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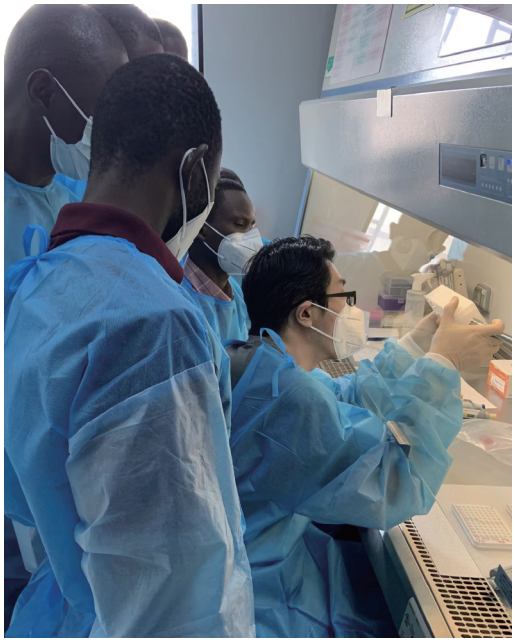

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Cover Photo: China CDC staff conducting laboratory training for local staff in Sierra Leone-China Friendship Biosafety Laboratory, Freetown, Sierra Leone, February 17, 2022.

Preplanned Studies

Assessing the Effectiveness of a Community-Based Smoking Cessation Intervention — Shenzhen City, Guangdong Province, China, 2022

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Summary

What is already known about this topic?

Research on community-based smoking cessation interventions in China is still in its early stages. Most existing studies have focused on a limited number of communities and have primarily examined interventions conducted by study teams rather than broader community initiatives.

What is added by this report?

The three-month continuous abstinence rate for the intervention group (21.61%) was significantly higher than that for the control group (8.98%). Comprehensive community-based smoking cessation interventions, administered by trained physicians at community health service centers and supported by community workers, have shown effectiveness in improving a variety of outcomes among community smokers.

What are the implications for public health practice?

The feasibility and effectiveness of comprehensive community-based smoking cessation interventions make them a valuable addition to existing cessation services in China. Wider implementation of these interventions should be pursued as a complementary approach to current efforts to reduce smoking rates in China.

In China, there are over 300 million smokers, with approximately 19.8% having attempted to quit within the past 12 months (*1*). While numerous studies have demonstrated that cessation medication and counseling can improve quit rates (*2*–*3*), a mere 4.6% and 3.2% of those who tried to quit within the past 12 months reported using smoking cessation medication or receiving cessation counseling, respectively (*1*). This suggests that current cessation services are insufficient in addressing the needs of smokers aiming to quit.

In this study, the effectiveness of a comprehensive, community-based cessation intervention model was evaluated through a two-arm, parallel, cluster-randomized controlled trial conducted between August 2022 and March 2023 in Shenzhen, China. Eligible smokers were recruited through community health service centers (CHSC) and were assigned to receive either the community-based cessation intervention or self-help smoking cessation materials. Participants were followed up via telephone for three months. A total of 492 eligible participants were included in the analysis. The intervention group demonstrated a significantly higher self-reported quit attempt rate, 7-day point prevalence of abstinence rate (PPAR), smoking reduction rate, and continuous abstinence rate (CAR) compared to the control group at the three-month follow-up (59.75% *vs.* 40.63%, 33.05% *vs.* 21.88%, 33.47% *vs.* 23.05%, and 21.61% *vs.* 8.98%; $P<0.05$). Furthermore, participants who received the community-based cessation intervention [adjusted odds ratio (a*OR*)=3.530, 95% confidence interval (*CI*): 1.942–6.413], had lower perceived difficulty of quitting scores (a*OR*=0.872, 95% *CI*: 0.773–0.984), and were married (a*OR*=2.203, 95% *CI*: 1.025–4.736) exhibited a higher likelihood of achieving continuous abstinence (CA).

Twenty matched communities in Shenzhen were selected and randomized (1∶1) into either the intervention or control group. Eligible participants were current smokers over 18 years of age who planned to quit within one month and signed an informed consent form; individuals with psychiatric or psychological disorders were excluded (Figure 1). For the intervention group, a "3+1" smoking cessation service model, developed based on the "5A" (Ask, Advise, Assess, Assist, Arrange) and "5R" (Relevance, Risks, Rewards, Roadblocks, Repetition) principles (*4*), was provided by trained and assessed CHSC physicians. The "3+1" model comprised three face-to-face interventions at the initial visit, 7 days, and 1

month after the quit date, as well as one telephone intervention on the quit date. The first visit featured a 40-minute face-to-face cessation counseling session, while the following two face-to-face interventions lasted for at least 20 minutes on the seventh day and first month after the quit date. The telephone intervention on the quit date typically spanned approximately 5 minutes.

Moreover, in the intervention group, community workers implemented advocacy initiatives and established smoke-free environments, including smoke-free schools, smoke-free households, and smoke-free government buildings, to foster an anti-tobacco atmosphere. Participants in this group also received self-help cessation materials. Conversely, the control group was solely provided self-help cessation materials, distributed by CHSC physicians. The study gained ethical approval from the Ethical Review Committee of the China CDC (202128) and was registered in the Chinese Clinical Trial Registry (ChiCTR2200 056242).

In the current study, participants lost to follow-up were treated as continuing smokers with no decrease in cigarette consumption relative to baseline. Outcome measures encompassed quit attempts, 7-day point prevalence of abstinence (PPA), CA, and smoking reduction at the three-month follow-up. Among these measures, a quit attempt referred to self-reported abstinence from smoking for 24 hours or longer; 7-day PPA denoted self-reported abstaining from smoking for the preceding seven consecutive days or more; CA described self-reported sustained abstinence from smoking since the designated quit date; and smoking reduction was characterized as self-reported reduction in daily cigarette consumption by half or greater in comparison to the initial visit, excluding participants who self-reported cessation. An intent-to-treat approach was employed for this study.

Data analysis was conducted using SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA). Continuous variables adhering to a normal distribution were described using means and standard deviations, whereas non-normally distributed variables were presented as medians and interquartile ranges. Categorical variables were summarized using frequencies and corresponding percentages. Unconditional logistic regression analysis was utilized to examine factors associated with 3-month continuous abstinence. Odds ratios (*OR*) and 95% *CI* were calculated. All *P*-values were two-sided, with $P<0.05$ indicating statistical significance.

In the current research, a sample of 492 participants was gathered, comprising 236 individuals from intervention communities and 256 from control communities. The mean age of the participants was 42.34±13.14 years, with a majority being male (95.12%). Most participants were married (81.91%) and employed (70.53%), and approximately one-third (33.13%) had obtained a college degree or higher educational attainment (Table 1).

At the three-month follow-up, successful smoking cessation was reported by 15.04% of participants. The CAR for the intervention group was 21.61%, which was significantly higher than the control group's 8.98% [unadjusted odds ratio (c*OR*)=2.793, 95% *CI*: 1.646–4.739]. Participants with a shorter smoking history were more likely to quit (c*OR*=0.975, 95% *CI*: 0.954–0.997). Lower nicotine dependence was associated with a higher CAR, as low, moderate, and severe nicotine dependence levels resulted in CARs of 19.84%, 9.63%, and 10.91%, respectively (c*OR*=0.644, 95% *CI*: 0.458–0.905). Furthermore, higher CARs were observed among participants who perceived greater importance in quitting (c*OR*=1.134, 95% *CI*: 1.000–1.286), reported lower difficulty in quitting (c*OR*=0.904, 95% *CI*: 0.823–0.994), and expressed higher confidence in quitting (c*OR*=1.161, 95% *CI*: 1.044–1.291) (Table 1).

A logistic regression model was employed to investigate the predictors associated with CAR (1: cessation maintained for three months, 0: cessation not maintained for three months). The results revealed that participants in the intervention group had a higher likelihood of achieving continuous abstinence (a*OR*=3.530, 95% *CI*: 1.942–6.413), while perceiving greater difficulty in quitting smoking was associated with a reduced probability of sustaining abstinence for three months (a*OR*=0.872, 95% *CI*: 0.773–0.984). Additionally, married participants were more likely to remain abstinent from smoking for three months (a*OR*=2.203, 95% *CI*: 1.025–4.736) (Table 2).

A similar method was utilized to investigate differences between the intervention group and the control group in relation to quit attempts, PPA, and smoking reduction at a three-month follow-up. The findings revealed that a higher proportion of participants in the intervention group (59.75%) attempted to quit smoking as compared to the control group (40.63%), with an a*OR* of 3.063 and a 95% *CI* of 1.965–4.774. Moreover, the intervention group displayed a higher PPA rate (33.05%) in contrast to the control group (21.88%), with an a*OR* of 2.364 and

TABLE 1. Characteristics of study participants and those who achieved 3-month continuous abstinence — Shenzhen City, China, 2022.

Characteristic	Total, *N* (%)	CAR, *N* (%)	c*OR*[*]	95% *CI*	*P*
Total	492	74 (15.04)			
Group			2.793	1.646–4.739	<0.001
Intervention group	236 (47.97)	51 (21.61)			
Control group	256 (52.03)	23 (8.98)			
Demographic characteristics					
Gender			1.961	0.751–5.116	0.169
Male	468 (95.12)	68 (14.53)			
Female	24 (4.88)	6 (25.00)			
Age (years) (M±SD)	42.34±13.14	42.46±14.46	1.001	0.982–1.020	0.934
Marital status			1.717	0.960–3.071	0.068
Married	403 (81.91)	55 (13.65)			
Unmarried/divorced/widowed	89 (18.09)	19 (21.35)			
Education level					
Primary or lower	19 (3.86)	3 (15.79)	1.160	0.937–1.437	0.173
Secondary school	77 (15.65)	9 (11.69)			
High school/secondary specialized school	118 (23.99)	14 (11.86)			
Specialized school	115 (23.37)	19 (16.52)			
College or higher	163 (33.13)	29 (17.79)			
Employment status			1.015	0.591–1.743	0.958
Employed	347 (70.53)	52 (14.99)			
Unemployed	145 (29.47)	22 (15.17)			
Perceived health status at the first visit			0.637	0.378–1.072	0.090
Very good/good	305 (61.99)	53 (17.38)			
Fair	182 (36.99)	20 (10.99)			
Very poor/poor	5 (1.02)	1 (20.00)			
Presence of chronic non-communicable diseases			1.111	0.662–1.862	0.691
Yes	183 (37.20)	26 (14.21)			
No	309 (62.80)	48 (15.53)			
BMI (kg/m^2)			0.889	0.710–1.112	0.303
Thin (<18.5)	12 (2.44)	0 (0.00)			
Normal (18.5–24)	250 (50.81)	41 (16.40)			
Overweight (24–28)	182 (36.99)	30 (16.48)			
Obese (≥28)	48 (9.76)	3 (6.25)			
Tobacco-related factors					
Age at initiation of smoking (years)			2.377	0.832–6.794	0.106
<18	55 (11.18)	4 (7.41)			
≥18	437 (88.82)	70 (15.98)			
Duration of smoking (years) ($\bar{X}$±SD)	16.46±12.67	13.419±12.560	0.975	0.954–0.997	0.026
Price per pack of cigarettes (CNY) ($\bar{X}$±SD)	24.34±11.36	26.014±12.951	1.013	0.994–1.033	0.183
Fagerström test for nicotine dependence			0.644	0.458–0.905	0.011
Low (0–3)	247 (50.20)	49 (19.84)			
Moderate (4–5)	135 (27.44)	13 (9.63)			
Severe (6–10)	110 (22.36)	12 (10.91)			

TABLE 1. (Continued)

Characteristic	Total, *N* (%)	CAR, *N* (%)	c*OR**	95% *CI*	*P*
Previous quit attempts			0.649	0.396–1.066	0.088
0	208 (42.28)	38 (18.27)			
≥1	284 (57.72)	36 (12.68)			
Perceived importance of quitting ($\bar{X}$±SD)	7.87±2.24	8.351±1.801	1.134	1.000–1.286	0.049
Perceived difficulty of quitting ($\bar{X}$±SD)	7.24±2.47	6.689±2.590	0.904	0.823–0.994	0.037
Perceived confidence of quitting ($\bar{X}$±SD)	6.54±2.52	7.284±2.180	1.161	1.044–1.291	0.006
Knowing that smoking causes 4 diseases			0.782	0.476–1.285	0.332
Yes	247 (50.20)	41 (16.60)			
Not all	245 (49.80)	33 (13.47)			
Knowing that second hand smoke causes 4 diseases			1.057	0.619–1.805	0.839
Yes	344 (69.92)	51 (14.83)			
Not all	148 (30.08)	23 (15.54)			
Should smoking be allowed in indoor areas of the following establishments					
Workplace			0.979	0.501–1.913	0.951
No	411 (83.54)	62 (15.09)			
Yes/not sure	81 (16.46)	12 (14.81)			
Public place			1.007	0.526–1.929	0.983
No	406 (82.52)	61 (15.02)			
Yes/not sure	86 (17.48)	13 (15.12)			
Public transportation			1.076	0.521–2.224	0.843
No	429 (87.19)	64 (14.92)			
Yes/not sure	63 (12.81)	10 (15.87)			
Home			0.724	0.414–1.268	0.259
No	338 (68.70)	55 (16.27)			
Yes/not sure	154 (31.30)	19 (12.34)			

Abbreviation: CAR=continuous abstinence rate; BMI=body mass index; *CI*=confidence interval; CNY=Chinese Yuan.
* c*OR*: unadjusted odds ratio; the results of a single factor analysis by logistic regression model (unadjusted for factors).

TABLE 2. Comparison of cessation outcomes between the intervention and control groups at a three-month follow-up — Shenzhen City, China, 2022.

Three-month follow-up	Intervention group (*N*=236)	Control group (*N*=256)	a*OR**	95% *CI*	*P*
	n (%)	*n* (%)			
Quit attempt rate	141 (59.75)	104 (40.63)	3.063	1.965–4.774	<0.001
7-day PPAR	78 (33.05)	56 (21.88)	2.364	1.481–3.773	<0.001
Smoking reduction rate	79 (33.47)	59 (23.05)	1.738	1.113–2.716	0.015
CAR	51 (21.61)	23 (8.98)	3.530	1.942–6.413	0.003

Abbreviation: *CI*=confidence interval; PPAR=point prevalence of abstinence rate; CAR=continuous abstinence rate.
* a*OR*: adjusted odds ratio; adjusted for characteristics and tobacco-related factors.

a 95% *CI* of 1.481–3.773. Lastly, there was a statistically significant difference between the proportions of participants who reduced daily cigarette consumption by half or more in the intervention group (33.47%) compared to the control group (23.05%), with an a*OR* of 1.738 and a 95% *CI* of 1.942–6.413 (Table 2).

DISCUSSION

This study revealed that the community-based smoking cessation intervention significantly enhanced quit attempt rates, 7-day PPAR, CAR, and smoking reduction rates among participants in the intervention group. These findings indicate that the community-

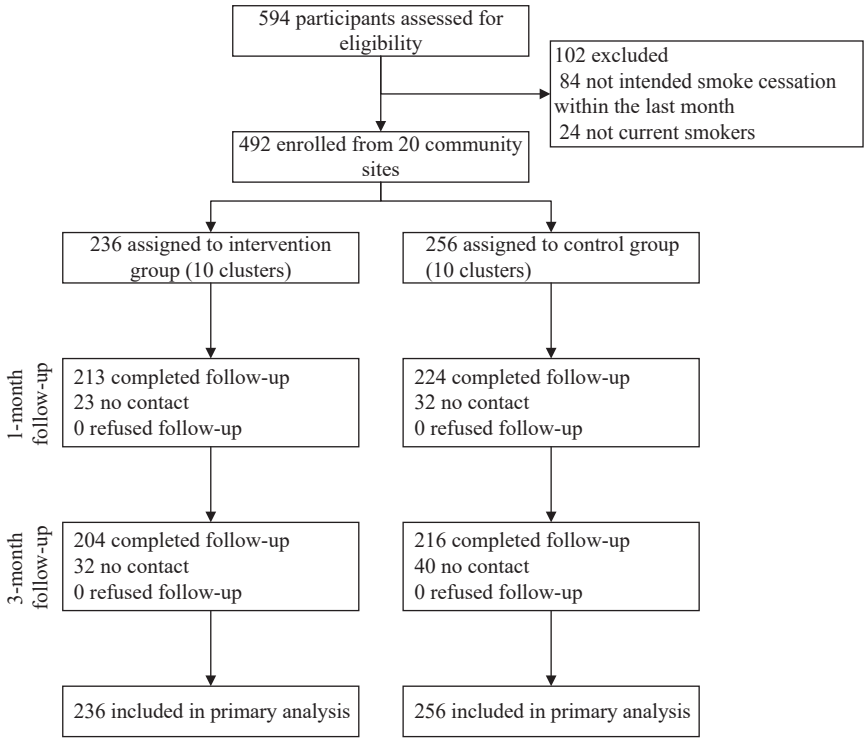

FIGURE 1. Flowchart of participant recruitment and progression throughout the study — Shenzhen City, China, 2022.

based cessation intervention, administered by trained physicians at CHSC and aided by community workers, is both effective and feasible in promoting smoking cessation. Moreover, this model complements the existing cessation service system and necessitates the integration of such services.

It is worth noting that the three-month CAR for the intervention group (21.61%) was significantly higher than that for the control group (8.98%). Results from the multivariable logistic regression analysis indicated that participants who received the community-based smoking cessation intervention had a 3.530 times greater likelihood of achieving three-month continuous abstinence compared to those in the control group (a*OR*=3.530; 95% *CI*: 1.942–6.413). These findings suggest that the community-based smoking cessation intervention was effective in supporting participants in their efforts to quit smoking.

Furthermore, the results from the multivariable logistic regression analysis demonstrated that an increased perceived difficulty of quitting among smokers was associated with a decreased likelihood of achieving three-month continuous abstinence (a*OR*=0.872, 95% *CI*: 0.773–0.984). This relationship might be partially explained by the concept of self-efficacy, as a lower perceived difficulty of quitting suggests greater self-efficacy, which subsequently enhances the probability of successful smoking cessation (*5*). These findings indicate that community-based interventions should emphasize reducing the perceived difficulty of quitting and boosting self-efficacy to further encourage smoking cessation at the community level.

Compared to the three-month CAR (19.3%) achieved in the psychological combined telephone follow-up intervention conducted by Wu et al. (*6*) at the Smoking Cessation Clinic (SCC) of the PLA General Hospital, our community-based smoking cessation intervention yielded similar cessation results. However, CHSCs are more accessible and convenient for smokers seeking professional assistance in quitting smoking due to their location within communities. Unlike SCCs in professional tertiary hospitals, CHSCs have stronger connections with residents and greater potential to improve smokers' willingness to quit through health education related to smoking.

Research has shown that varenicline can effectively increase the CAR among smokers (*2*,*7*). However, in CHSCs, cessation medications remain inaccessible due to high costs and inadequate insurance coverage. Consequently, the CAR in our study was notably lower than the rate reported by Jiang Bin and colleagues (31.3%) (*8*), who examined a combination of varenicline use and psychological intervention. To

enhance cessation efficacy, integrating smoking cessation medications into community-based intervention programs is recommended.

In the present study, the average age of participants was over 40 years, which resulted in limited representation of young smokers. However, past research suggests that quitting smoking at an earlier age can yield significant health benefits (*9*–*10*). Therefore, it is critical to increase the involvement of young smokers in community-based smoking cessation interventions to promote public health. Future studies should investigate the factors that prevent younger smokers from participating in such interventions and develop strategies tailored to their unique needs. This may potentially involve the use of smoking cessation apps or other mobile technologies to increase the appeal and accessibility of the intervention for younger individuals.

The present study has several limitations. Firstly, the cessation rate was based on self-reported data, without biochemical validation. Secondly, intermediate and long-term cessation outcomes, including those at 6 and 12 months, were not available for evaluation. Finally, smokers who missed appointments were considered unsuccessful quitters, which might result in an underestimation of the quit rate for the community-based cessation intervention service.

The community-based smoking cessation intervention effectively promoted and supported quit attempts among smokers, significantly enhancing their success rates in discontinuing smoking. Moreover, the intervention facilitated a reduction in daily cigarette consumption for those who were unable to quit successfully. These findings indicate that the community-based smoking cessation intervention is efficient in endorsing smoking cessation. Given its feasibility and effectiveness, the government should advocate for and establish a community-based smoking cessation service system that integrates daily tobacco control activities as part of community health services. Additionally, the CHSC should enhance physicians' capacity to provide smoking cessation services to community smokers seeking to quit.

Conflicts of interest: No conflicts of interest.

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Vital Surveillances

Genotypes Diversity of Acute Gastroenteritis Outbreaks Caused by Human Sapovirus — Beijing Municipality, China, 2015–2021

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ABSTRACT

Introduction: Human sapovirus (HuSaV) is an enteric virus responsible for sporadic cases and outbreaks of acute gastroenteritis (AGE) globally. A seven-year active surveillance study was conducted to investigate the molecular epidemiology of HuSaVs associated with AGE outbreaks in Chaoyang District of Beijing Municipality, China from January 2015 to December 2021.

Methods: Fecal and anal swab samples were obtained from patients experiencing AGE outbreaks. HuSaVs were identified through reverse transcription polymerase chain reaction (RT-PCR), and partial viral protein 1 (VP1) sequences (approximately 434 base pairs) were utilized for genotyping, single nucleotide polymorphism (SNP) analysis, and phylogenetic examination.

Results: HuSaVs were identified in 71 AGE outbreaks, demonstrating a detection rate of 10.5%, second only to norovirus. The primary demographic affected by HuSaV were children under the age of 5 in kindergarten settings. Infection rates tended to peak during two distinct periods: May to June and September to December. Upon genotyping, seven distinct genotypes emerged. GII.3 was the most prevalent, accounting for 54.9% of cases, followed by GI.1 (12.7%), GI.2 (9.9%), GII.5 (7.0%), GI.5 (2.8%), GI.6 (1.4%), GII.1 (1.4%), and untyped cases (9.9%). A phylogenetic analysis of GII.3 identified three distinct groups, with 15 notable SNPs observed.

Conclusions: This study offers a comprehensive analysis of the persistent prevalence of HuSaV outbreaks in Chaoyang District, Beijing Municipality, China. Over time, the diversity of HuSaV subtypes has shifted, and it is now recognized as the second leading viral agent responsible for AGE outbreaks. This highlights the importance of ongoing surveillance in the future.

Human sapovirus (HuSaV) is an enteric virus responsible for sporadic cases and outbreaks of acute gastroenteritis (AGE) globally. The prevalence of HuSaV ranges from 1% to 17% of diarrheal occurrences worldwide (*1*). However, the majority of studies concerning AGE outbreaks have centered on norovirus (NoV), leaving the epidemiological features of HuSaV outbreaks relatively unclear. HuSaV is a member of the *Caliciviridae* family, similar to human NoV, causing milder yet virtually identical symptoms, such as vomiting, diarrhea, and fever. The virus has a positive-sense, single-stranded ribonucleic acid (RNA) genome and is non-enveloped. Its approximately 7.5 kb RNA genome consists of two or three open reading frames (ORFs). SaV viruses are classified into 19 genogroups (GI to GXIX) based on the viral protein 1 (VP1) sequence and are further divided into 18 well-established genotypes: GI.1 to GI.7, GII.1 to GII.8, GIV.1, GV1, and GV.2, along with a tentative GII genotype GII.NA 1 (*2–3*). GI and GIV groups have been predominantly reported between October 1976 and April 2016 (*4*). Currently, there is a scarcity of data on the molecular epidemiology of HuSaV outbreaks in Beijing Municipality, China.

The initial identification of HuSaV in an AGE outbreak in Chaoyang District, Beijing, transpired in December 2015. Comprehending genotype distribution offers valuable information regarding transmission patterns, accurate diagnosis of circulating strains, population immunity, and potential vaccine development. Consequently, this study investigated the epidemiological trends and genetic features of HuSaV in AGE outbreaks. These findings may contribute to an enhanced understanding of HuSaV infection in Beijing.

METHODS

Data Sources

This study examines AGE outbreaks from January

2015 to December 2021 in Chaoyang District of Beijing. AGE cases were defined as patients exhibiting diarrhea (three or more loose stools within a 24-hour period) and/or vomiting (one or more episodes). An outbreak was characterized by the occurrence of three or more epidemiologically linked AGE cases within a 3-day period. Outbreaks were reported to community health centers by schools, kindergartens, and other institutions, and subsequently to the Chaoyang District CDC.

The Chaoyang District CDC conducted investigations within 24 hours of receiving a report, and fecal specimens or anal swabs were collected from cases within 48 hours of initiating the investigation to detect pathogens. An AGE outbreak surveillance network is already established in Beijing (*5*). In the Chaoyang CDC, AGE outbreak diagnostic tests include detection of NoV, sapovirus (SaV), rotavirus (RV), astrovirus (AstV), and enteric adenovirus (AdV). A HuSaV outbreak was confirmed if two or more AGE cases tested positive for HuSaV via real-time polymerase chain reaction (PCR).

Sequencing and Data Analysis

HuSaV-positive samples were amplified using primers SLV531/SLV574, yielding a 434-bp PCR product. PCR products were purified and sequenced. All sequences were aligned using BioEdit (version 7.0.5.3, Borland, Scotts Valley, USA). Nucleotide sequences from this study and reference strains were compared to examine their differences using the MegAlign module of the Lasergene software package (version 7.1, DNASTAR, Madison, WI). The number of single nucleotide polymorphism (SNP) sites was calculated based on the alignments. Fisher's exact test was employed to evaluate the significance of frequency differences among the three groups of bases. *P*-values were corrected using the Benjamini-Hochberg (BH) method. Genotypes were determined through phylogenetic analysis, utilizing the neighbor-joining method and a bootstrap test with 1,000 iterations, implemented in MEGA software (version 6.0, Mega Limited, Auckland, New Zealand). Reference strains for different HuSaV genotypes were selected and obtained from the GenBank database.

RESULTS

Detection and Epidemical Distribution of HuSaV

In a review of 678 AGE outbreaks from 2015 to 2021 in Chaoyang District, 71 (10.5%) were laboratory-confirmed as HuSaV-associated. HuSaV ranked second among the five most common diarrhea-associated viruses, including NoV (59.3%), SaV (10.5%), AstV (2.4%), enteric AdV (0.9%) , and RV (0.2%). Furthermore, no pathogens were detected in 26.7% of the samples. Of the HuSaV-associated cases, 67 (94.4%) had single HuSaV infections, two had co-infections with AstV, one had a co-infection with AdV, and one had a triple infection, which involved GII NoV and AstV.

HuSaV predominantly infected children under 5 years of age in kindergarten settings among the enrolled subjects. Out of the 71 HuSaV outbreaks, 90.1% (64/71) occurred in kindergartens, while 9.9% (7/71) took place in primary schools. The median age of the HuSaV-positive individuals was 4 years (range: 2–10 years). The highest proportion of infected individuals was found in the 4-year-old age group (39.0%), followed by the 3-year-old (28.6%) and 5-year-old (18.2%) age groups. The proportion of HuSaV-positive $P<0.001$, Chi-square test).

The modes of transmission were identified for 63 outbreaks. The predominant mode of transmission was person-to-person, accounting for 62 of the 63 outbreaks (98.41%). Water-borne transmission was the next most common, contributing to one of the 63 outbreaks (1.59%).

HuSaV-caused AGE outbreaks occurred every year, and the constituent ratio ranged from 5.9% to 15.0%. Outbreaks typically occurred in two periods: May to June and September to December, with the exception of 2020 (Table 1). A few cases occurred in March and April, while no cases were reported in January, February, July, and August.

Distribution of HuSaV Genotypes

The genetic diversity of AGE-causing HuSaVs was analyzed in this study. Out of 71 HuSaV outbreaks, the partial VP1 gene was successfully amplified and sequenced in 64 outbreak samples. Seven genotypes were identified, with 19 (26.8%) belonging to the GI genogroup, 45 (63.4%) to the GII genogroup, and 9.9% remaining untyped. The specimens from these untyped outbreaks had low viral loads and could not be sequenced. HuSaV outbreaks caused by the GII genogroup were predominant in all reported settings, including 40 outbreaks in kindergartens and 5 outbreaks in primary schools. In contrast, all GI genogroup outbreaks occurred exclusively in kindergartens. No significant differences were observed

in the gender of infected individuals, month of infection, or median age between HuSaV GI and GII outbreaks.

Furthermore, it is worth noting that GII.3 emerged as the most predominant genotype, accounting for 54.9% of the cases and being detected in almost every month. Other genotypes observed included GI.1 (12.7%), GI.2 (9.9%), GII.5 (7.0%), GI.5 (2.8%), GI.6 (1.4%), GII.1 (1.4%), and untyped (9.9%). Additionally, the composition of genetic diversity exhibited variance across different years (Table 2). However, no instances of multiplex HuSaV infections involving distinct genotypes within a single outbreak were identified in this study.

Phylogenetic Analysis of HuSaV Outbreaks

In order to determine the genetic relationships of the partial VP1 genes of the HuSaV detected in this study with those previously reported, phylogenetic trees were constructed and analyzed (Figure 1). Among the strains detected in this study, nine strains clustered with GI.1 reference sequences from Japan, China, the Republic of Korea, and the Republic of Tunisia, exhibiting sequence similarity ranges of 99.0%–100%,

TABLE 1. Number and proportion of HuSaV outbreaks by genotype and by year.

Genotype	Proportion of outbreaks (%)							
	2015 (*n*=2)	2016 (*n*=3)	2017 (*n*=18)	2018 (*n*=18)	2019 (*n*=16)	2020 (*n*=1)	2021 (*n*=26)	Total (*n*=71)
GI.1	–	33.3	16.7	20.0	–	100	11.5	12.7
GI.2	–	–	11.1	20.0	6.3	–	11.5	9.9
GI.5	–	–	–	–	12.5	–	–	2.8
GI.6	–	–	–	–	–	–	3.9	1.4
GII.1	–	–	5.6	–	–	–	–	1.4
GII.3	100	33.3	44.4	40.0	62.5	–	61.5	54.9
GII.5	–	33.3	–	–	18.8	–	3.9	7.0
Untyped	–	–	22.2	20.0	–	–	7.7	9.9

Note: "–" means that no related outbreak was detected.
Abbreviation: HuSaV=human sapovirus.

TABLE 2. Monthly distribution of HuSaV AGE outbreaks in Chaoyang District of Beijing, from January 2015 to December 2021.

Time	2015	2016	2017	2018	2019	2020	2021	Total
January	–	–	–	–	–	–	–	–
February	–	–	–	–	–	–	–	–
March	–	–	GII.3 (1), GI.2 (1)	–	GII.5 (1)	–	–	GII.3 (1), GII.5 (1), GI.2 (1)
April	–	–	–	–	–	–	GI.1 (1), GI.2 (1)	GI.1 (1), GI.2 (1)
May	–	GII.3 (1)	–	–	–	–	GII.3 (6), GI.1 (1), GI.2 (1), GI.6 (1), GII.5 (1)	GII.3 (7), GI.1 (1), GI.2 (1), GI.6 (1), GII.5 (1)
June	–	–	GI.1 (1)	GII.3 (1), untyped (1)	GII.3 (1), GI.2 (1)	–	GII.3 (7), GI.1 (1), GI.2 (1), untyped (1)	GII.3 (8), GI.1 (2), GI.2 (2), untyped (2)
July	–	–	GII.3 (1)	–	–	–	GII. 3(1), untyped (1)	GII.3 (2), untyped (1)
August	–	–	–	–	–	–	–	–
September	–	GII.5 (1)	GII.3 (3), GI.1 (1), untyped (1)	–	GII.3 (3)	–	GII.3 (1)	GII.3 (8), GII.5 (1), GI.1 (1), untyped (1)
October	–	GI.1 (1)	GII.3 (2), GI.1 (1), GII.1 (1)	–	GII.3 (1), GI.6 (1)	–	–	GII.3 (3), GI.1 (2), GII.1 (1), GI.6 (1)
November	GII.3 (2)	–	GI.2 (1)	GII.3 (1), GI.1 (1), GI.2 (1)	GII.3 (3), GII.5 (1)	GI.1 (1)	GII.3 (1)	GII.3 (7), GII.5 (1), GI.1 (2), GI.2 (2)
December	–	–	Untyped (3)	–	GII.3 (2), GI.5 (1), GII.5 (1)	–	–	GII.3 (2), GI.5 (1), GII.5 (1), untyped (3)

Note: The numbers in parentheses represent the number of samples classified to the corresponding genotype.
"–"means that no related outbreak was detected.
Abbreviation: HuSaV=human sapovirus; AGE=acute gastroenteritis.

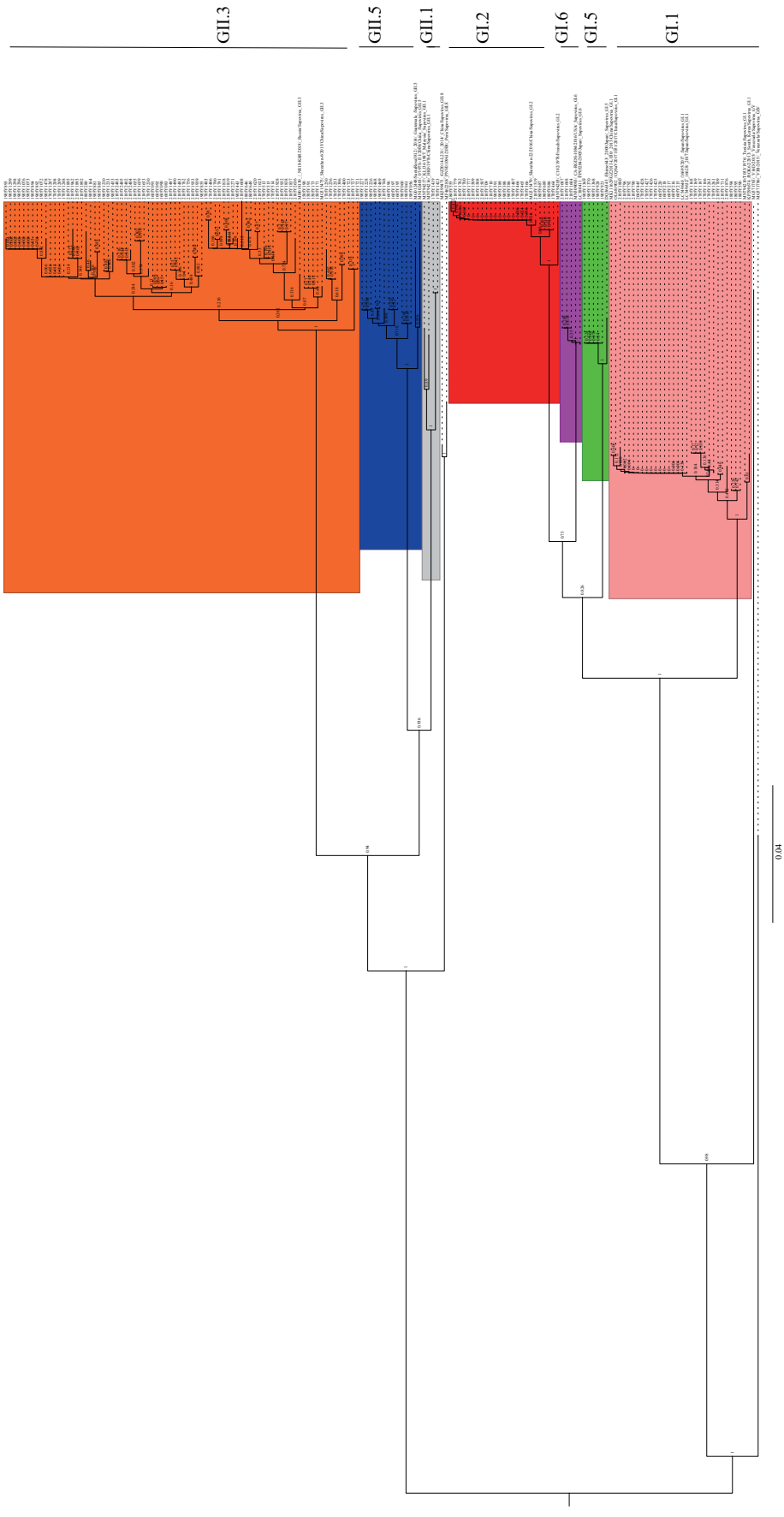

FIGURE 1. Phylogenetic analysis of HuSaV based on partial VP1 nucleotide sequences.
Note: The phylogenetic tree was constructed using the neighbor-joining method and a bootstrap test with 1,000 iterations.
Abbreviation: HuSaV=human sapovirus; VP1=viral protein 1.

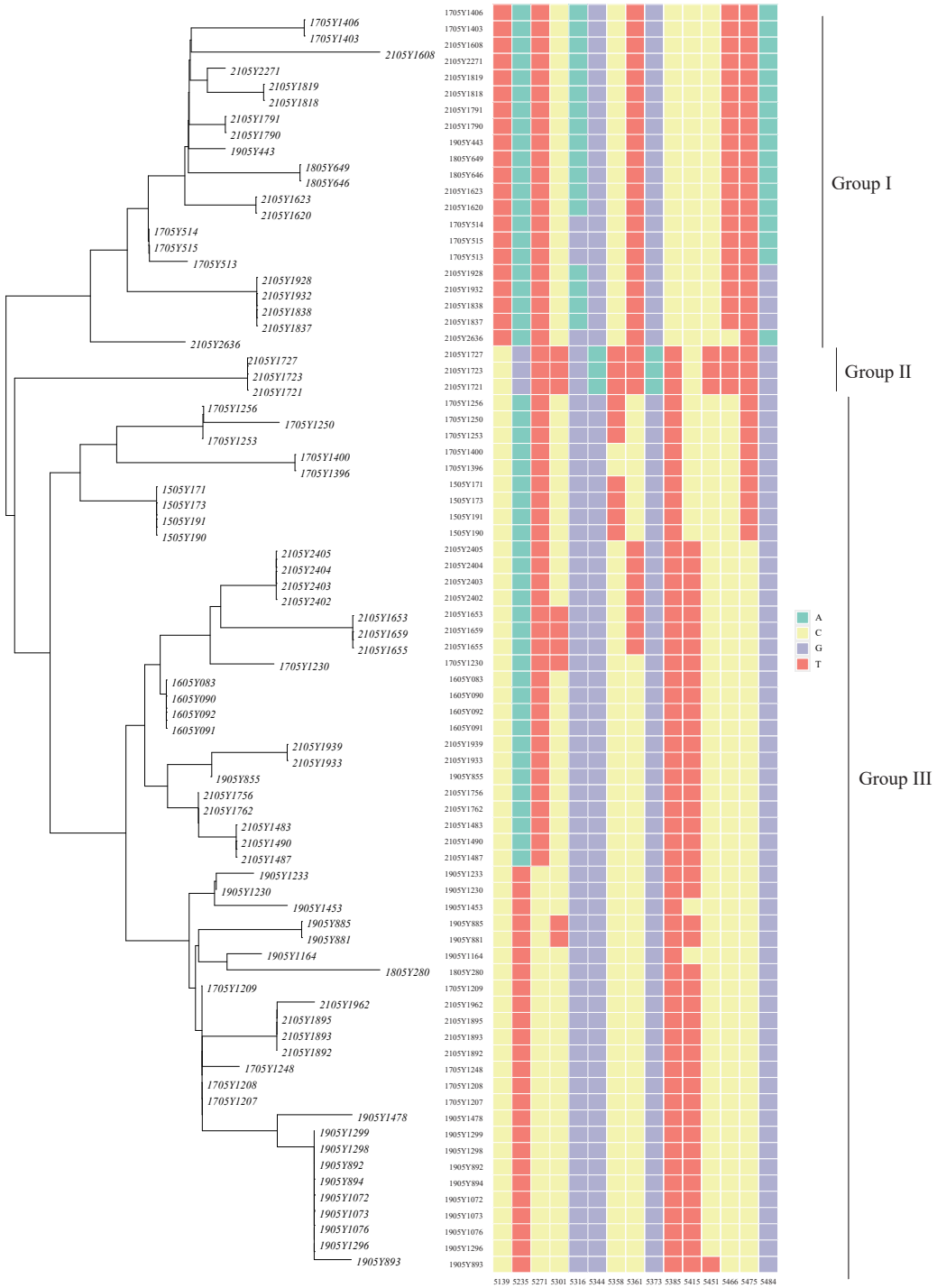

FIGURE 2. Phylogenetic and SNP analysis of partial HuSaV VP1 genes from all GII.3 isolates in the present study.
Abbreviation: HuSaV=human sapovirus; VP1=viral protein 1; SNP=single nucleotide polymorphism.

98.8%–99.8%, 97.4%–98.6%, and 96.6%–97.8%, respectively. Seven strains clustered with GI.2 sequences from China and French Guiana, with similarity ranges of 99.5%–100% and 97.4%–97.6%, respectively. Seven strains clustered with GI.5 sequences from Japan, displaying a similarity of 98.3%. Two strains clustered with GI.6 sequences from Japan and the United States, with a similarity of 99.5%. One strain clustered with GII.1 sequences from Malaysia and China, with similarities of 94.0% and 94.2%. Thirty-nine strains clustered with GII.3 sequences from Russia, with similarity ranging from 96.4%–98.6%. Five strains clustered with GII.5 sequences from the Republic of Guatemala and

Taiwan, China, with similarities of 95.4%–96.2% and 93.0%–93.5%, respectively.

As previously mentioned, GII.3 was responsible for 86.7% (39/45) of HuSaV outbreaks within the GII genogroup. A phylogenetic tree was constructed using only the GII.3 HuSaV isolates from this study, revealing that the 39 GII.3 outbreak strains could be categorized into three distinct groups: I, II, and III. These groupings were further supported by SNP analysis, which identified a total of 15 SNPs with significant differences among the three groups (Figure 2). Of the 39 GII.3 outbreaks analyzed, 12 (30.8%) were attributed to Group I, 1 (2.6%) to Group II, and 26 (66.7%) to Group III.

CONCLUSIONS

Viral infections continue to be a significant cause of AGE in children globally. The extensive viral diversity presents a substantial challenge for the development of effective vaccines. Currently, only RV vaccines have been commercialized for worldwide use. Consequently, monitoring the genetic diversity of AGE-associated viruses, particularly uncommon or neglected ones such as HuSaV, is crucial. The findings from this study indicate that HuSaV was the second most prevalent cause of AGE outbreaks, following NoV, in the Chaoyang District of Beijing, China, between 2015 and 2021, which is consistent with observations in some other countries (*6*–*7*). Nonetheless, the genetic diversity of HuSaV found in this study differs from previous reports in both China (*8*–*9*) and other countries (*10*–*13*), where the GI genogroup was predominant.

The predominant genotype in Chaoyang District is GII.3, which contrasts with the previously reported genotype GI in both China and other countries. Our study documents an increase in HuSaV outbreaks attributed to genogroup II, particularly GII.3, for the first time, as compared to sporadic occurrences in other regions of China (*14*–*16*). Consequently, we hypothesize that GII.3 HuSaV underwent a transition from quantitative to qualitative change due to rapid evolution in China. This assumption is supported by evolutionary dynamics analysis, which suggests that the less common GII.3 HuSaVs evolved at a faster rate than the predominant GI.1 HuSaVs (*16*).

The epidemic seasons in Chaoyang District occur in May to June and September to December, which deviate from those observed in southern China. In contrast to other reports on the seasonal distribution of HuSaVs, outbreaks in Beijing typically take place during two periods, as depicted in Figure 1. This variation significantly differs from the early spring occurrences reported in southern China (*9*), which may result from the considerable difference in latitudes between the two regions. In Japan, researchers have also discovered that the detection rate of HuSaV within a month varied across different years (*6*). As attention towards HuSaV increases and detection methods improve, fluctuations in the distribution patterns of HuSaVs from place to place and year to year could change. Consequently, the factors contributing to these variations may become more evident in future studies.

There were several limitations observed in our study. First, insufficient data and resources were available to establish a relationship between SNPs and epidemiological features among different groups of GII.3 HuSaVs. Second, due to the emergence of coronavirus disease 2019, only one HuSaVs outbreak was observed in 2020, resulting in a discontinuity in our study's data.

In conclusion, HuSaV is a primary cause of diarrhea, with GII.3 emerging as the predominant strain, potentially leading to an increase in cases and outbreaks of diarrhea in the near future. Continuous surveillance and timely data updates on HuSaV within China are essential to inform adjustments to prevention and control strategies against HuSaV-induced AGE during this potential transition period.

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Perspectives

Sustainable Laboratory Capacity Building in Sierra Leone: From Ebola to COVID-19

Lili Wang[1]; Alie Brima Tia[2]; Bing Xu[1]; Xiaopeng Qi[1,#]; Doris Harding[3,#]

The onset of the Ebola outbreak in 2014 originated in Guinea and proceeded to swiftly reach Sierra Leone and Liberia (*1*). Unfortunately, these nations were critically deficient in their capacity for pathogenic testing and diagnostics, the availability of healthcare workers, and their supply of epidemic prevention materials, thereby impeding their ability to address the outbreak efficiently (*2*). This grave scenario underscored the need for support from the worldwide community.

At the time, Sierra Leone lacked the domestic capacity for laboratory testing, including that of biosafety level 3 (BSL-3). Recognizing this constraint, the Chinese government hastily dispatched a mobile BSL-3 laboratory and corresponding technicians to Sierra Leone to facilitate testing within the country. Furthermore, the Chinese government expedited the establishment of the Sierra Leone-China Friendship Biosafety Laboratory (BSL-3) within a span of three months, observed to be operational by March 2015. It is important to note that this laboratory also conducted investigations centered on the detoxification of body fluids in Ebola survivors, fueling advancements in the understanding and management of the Ebola virus (*3*). The laboratory has played a significant role in the prevention and control measures during the Ebola epidemic.

The Ebola outbreak in West Africa underscored a lack of ability to identify and diagnose emerging and re-emerging infectious diseases. While the outbreak has since ended, it underscored the necessity of strengthening the health system to better respond to future public health crises. This was underscored to the government and health practitioners. Sierra Leone, in particular, experienced a high mortality rate due to diseases such as malaria, pneumonia, diarrhea, cholera, Lassa fever, and measles. The nation continues to bear the brunt of public health crises, experiencing significant morbidity and mortality. Consistent international efforts are crucial to build the resilience and capacity of the public health system. To this end, China CDC, in collaboration with the Ministry of Health and Sanitation, Sierra Leone, implemented a multi-year capacity building program that incorporated laboratory operational capacity development and personnel capacity building (*4*). This article evaluates the performance and outcomes of this program (Table 1).

Improved Laboratory Operating Capacity

When the Sierra Leone-China Friendship Biosafety Laboratory was founded in 2015, it initially lacked operational capabilities. This deficiency was addressed through a comprehensive program that furnished the laboratory with skilled staff, necessary facilities, and a relevant management system — an essential

TABLE 1. Framework of the program.

Framework	Laboratory operational capacity building	Personnel capacity building
Inputs	Staff	Mentors
	Technical support	Short/long-term training
	Financial resources	
	Facilities	
Process	Developing a laboratory management system	Developing a training plan
	Extending testing capacity	Mentor instruction
	Developing a sentinel surveillance system	Learning from practice
	Maintaining operation of the lab	Encouraging further education
Outcomes	Sustained operation of the laboratory	Established professional workforce in the lab
	Increased testing scope of pathogens	Improved competency of public health personnel
	Enhanced surveillance capacity	

foundation for maintaining laboratory operations.

The implementation of this program gradually enhanced the laboratory's testing capacity, expanding from initial testing for only the Ebola virus to include several other types of pathogens by the end of 2022 (Figure 1). This laboratory, designated as the National Reference Laboratory for Viral Hemorrhagic Fevers in Sierra Leone, was responsible for testing samples from unexplained severe cases or suspected instances of hemorrhagic fever to determine the responsible pathogens. Concurrently, the program instituted a sentinel surveillance system to manage surveillance of hemorrhagic fever viruses, diarrheal pathogenic bacteria, and mosquito vectors. This bolstered disease diagnosis and early warning capabilities for infectious disease outbreaks (*5*–*7*), with all gathered samples tested within the laboratory. Given the persistent risk of severe diseases like Lassa fever and Ebola within the country, in addition to the identification of new pathogens (*8*–*9*), this sentinel surveillance system facilitates early detection of outbreaks and enhances the country's preparedness for such situations.

The continued capacity enhancement in the laboratory facilitated the swift establishment of testing for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus at the pandemic's early stage. As of February 5, 2020, the Sierra Leone-China Friendship Biosafety Laboratory has fully developed both sequencing and reverse transcription-polymerase chain reaction (RT-PCR) detection capabilities for the SARS-CoV-2 virus, which positioned Sierra Leone among the first African countries with this testing ability. The laboratory, serving as a public health facility, was appointed the national testing site for the SARS-CoV-2 virus. It began testing its first suspected coronavirus disease 2019 (COVID-19) sample on February 14, 2020, signifying the onset of emergency COVID-19 testing in Sierra Leone. The first positive nucleic acid sample was detected in the laboratory at 2:00 a.m. on March 31, 2020. The Sierra Leone government announced the country's first COVID-19 case that same day (*10*). Up to December 2022, the laboratory had tested 131,708 samples from suspected COVID-19 cases, which constituted 34.4% of the national testing volume in Sierra Leone (Figure 2). Among these, 2,697 were positively confirmed, representing 34.7% of all confirmed cases in the country.

Improving the Competency of Local Public Health Personnel

This multi-year program is focused on providing long-term training in laboratory biosafety, quality management, and testing techniques related to viruses,

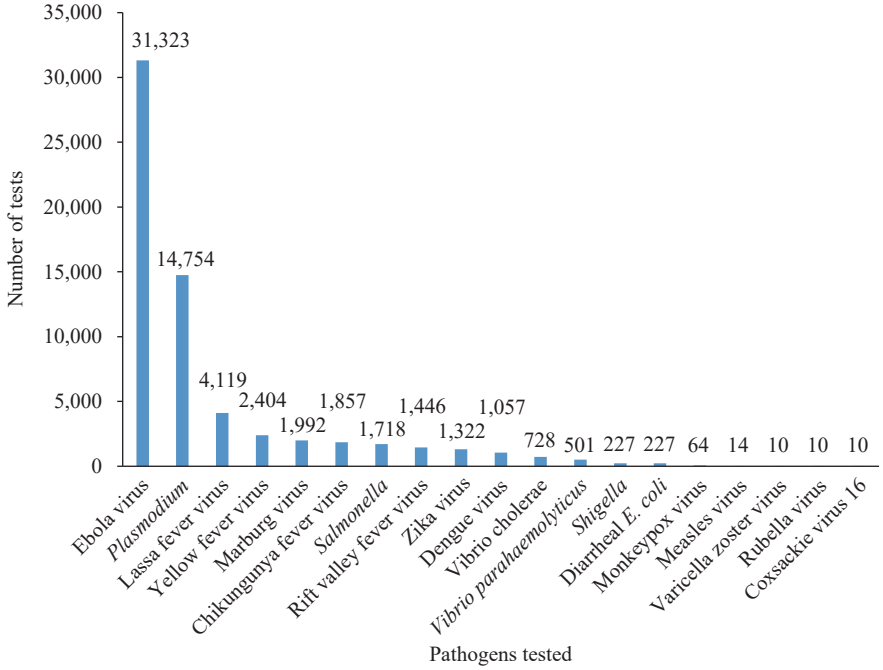

FIGURE 1. Distribution of pathogen types tested and the corresponding number of tests conducted in the laboratory, 2015−2022.

bacteria, and parasites. A training plan was developed specifically for personnel working in the laboratory, additionally, long-term mentors were dispatched to Sierra Leone. The comprehensive training curriculum covered areas such as pathogen characteristics, specimen collection, data entry, operational standards, personal protection, correct utilization of equipment, materials management, and biosafety. Training was delivered through a combination of lectures, simulation exercises, and practical sessions under the supervision of skilled Chinese technicians.

When operations began in the lab in 2015, a total of five local staff members were recruited for training. Following an eight-year period, 19 local lab technicians have undergone training in the laboratory. Among this group, four have successfully secured scholarships for master's or doctoral programs outside Sierra Leone. Ongoing training has been used to bolster the competency of local staff in areas such as biosafety and biosecurity, quality management, disease surveillance, and laboratory diagnostics (Table 2). This trained local workforce played a significant role in laboratory testing and diagnostic processes throughout the COVID-19 pandemic. This team ensured that the laboratory was able to conduct tests on a daily basis and report results to the Sierra Leone Ministry of Health and Sanitation

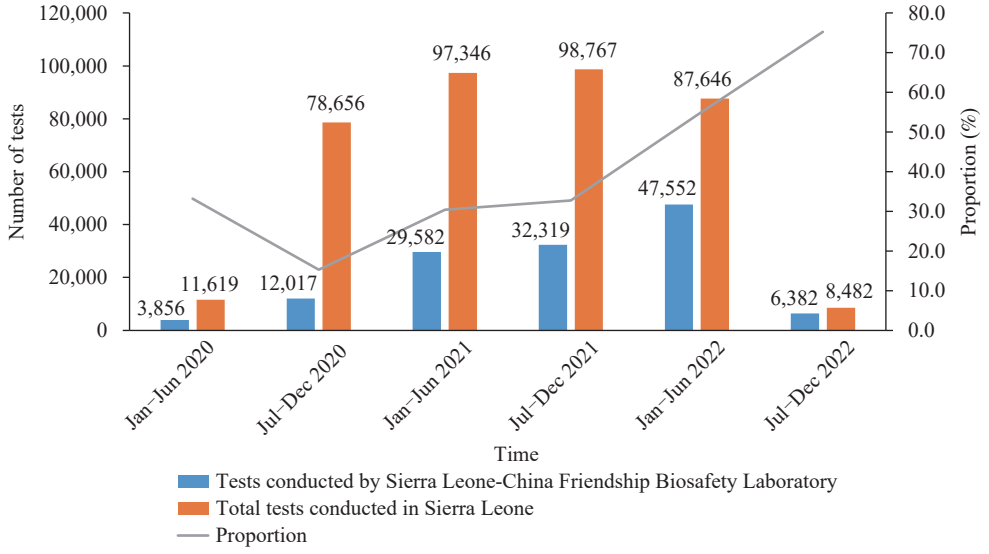

FIGURE 2. Comparison of coronavirus disease 2019 (COVID-19) polymerase chain reaction (PCR) tests conducted by Sierra Leone-China Friendship Biosafety Laboratory with the total tests conducted in Sierra Leone.

TABLE 2. Comparison of laboratory personnel competency in 2015 and 2023.

Category	Ability	March 2015	March 2023
Biosafety	Operating in biosafety level 3	None	Competent
	Using personal protective equipment properly	None	Competent
	Biological waste management & disposal	None	Competent
	Biosafety awareness	None	Acquired
Quality management	Sample collection, transportation and preservation	None	Competent
	Process control	None	Competent
	Documents and records	None	Competent
	Data management	None	Competent
Disease surveillance	Perception of active monitoring	None	Acquired
	Process of surveillance	None	Competent
Lab diagnosis	Nucleic acid testing	None	Competent
	Enzyme-linked immunosorbent assay	None	Competent
	Sequencing	None	In training

within 24 hours of receiving a sample.

In the past years, the program successfully conducted or sponsored 37 short-term training courses, collectively reaching 1,061 participants from across the country. This initiative significantly strengthened Sierra Leone's capacity for pathogenic testing at the district level. The curriculum spanned diverse topics such as pathogen collection, biosafety, surveillance, quality control, pathogenic diagnosis, and disease control. It encompassed diseases such as Ebola, Lassa fever, plague, anthrax, monkeypox, Marburg, malaria, typhoid fever, and SARS-CoV-2, among others (Figure 3).

DISCUSSIONS

The development of Sierra Leone-China Friendship Biosafety Laboratory during the Ebola outbreak and its subsequent crucial involvement in managing the COVID-19 pandemic have significantly fortified Sierra Leone's laboratory capabilities in both preventing and controlling infectious diseases, as well as responding to health emergencies. The COVID-19 pandemic reiterated the critical necessity to uphold global health security. This is particularly true in terms of enhancing all countries' capacities in observing, detecting, and responding to infectious diseases, which are vital measures in the successful containment of such pandemics.

The program is aligned with the Sierra Leone National Action Plan for Health Security (2018–2022) (*11*). Successful execution of this initiative strengthens Sierra Leone's disease surveillance processes, emergency preparedness, and personnel proficiency. Consequently, the capacity of the public health sector to prevent, detect, validate, and report to both local and international bodies, and respond to incidents or outbreaks of emerging or re-emerging infectious diseases of significant public health concern, is substantially improved. This enhancement ultimately benefits the health and well-being of the people of Sierra Leone, as well as the global community.

Given the constraints of limited resources, additional measures must be taken to sustain the operation of this laboratory. This includes nurturing a competent local workforce and maintaining ongoing surveillance. These efforts are crucial for bolstering the country's integral capacities as stipulated under the International Health Regulations (IHR) 2005, thereby augmenting the health security of both the country and the wider sub-region.

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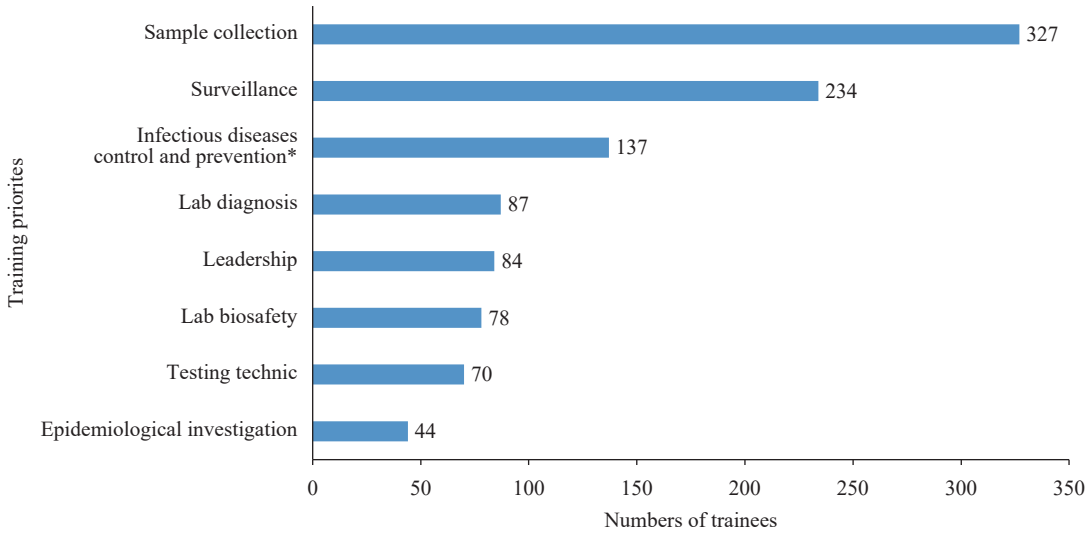

FIGURE 3. Numbers and priorities of brief training workshops conducted from 2015 to 2022.
* Workshops centered on the control and prevention of infectious diseases, such as malaria, cholera, measles, and hepatitis, are being implemented in Sierra Leone.

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Notes from the Field

A Case of Psittacosis — Qingdao City, Shandong Province, China, May 2023

Ying Li[1,2,&]; Bei Pan[3,&]; Wenzhe Xiao[4]; Lizhu Fang[3]; Lijie Zhang[1]; Fachun Jiang[3]; Jing Jia[1,3,#]; Huilai Ma[1,#]

A case of psittacosis was identified in Qingdao City, Shandong Province on May 4, 2023. The patient, a 69-year-old retired male, was admitted to a medical facility on April 22, 2023. His symptoms, which had persisted for two days prior to his admission, included a fever (reaching 38.4 °C at its peak), an escalating cough, and wheezing, none of which had been previously treated. Upon admission, the patient was placed on continuous low-flow oxygen, administered piperacillin/tazobactam as antibacterial therapy from April 22 to May 1, given ambroxol hydrochloride injections as an expectorant from April 22 to April 27, and provided panting and hydroprednisone as spasmolytic and anti-inflammatory treatment. He maintains a flock of carrier pigeons year-round, which he regularly releases.

On April 23, sputum samples from the patient were processed using 198 targeted next-generation sequencing (NGS) tests for respiratory pathogens. These tests detected *Chlamydia psittaci (C.psittaci)*, with two reads number exceeding 500. As illustrated in Figure 1, the results corresponded to the CP3 strain (*1*). The Shandong Provincial Center for Disease Control and Prevention subsequently confirmed the presence of *C. psittaci* in these samples using metagenomic NGS. Following these results on April 24, oral doxycycline was incorporated into the patient's treatment regimen, leading to an improvement in his condition. The patient was discharged on May 3. Meanwhile, fecal samples from the pigeons were collected for real-time polymerase chain reaction analysis, which returned positive results. It is noteworthy that the patient's family members, who had no close contacts with the pigeons, remained healthy and did not exhibit any signs of illness.

Psittacosis, a zoonotic disease attributable to *C. psittaci*, impacts humans, birds, and diverse animal populations (*2*). Some instances of psittacosis can exacerbate rapidly, leading to fatalities if the treatment is not administered promptly. Prominent reservoirs of infection encompass birds, especially parrots, seagulls, and pigeons. *C. psittaci* is categorized into nine distinct genotypes, reliant on the sequences of the outer membrane protein A gene (*3*). The disease mainly transmits to humans when they inhale bacteria present in bird feces or secretions during close contact, while direct transmission from human to human is uncommon. Despite its rarity (*4*), fewer than 10 cases of psittacosis have been reported annually in the US since 2010 (*5*). In China, most psittacosis cases are sporadic, with a small number of clusters reported in recent years (*6*–*7*). However, it is speculated by experts that there is significant underreporting and potential misdiagnosis of psittacosis (*5*), possibly owing to insufficient awareness about the disease and limited testing resources. In the present diagnostic case, NGS technology was deployed, factoring in the clinical manifestations and the patient's history of pigeon keeping.

This report denotes the inaugural case of psittacosis in Qingdao City, thereby necessitating an immediate call to action for implementing a prevention and control program within the region. This program must include systematic surveillance of pigeons, parrots, zoological gardens, and avian trading markets, with a particular focus on seagulls in Qingdao during the winter and spring seasons. Similarly, it is essential to promote awareness and education about psittacosis in the community.

Employment of multi-pathogen detection methodologies, including NGS, is recommended in scenarios of unexplained fevers. Clinicians need more training in the diagnosis and treatment of such conditions, as well as increased awareness of rare or newly emerging infectious diseases.

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A

Chlamydia psittaci WC, complete genome

Sequence ID: CP003796.1 Length: 1172265 Number of matches: 1

Range 1: 684766 to 684866 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
187 bits(101)	3e-43	101/101(100%)	0/101(0%)	Plus/Plus

```
Query  1       ATCGCACATTGCTTTAGAATGCACTCTACAAACCCATCCAAATATCACTCTTATAGGAGA  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  684766  ATCGCACATTGCTTTAGAATGCACTCTACAAACCCATCCAAATATCACTCTTATAGGAGA  684825

Query  61      AGAAATCGCAGAAAAGAATGTGCCTCTAAATACGATAATCC  101
               |||||||||||||||||||||||||||||||||||||||||
Sbjct  684826  AGAAATCGCAGAAAAGAATGTGCCTCTAAATACGATAATCC  684866
```

B

Chlamydia psittaci CP3, complete genome

Sequence ID: CP003797.1 Length: 1168150 Number of matches: 1

Range 1: 58905 to 59005 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
187 bits(101)	3e-43	101/101(100%)	0/101(0%)	Plus/Minus

```
Query  1       GCAGCCTTCCTAGCCTTAAACATTTGGGATCGCTTCGACATTTTCTGCACCTTAGGGGCA  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  59005   GCAGCCTTCCTAGCCTTAAACATTTGGGATCGCTTCGACATTTTCTGCACCTTAGGGGCA  58946

Query  61      TCCAATGGATACTTCAAATCAAGTTCGGCTGCATTCAACTT  101
               |||||||||||||||||||||||||||||||||||||||||
Sbjct  58945   TCCAATGGATACTTCAAATCAAGTTCGGCTGCATTCAACTT  58905
```

FIGURE 1. Comparatively favorable BLAST results gained from targeted next-generation sequencing, with read numbers surpassing 500; results pertain to a psittacosis case identified in Qingdao City, Shandong Province, 2023. The BLAST results are delineated for (A) the most abundant and (B) the second most abundant read counts.

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