

Vital Surveillance

Epidemiological Characteristics of Anthrax and *Bacillus anthracis* Strains — China, 2020–2024

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ABSTRACT

Introduction: The incidence of anthrax in China has declined since 1990 but has resurged in recent years. The purpose of this study was to describe the epidemiology of anthrax and *Bacillus anthracis* (*B. anthracis*) in China from 2020 to 2024 to inform control strategies.

Methods: A descriptive analysis was performed of case characteristics. *B. anthracis* strains underwent whole-genome sequencing, and phylogenetic relationships were determined by core-genome single nucleotide polymorphism (cgSNP) and canonical single nucleotide polymorphism (canSNP) analysis.

Results: Between 2020 and 2024, 1,870 anthrax cases were reported, with a 0.59% case fatality rate. The majority of cases were cutaneous and the incidence increased from year to year. Cases were distributed across 12 provincial-level administrative divisions (PLADs), with most regions showing an increased incidence between 2021 and 2024. Farmers and herders were the most affected occupational groups, and farmers accounted for an increasing proportion of cases. Cases were primarily concentrated among young and middle-aged men and peaked between July and September. A total of 185 *B. anthracis* strains were identified in the 12 PLADs and were classified into four sublineages (A.Br.001/002, A.Br.Ames, A.Br.Vollum, and A.Br.005/006). A.Br.001/002 was the most prevalent and widely distributed sublineage. Phylogenetic analysis revealed 14 branches, demonstrating population diversity. Cross-clustering was present among strains from different PLADs.

Conclusions: From 2020 to 2024, anthrax cases generally followed historical patterns but the incidence increased, with broader geographical spread, more diverse clinical types, and interprovincial transmission. These findings highlight the need to strengthen anthrax control efforts, particularly in the central and eastern PLADs.

Anthrax is an acute zoonosis caused by *Bacillus anthracis* (*B. anthracis*) that has historically posed a serious threat to human health and hindered the development of animal husbandry. The global incidence of anthrax decreased by the 1990s. However, bioterrorism-related incidents have drawn widespread attention to this disease (1). Furthermore, owing to factors such as global warming and rapid socioeconomic development, anthrax is re-emerging and its threat to human health is increasing (2–4). Over the past two decades, anthrax cases in China have primarily been distributed across the western and northeastern regions; however, recent surveillance data have indicated an increased incidence in the central and eastern provincial-level administrative divisions (PLADs) of the country (5).

B. anthracis is a genetically homogeneous pathogen. In epidemiological investigations, genotyping techniques such as canonical single nucleotide polymorphism (canSNP) analysis and multiple-locus variable-number tandem repeat analysis (MLVA) are often used to trace infection sources and determine routes of transmission. However, these methods have limitations in terms of resolution (6–7). In recent years, advancements in whole-genome sequencing technologies have provided new tools for the in-depth analysis of the *B. anthracis* genome. Novel approaches such as core-genome single nucleotide polymorphism (cgSNP) analysis and core-genome multilocus sequence typing (cgMLST) have been applied to the epidemiological investigation and phylogenetic analysis of *B. anthracis* (8–9).

In view of the increasing incidence of anthrax, this study analyzed the epidemiological characteristics of anthrax in China from 2020 to 2024. Whole-genome sequencing and bioinformatics analysis of *B. anthracis* strains isolated during this period were performed to provide a more comprehensive understanding of the current characteristics of anthrax spread and transmission in China.

METHODS

Human anthrax surveillance data for 31 PLADs in China were obtained from the National Notifiable Disease Reporting System (NNDRS) for the years 2020 to 2024. *B. anthracis* strains isolated during this period were collected using the Chinese Pathogen Identification Net. All strains were identified via phage lysis, penicillin susceptibility testing, and the detection of specific *pagA* and *capC* genes.

A descriptive analysis was performed of the number of reported cases and deaths associated with anthrax, and the incidence, mortality, and case fatality rates. The geographical, demographic, and temporal distributions of the reported cases and the proportion of each clinical type of anthrax were described.

DNA was extracted from the strains using the Wizard Genomic DNA Purification Kit (Promega Beijing Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's recommendations. The DNA extract was filtered through a 0.22 µm filter and verified via a sterility test before use. All procedures were performed in a BSL-3 laboratory. The canSNP analysis was conducted as described previously (10).

Long-read and short-read sequencing libraries of genomic DNA were constructed and all isolates were sequenced using Oxford Nanopore third-generation sequencing technology and Illumina second-generation high-throughput sequencing technology. The average sequencing coverage depth for each sample was greater than 100-fold. Hifiasm (v0.19.5; developed by Harvard University, Cambridge, MA, USA) was used to assemble the sequences, Pilon (v1.24; developed by the Broad Institute of MIT and Harvard, Cambridge, MA, USA) was used to correct errors, and CheckM

(v1.2.2; developed by the Australian Centre for Ecogenomics, The University of Queensland, St. Lucia, QLD, Australia) was used to evaluate the quality and completeness of the assembled sequences.

Genome-wide single-nucleotide polymorphisms (SNPs) were detected using GATK4 HaplotypeCaller with the *B. anthracis* "Ames Ancestor" (GCF_000008445.1) as the reference. cgSNPs were extracted using Snippy (v4.6.0). To infer the population structure and phylogenetic relationships, a maximum-likelihood phylogeny was reconstructed in IQ-TREE (v2.3.4) using the GTR model, with branch support evaluated using 1,000 ultrafast bootstrap (UFBoot2) replicates. Nodes with bootstrap support (BS) $\geq 70\%$ were considered well-supported, and those with BS $\geq 95\%$ as strongly supported, according to standard practice.

RESULTS

Epidemiological Characteristics

From 2020 to 2024, a total of 1,870 anthrax cases were reported, with 224, 392, 349, 434 and 471 cases reported in 2020, 2021, 2022, 2023, and 2024, respectively. The number of cases and incidence were the lowest in 2020 (224 cases, 0.016/100 000) and highest in 2024 (471 cases, 0.034/100 000), indicating an increase in both the number of cases and the incidence (Figure 1). Eleven deaths were reported, corresponding to a case fatality rate of 0.59%. Of the 1,870 reported cases, 1,852 (99.04%) were cutaneous anthrax. Four cases of pulmonary anthrax and 14 untyped cases (including meningitis and intestinal anthrax) were reported. Seven of the 14 untyped cases were fatal, a 50% case fatality rate. From 2020 to 2024 the annual average of cases reported was 374, an

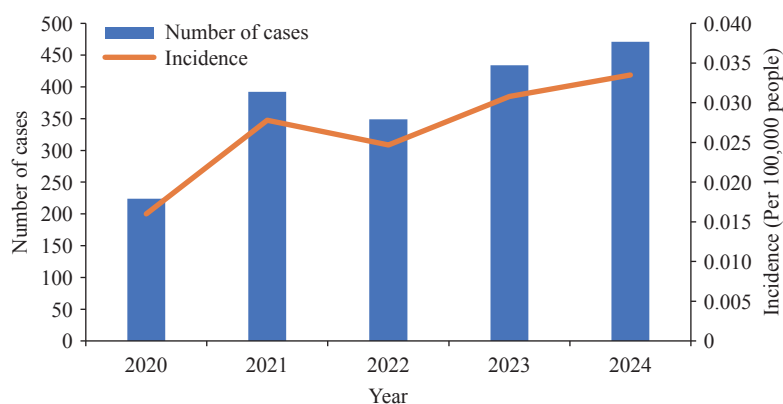


FIGURE 1. The number of cases and incidence of anthrax in China from 2020 to 2024.

increase from 289 in the previous decade (2010–2019). In addition, the reporting of non-cutaneous types, such as pulmonary anthrax (only one reported previously), revealed changes in the epidemiology of anthrax (5).

From 2020 to 2024, 20 of the 31 PLADs reported anthrax cases, with 96.36% (1,802/1,870) of these cases distributed across 12 PLADs, namely Qinghai, Gansu, Sichuan, Ningxia, Inner Mongolia, Hebei, Tibet, Shanxi, Henan, Liaoning, Shandong, and Xinjiang; of which Qinghai, Gansu, Sichuan, Ningxia, Inner Mongolia, and Hebei accounted for 78.88% (1,475/1,870) of the reported cases. Between 2021 and 2024, the number of anthrax cases increased in most PLADs (Figure 2A). The number of cases in Hebei, Shanxi, Shandong, and Ningxia PLADs showed a marked increase compared with the number reported during the previous decade.

From 2020 to 2024, herders and farmers were the most commonly affected occupational groups, accounting for 50.0% and 39.2% of the cases, respectively. The proportion of cases among farmers exceeded that among herders in 2021, and the number of cases among farmers and herders was approximately equal in 2024 (Figure 2B). The majority of anthrax cases were in the 30–59-years age group, and the male-to-female ratio was 2.61:1 (Figure 2C).

From 2020 to 2024, anthrax cases were reported throughout the year but exhibited a distinct seasonal pattern, with 52.46% (981/1,870) of all cases occurring in the summer months between July and September (Figure 2D).

canSNP and cgSNP Genotyping

A total of 185 *B. anthracis* strains isolated from patients (96 strains), animals (56 strains), and the environment (33 strains) were collected from the 12 most affected PLADs from 2020 to 2024. The characteristics of the *B. anthracis* isolates are shown in Table 1.

A maximum likelihood tree was constructed based on 2,064 SNPs. The population structure and phylogenetic relationships of the 185 strains were analyzed using a phylogenetic tree combined with traditional canSNP (based on 13 SNPs) characteristics (7). The cgSNP results were consistent with those of the canSNP analysis but provided a higher resolution. The strains were distributed among four sublineages of *B. anthracis* lineage A: A.Br.001/002, A.Br.Ames, A.Br.Vollum and A.Br.005/006 (Figure 3A). A.Br.001/002 was the most prevalent sublineage,

accounting for 90.3% (167/185) of the isolates, followed by A.Br.Ames and A.Br.005/006, which accounted for 3.8% (7/185) each. A.Br.Vollum was the least abundant sublineage, accounting for 2.2% (4/185) of the isolates. A.Br.001/002 was widely distributed across all PLADs. A.Br.Vollum occurred in Hebei Province; A.Br.005/006 occurred in Qinghai and Sichuan Provinces; and A.Br.Ames occurred in Inner Mongolia, Hebei, Shanxi, and Shandong PLADs.

Among the 185 strains, the 11 strains belonging to the A.Br.Vollum and A.Br.005/006 sublineages showed higher levels of genetic variation and divergence than those of the other strains. The remaining 174 strains belonged to the A.Br.001/002 and A.Br.Ames sublineages. These two sublineages are difficult to distinguish using the canSNP method; As the majority of strains in China belong to the A.Br.001/002 sublineage, a more discriminatory method is required. This study demonstrates the crucial role of cgSNP typing in distinguishing between the A.Br.001/002 and A.Br.Ames sublineages. Further analysis of the 174 strains identified 1,471 SNPs, allowing these two sublineages to be further divided into 14 major phylogenetic branches, demonstrating the population diversity of *B. anthracis* in China. The BS values for these major branches ranged from 92% to 100%, with 8 of 14 branches having BS values of 100%. The clustering of strains by geographic region and the close genetic proximity of strains within each cluster are shown in Figure 3B. For example, strains from Qinghai and Sichuan Provinces exhibited distinct regional clustering, and some strains from Hebei and Ningxia PLADs showed very close genetic proximity, suggesting a possible common source. In addition, strains isolated from different regions during the same period showed close genetic proximity. For example, in 2024, strains from Shanxi, Hebei, and Shandong Provinces clustered within the A.Br.Ames sublineage, suggesting the possibility of transmission between PLADs. Overall, with the exception of strains from Qinghai and Sichuan Provinces, strains from the other PLADs exhibited cross-clustering patterns, with the strains from Hebei and Inner Mongolia, in particular, showing cross-clustering with strains from other PLADs.

DISCUSSION

From 2020 to 2024, the incidence of anthrax in China generally remained below 0.05 per 100,000.

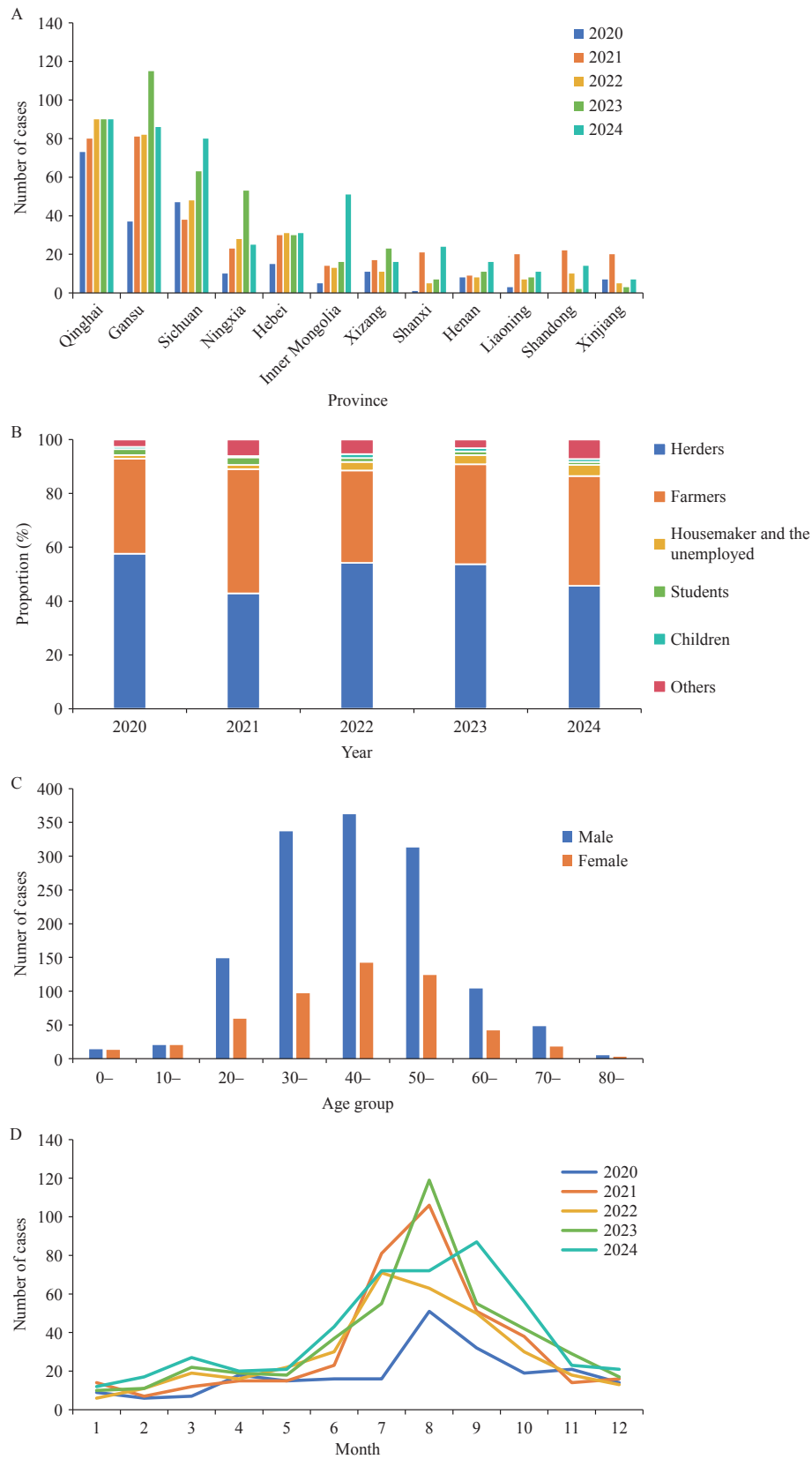


FIGURE 2. Epidemiological characteristics of anthrax cases in China from 2020 to 2024. (A) The number of anthrax cases in the 12 most affected PLADs from 2020 to 2024. (B) Distribution by occupational group. (C) Distribution by age and sex. (D) Distribution by month and year.

TABLE 1. Distribution of *Bacillus anthracis* isolates in China by PLAD, 2020–2024.

PLAD	2020	2021	2022	2023	2024	Total	canSNP subgroups (n)
Hebei	9	4	5	12	22	52	A.Br.001/002 (44), A.Br.Vollum (4), A.Br.Ames (4)
Qinghai	2	7	0	26	1	36	A.Br.001/002 (32), A.Br.005/006 (4)
Sichuan	7	0	14	10	0	31	A.Br.001/002 (28), A.Br.005/006 (3)
Ningxia	3	5	8	9	0	25	A.Br.001/002 (25)
Shaanxi	2	6	1	0	3	12	A.Br.001/002 (12)
Liaoning	0	4	0	3	0	7	A.Br.001/002 (7)
Shandong	0	0	7	0	0	7	A.Br.001/002 (6), A.Br.Ames (1)
Inner Mongolia	3	0	0	0	3	6	A.Br.001/002 (5), A.Br.Ames (1)
Henan	0	3	0	2	0	5	A.Br.001/002 (5)
Anhui	0	2	0	0	0	2	A.Br.001/002 (2)
Xinjiang	0	1	0	0	0	1	A.Br.001/002 (1)
Shanxi	0	0	0	0	1	1	A.Br.Ames (1)
Total	26	32	35	62	30	185	

Abbreviation: SNP=Single nucleotide polymorphism; PLAD=Provincial-level administrative division.

Cutaneous anthrax was the predominant clinical type of anthrax, with a case fatality rate below 1%. The epidemiological characteristics, including the geographic, temporal, and demographic distribution, were largely consistent with those of previous anthrax cases in China.

However, some new characteristics emerged among anthrax cases reported between 2020 and 2024. The number of anthrax cases has increased annually, and in 2024, reached the highest level since 2006. The geographic distribution of cases expanded, shifting from the western and northeastern regions to the western and central regions (5). In recent years, Hebei, Shanxi, Shandong, and Henan Provinces have reported relatively high numbers of cases and have been ranked among the top 12 PLADs. Moreover, cases have been reported in PLADs such as Tianjin, Anhui, Guangdong, and Jilin PLADs, in which no cases had been reported for many years. Among the primary affected occupational groups, the ratio of cases among farmers to herdsmen increased, which was associated with an increase in the number of cases reported in the agricultural PLADs in central China. Clinical types such as pulmonary anthrax, anthrax meningitis, and intestinal anthrax have emerged. This emergence might be related to the development of improved diagnostic technologies which improved the detection rate of *B. anthracis* and the diagnosis rate of anthrax. Specifically, high-throughput sequencing and metagenomic sequencing provide valuable information for the diagnosis of pulmonary anthrax and anthrax meningitis (11–12). These clinical types are frequently associated

with diagnostic delays and suboptimal therapeutic interventions, which may have contributed to the reported 50% case fatality rate.

The strain distribution of *B. anthracis* differed from that reported in previous studies. In a study of *B. anthracis* isolates collected in China between 1990 and 2016, the strains were classified into five sublineages: A.Br.001/002, A.Br.Ames, A.Br.Vollum, A.Br.008/009, and A.Br.Aust94 (13). A.Br.001/002 was widely distributed and represented the dominant sublineage in China. A.Br.Ames was found primarily in Inner Mongolia and Liaoning, whereas A.Br.008/009, A.Br.Aust94, and A.Br.Vollum were found mainly in Xinjiang. In this study, A.Br.001/002 remained the predominant and most widely distributed sublineage. However, new sublineages emerged, and previously reported sublineages spread geographically. Strains belonging to the A.Br.005/006 sublineage were first isolated in Liaoning Province in 2019 and were later detected in Qinghai and Sichuan Provinces. The A.Br.Ames sublineage was isolated not only in Inner Mongolia and Liaoning but also in Hebei, Shandong, and Shanxi Provinces. Strains belonging to the A.Br.Vollum sublineage were isolated in Hebei Province in 2020, 2022, and 2024, but were not identified in Xinjiang or other regions during this period, suggesting that these strains may have originated from raw wool materials imported from South or Central Asia. Notably, no strains of the A.Br.Aust94 and A.Br.008/009 sublineages, which were relatively common in Xinjiang Uygur Autonomous Region between 1980 and 2000, were

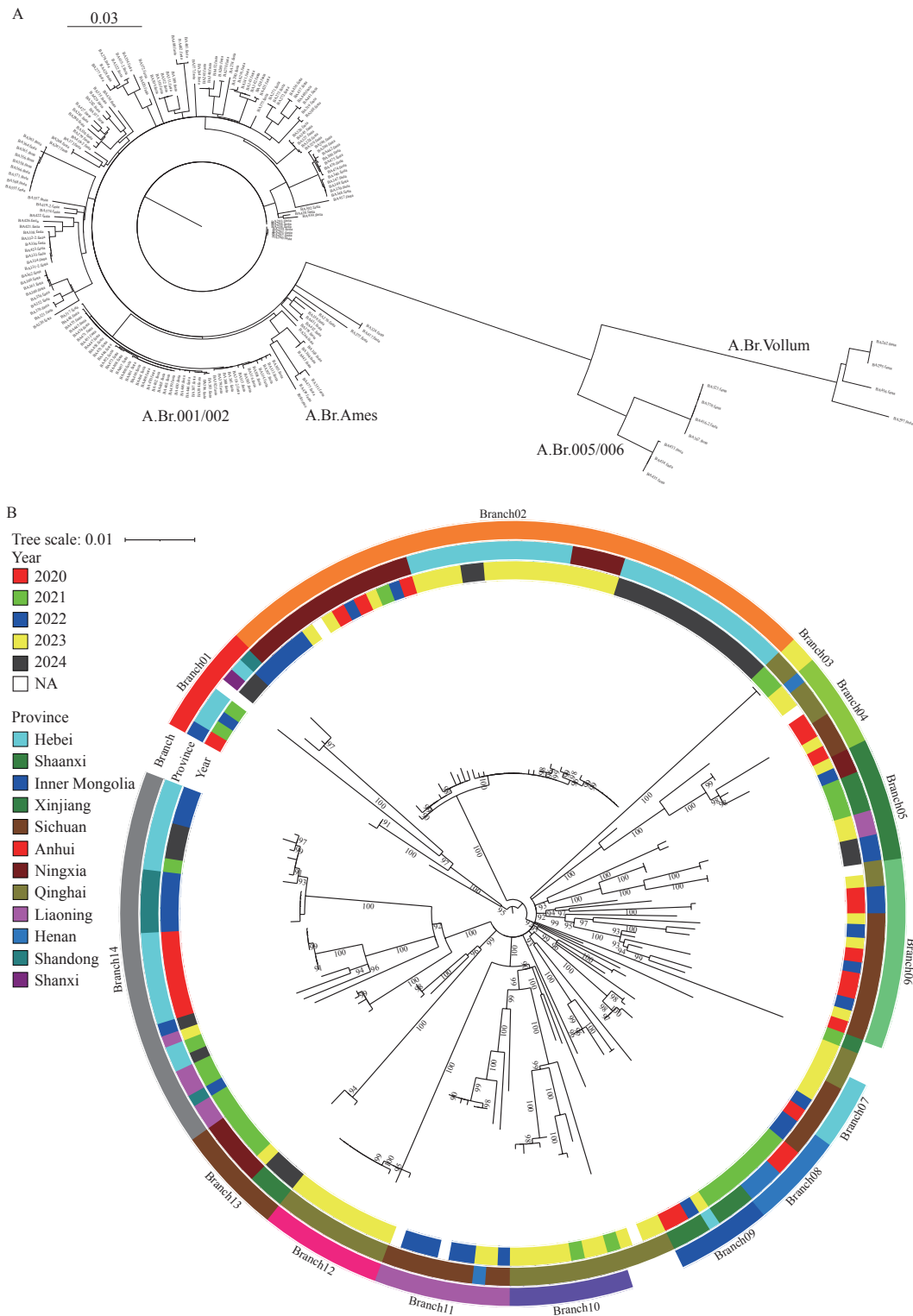


FIGURE 3. Maximum likelihood tree constructed based on the complete genomes of *Bacillus anthracis* strains. (A) Maximum likelihood tree constructed based on the complete genomes of 185 *B. anthracis* strains. (B) Maximum likelihood tree constructed based on the complete genomes of 174 *B. anthracis* strains belonging to the A.Br.001/002 and A.Br.Ames sublineages.

Note: The inner circle color indicates the PLAD in which the strain was isolated, the middle circle color indicates the year of strain isolation, and the outer circle color indicates the phylogenetic branch number. BS values are shown at the nodes (only values $\geq 90\%$ are displayed). The scale bar indicates 0.01 substitutions per site.

Abbreviation: PLAD=Provincial-level administrative division; BS=Bootstrap support.

detected in recent years. Furthermore, phylogenetic analyses of strains from different PLADs revealed that most isolates from Sichuan and Qinghai Provinces were distantly related to those from other regions and exhibited diversity within each PLAD. This pattern may be because most of these strains were isolated from sporadic cases of anthrax among Tibetan herders whose geographical environment, lifestyle, and cultural practices may have limited the spread of anthrax outside their communities. In contrast, strains from Inner Mongolia and Hebei appeared to be epidemiologically linked to those from multiple PLADs, probably because of the extensive livestock farming, slaughtering, and trading networks in these areas. This study demonstrates the effectiveness of integration of canSNP and cgSNP genotyping methods for population-level analysis and transmission source tracing of *B. anthracis*; however, further refinement is needed.

Anthrax is a re-emerging infectious disease that poses a threat to human and animal health (2–3,8). This study revealed a geographic expansion of anthrax cases in recent years, particularly in agriculturally dependent PLADs, where the number of cases has increased substantially. Possible reasons include economic globalization, population mobility, and the trade of livestock and livestock products, which have facilitated the spread and transmission of anthrax. In addition, climate change may promote anthrax transmission (14–16). In this study, genome-wide phylogenetic analyses provided an enhanced understanding of *B. anthracis* strain relationships, and the observed genotypic changes and phylogenetic relationships provided biological evidence of transnational or inter-provincial transmission. The evolving epidemiology of anthrax poses new challenges for its prevention and control. Compared with pastoral regions, the largely agricultural central and eastern PLADs differ in terms of livestock practices, which may complicate efforts to control anthrax. An effective control strategy requires integration of epidemiological investigations, source tracing, disinfection, livestock control, public education, and professional training.

This study has several limitations. First, the sampling of strains was geographically and temporally uneven; therefore, analysis of a larger number of isolates is warranted to validate our findings. Second, cross-provincial phylogenetic clustering was correlative; therefore, same-clade clustering does not necessarily indicate a common source and does not constitute causal evidence of transmission. Third, we did not

collect data on socioeconomic or climatic factors and therefore could not assess their potential impact on anthrax incidence and transmission.

In conclusion, this study describes the recent re-emergence of anthrax in China, and its evolution from a disease characterized by local outbreaks to a more threatening and complex public health challenge. The rising incidence, expanding geographic range, and similarities of strains between PLADs indicate that epidemic foci are widening and becoming more difficult to control through isolated local efforts. The diversification of clinical types and the emergence of novel genotypes not only reflect the increased detection rate of atypical anthrax cases enabled by the application of new technologies but also reveal the increasing complexity of routes of exposure, suggesting that the incidence of atypical cases of anthrax is likely to increase in the future, and poses greater challenges for prevention and control. This situation necessitates a comprehensive change to existing control measures, with a shift from individual case management to an integrated defense network that encompasses climate change monitoring, quarantine of animals moving between regions, and genotypic surveillance to detect emerging strains.

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