Short Report: Molecular Characterization of *Bacillus anthracis* Directly from Patients' Eschar and Beef in an Anthrax Outbreak in Jiangsu Province, China, 2012

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Abstract. An outbreak of anthrax was reported in Lianyungang, Jiangsu Province, China in 2012. Laboratory confirmation of cases was made by detection of *Bacillus anthracis* genes *rpoB*, *pagA*, and *cap* using real-time polymerase chain reaction (PCR); source tracking was conducted by multiple locus variable-number tandem-repeat analysis (MLVA) and *pagA* sequencing using DNA extracted from case specimens and meat from a suspected slaughtered cow. The genotypes were MLVA type 57 and *pagA* genotype I. Combined with the field epidemiological data, the four cutaneous anthrax cases most likely were caused by butchering of the sick cow. Backward tracing of animal cases identified the region of origin, and some public health measures, such as reactive or preventative animal vaccination for cattle, intersectoral cooperation, ensuring proper pre-slaughter inspection, and educating butchers and villagers about this disease, could be used to prevent *B. anthracis* infection.

Bacillus anthracis, the etiological agent of anthrax, is a Gram-positive, spore-forming bacterium that can cause serious and often fatal disease among livestock and humans. Animals are infected by contact with contaminated grass or soil. Human infections may result from contact with infected animals or products made from or derived from those animals, including hair, wool, leather, hide, hoof, and bone. Four types of human anthrax are currently recognized: cutaneous, gastrointestinal, pulmonary (inhalation), and injectional anthrax.¹ The most common form, cutaneous anthrax, generally results from infection through abrasions of the skin that often occur when handling animal products contaminated with *B. anthracis* spores. In China, the main form of infection is cutaneous anthrax, resulting from butchering and subsequent consumption of infected livestock.²

On July 25, 2012, 200 cattle were transported to the slaughter house by trucks in Lianyungang, Jiangsu Province. The cattle originated from a farm in Liaoning Province in the northern part of China, where cutaneous anthrax has been previously reported.^{3,4} On the same day, a sick cow from this transported cohort from Liaoning was found just after arriving in Ganyu County, unloaded from the truck, and slaughtered by several villagers in Lianyungang. On July 26, four individuals who participated in the slaughter presented to local medical facilities with a variety of symptoms. These symptoms included lesions, fever > 39°C, dizziness, headache, cough, chest pain, fatigue, and armpit lymphadenectasis or submaxillary lymph node intumescence after onset of the disease. An additional four cases presented with mild symptoms. On August 2, a hospital doctor reported a case of anthrax (case 1) with three lesions on both of his hands after slaughtering a sick cow in Banlu village. After confirming the information by telephone, an outbreak response team from Lianyungang Municipal Center for Disease Control and Prevention (CDC) went to the village to conduct an investigation as previously reported.⁵

Clinical specimens (including blood and eschar) from the four severe cases and uncooked meat from the slaughtered

cow were collected, total DNA was extracted from each sample, and the DNA was assayed for the *rpoB*, *pag*, and *cap* genes of *B. anthracis* by real-time polymerase chain reaction (PCR) using previously described methods.^{6–8} Eschar samples from all four cases as well as the meat sample were positive for all three *B. anthracis* genes; none of the blood samples from cases were real-time PCR-positive.

Although *B. anthracis* is a genetically conserved species, a previous study of 426 isolates showed that multiple locus variable-number tandem-repeat analysis (MLVA) could separate isolates into two main groups (A and B); these groups could be further separated into six unique clusters consisting of 89 distinct genotypes.⁹ Group A isolates formed four of these clusters (A1–A4) and were found to be responsible for most epidemics and outbreaks. In this study, MLVA was used to provide a genotype of *B. anthracis* directly from the human and meat samples. All of them, independent of origin, were identified as type 57 (*vrnA* = 313, *vrnB*₁ = 229, *vrnB*₂ = 162, *vrnC*₁ = 583, *vrnC*₂ = 532, *CG*₃ = 158, *pXO*₁ = 123, and *pXO*₂ = 141); MLVA also confirmed that all of the *B. anthracis* associated with this outbreak harbored both plasmids *pXO*₁ and *pXO*₂.

Additional analysis of the MLVA type from the outbreak-associated *B. anthracis* with isolates from mainland China, Hong Kong Special Administrative Region,^{10,11} and the well-described reference strains Ames¹² and Sterne⁹ confirmed that MLVA type 57 belongs to the dominant branch A3.b, which includes almost all *B. anthracis* isolates characterized from China¹¹ and strains Ames and Sterne (Figure 1). MLVA type 57 has been associated with *B. anthracis* isolates recovered from Beijing, Guangxi, Hebei, Shandong Province, and Xinjiang Uygur Autonomous Region. However, *B. anthracis* isolates from Liaoning, Jiangxi, and Shandong Provinces have previously been typed as MLVA type 58,¹¹ and MLVA type 59 isolates have been recovered from Gansu, Guangxi, Hebei, Henan, Shandong Province, and Inner Mongolia Autonomous Region. Additional accumulation of MLVA data, including data from other provinces, might reveal the potential relationship between strains isolated during the same time period in China or rapidly and easily distinguish non-outbreak isolates, helping to elucidate the geographical distribution of *B. anthracis*.

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FIGURE 1. Dendrogram based on the MLVA genotyping assay showing relationships of the *B. anthracis* isolates in this study. The dendrogram was constructed using unweighted pair-group method arithmetic average cluster analysis of diverse *B. anthracis* genotype scores from the China mainland, Hong Kong Special Administrative Region, and well-known reference strains, including Ames and Sterne by BioNumerics version 4.0 software (Applied Maths, Sint-Martens-Latem, Province of East Flanders, Belgium). Genotype numbers are listed as previously described.^{9,12} GER = Germany; INDO = Indonesia; SAF = South Africa.

Protective antigen (PA) is encoded by the *pagA* gene and one of three toxins produced by isolates of *B. anthracis*; it is a critical component in the development of immunity against anthrax.¹³ Previous studies have documented six *pagA* genotypes.¹⁴ *PagA* (2,294 bp) was amplified from real-time PCR-positive samples and sequenced as described.¹⁴ All of them in this study were *pagA* genotype I, identical to *B. anthracis* strains Ames and Sterne.

Collectively, the genotyping results provided evidence that four human cases and the meat obtained from the slaughtered sick cow may be infected from a common source. Together with the epidemiological investigation, our evidence indicated the link between the four human cases and the slaughtered sick cow. In conclusion, our results provide evidence supporting the usefulness of genotyping for epidemiological investigation.

We recommend that some public health measures should be taken to prevent *B. anthracis* infection, including identification of the region of origin by backward tracing of animal cases, reactive or preventative animal vaccination for cattle, intersectoral cooperation between medical and veterinary authorities, ensuring proper pre-slaughter inspection to ensure that only healthy animals are used for meat sources, and providing education to butchers and villagers about this disease. This latter point addresses refraining from butchering and eating meat from sick livestock or carcasses and leaving the disposal of these animals to veterinary or other trained personnel.

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