Molecular Investigation of an Anthrax Epidemic in Guizhou Province, China

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To the Editor: Anthrax is a zoonosis to which most mammals, especially herbivores, are considered susceptible.[1] Humans almost invariably contract anthrax from handling infected animals, from carcasses of animals that have died from the disease, or from the meat, skin, hair, bones, etc., of such animals.[1,2] Guizhou Province is a historical epicenter of anthrax in the southwest of China.[3,4] In June 2013, an epidemic suspected to be anthrax consisting of five human cases occurred in Wengan County. The clinical symptoms included erythema, papules, and vesicles on the skin of the hands and forearm, accompanied by tissue swelling, infiltration, central cutaneous necrosis, and ulcerative scabs, which are consistent with the Diagnostic Criteria for Anthrax (WS 283-2008). Five strains suspected to be Bacillus anthracis were collected from one of the five patients as well as from soil samples from this epidemic. To provide an etiologic basis for the diagnosis of patients and epidemics and to enhance our understanding of the genetic characteristics of B. anthracis in Guizhou Province, a retrospective molecular epidemiological investigation was carried out on the five isolates collected in this epidemic.

DNA templates were prepared directly from the bacterial colonies by the boiling method, and the protective antigen (PA) or capsule (CAP) genes of pathogenic B. anthracis were identified.[5] Multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA-8) was utilized to identify the MLVA type (MT). The BioNumerics software package version 4.0 (Applied Maths, Kortrijk, Belgium) was applied for clustering analysis using the unweighted pair group method analysis (UPGMA) and the construction of a minimum spanning tree (MST) to determine phylogenetic patterns. The MLVA data of B. anthracis from other regions in Guizhou Province were used for genetic comparison. All the experiments were performed according to the General Requirements for Biosafety (GB 19489-2008) and approved by the Biosafety Committee of Guizhou Provincial Centre for Disease Control and Prevention; they were also approved by the Ethics Review Committee of Guizhou Provincial Centre for Disease Control and Prevention.

One strain of B. anthracis was isolated from the human skin vesicle exudates, and four strains were isolated from the soil samples [Table 1]. The polymerase chain reaction results showed that both the PA and CAP genes were positively identified in the five isolates of the B. anthracis strains. The VNTR profiles of the five strains were completely consistent [Supplementary Table 1]. The five B. anthracis isolates belonged to one MT, which was nominated as MT29. The MLVA profiles were different from the isolates of the 28 profiles (MT1–M28) of B. anthracis collected from other regions in Guizhou Province [Figure 1A].

The MLVA cluster tree based on the MLVA data of the five isolates and the 28 profiles of isolates from other regions of Guizhou Province showed high diversity, with a gross coefficient of similarity of 55% [Figure 1A]. All the MLVA profiles including MT29 of the five isolates and MT1–M28 of the isolates from other regions in Guizhou were grossly divided into clusters a-e. The five isolates of this study belonged to cluster c, which could be further divided into several clades; the five isolates from Guizhou Province were grouped into a single clade, with a coefficient of similarity of 100%. In addition, the cluster tree showed that the five isolates collected from the epidemic were genetically close to the strain 2010011 from Anlong County and 2011002 from Sinan County. The MST showed that the MLVA profiles of the five isolates and the 28 MLVA profiles of the isolates from other regions of Guizhou Province formed five clonal complexes (CCs) (CCG1, CCG6, CCG14, CCG21, and CCG25) and three singletons (G4, G27, and G28). The five isolates from the epidemic belonged to CCG6, which contains G5, G6, G7, G8, G9, G10, G11, G12, G28, and G29 [Figure 1B].

The results of this study suggested that the epidemic was caused by B. anthracis, which provided the etiologic basis for the diagnosis of patients and the investigation of this epidemic. In addition, the five B. anthracis isolates recovered from the epidemic were genetically different from the isolates from other regions. Our results will not only help to understand disease origins and transmission patterns but also facilitate the development of various measures to control anthrax.

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Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. The patients’ guardians have given their consent for reporting their images and other clinical information in the journal. The patients’ guardians understand that their names and initials will not be published and due efforts will be made to conceal their identity.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Table 1: Background information and identification results of B. anthracis isolates from epidemic in Wengan County of Guizhou Province, 2013

| Number | Strains   | Source | Prefecture | County | Year | Phage lysis | Penicillin inhibition | pXO1 | pXO2 |
|--------|-----------|--------|------------|--------|------|-------------|-----------------------|------|------|
| 1      | 2013001   | Human skin | Zunyi | Wengan | 2013 | +           | +                     | +    | +    |
| 2      | 2013002   | Soil    | Zunyi | Wengan | 2013 | +           | +                     | +    | +    |
| 3      | 2013003   | Soil    | Zunyi | Wengan | 2013 | +           | +                     | +    | +    |
| 4      | 2013004   | Soil    | Zunyi | Wengan | 2013 | +           | +                     | +    | +    |
| 5      | 2013005   | Soil    | Zunyi | Wengan | 2013 | +           | +                     | +    | +    |
| 6      | Positive control | /        | /      | /       | /    | /           | /                     | +    | +    |

+: Positive; B. anthracis: Bacillus anthracis; /: Unknown.

Figure 1: MLVA analysis results of B. anthracis isolated in Guizhou Province. (A) Relationships of the B. anthracis isolates based on MLVA. The dendrogram based on the MLVA profiles of isolates from the epidemic in Wengan and other regions in Guizhou were constructed using UPGMA (a-e indicates different clusters, respectively). (B) Genetic relationships based on a minimum spanning tree of the five MLVA profiles of B. anthracis isolates. Each circle corresponds to an MLVA profile. The shadow zones in different colors correspond to different clonal complexes. The size of the circle is proportional to the number of isolates. MLVA: Multiple-locus variable-number tandem repeat analysis; UPGMA: Unweighted pair group method analysis.

Conflicts of interest
There are no conflicts of interest.

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Supplementary Table 1: MLVA profiles of the *B. anthracis* strains isolated from the epidemic in Wengan County of Guizhou Province, 2013

| Number | Strain   | vrrA | vrrB1 | vrrB2 | vrrC1 | vrrC2 | CG3 | pXO1-at | pXO2-at |
|--------|----------|------|-------|-------|-------|-------|-----|---------|---------|
| 1      | 2006001  | 313  | 229   | 162   | 581   | 532   | 158 | 132     | 141     |
| 2      | 2006002  | 313  | 229   | 162   | 581   | 532   | 158 | 132     | 141     |
| 3      | 2006003  | 313  | 229   | 162   | 581   | 532   | 158 | 132     | 141     |
| 4      | 2006004  | 313  | 229   | 162   | 581   | 532   | 158 | 132     | 141     |
| 5      | 2006005  | 313  | 229   | 162   | 581   | 532   | 158 | 132     | 141     |

MLVA: Multiple-locus variable-number tandem repeat analysis; *B. anthracis*: *Bacillus anthracis*. 