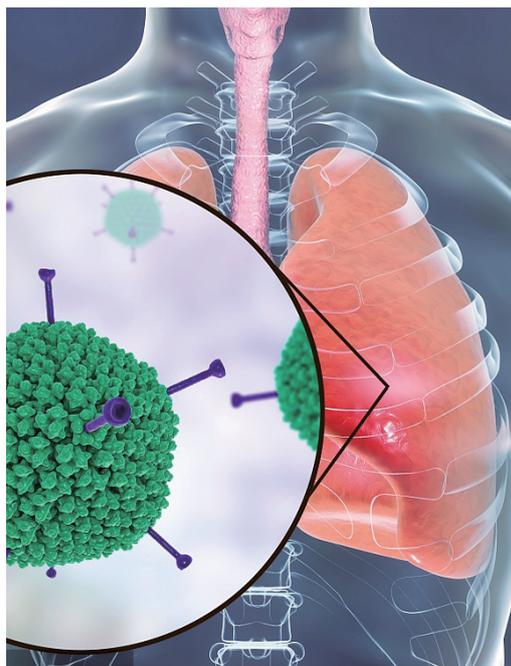


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Preplanned Studies

Outbreaks of Acute Respiratory Disease Associated with Human Adenovirus Infection in Closed Camps, China, December 2011–March 2014

Juan Du^{1,✉}; Xiaodong Zhao^{2,✉}; Fang Tang³; Doudou Huang⁴; Guangqian Pei¹; Xiaoi Zhang¹; Baogui Jiang¹; Qingbin Lu^{1,✉}; Wei Liu^{1,2,✉}; Yigang Tong^{2,5,✉}

Summary

What is already known on this topic?

Human adenovirus (HAdV) was frequently associated with acute respiratory disease (ARD) outbreaks in military camps.

What is added by this report?

HAdV-B7, HAdV-B14, and HAdV-B55 were determined to be responsible for 3, 2, and 4 ARD outbreaks in military camps, China, respectively, with a total attack rate of 28.0% during 2011 to 2014.

What are the implications for public health practice?

The findings suggest that vaccine development and administration in military camps must be prioritized. Quarantining among new recruits before entering into the military and the identification of the major responsible genotypes at the current stage is warranted.

Human adenovirus (HAdV) was frequently associated with acute respiratory disease (ARD) outbreaks in closed environment, especially in military camps. In the current study, we investigated the genetic characteristics and epidemiological characteristics of HAdV strains that were responsible for the ARD outbreaks in military camps in China. Among HAdV-related outbreaks that were reported from 2011 to 2014, a total of 3,813 patients were diagnosed from 13,622 camp members, with an overall attack rate of 28.0%. HAdV-B7, HAdV-B14, and HAdV-B55 were determined to be responsible for 3, 2, and 4 outbreaks, respectively. The total attack rate of HAdV-related ARD was 28.0%, ranging from 10.9% to 39.9% among various outbreaks. HAdV-B14 related outbreaks had a higher attack rate than outbreaks of the other two genotypes. Phylogenetic analysis revealed no obvious relationship between outbreak strains and locally circulating strains, indicating the introduction of the strain from a new recruit's hometown might be

responsible for the outbreaks. The findings suggest that vaccine development and administration in military camps must be prioritized. Quarantining among new recruits before entering into the military and the identification of the major responsible genotypes at the current stage is warranted.

Traditionally, there are 51 HAdV serotypes, which are assigned to seven subgroups (A–G) according to biophysical, biochemical, and genetic characteristics. In recent years, new serotypes or subspecies were increasingly recognized by use of phylogenetic analysis, which arised from genome recombination between the hexon gene, fiber, and penton genes. These newly emerged HAdV types resulted in continuous outbreak events on campuses, such as in university campuses or at recruit training centers (1–2), posing severe threats to public health. In China, the ARD outbreak had been constantly reported in the closed community, such as in military camps; however, their etiological agents are rarely determined, in part due to the lack of molecular typing in field investigations. The first known HAdV-associated ARD outbreak in the military was reported from a recruit training camp in 2009, which was determined to be caused by HAdV-B55 infection (3). In this study, we described the HAdV-related ARD outbreaks that had been reported in military camps from 2011–2014, investigating the genetic characteristics of the responsible HAdV strains and their related epidemiological characteristics.

The field investigation and laboratory tests were carried out in accordance with the approved guidelines. Briefly, after the report of an outbreak, nasopharyngeal aspirate (NPA) samples were collected from the index case and from at least 30% of the ARD patients and tested for the commonly seen viral pathogens, i.e., HAdV, influenza virus, respiratory syncytial virus, human rhinovirus, human enterovirus, human coronavirus, and human metapneumovirus. All tests were performed by polymerase chain reaction (PCR) or

real-time PCR. HAdV infection from the outbreak was identified by PCR assay using HAdV universal and serotype-specific conventional PCR assays (4–5). To validate the causal relationship between HAdV and the outbreak disease, seroconversion evidence was further determined from paired samples using the neutralizing antibody test. For each outbreak, the responsible HAdV strains were isolated. Briefly, NPA specimens were inoculated into A549 cells incubated at 36 °C and observed for cytopathic effects (CPEs) for 21 days. Selected paired samples (acute-phase samples and convalescent-phase samples that were collected over one month apart) were tested for the presence of anti-HAdV antibodies by a colorimetric serum microneutralization assay, using the strains isolated from the outbreak event. All the subjects who were residing in the military camps over the outbreak duration were interviewed using a standard questionnaire to acquire the following information: date of birth, sex, clinical symptoms, date of contact with the patients, and date of onset of symptoms. All data were collected as part of the emergency response to the outbreak events. Data were de-identified for analysis, and the informed consent requirement was waived.

During the period from December 2011 to March 2014, 9 HAdV-related outbreak events of ARD were determined from military camps or recruit training centers, based on both molecular and serological evidences. In each of the outbreaks, other respiratory viruses than HAdV, including influenza A virus or parainfluenza virus, was only occasionally tested as positive, and were not considered as the causative agents of the outbreaks. According to the standard criteria to define ARD, a total of 3,813 patients were diagnosed from 13,622 camp members, with an overall attack rate of 28.0%. The attack rates ranged from 10.9% to 39.9% among various outbreaks (Table 1). Four camps had only male recruits. The other four camps had both male and female recruits, where female patients were also reported, but with a lower attack rate than in males (21.0% vs. 39.4%, $P<0.001$). The patients were aged from 18 to 24 years. All the HAdV-associated ARD were reported among cold season from December to next March, which coincided with the training period for recruits. The outbreaks were identified to start from 1 to 6 weeks (median 3 weeks) after the initiation of the training activity. The peak outbreak occurred mostly from 11 to 25 days after the disease onset of index case.

HAdV-positive results were further confirmed by

TABLE 1. Epidemiological characteristics of HAdV-related outbreak events of acute respiratory disease in military camps during December 2011–March 2014.

Characteristic	All participants (N=13,622)	
	ARD patients (n=3,813)	P
Gender		
Male	3,720 (28.35)	<0.001
Female	93 (18.56)	
Age of soldier		
New recruits	2,722 (31.86)	<0.001
Veterans	1,091 (21.48)	
HAdV genotype		
HAdV-B7	688 (24.77)	<0.001
HAdV-B14	1,016 (37.70)	
HAdV-B55	2,109 (25.88)	
Outbreaks areas, province		
HuBei/2013/HAdV-B7	376 (25.53)	<0.001
ShanXi/2013/HAdV-B7	107 (16.54)	
HuBei/2013/HAdV-B7	205 (31.16)	
LiaoNing/2012/HAdV-B14	185 (30.32)	
GanSu/2013/HAdV-B14	831 (39.86)	
HeBei/2012/HAdV-B55	650 (28.54)	
ShanXi/2011/HAdV-B55	309 (10.92)	
TianJin /2012/HAdV-B55	1,150 (37.80)	
Total	3,813 (27.99)	

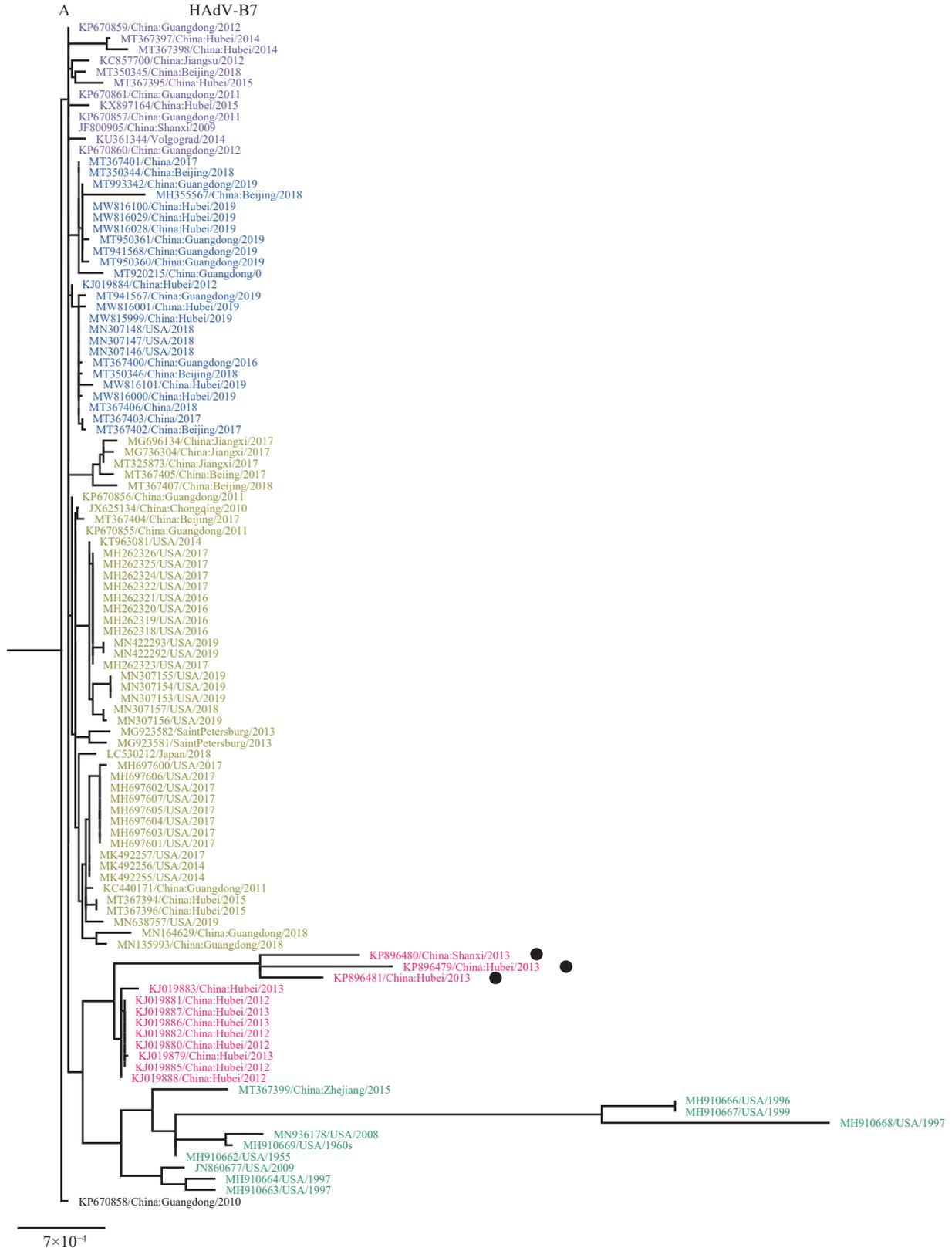
Note: The information on the ninth outbreak of Liaoning /2013/ HAdV-B55 was missing.

sequencing of hexon gene, penton, and fiber genes. The genotype was determined by alignment and phylogenetic tree analysis. HAdV-B7, HAdV-B14, and HAdV-B55 were reported to be responsible for 3, 2, and 4 outbreaks, respectively. A higher attack rate was identified from HAdV-B14 (37.7%), significantly higher than that of HAdV-B7 (24.8%) and HAdV-B55 (25.9%) ($P<0.001$, Table 1). The mean age of the patients was highly comparable among all the outbreak episodes regardless of the HAdV types. The incubation period ranged from 4 to 8 days, showing no difference among outbreaks associated with various genotypes. Among 3,813 ARD patients, 736 (19.3%) developed pneumonia, and the patients with HAdV-B7 (164, 23.8%) and HAdV-B55 (518, 24.6%) had a higher chance of developing pneumonia than those with HAdV-B14 (54, 5.3%; $P<0.001$).

To investigate the genetic characteristics of the responsible virus, the whole genome sequence of one strain for each outbreak was obtained by deep-

sequencing of the HAdV isolates or the raw positive samples. Previously published sequences of HAdV-B7, HAdV-B14, HAdV-B55, and HAdV-B11 were recovered from GenBank and used for the alignment

with the current outbreak strains. For each genotype, the phylogenetic trees were respectively constructed based on the HAdV complete genome and 3 segments (penton, hexon, and fiber) by maximum likelihood



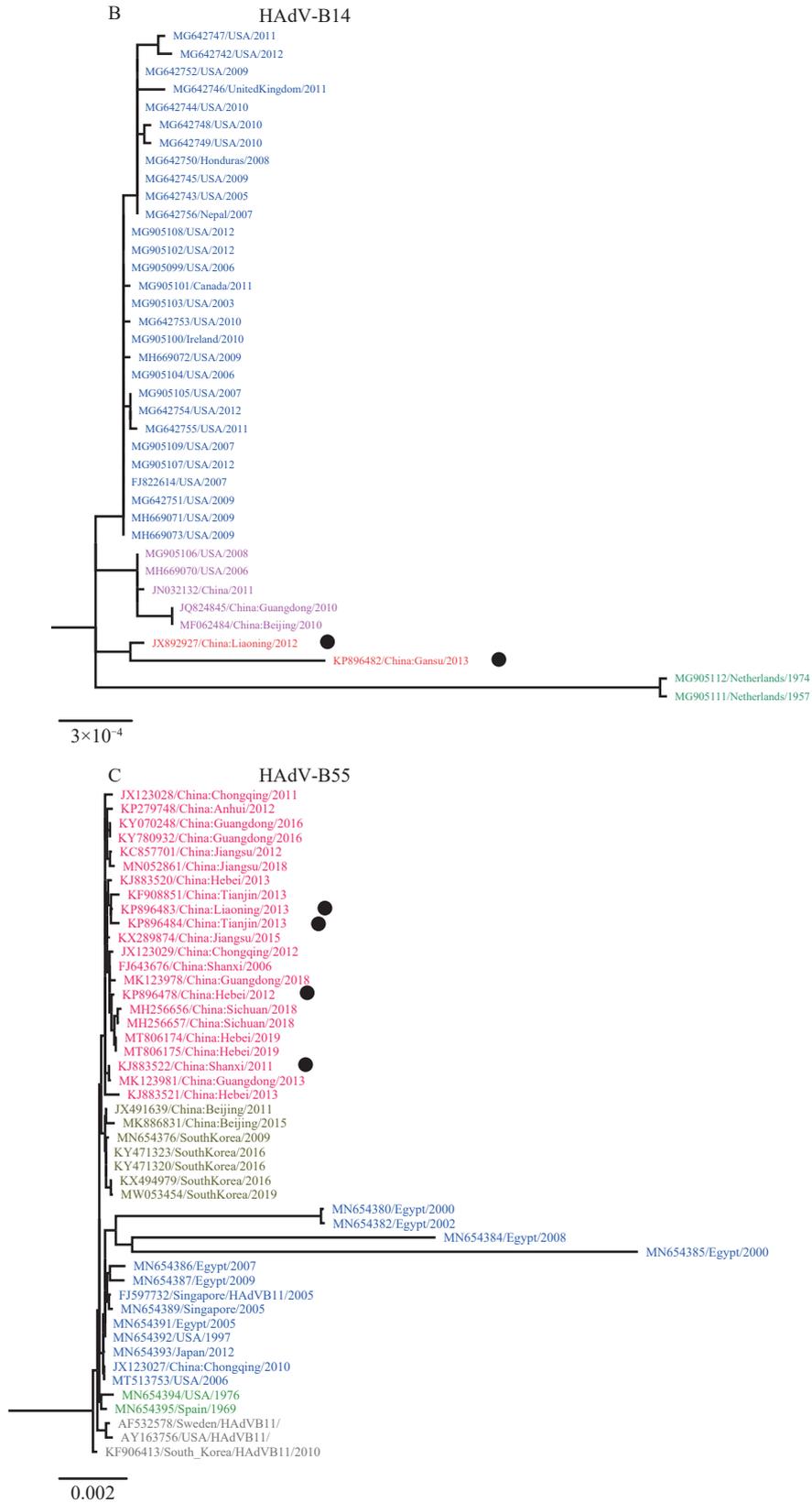


FIGURE 1. Phylogenetic tree was constructed based on the full genome sequences using maximum likelihood method with 1,000 bootstraps by MEGA 7.0. (A) HAdV-B7; (B) HAdV-B14; (C) HAdV-B55. Note: The strains labeled with black dots were obtained from the current study.

method using the MEGA 7 software (version 7.0.26, USA).

For alignment of HAdV-B7 genotype, a total of 109 sequences were obtained from GenBank. Based on the alignment with these available sequences, most of the Chinese strains could be clustered in 5 branches that were distinct from the strains of other countries (Figure 1A, Supplementary Figure S1A, S2A, and S3A, available in <http://weekly.chinacdc.cn/>). Interestingly all 3-outbreak related HAdV-B7 strains in the current study and the strains from Hubei in the year of 2012 and 2013 formed a separate cluster within the Chinese lineage, irrespective of the geographic location of the outbreak events, possibly suggesting genetic characteristics specific to outbreaks.

For HAdV-B14, a total of 50 sequences were recovered from GenBank, including 38 full genome sequences, 5 hexon, and 7 fiber gene sequences. The phylogenetic trees based on full genome sequences demonstrated a clear divergence of the 2 lineages that were derived from the year before 2012 and after 2012 for the strains from China, respectively; while for the hexon gene, the 2 lineages from China became 1 lineage, and for fiber and penton genes, the geographical distribution disappeared (Figure 1B, Supplementary Figures S1B, S2B, and S3B). The strains from China between 2010–2011 were clustered with those from the USA between 2006 and 2008. According to alignment of complete genome sequences, the current outbreak strain (KP896482/China:Gansu/2013) was most closely related to the strain from Liaoning (JX892927/China:Liaoning/2012) (99.9% nucleotide similarity and 99.6% amino acid similarity) while differing from the outbreak strain in the same region that were isolated in 2011 (for hexon gene, JX310315/China:Gansu/2011, 99.8% nucleotide similarity and 98.1% amino acid similarity; for fiber gene, JX310316/China:Gansu/2011, 99.7% nucleotide similarity and 98.1% amino acid similarity). This might reflect that the outbreak event was caused by a strain introduced into the camp by recruits and that the strain did not locally circulate.

For HAdV-B55, a total of 59 sequences were recovered from GenBank, including 47 full genome sequences, 6 hexon, and 6 fiber gene sequences. Based on the full genome sequences, all Chinese strains had high homologies, showing spatial specific distribution patterns and clustering into one branch (Figure 1C). Two strains from Beijing were clustered with strains from the Republic of Korea. While for hexon, fiber, and penton genes, the Chinese strains formed several

branches (Supplementary Figures S1C, S2C, and S3C). The strains detected during the recent military outbreaks from 2011 to 2013 were interspersed in the strains isolated from the community strains between 2010 and 2012. This might indicate that the most recent descendant from the early isolates were responsible mainly for the current outbreaks in military camps.

DISCUSSION

In summary, we have determined HAdV-B7, HAdV-B14, and HAdV-B55 to be the most common HAdV genotypes that were responsible for outbreaks in military camps or recruit training centers in China. This is different from the situation in the American military, where HAdV-E4, HAdV-B7, and HAdV-B14 act as the predominant strains sequentially isolated from the outbreaks (6–9). According to prior epidemiological investigation performed in community population in China, HAdV-B3 and HAdV-B7 were most frequently detected among ARD patients, while only in recent years HAdV-B55 emerged with increasing prevalence (10). In the absence of herd immunity against the uncommonly seen HAdV-B14 and newly emerging HAdV-B55 in the local community populations, military recruits were highly susceptible to outbreaks if these 2 types of HAdV were introduced into the susceptible population. An HAdV vaccination program against these three outbreak-related genotypes is urgently needed in this high-risk population.

By comparing various outbreaks, a higher attack rate was identified from HAdV-B14-related outbreaks when compared to those from HAdV-B7 and HAdV-B55, possibly indicating a higher susceptibility and transmissibility of HAdV-B14 in closed settings than the other two types. This conclusion, however, could differ in terms of geographic origin and immunity background of the population. Similar with previous findings (8), younger age and male gender were associated with an increased risk for HAdV infection during outbreak events, irrespective of the HAdV genotypes. Although disease severity varied among genotypes, we observed that infection with HAdV-B7 and HAdV-B55 caused more pneumonia than HAdV-B14 from the outbreak events. The infection source of the HAdV outbreak was preliminarily explored by genetic alignment of the viral strains. For HAdV-B14, no obvious phylogenetic relationship was derived between the HAdV responsible for the outbreak and

the locally circulating strain, likely indicating the introduction of the strain from a new recruit's hometown as the major infectious source. However, whether this hypothesis holds true and how the disease was introduced into the camp are hard to be determined, since the infected individual might still be in the incubation period at the time of entering camp, and thus might be missed even with mandatory quarantine for febrile illness. Quarantine among the new recruits before entering into the military and the identification of the major responsible genotype at the current stage is warranted.

This study was subject to some limitations. The severity of ARD caused by different HAdV genotypes was not evaluated as no individual-level data were collected. The study did not include all the outbreaks of HAdV in closed camps during December 2011–March 2014 in China.

Conflicts of interest: No conflicts of interest declared.

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Corresponding authors: Qingbin Lu, qingbinlu@bjmu.edu.cn; Wei Liu, liuweib@bmi.ac.cn, lwbieme@163.com; Yigang Tong, yigang.tong@gmail.com.

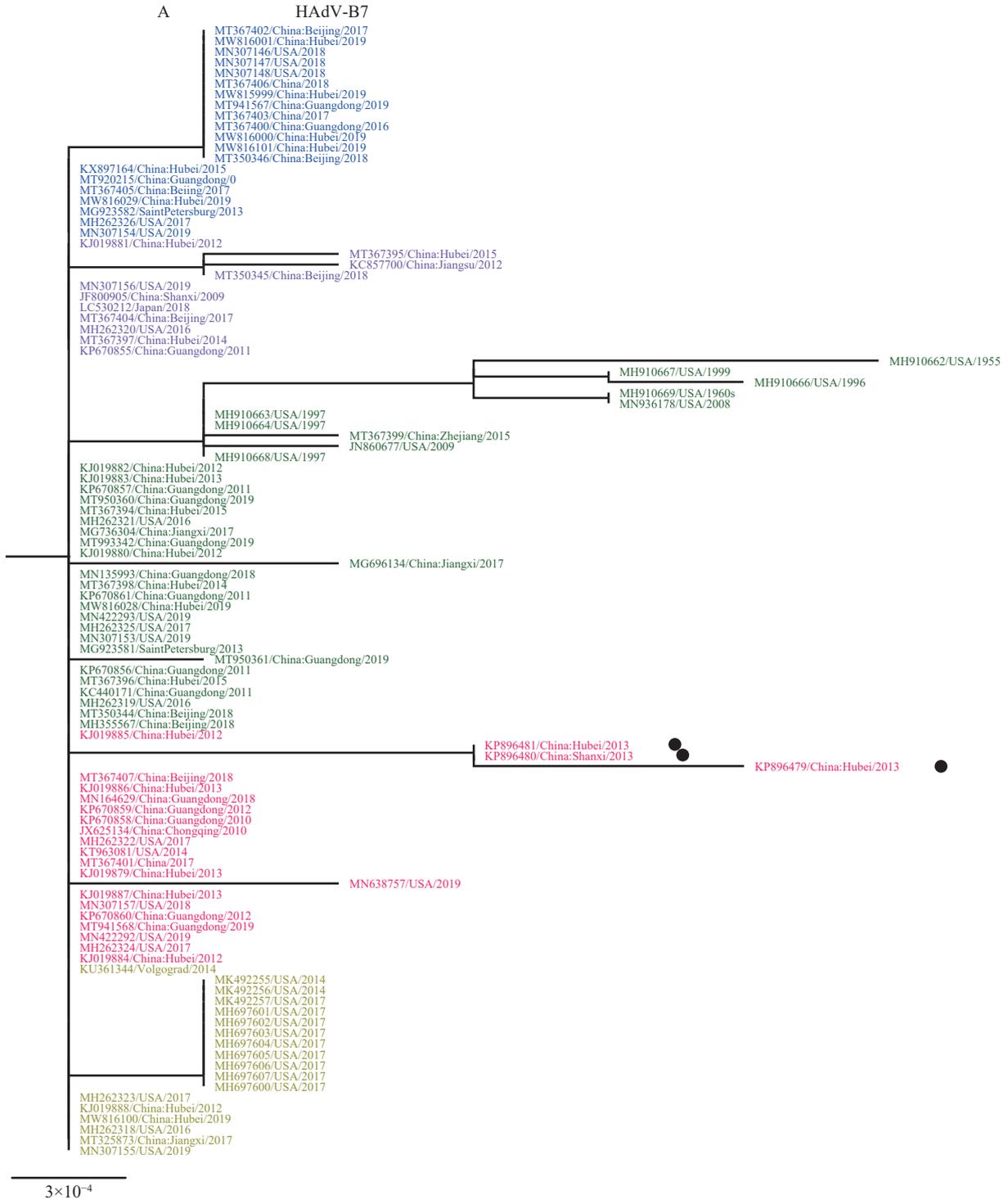
¹ Department of Laboratorial Science and Technology, School of Public Health, Peking University, Beijing, China; ² State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China; ³ Center for Diseases Control and Prevention of Chinese People's Armed Police Forces, Beijing, China; ⁴ The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China; ⁵ College of Life Science and Technology, Beijing University of Chemical Technology,

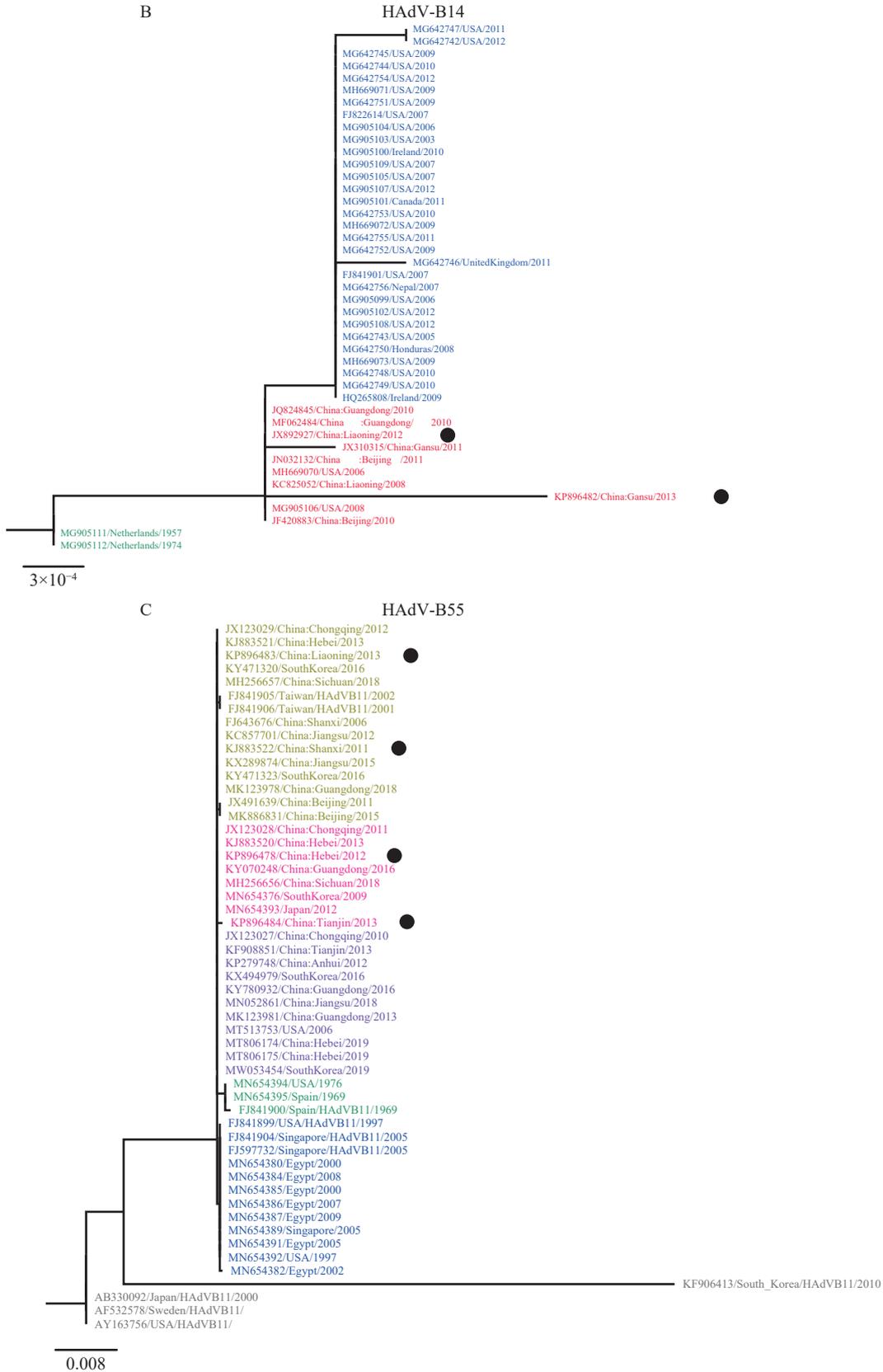
Beijing, China.
& Joint first authors.

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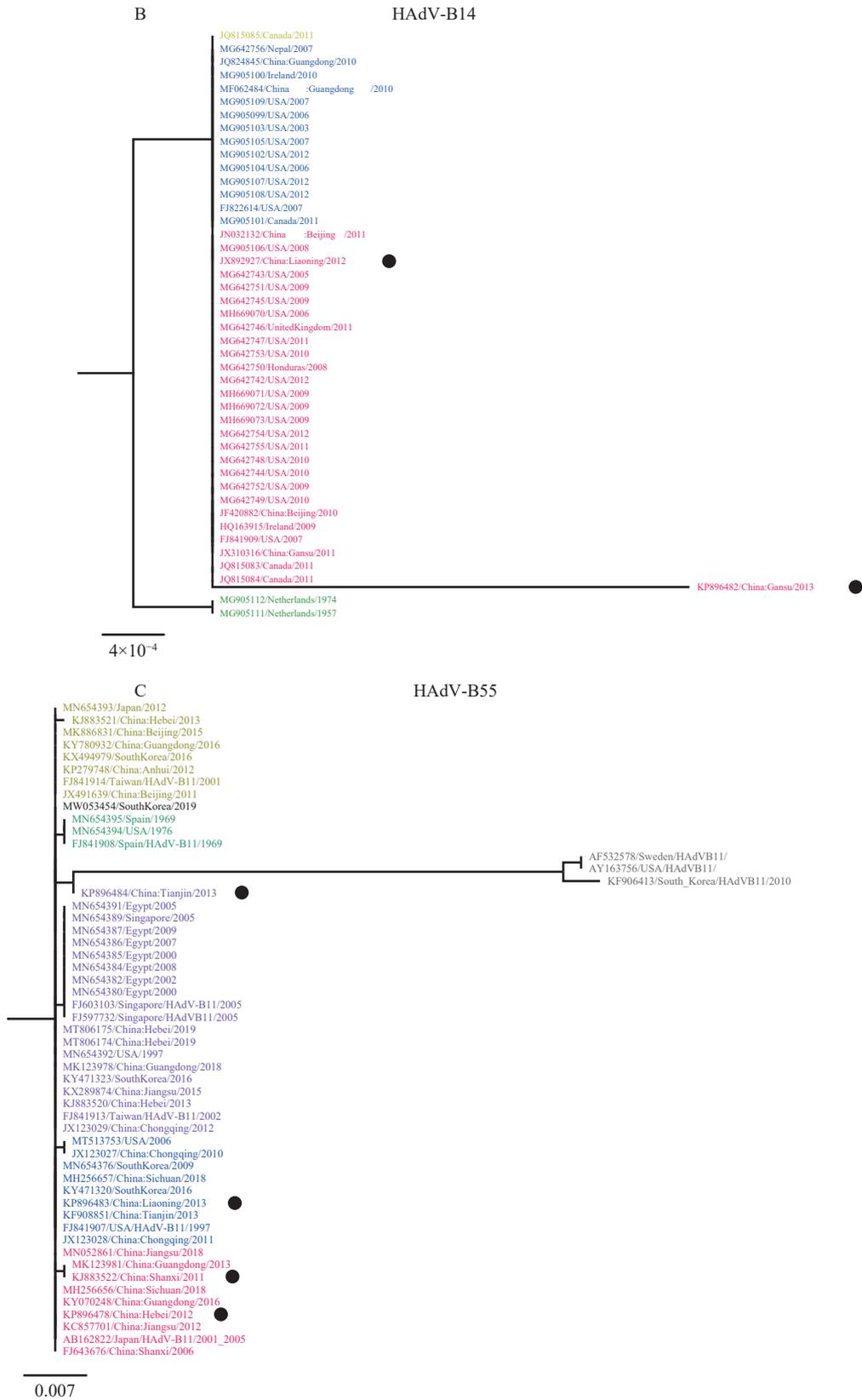




SUPPLEMENTARY FIGURE S1. Phylogenetic tree was constructed based on the hexon gene using maximum likelihood method with 1000 bootstrap by MEGA 7.0. (A) HAdV-B7; (B) HAdV-B14; (C) HAdV-B55.

Note: The strains labeled with black dots were obtained from the current study.





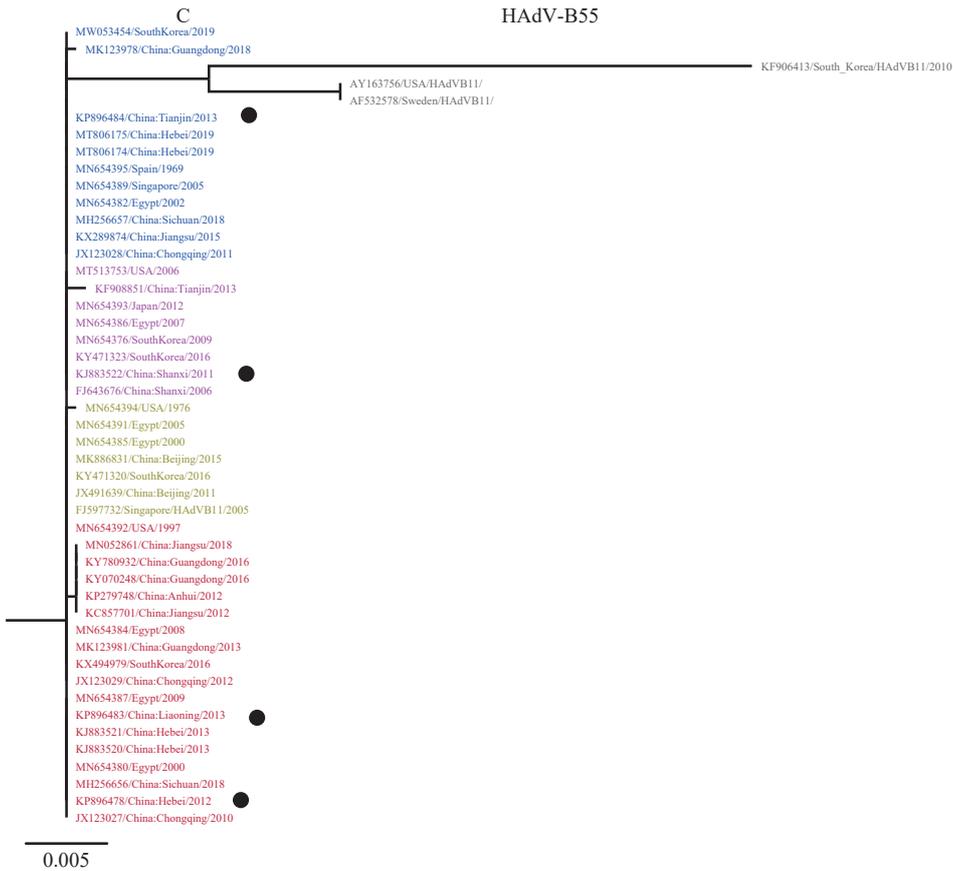
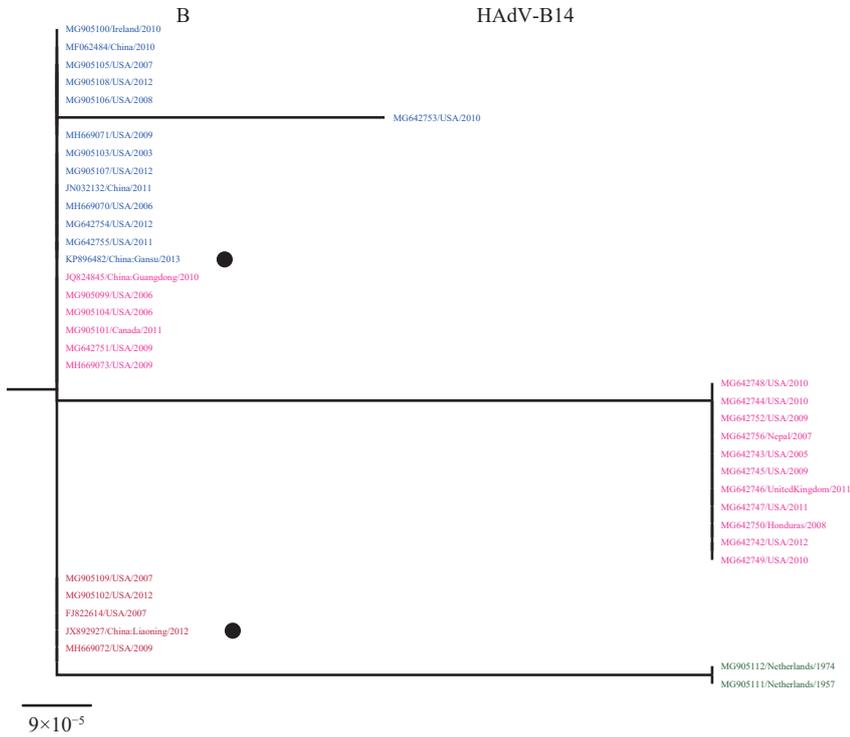
SUPPLEMENTARY FIGURE S2. Phylogenetic tree was constructed based on the Fiber gene using maximum likelihood method with 1,000 bootstrap by MEGA 7.0. (A) HAdV-B7; (B) HAdV-B14; (C) HAdV-B55.

Note: The strains labeled with black dots were obtained from the current study.

A

HAdV-B7





SUPPLEMENTARY FIGURE S3. Phylogenetic tree was constructed based on the Penton gene using maximum likelihood method with 1,000 bootstrap by MEGA 7.0. (A) HAdV-B7; (B) HAdV-B14; (C) HAdV-B55.

Note: The strains labeled with black dots were obtained from the current study.

Preplanned Studies

Malaria Surveillance of Entry People During the COVID-19 Epidemic — Guangdong Province, China, October 2020–May 2021

De Wu¹; Zhuohui Deng¹; Rongxin Lin¹; Qiang Mao¹; Wenchen Lu¹; Caiwen Ruan¹; Yongzhen Cen¹; Ning Xiao²; Tie Song^{1,†}

Summary

What is already known about this topic?

Malaria control was affected by the coronavirus disease 2019 (COVID-19) pandemic. This study conducted active case finding for key flights and key populations to determine malaria transmission.

What is added by this report?

Surveillance for malaria was conducted for entry personnel coming from areas affected by malaria. It is estimated that at least 100,000 tests were conducted in Guangdong Province; 154 cases were confirmed during the surveillance.

What are the implications for public health practice?

To maintain the malaria elimination status, comprehensively maintaining a sensitive and effective surveillance response system is especially important.

Malaria is a parasitic infection transmitted by *Anopheles* mosquitoes that is a major threat to global health. There were an estimated 229,000 cases and 409,000 deaths globally in 2019 (1). Historically, Guangdong is one of the most affected provinces in China by the threat of malaria. The average annual incidence rate in the whole province was between 1.22–183.23 per 10,000 from 1950–1980 (2), and the most historically dominant strains were *Plasmodium falciparum* and *P. vivax* in Guangdong. After more than half a century of effort to control the spread of malaria, indigenous cases had been completely eliminated in Guangdong Province since 2010 (3). Since then, as malaria is one of the main imported vector-borne diseases in Guangdong Province, the main goal of malaria prevention and control is to prevent local transmission caused by imported cases. From 2011 to 2019, the average number of imported malaria cases ranged from 136–206 per year (4). Thus the threat of imported cases cannot be ignored. However, since the coronavirus disease 2019

(COVID-19) pandemic emerged in December of 2019 (5), the early diagnosis and control of malaria has been difficult because it has similar clinical symptoms to COVID-19 in the early stages of the disease. For this reason, we carried out malaria detection and surveillance research in COVID-19 quarantine sites and other key places.

In order to strengthen the surveillance of malaria at COVID-19 quarantine sites and entry ports, to detect malaria cases early, and ultimately achieve the goal of accurately blocking the spread of malaria, we have required the following 3 categories of entry personnel from malaria-endemic areas to be screened for malaria since the resumption of international flights in October 2020: 1) people who have symptoms, including fever, headache, chills, etc.; 2) people who have accompanied confirmed malaria cases, such as those who work and live together abroad; and 3) people on flights with more than one confirmed malaria case. The third category was encouraged to be expanded in scope in qualified areas to include all inbound personnel in highly affected endemic areas. People grouped in any of the 3 categories would undergo the first screening test on the first day of entering China and undergo the second test on the day before terminating their quarantine period, i.e., the 14th day of quarantine. The samples were collected via peripheral blood through a finger prick, and the detection reagent was *Plasmodium* antigen rapid detection test (RDT). If the test was positive, the patient would be sent to designated hospitals to collect 3 mL of venous blood, and further microscopic examination was conducted in the hospital. Samples with positive microscopic examinations were sent to the responsible CDC for microscopic and nucleic acid re-examination and species identification.

To effectively control the spread of malaria, epidemic point disposal and vector control have been strengthened further. Response measures were implemented for quarantine sites, designated hospitals,

and their surrounding areas and included surveillance of *Anopheles* vectors and mosquito vector density and control of suspected breeding places. Anti-mosquito isolation was carried out for suspected screening person. No *Anopheles* vectors were found, and no secondary cases were detected in the isolated areas where cases occurred.

From October 2020 to May 2021, it was estimated that at least 100,000 tests were conducted in Guangdong Province; 154 cases were confirmed, of which 3 cases were also infected with coronavirus disease 2019 (COVID-19). A total of 151 samples (excluding 3 cases of COVID-19 coinfection) were sent to Guangdong CDC. The time from case discovery to diagnosis and species identification was completed within 3 days, and the samples were sent to Guangdong CDC for further detection and analysis within 5 days. The results of species identification showed that falciparum malaria was the most common (87.7%; 135/154 of cases), followed by ovale malaria (7.1%; 13/154), vivax malaria (3.9%; 6/154), malariae malaria (0.6%; 1/154), and mixed infection (0.6%; 1/154) (Table 1).

All cases originated from at least 23 distinct African countries, of which the Democratic Republic of Congo, Cameroon, Nigeria, the Republic of Congo, and Uganda were the top 5 countries with the largest number of cases, with a combined total of 95 cases, accounting for 61.7% of all cases. According to the registered residence of the infected patients, the imported cases were residents of 22 provincial-level administrative divisions (PLADs). Among them, Henan, Guangdong, Shandong, Hubei, and Hunan were the 5 provinces of residence with the most cases, accounting for 51.3% of all cases. All imported cases were concentrated in the COVID-19 quarantine sites

of 8 cities, of which Guangzhou had the most cases with 120 (77.9%), followed by Foshan and Shenzhen (Figure 1). The main occupation types of infected patients were workers and migrant workers; the age distribution was mainly concentrated in adults aged 20–60 years. In addition, these imported cases were found through active case finding: 3 cases were found at the port of entry, 5 cases were found by close contacts, and the other cases were found in COVID-19 quarantine sites.

DISCUSSION

After 2017, China has achieved the goal of zero local cases (6), and imported malaria has become the main target of malaria control in China. From 2017 to 2019, 8,202 malaria cases were imported into China, including 610 cases in Guangdong, accounting for 7.4% of all cases (4,6). Therefore, Guangdong Province is one of the main malaria import provinces in China. According to surveillance data in 2002, *An. anthropophagus*, *An. sinensis*, *An. minimus*, and *An. riyuetan* were confirmed as natural vectors of malaria transmission in Guangdong province, of which *An. anthropophagus* and *An. minimus* were the highest contributing vectors of the malaria epidemic in Guangdong Province, followed by *An. sinensis* (7). Due to the high annual average temperature, heavy rainfall, and long annual average activity time of vectors in Guangdong Province, the risk of local transmission or secondary cases caused by imported malaria is high. Since most of the prevention and control resources have been applied to COVID-19 prevention and control work, other infectious diseases were overlooked. For this reason, Guangdong Province began screening blood samples from people returning

TABLE 1. Distribution of *Plasmodium* types in Guangdong Province from October 2020 to May 2021.

Time	<i>P. vivax</i>		<i>P. falciparum</i>		<i>P. malariae</i>		<i>P. ovael</i>		Mixed infection		Total	
	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death
October, 2020	0	0	19	1	0	0	1	0	0	0	20	1
November, 2020	1	0	21	0	0	0	2	0	0	0	24	0
December, 2020	3	0	20	0	1	0	0	0	0	0	24	0
January, 2021	0	0	16	0	0	0	3	0	0	0	19	0
February, 2021	1	0	13	0	0	0	1	0	0	0	15	0
March, 2021	0	0	15	0	0	0	0	0	0	0	15	0
April, 2021	0	0	16	0	0	0	3	0	1	0	20	0
May, 2021	1	0	15	0	0	0	1	0	0	0	17	0
Total	6	0	135	1	1	0	11	0	1	0	154	1

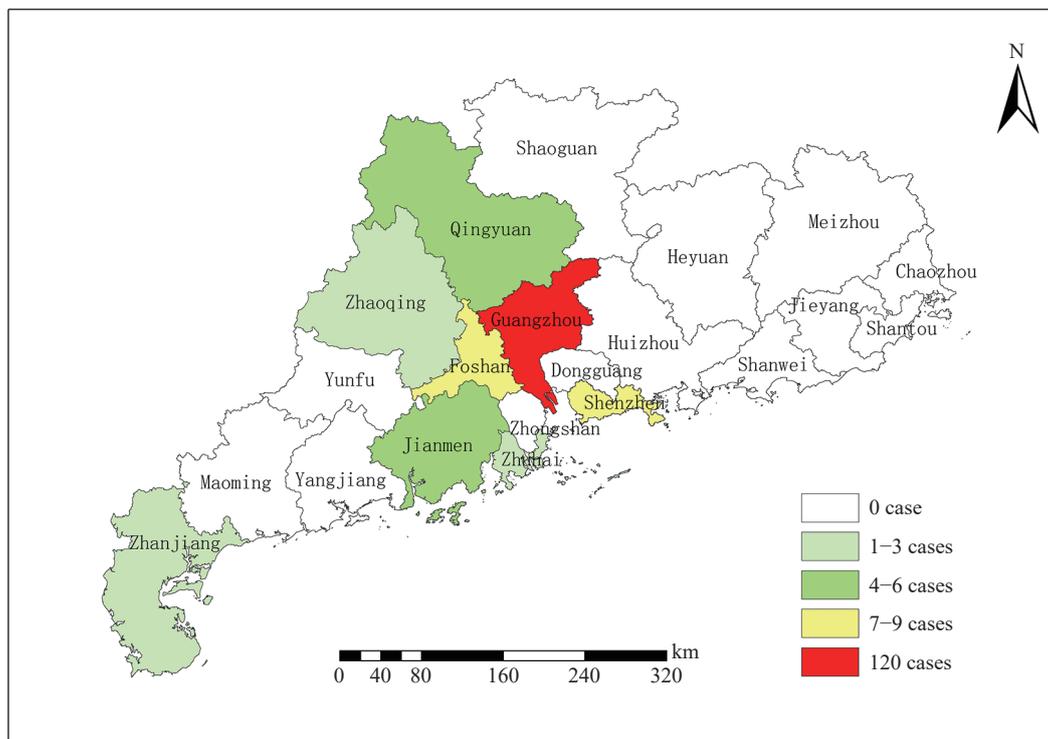


FIGURE 1. Distribution of reported malaria cases in Guangdong Province from October 2020 to May 2021.

from malaria-endemic areas such as Africa and Southeast Asia for RDT screening at all COVID-19 quarantine sites. Following the discovery of cases, the target population has gradually expanded to include travel companions and people on the same flight as infected patients. The number of screenings has been increased from one to two, greatly increasing the rate of early detection of cases.

The limitations of this study mainly include the following two points. First, the monitoring time is short, which can not fully reflect the epidemic characteristics of imported malaria cases. Second, there is a lack of mosquito vector monitoring data at quarantine points, which can not well analyze the transmission risk after malaria input.

Falciparum malaria has the highest fatality rate and is the main species of malaria imported into China. Among Chinese travellers, the mortality rate of *Plasmodium falciparum* infection was between 1%–2% (8). In this active monitoring process, 135 cases of falciparum malaria were found. Due to timely detection and treatment, the case-fatality rate was less than 1%. In addition, we have successfully diagnosed 3 cases of COVID-19 complicated with *Plasmodium* infection. Because COVID-19 is caused by a highly pathogenic microorganism (9), biosafety lab 3 (BSL-3) personal protection is necessary for the process of

malaria screening and testing. Through the effective development of rapid screening and confirmation of malaria in COVID-19 quarantine sites, so far, all detected malaria cases had been diagnosed and treated in time, and the malaria epidemic situation has been handled quickly and effectively. No second-generation cases caused by imported malaria and local epidemic spread occurred. The results showed that the measures to prevent imported and re-transmitted malaria were powerful and effective in Guangdong Province during the COVID-19 pandemic.

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Corresponding author: Tie Song, tsong@cdcp.org.cn.

¹ Institute for Parasitic Disease Control and Prevention, Center for Disease Control and Prevention of Guangdong, Guangzhou, Guangdong, China; ² National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China.

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Preplanned Studies

Identifying the Key Nodes of HIV Molecular Transmission Network Among Men Who Have Sex with Men — Guangzhou, Guangdong Province, China, 2015–2017

Juan Yang¹; Zhigang Han²; Huifang Xu²; Hui Xing¹; Peng Xu¹; Weibin Cheng²; Yuzhou Gu²; Fan Lyu^{1,†}

Summary

What is already known about this topic?

Identifying the most influential spreaders in human immunodeficiency virus (HIV) transmission networks is crucial for developing effective prevention strategies.

What is added by this report?

This study identified key nodes of the HIV molecular transmission network among men who have sex with men (MSM) by utilizing linkages between sequences to reconstruct the transmission network at the molecular level.

What are the implications for public health practice?

This study could act as an important supplement of laboratory results to epidemiological studies and suggests that interdisciplinary research could inspire new ideas for finding breakthroughs on HIV/acquired immunodeficiency syndrome (AIDS) prevention and control.

Based on reports of the acquired immunodeficiency syndrome (AIDS) epidemic in China in December 2017, sexual transmission accounted for more than 90% of total infections, and 26.86% of the sexual transmission infections were men who have sex with men (MSM) (1). According to the research conducted by Ethan Morgan and his colleagues, it is necessary to conduct investigations that focus on networks of target populations rather than traditional epidemiological factors such as geographic areas of high incidence (2). Identifying the most influential spreaders of the human immunodeficiency virus (HIV) transmission networks is crucial to develop effective prevention strategies.

Analyzing the structure of networks provides an optimal way to confirm the location and the role of key nodes that play key roles in accelerating HIV transmission in the network. Given the hidden nature of the MSM population, it is difficult to confirm the relationship ties between any two members of the

community, which is the first step to analyze network structure in traditional epidemiologic field investigations. Phylogenetics provides probabilities for network structure analysis in HIV research. Inferring putative transmission is the process of utilizing molecular phylogenetics analyzed by using HIV sequences to identify transmission events in groups of individuals (3). This study identified key nodes of the HIV molecular transmission network among MSM by utilizing linkages between sequences to reconstruct the HIV transmission network at the level of molecular genetics.

A total of 184 sequences of the HIV-1 pol full-length gene were assessed and stratified over 2 periods based on the year of sample collection (2015–2017). All 184 sequences were aligned with all known sequences in the HIV database (<http://hiv-web.lanl.gov/content/index>, operated by Triad National Security, LLC for the U.S. Department of Energy's National Nuclear Security Administration) using the Basic Local Alignment Search Tool (BLAST) before analysis. The length of the HIV-1 pol gene was 3,045 base pairs (bp) and the nucleotide positions of pol were 2,147–5,192 according to HXB2 subtype B reference strain (GenBank accession number K03455). The sequences were edited with the software Sequencher (version 5.0, Gene Codes Corporation, Ann Arbor, MI, USA). The reference sequences that were available on HIV Database) covered the major HIV-1 subtypes/CRFs. Among the 184 successfully amplified pol full-length sequences, 44.02% (81/184) were CRF07_BC, 33.15% (61/184) were CRF01_AE, 13.04% (24/184) were 01_B, 3.80% (7/184) were B, and 5.98% (11/184) were others.

HIV molecular transmission network was based on genetic distance (4). Putative transmission links in the network were identified with dichotomized data, which was determined by whether the pairwise genetic distance was less than 0.015 substitutions per site within all sequences (5). In our study, the Tamura-Nei 93 pairwise genetic distances were calculated by Mega

[Mega 7.0: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar S, Stecher G, and Tamura K 2016)] (6).

All social network analyses was conducted by UCINET 6.0 (version 6.05; Borgatti, Everett, and Freeman, 2002). The methods were described in the Supplementary Materials (available in <http://weekly.chinacdc.cn/>). All statistical analyses were performed with SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Multivariate logistic regression model was used to analyze the demographic characteristics of the key nodes.

Of the 184 HIV-1 sequences that were of patients diagnosed between 2015–2017, 75 sequences had at least one relationship tie with another patient (Figure 1). The characteristics of the participants are presented in Table 1. Social network analysis demonstrated that there were 14 cliques that included at least 3 nodes (Supplementary Materials, Supplementary Table S1, available in <http://weekly.chinacdc.cn/>). The biggest clique includes 24 members, and there were some cliques sharing the same members. Cliques 1–8 shared a lot of same members, and clique 9 only included 4 members that did not share any member with others.

The clique co-membership method yields a large

subgroup consisting of cliques 1–8 with a median subgroup of cliques 10 and 12, 4 smaller groups including cliques 9, 11, 13, and 14, and the outsiders. We denoted the 6 subgroups as A, B, C, D, E, and F. M026 acted as a broker between Subgroup B and F, E and F, D and F, as well as D and E. Subgroup B and D shared 2 actors {30, M026} acting as brokers between them. There were 3 shared members between groups B and E, respectively: {R12, M026, M056}.

From the result of lambda analysis (Supplementary Table S2, available in <http://weekly.chinacdc.cn/>), there were 17 lambda sets with $\lambda = 1$ that have a minimum of 1 independent path linking for any two actors. The largest λ was 19; it include 2 actors {27, M057}. A little bit smaller λ were 15 and 10, the actors in the lambda sets were {4, 27, M057} and {26, M050, 4, 27, M057} respectively. All of the above 5 nodes were nested hierarchically in the set with $\lambda = 1$, which has the largest number of members. These five nodes have the most relationship ties in the set and were in the most active central position.

Finally, we identified 9 key nodes by using cohesive subgroup analysis in the HIV molecular transmission network; {30, M026, R12, M056} acted as brokers between subgroups, and {26, M050, 4, 27, M057} were confirmed as the most active nodes in one

TABLE 1. Characteristics of the study population according to categories of number of connections in Guangzhou, Guangdong Province, China, 2015–2017.

Characteristics	Number of respondents N (%)	Number of connections			P value*
		0	1	≥2	
Total	184 (100.00)	109 (59.24)	36 (19.57)	39 (21.20)	
Age (years)					0.21
18–25	69 (37.50)	47 (68.12)	10 (14.49)	12 (17.39)	
26–35	71 (38.59)	37 (52.11)	19 (26.76)	15 (21.13)	
≥36	44 (23.91)	25 (56.82)	7 (15.91)	12 (27.27)	
Educational level					0.53
Primary school	44 (23.91)	27 (61.36)	6 (13.64)	11 (25.00)	
Junior and senior high school	44 (23.91)	29 (65.91)	7 (15.91)	8 (18.18)	
College and above	96 (52.17)	53 (55.21)	23 (23.96)	20 (20.83)	
Marital status					0.26
Married	29 (15.76)	13 (44.83)	7 (24.14)	9 (31.03)	
Unmarried	143 (77.72)	89 (62.24)	28 (19.58)	26 (18.18)	
Divorced	12 (6.52)	7 (58.33)	1 (8.33)	4 (33.33)	
Time of diagnosis					0.01
2015–2016	100	53 (53.00)	28 (28.00)	19 (19.00)	
2016–2017	84	56 (66.67)	8 (9.52)	20 (23.81)	

Note: All percentages are line percentages.

* P value for chi-square test for categorical variables.

subgroup. We analyzed the demographic characteristics of these key nodes. From the results of multivariate logistic regression model, young MSM born in the 1990s (aged 18–25) and 1980s (aged 26–35) was 0.06 and 0.12 times, respectively, likely to be a key node than older MSM born in the 1970s (aged 36 and older) or before (Table 2).

DISCUSSION

Of 184 newly-HIV diagnosed MSM, 40.76% were linked to other MSM. Social network analysis demonstrated that 9 key nodes were detected.

By using the clique co-membership method, there were four key nodes acting as brokers between subgroups. It could be inferred that there were a lot of subgroups connected by sharing co-members in the HIV molecular transmission network among MSM in Guangzhou City, Guangdong Province, China.

The four nodes that occupied important bridge locations were critical in controlling and understanding the spread processes as well as for developing effective prevention strategies.

Selecting candidates who connect across groups of otherwise disconnected individuals (such individuals are known as “bridging actors”) based on their network positions was shown to be more likely to enhance the

diffusion of innovative HIV prevention interventions when compared to other centrally located popular opinion leaders (7). Some HIV-infected MSM called as key nodes mediated the transmission of HIV among different subpopulations. Young MSM were less likely to promote HIV transmission than older MSM.

Based on connectivity cohesive subgroup analysis, known as the lambda sets method, we detected 5 key nodes. They were possibly taking on some kind of leadership role. In fact, they were active only in several subgroups of the transmission network in this study, rather than participating in the whole network of HIV transmission. In our study, there were at least three independent subgroups with members closely connected to each other within them. Therefore, it is immensely vital for HIV prevention and control to determine subgroups with different characteristics in HIV transmission network among MSM.

In recent years, HIV incidence in young Chinese MSM was significantly higher than that of older MSM (8). However, based on our results, MSM who were younger than 25 years old were less likely to promote the wide spread of HIV than older MSM. The results of our survey on the social interaction patterns of this group also confirmed this point: MSM aged about 30 and above were more likely to have condomless anal intercourse (CAI) with those of different ages (9). The

TABLE 2. The demographic characteristics of key nodes in the HIV transmission network in Guangzhou, Guangdong Province, China, 2015–2017.

Characteristics	Key nodes N (%)	Others N (%)	Adjusted OR 95% CI	P value
Total	9 (4.89)	175 (95.11)		
Age (years)				
18–25	1 (1.45)	68 (98.55)	0.06 (0.01–0.74)	0.03
26–35	2 (2.82)	69 (97.18)	0.12 (0.02–0.84)	0.03
≥36	6 (13.64)	38 (86.36)	1.00	
Educational level				
Primary school	3 (6.82)	41 (93.19)	0.42 (0.06–2.95)	0.38
Junior and senior high school	1 (2.27)	43 (97.73)	0.28 (0.03–3.07)	0.29
College and above	5 (5.21)	91 (94.79)	1.00	
Marital status				
Married	3 (10.34)	26 (89.66)	1.35 (0.11–16.12)	0.81
Unmarried	5 (3.50)	138 (96.50)	1.40 (0.09–22.93)	0.81
Divorced	1 (8.33)	11 (91.67)	1.00	
Sample resource				
NHS	4 (4.00)	96 (96.00)	0.61 (0.14–2.75)	0.52
VCT	5 (5.95)	79 (94.05)	1.00	

Abbreviations: HIV=human immunodeficiency virus; OR=odds ratio; CI=confidence interval; NHS= the National HIV sentinel Surveillance; VCT=HIV voluntary counseling and testing clinics.

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Supplementary Materials

Cohesive subgroups analysis is a powerful and mathematically rigorous method to characterize network robustness. The strength lies in the capacity to detect strong connections among nodes that not only have no neighbors in common, but that may be distantly separated in the graph (1).

Cliques

A clique is a subgroup of actors in which each actor is adjacent to any other actors in it, and it is impossible to add any other actors to the clique without violation of this condition (2). In our study, we constrain the minimum size of any clique to three.

When there are many cliques, it is difficult to interpret the result of cohesive subgroups for the overlap between cliques, which can result in hidden features of the structure. A method to solve this issue would be to try to remove or reduce the overlap by performing additional analysis such as clique co-membership(2). The first step is to combine cliques who have more than 2/3 of all actors being shared. After the first step, if there are still too many cliques, those that share more than 1/3 of the same members can be merged (3). From a small number of cliques, we can detect a set of key nodes acting as the bridge between subgroups.

Lambda Sets

Lambda sets, based on the property that members of the set have greater edge connectivity with other members than with non-members, have been shown to correspond to a particular hierarchical clustering of the nodes in a network (4). It is a maximal subset of actors who have more edge-independent paths connecting them to each other than to outsiders since actors in lambda sets with connectivity λ have a minimum of λ independent paths linking any one to any other. When λ is large, a lambda set describes a subset that is relatively difficult to disconnect by means of edge removals (4). In infectious disease research, we can detect those who are the most active in the subgroup, which is the most important for disease control.

SUPPLEMENTARY TABLE S1. Clique analysis in HIV transmission network with 184 nodes in Guangzhou, Guangdong Province, China, 2015–2017.

Cliques	Number of nodes	ID
1	24	10 14 15 20 26 27 34 37 4 41 62 80 R10 R3 M003 M004 M010 M011 M050 M057 M060 M064 M100 M107
2	22	14 15 20 26 27 34 37 4 59 62 80 R3 M003 M004 M010 M011 M050 M057 M060 M064M100M107
3	22	14 15 20 26 27 34 37 4 47 59 80 9 M003 M004 M010 M011 M050 M057 M060 M064M100M107
4	24	10 14 15 20 26 27 34 37 4 41 62 80 R10 R3 M003 M004 M010 M011 M013 M050 M057 M060 M064 M100
5	22	14 15 20 26 27 34 37 4 59 62 80 R3 M003 M004 M010 M011 M013 M050 M057 M060 M064 M100
6	17	10 20 26 27 34 4 41 62 R10 R3 M003 M010 M017M050 M057 M060 M107
7	12	2 20 26 27 4 59 62 R3 M013 M050 M057 M060
8	5	34 4 41 M057 M073
9	4	25 M101 M103 M104
10	6	30 75 R12 M026 M048 M056
11	4	30 M026 M037 M065
12	5	75 R12 M026 M056 M108
13	5	8 R12 M026 M056 M109
14	3	M026 M048 M068

SUPPLEMENTARY TABLE S2. Lambda sets in HIV transmission network with 184 nodes in Guangzhou, Guangdong Province, China, 2015–2017.

λ	The number of sets	Actors
1	17	1: (3, 13); 2: (44, M009); 3: (R11, M016); 4: (M024, M025); 5: (M045, M046); 6: (M034, M051); 7: (6, M069); 8: (M019, M083); 9: (M078, M086); 10: (M079, M087); 11: (M080, M088); 12: (M028, M090); 13: (M084, M092); 14: (M098, M099); 15: (25, M101, M103, M104); 16: (47, 9, M017, M073, 14, 2, 37, 41, 59, 80, M003, M004, M011, M013, 15, 34, M010, 10, R10, 20, 62, R3, 26, M050, 4, 27, M057, M060, M064, M100, M107); 17: (8, M048, 75, R12, M026, M056, 30, M037, M065, M068, M108, M109, M110, M111)
2	4	1: (25, M101, M103, M104); 2: (14, 2, 37, 41, 59, 80, M003, M004, M011, M013, 15, 34, M010, 10, R10, 20, 62, R3, 26, M050, 4, 27, M057, M060, M064, M100, M107); 3: (75, R12, M026, M056); 4: (30, M037, M065)
3	3	1: (25, M101, M103, M104); 2: (41, 59, 80, M003, M004, M011, M013, 15, 34, M010, 10, R10, 20, 62, R3, 26, M050, 4, 27, M057, M060); 3: (M026, M056)
4	1	1: (15, 34, M010, 10, R10, 20, 62, R3, 26, M050, 4, 27, M057, M060)
5	1	1: (M010, 10, R10, 20, 62, R3, 26, M050, 4, 27, M057, M060)
6	1	1: (10, R10, 20, 62, R3, 26, M050, 4, 27, M057, M060)
8	1	1: (20, 62, R3, 26, M050, 4, 27, M057)
10	1	1: (26, M050, 4, 27, M057)
15	1	1: (4, 27, M057)
19	1	1: (27, M057)

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Notes from the Field

Two Confirmed Patients of Anthrax — Binzhou City, Shandong Province, China, August 2021

Xiaolin Yu^{1,✉}; Lifang Zhang^{2,✉}; Ming Fang¹; Hao Liang³; Qi Zhang³; Xiusheng Yin²; Zengqiang Kou¹; Wenwu Yin⁴; Zhong Li¹; Enmin Zhang^{4,✉}; Shujun Ding^{1,✉}

As of August 15, 2021, 2 confirmed cases of anthrax were reported to Shandong CDC, one of which was a 14-year-old student (Patient A) and the other was a 35-year-old man (Patient B) engaging in the slaughter of cattle. Patient A died on August 6, and Patient B has been transferred to an infectious disease hospital for isolation and treatment. The Shandong CDC formed a joint investigation team to determine the source of infection and prevent any further spread.

On July 28, 2021, Patient A suddenly experienced fever, fatigue, retching, diarrhea, and convulsions and then was taken to the health clinic of Liumiao Village in the evening of the same day and then again on July 30. On July 31, Patient A was transferred to Binzhou Medical University Hospital due to sudden unconsciousness, lockjaw, and nuchal rigidity during infusion in the village clinic. On August 6, Patient A was voluntarily discharged from the hospital and died the same day. The corpse had been treated under the anthrax disposal regulations, and the burial site had been strictly terminally disinfected. Patient A's cerebrospinal fluid tested positive for *Bacillus anthracis*, and serum was tested positive for the related IgG. Additionally, the blood culture also tested positive for

Bacillus anthracis by polymerase chain reaction (PCR). Experts preliminarily judged Patient A as intestinal anthrax or meningitis anthrax caused by sepsis. The genome size of *Bacillus anthracis* isolated from Patient A was 5,480,063 bp, and guanine-cytosine (GC) content was 35.07%; 5,875 genes were predicted, and the non-coding region accounted for 16.67% of the total genome (Figure 1). The number of genes annotated to function was 2,679 (45.6%) (Figure 2). Additionally, this strain included 38 virulence genes (*pagA*, *cya*, *lef*, and *atxA*, etc.) and complete capsular gene island (*capA*, *capB*, *capC*, *capD*, and *capE*); 6 drug-resistant genes were found in this genome, including *FosB*, *FosB2*, *mphL*, *bla1*, *bla2*, and *SatA*, all of which were inherent. Mobile genetic elements of this genome included *MICBan1*, *IS231L*, *ISBce14*, *IS231T*, and *ISBce17*.

On August 8, Patient B was identified initially as a suspected case during the active investigation and was subsequently diagnosed as cutaneous anthrax by Shandong CDC. Respondents concealed the truth in the early stage of case tracking. On August 12, with the assistance and verification of public security department staff, the investigation team found that

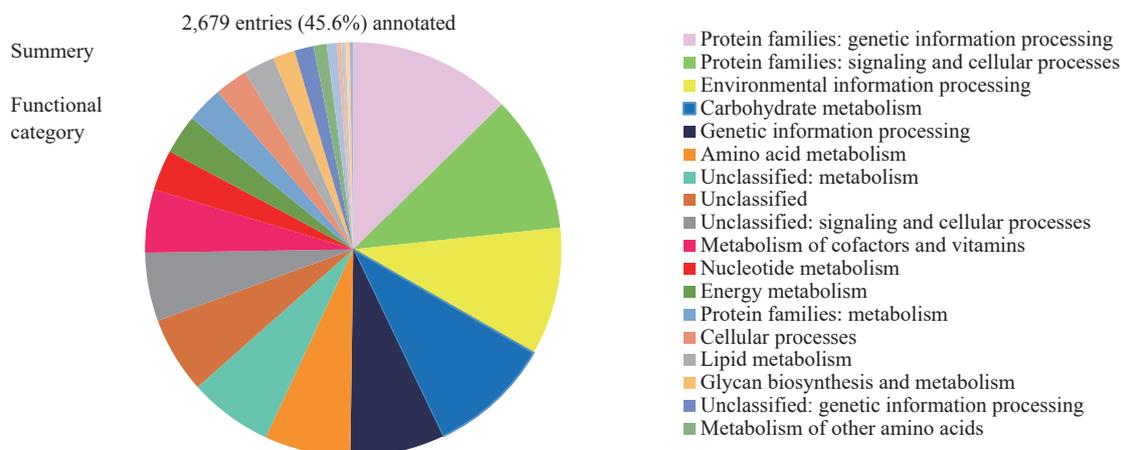


FIGURE 1. Gene function annotation of *Bacillus anthracis* isolated from Patient A.

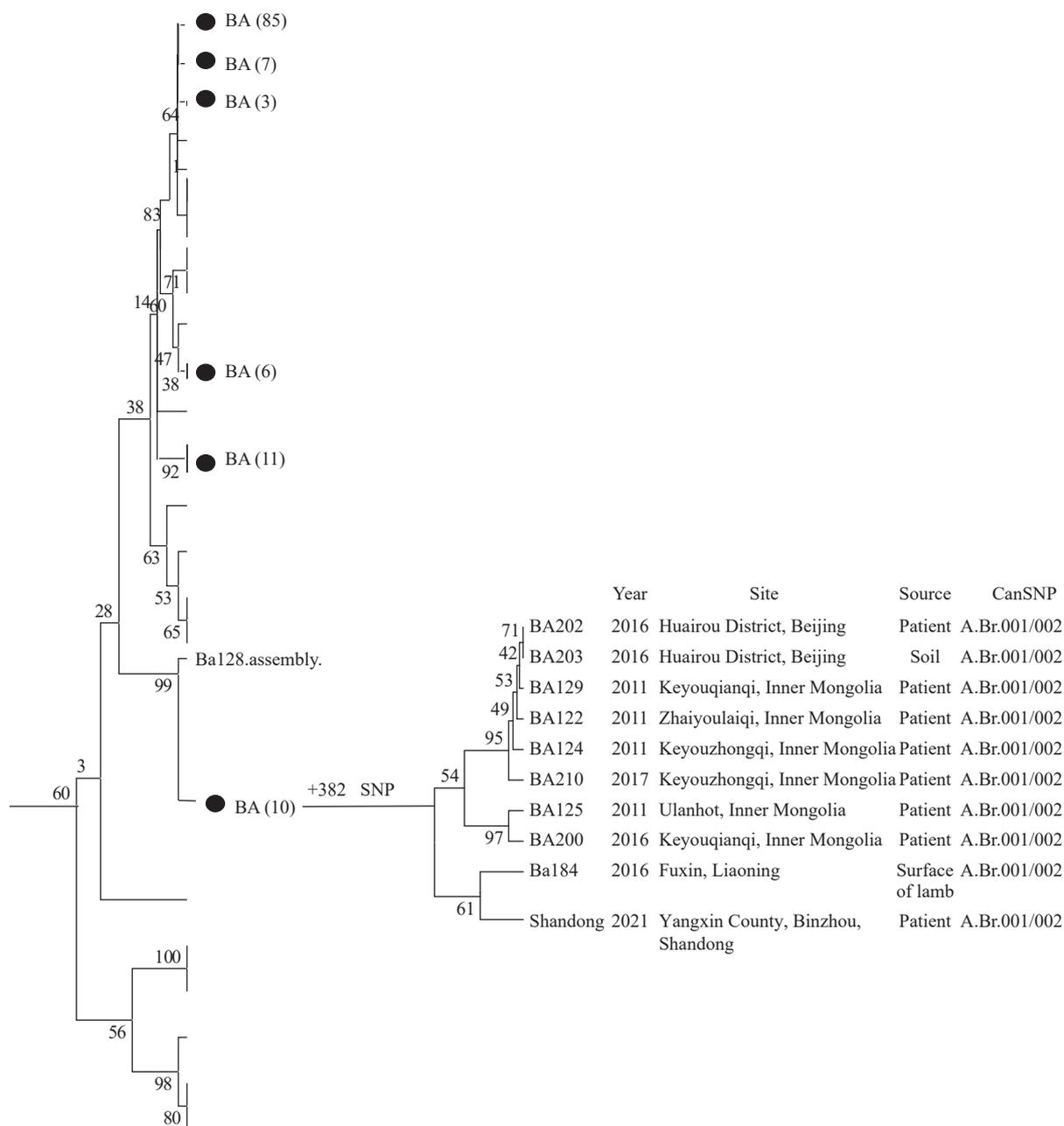


FIGURE 2. Phylogeny of *Bacillus anthracis* strains based on the unweighted pair-group method with arithmetic mean method.

Patient B slaughtered sick cattle bought from Wendian Town in Yangxin County at Patient A's home on July 25. The cattle seller said that these cattle were purchased from Jilin Province in early July. According to the respondents, black blood flowed on the ground, and internal organs emitted a peculiar smell during the slaughter process. These incidents reflected their lack of awareness of anthrax prevention and control and, when considering their economic burden and the chaotic private trading of livestock (1), laid a considerable risk for anthrax outbreaks.

Shandong CDC and related health agencies implemented a strict 12-day quarantine requirement for all close contacts of the 2 confirmed cases, including 24 that quarantined at home and 9 at centralized isolation facilities. The body temperature and health status of each close contact were monitored daily; meanwhile, 78 people have been screened for fever, diarrhea, vomiting, and skin symptoms in primary medical institutions, and no new cases have yet been found as of August 24. Preventive medications (ofloxacin, ciprofloxacin, etc.) were

provided to more than 4,700 close contacts and key personnel.

On August 8, a total of 10 serum samples were collected from family members and close contacts of Patient A and other suspected cases, all of which tested negative by enzyme-linked immunosorbent assay test and real-time quantitative PCR. In addition, 13 samples were collected from home environments and smears on the eschar lesions of a suspected cutaneous anthrax case, 8 of which were positive. On August 10, *Bacillus anthracis* was detected from the ground swab specimens collected from cold storage. For Patient B, investigators collected 160 environment samples (home environment: 21; processing plant: 104; slaughterhouse: 20; feedlot: 15) on August 11, of which 5 samples tested positive for anthrax-specific genes (protective antigen and capsule-related genes). On August 12, nucleic acid testing found that 2 samples collected from the slaughtered sick cattle tested positive.

Investigators checked the cattle ranches and sheep farms simultaneously. As of August 13, 39 blood smear specimens collected from the main testing area involving 8 sheep farms and 39 cattle ranches were all sent to the provincial animal department for testing, and all samples were negative. In addition, 54 households in the key testing area and 69 households in the general testing area were investigated, and investigators found no suspected sick animals. Meanwhile, the county carried out disinfection of

farms and slaughterhouses covering 1,254,564 square meters, and the beef products from the families whose samples were previously positive were disposed of properly. On August 14, animal immunization was carried out through Yangxin County.

To find the source of anthrax, prevent the further spread, and eliminate potential risks, the continued investigation and treatment of the current epidemic will be urgent and necessary. To better address the public's concern for anthrax, recommendations have been made to raise awareness for the prevention of anthrax among the public, further strengthening the training and guidance of medical staff and health workers from primary medical and healthcare services.

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Corresponding authors: Enmin Zhang, zhangenmin@icdc.cn; Shujun Ding, dsj_jn@126.com.

¹ Shandong Center for Disease Control and Prevention, Jinan, Shandong, China; ² Binzhou Center for Disease Control and Prevention, Binzhou, Shandong, China; ³ Shandong University, Jinan, Shandong, China; ⁴ Chinese Center for Disease Control and Prevention, Beijing, China.

& Joint first authors.

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Notes from the Field

HIV-2 Seroepidemiological Evidence in Hunan Province — China, 2003–2020

Jinyu Peng¹; Jianmei He¹; Jun Zheng¹; Xiaobai Zou¹; Yang Jiang¹; Xiaoling Su²; Xi Chen^{1,*}

The human immunodeficiency viruses-2 (HIV-2) is endemic in West Africa and is a rarely imported infectious disease in China due to the small number of cases having had a history of living abroad (1–3). In 2007, through routine HIV surveillance, 2 HIV-2 infected individuals were confirmed by serology and nucleic acid testing, becoming the first report of HIV-2 in China. Epidemiological case investigation showed that the two infected individuals had sexual contact, which were transmitted by heterosexual contact (4). In order to understand whether there are more HIV-2 infected individuals and the epidemiological characteristics of HIV-2, we conducted HIV-2 antibody detection and epidemiological investigation on the sera of individuals previously suspected of contracting HIV-2 in Hunan Province.

Stock blood samples with HIV-2 indicative bands in the original records of 15 AIDS laboratories in Hunan Province from 2003 to 2020 were collected. The laboratory of Hunan Provincial Center for Disease Control and Prevention used the approved and marketed kit [Germany Mikrogen GmbH (HIV 1+2) antibody testing kit] for simultaneous diagnosis of HIV-1 and HIV-2. Epidemiological data of the first test and of follow-up records of patients that could be contacted were also collected.

A total of 378 samples with HIV-2 indicative bands were collected, involving 363 individuals. Serological test results showed that 326 cases (89.81%) were HIV-1 antibody positive, 18 cases (4.96%) were HIV-2 antibody positive, 12 cases (3.31%) were HIV antibody positive but could not be typed, 6 cases (1.65%) were HIV antibody uncertain and 1 case (0.28%) was negative for HIV-1/2 antibody. Among the 18 HIV-2 antibody positive individuals, there were 15 cases from Xiangtan City and 3 cases from Changsha City. The epidemiological investigation showed that the cases consisted of 12 males (66.67%) and 6 females (33.33%), with a male-to-female ratio of 2:1. The average age of the first detection was (56.94±12.52) years old, with the male age ranging

from 33 to 76 years old and the female age ranging from 44 to 66 years old. There were 10 married patients (55.56%) including 1 couple, 6 patients who were divorced or widowed (33.33%), and 2 (11.11%) with unknown marital status. There were 13 cases found from hospital monitoring (72.22%), 2 from prison detention, 2 from blood screening in volunteer donors (22.22%), and 1 from spouse screening (5.56%). These samples were tested and 1 case was found in each year of 2005, 2009, 2013, 2014, 2018, and 2020, 6 cases in 2016, 2 cases in 2017, and 4 cases in 2019. By the end of 2020, 9 cases were alive and 9 cases were dead or missing; 3 cases have accepted antiviral treatment. Of the 7 cases that could be followed up with, all admitted to heterosexual transmission, 6 cases denied any history of travelling abroad, and 1 case denied any history of foreign sexual intercourse.

The HIV-2 infection epidemic has lasted for a long time in Hunan Province, and local transmission cases have already existed. The sites of infection are concentrated in Xiangtan and Changsha City, and there is a possibility of cluster infection. The initial source of HIV-2 transmission in Hunan Province needs to be identified, and a deeper epidemiological investigation of these infected individuals should be conducted as soon as possible. The clinical symptoms of these cases should be closely monitored, and the risk assessment of the spread of transmission should be strengthened to prevent further spread of the HIV-2 epidemic. There is a risk of HIV-2 local transmission in China, and the government should pay attention to it.

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* Corresponding author: Xi Chen, chenxi@hncdc.com.

¹ Hunan Provincial Center for Disease Control and Prevention, Changsha, Hunan, China; ² Xiangtan Center for Disease Control and Prevention, Xiangtan, Hunan, China.

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