environ Microbiol 74(9):2928–2931
32. Fasanella A, Garofolo G, Galante D, Quaranta V, Palazzo L, Lista F, Adone R, Jones MH (2010) Severe anthrax outbreaks in Italy in 2004: considerations on factors involved in the spread of infection. New Microbiol 33(1):83–86