ISSN 2096-7071
CN 10-1629/R1

CHINA CDC WEEKLY

Vol. 5 No. 14 Apr. 7, 2023

中国疾病预防控制中心周报

ISSN 2096-7071

China CDC Weekly

Cover Image: adapted from the World Health Organization, https://www.who.int/campaigns/world-health-day.

Preplanned Studies

Multiple Center Research on Relationship Between Screening Quality and Detection of Cervical Cancer — Six Provinces, China, June–December 2021

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Summary

What is already known about this topic?

The effective implementation of cervical cancer examination programs requires improved cervical cancer screening coverage and quality.

What is added by this report?

The detection rate of ≥high-grade squamous intraepithelial lesions (HSIL) in 6 hospitals was 19.6%. Not having undergone screening in the last 5 years and abnormal screening results had a negative association with detection of ≥HSIL, and abnormal screening results would increase the risk of detection by 75% compared with normal screening results. Additionally, low-grade, high-grade, and cancer of colposcopic impression were associated with a higher risk for detecting ≥HSIL.

What are the implications for public health practice?

It is essential to disseminate health knowledge about cervical cancer control to women in order to increase their awareness and screening rates. Additionally, it is necessary to further strengthen the training of professional staff to improve the quality of cervical cancer prevention, including screening, colposcopic examination, and follow-up for target female populations.

The coverage and quality of screening are essential for reducing the incidence of cervical precancers and cervical cancer. This multicenter study aimed to investigate the relationship between screening quality and the detection of cervical precancers and cervical cancer. The study was conducted from June to December 2021 in six hospitals across six provinces. The 2,945 participants were non-pregnant women who underwent colposcopy examinations. The average age of participants was 40.9±11.5 years old. Only 6.9% of participants had received human papillomavirus (HPV) vaccination. A total of 92.6% of participants had abnormal cervical screening results. Of the participants, 577 had high-grade squamous intraepithelial lesions (HSIL) or worse (≥HSIL), with a detection rate of 19.6%. Univariate analysis indicated that a lack of cervical cancer screening history in the past five years, as well as positive cervical screening and abnormal colposcopic impression, were independent associated factors of the ≥HSIL detection rate. A multivariable logistic regression showed that positive cervical screening [odds ratio (*OR*) =1.75, 95% confidence interval (*CI*): 1.07–2.86] was a risk factor for detecting ≥HSIL. Low-grade, high-grade, and cancer of colposcopic impression were associated with a higher risk for detecting ≥HSIL (*OR*=2.94, 95% *CI*: 2.13–4.08; *OR*=36.64, 95% *CI*: 26.07–51.48). It is important to disseminate health knowledge to improve public awareness of cervical cancer prevention and to enhance the capacity building of professional staff to improve the quality of cervical cancer screening.

Cervical cancer was the second most common cancer and the second leading cause of cancer-related death among women of reproductive age worldwide in 2020, with 604,127 new cases and 341,831 deaths (*1*). Of these, 88.1% of the new cases and 91.4% of the deaths occurred in low- and middle-income countries. In 2016, China reported 98,900 new cases and 30,500 deaths due to cervical cancer (*2*). Cervical cancer can be prevented through vaccination and screening with appropriate follow-up and treatment (*3*). Early detection and treatment of cervical precancers are also key to successful prevention. Cervical precancers include HSIL and adenocarcinoma in situ (AIS). Cervical cancer screening, colposcopy, and pathology are three steps for diagnosing cervical precancers. Therefore, the coverage and quality of screening are essential to reducing the incidence of cervical precancers. The objective of this multicenter study was to investigate the relationship between screening quality and the detection of cervical precancers and cervical cancer in hospitals, providing evidence to

improve cervical control for the target population in health facilities.

The study sites were six hospitals in six provincial-level administrative divisions (PLADs): Peking University First Hospital, Sichuan Provincial Maternity and Child Health Care Hospital, Women's Hospital School of Medicine Zhengjiang University, Yanbian Maternal and Child Health Care Hospital, Changzhou Maternal and Child Health Care Hospital, and Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region. The participants were all non-pregnant women who underwent colposcopy examination in the study hospitals from June to December 2021. A biopsy was performed after colposcopy examination, followed by biopsy specimens for pathological diagnosis. The collected data included age, cervical cancer screening history, HPV vaccination, cervical cancer screening, colposcopy examination, and pathology results. All the colposcopy doctors in the six hospitals had received training in colposcopy operations. The study was approved by the Biomedical Research Ethics Committee of Peking University First Hospital, with the ethic code 2020[321]. Finally, among 3,637 women, 2,945 participants were analyzed. The exclusion criteria included incomplete data collection. The outcome of this study was the detection of cervical precancers and cervical cancer (≥HSIL). SPSS software (version 26.0, IBM, Armonk, NY, USA) was used for statistical analyses, and $P<0.05$ was considered a statistically significant difference. Continuous variables were expressed as mean±standard deviation (SD); categorical variables were expressed as numbers and percentages; comparisons among groups were performed by Chi-square tests or Fisher exact tests as appropriate. Multivariable logistic regression models were used to evaluate the association between risk factors and detection of ≥HSIL.

The average age of participants was 40.9±11.5, ranging from 19 to 80 years old; 17.4% (511/2,945) were ≤29 years, 34.1% (1,003/2,945) were 30–39 years, 23.6% (696/2,945) were 40–49 years, 18.2% (536/2,945) were 50–59 years, and 6.8% (199/2,945) were ≥60 years. Only 6.9% (204/2,945) of participants had received HPV vaccination, with 4.3% (127/2,945) having completed the three-dose regimen. Additionally, 50% (1,473/2,945) of participants had a cervical cancer screening history within the past five years.

Among 2,945 participants, 80.6% (2,373/2,945) received HPV combined cytology co-testing screening, while 19.4% (572/2,945) received cytology screening. Overall, 92.6% had abnormal cervical screening results, including cytology ≥atypical squamous cells of undetermined significance (ASC-US) or HPV positive, or both abnormal cytology results and HPV positive. Only 7.4% underwent colposcopic examinations for abnormal symptoms. According to the colposcopic impression, 34.1% (1,004/2,945) of participants were normal/benign, 48.4% (1,426/2,945) were low-grade, 16.0% (470/2,945) were high- grade, and 1.5% (45/2,945) were cancer. Through biopsy and pathology diagnosis, 80.4% (2,368/2,945) were ≤low-grade squamous intraepithelial lesions (LSIL); 537 participants were HSIL, and the percentage was 18.2%; 13 participants were AIS, and the percentage was 0.4%. Additionally, 27 women (0.9%) were diagnosed with invasive cervical cancer (ICC). The participants with ≥HSIL totaled 577, and the detection rate was 19.6%. Of these 577 participants with ≥ HSIL, 471 (81.6%) had HPV combined cytology co-test for cervical cancer screening and 106 (18.4%) had cytology tests for cervical cancer screening (Figure 1).

The results of Table 1 indicated that a lack of

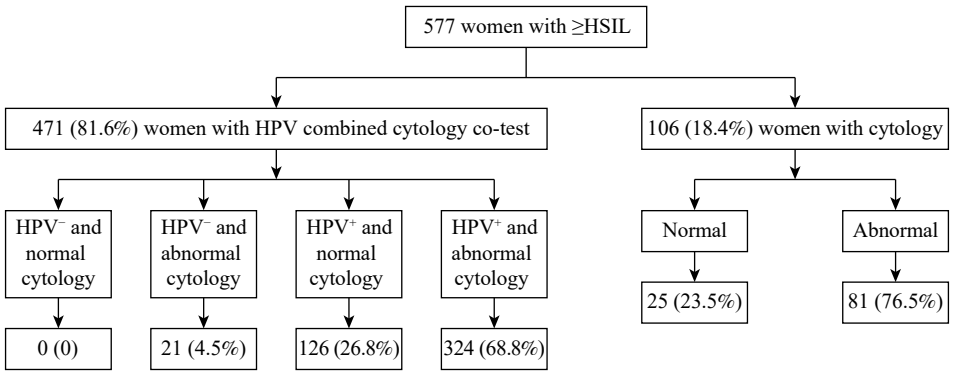

FIGURE 1. Distribution of ≥HSIL with different screening methods (*n*, %) — six provinces, China, June–December 2021.
Abbreviation: HSIL=high-grade squamous intraepithelial lesion; HPV=human papillomavirus.

cervical cancer screening history within the past 5 years, as well as a positive cervical screening and an abnormal colposcopic impression, were independent associated factors of ≥HSIL detection. The results of multivariable logistic regression to evaluate the associations between clinical risk factors and ≥HSIL are presented in Table 2. Positive cervical screening was found to be a risk factor for detecting ≥HSIL (*OR*=1.75, 95% *CI*: 1.07–2.86). Additionally, low-grade, high-grade, and cancer of colposcopic impression were associated with a higher risk for detecting ≥HSIL (*OR*=2.94, 95% *CI*: 2.13–4.08; *OR*=36.64, 95% *CI*: 26.07–51.48).

DISCUSSION

In our study, the detection rate of ≥HSIL in six hospitals was 19.6%. Having no cervical cancer screening history in the past five years, a positive cervical screening, and an abnormal colposcopic impression were identified as clinical risk factors for detecting ≥HSIL.

Our study showed that the detection rate of ≥HSIL was 19.6%, which is similar to the results of other studies. A study from Chongqing Hospital reported that among 1,055 participants, 211 cases were ≥ cervical intraepithelial neoplasia 2 (CIN2), resulting in a detection rate of 20% (*4*). Previous studies have reported that the detection of CIN and ICC ranged from 4.1 to 6.0 per 1,000 in different age groups with different cervical cancer screening methods in the general population (*5*). In hospitals, most patients had abnormal cervical screening results, making them a high-risk population, thus the detection rate of cervical cancer was much higher than in the general

TABLE 1. Characteristics of participants by detection of ≥HSIL (*n*, %) — six provinces, China, June–December 2021.

Clinical risk factor	≤LSIL*, *n* (%)	≥HSIL*, *n* (%)	χ^2	*P*
Age (years)			5.38	>0.05
≤29	405 (79.3)	106 (20.7)		
30–39	792 (79.0)	215 (21.0)		
40–49	572 (82.2)	124 (17.8)		
50–59	443 (82.6)	93 (17.4)		
≥60	156 (77.9)	43 (22.1)		
HPV vaccination			4.93	>0.05
Yes	163 (79.9)	41 (20.1)		
No	2,205 (80.4)	536 (19.6)		
Cervical cancer screening history in 5 years			32.82	<0.001
Yes	1,137 (82.7)	217 (17.3)		
No	1,158 (78.1)	320 (21.9)		
Unknown	73 (64.5)	40 (35.4)		
Cervical cancer screening method			0.51	>0.05
Cytology	466 (81.5)	106 (18.5)		
HPV combined cytology co-test	1,902 (80.2)	471 (19.8)		
Cervical cancer screening results this time			13.10	<0.001
Abnormal	2,018（79.4）	525 (20.6)		
Normal	350（87.1）	52 (12.9)		
Colposcopic impression†			759.68	<0.001
Normal/benign	953 (94.9)	51 (5.1)		
Low-grade	1,237 (86.7)	189 (13.3)		
High-grade	177 (37.7)	293 (62.3)		
Cancer	1 (2.2)	44 (97.8)		

Abbreviation: HSIL=high-grade squamous intraepithelial lesion; LSIL=low grade squamous intraepithelial lesion; HPV=human papillomavirus.

* ≤LSIL includes normal cervix and LSIL; ≥HSIL included HSIL and ICC.

† The Fisher exact test was used due to the small sample size of one cell (<5).

TABLE 2. Multiple logistic regression analysis of related clinical factors on detection of ≥HSIL — six provinces, China, June–December 2021.

Risk factor	Detection of ≥HSIL		
	*OR**	95% *CI*	*P*
Cervical cancer screening results			
Normal	Ref.		
Abnormal	1.75	1.07–2.86	<0.050
Colposcopic impression			
normal/benign	Ref.		
low-grade	2.94	2.13–4.08	<0.001
High-grade and malignant cancers	36.64	26.07–51.48	<0.001

Abbreviation: HSIL=high-grade squamous intraepithelial lesion; *CI*=confidence interval; *OR*=odds ratio.
* Adjusted for age, HPV vaccination, and cervical cancer screening history in the past 5 years.

population.

Our study revealed that a lack of cervical cancer screening within the past 5 years and abnormal screening results are associated with the detection of ≥ HSIL. Specifically, abnormal screening results were found to increase the risk of ≥HSIL detection by 75% compared to normal screening results. Cervical cancer screening history was a risk factor for advanced stages of cervical cancer; a study from Denmark found that the less advanced invasive cervical cancer stage (stage I) was 3.14 times higher given adequate attendance to cervical cancer screening programs, with 61.6% of patients having deficient screening histories (*6*). An American research also indicated that 60% of women aged 21 years and older who were diagnosed with invasive cervical cancer had no cervical cancer history among 367 participants (*7*). Cervical cancer screening programs have been conducted by the Chinese government since 2009, but only half of the participants attended screening in the last 5 years in our study. The percentage of deficient screening histories in our study was slightly lower than those of previous studies (*6*–*7*), which may be related to the different age range and observation indicators. The study reported that abnormal cervical cancer screening results were a risk factor for the detection rates of HSIL and ICC (*OR*=1.75). For a woman who has cervical cancer screening just once in her life after 35 years old, her risk of dying from cervical cancer would decrease by 70%. If she is screened every 5 years, her risk of dying from cervical cancer drops by more than 85% (*8*). Therefore, cervical cancer screening is an efficient secondary preventable measure; it is not only about screening on time, but also a long-term management strategy for controlling cervical precancers and cancer. According to the report about cervical cancer screening coverage estimates of 202 countries, 1.6 billion (67%) of 2.3 billion women aged 20–70 years had never been screened for cervical cancer (*9*). In China, the population-based screening rate is lower (*10*). It is necessary to conduct more extensive health education to enhance awareness of cervical cancer among the target population, and then to increase the initiative screening rate.

Colposcopy examination is a key method for diagnosing cervical precancer as a second step, and it requires a more professional and trained doctor. According to the quality control manual of cervical screening, the high-grade coincidence rate between colposcopy impression and biopsy should be ≥60% (*11*). The coincidence rate in our study was 62.3% (HSIL), meeting the quality control requirement. The doctors in the six study hospitals had received training, which enabled them to provide high-quality colposcopy examinations and improve the detection rate of cervical precancer.

HPV vaccination is an important primary prevention method; however, due to the low vaccine coverage rate, it has not been a significant factor in our study. Most women were screened by HPV combined cytology co-test, but the detection rate of cervical precancers did not differ significantly between different screening methods. The detection rate was mainly influenced by the quality of colposcopy and the screening results. There were several limitations to our study. Firstly, we focused mainly on clinical factors affecting the detection rate of cervical precancers in medical facilities, not including social factors. Secondly, most participants underwent colposcopy examination due to abnormal cervical cancer screening results, and few participants underwent colposcopy examination for abnormal symptoms; however, we did

not collect more detailed information on abnormal symptoms. Finally, in this study, we only collected data from participants who underwent colposcopy examination, and the cervical cancer screening results may have come from different health facilities or different screening methods, so we could not compare the relationship between screening quality and detection of ≥HSIL. Therefore, further research with larger populations, higher quality, and multiple sites is needed in the future.

The World Health Organization's cervical cancer elimination campaign must increase both screening coverage and treatment of detected cervical precancers (*9*). Our study indicated that cervical cancer screening and colposcopy impression were the main clinical influencing factors in health facilities. To achieve elimination goals, it is essential to disseminate health knowledge to improve public awareness of cervical cancer prevention and to enhance capacity building of professional staff to improve the quality of cervical cancer screening.

Acknowledgement: All the participants and investigators from 6 hospitals.

Conflicts of interest: The authors do not have any competing interests.

doi: 10.46234/ccdcw2023.038

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Submitted: February 02, 2023; Accepted: February 28, 2023

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Preplanned Studies

Changing Patterns of Heart Disease Mortality in Rural and Urban Areas — China, 1987–2021

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Summary

What is already known about this topic?

The burden of heart disease is increasing rapidly due to the aging population and changing lifestyles in China.

What is added by this report?

This study investigated the evolution of mortality rates due to heart disease in urban and rural areas of China over the past 35 years, and identified the age-period-cohort effects on mortality changes.

What are the implications for public health practice?

Healthcare providers should prioritize attention to heart disease among older males living in rural areas.

Heart disease (HD) is an important component of the spectrum of cardiovascular diseases (CVDs). China has the highest number of new HD cases in the world (*1*), posing significant health and economic burdens to society. However, its mortality trends at the national level and urban-rural differences remain unclear. This study, which identifies the age, period, and cohort effects of HD mortality trend changes, would assist the government in understanding future trends and optimizing public health policies. The HD mortality data by age, gender, and region were extracted from China's National Health Commission's death registration system, after quality control of ID and duplicate removal (*2*). The age-standardized mortality rate was calculated based on the world standard population using the direct method. Joinpoint regression was employed to determine HD mortality change patterns across time (*3*). The age-period-cohort model was used to estimate cohort and period effects (*4*). In this model, age refers to the individual's age at a given time, period refers to the time at which the outcome is measured, and cohort refers to the group of individuals born in a particular period. This study revealed that HD mortality in China has rapidly increased over the past two decades, with men increasing faster than women and rural areas increasing faster than urban areas. It is essential for healthcare providers to prioritize their attention towards heart disease among older males living in rural areas.

Figure 1 shows the long-term trends in crude and age-standardized mortality rates for HD in China's urban and rural populations by gender from 1987–2021. The crude mortality rates from HD showed a significant upward trend in all gender groups and areas during the study period, particularly in rural areas, where the mortality from HD rose sharply since 2005. In metropolitan areas, the crude HD mortality increased by 1.78-fold, with increases of 1.95-fold for men and 1.62-fold for women. In rural regions, the mortality rate increased by 2.11-fold, with 2.22-fold increases for males and 1.99-fold increases for women. However, the increasing trend of mortality after standardization was not as pronounced, showing a stable fluctuation. In general, men had a higher HD mortality than women, and rural areas had a higher mortality than cities.

The results of the joinpoint regression describing the mortality trends are presented in Table 1. The results indicate that the fastest increasing interval for heart disease mortality in urban areas of China was from 2002–2010, while the fastest increasing period in rural areas was from 2005–2011, which corresponded to the time of the fastest economic growth rate in China. The age-standardized mortality in urban areas showed a small downward trend, while rural mortality rates varied more but remained relatively stable over the last decade.

Figure 2 (A–B) shows HD net- and local-drift. Net-drift represents the average yearly trend for the entire population over the previous three decades, while local-drift refers to the average annual trend for distinct age groups. In this study, the overall net-drift pattern was different in rural and urban China. HD mortality decreased significantly in the urban population across the whole study period {–0.84% [95% confidence interval (*CI*): –1.40 to –0.28]}, while the general tendency was not noticeable in rural regions [–0.51% (95% *CI*: –1.23 to –0.21)]. Local-drift reflects the further age variability in mortality trends. The data

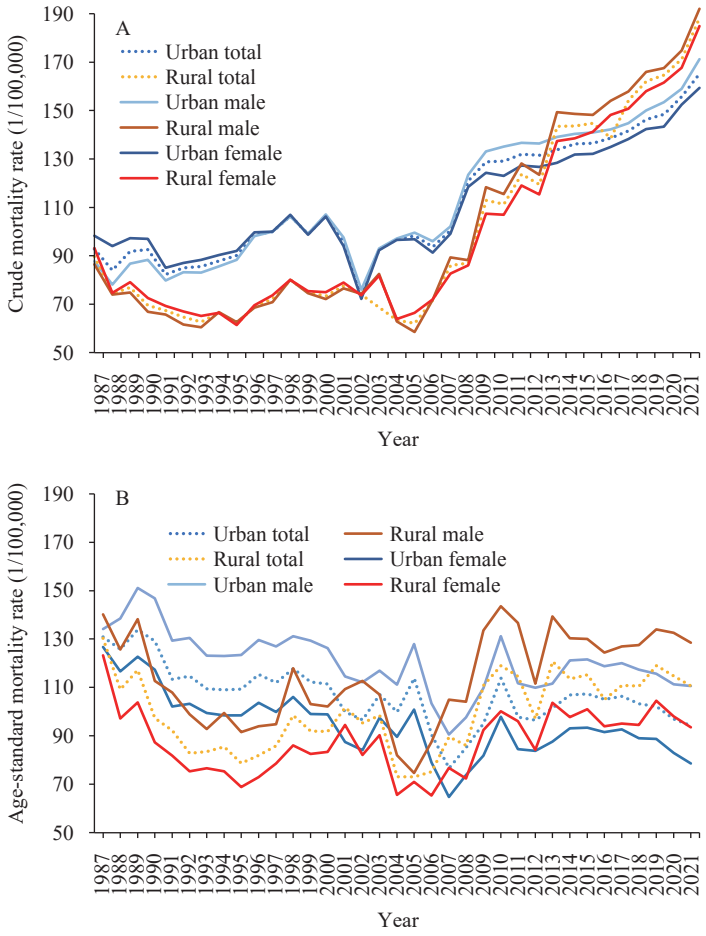

FIGURE 1. Trend of crude and age-standardized mortality rates from heart disease in China from 1987 to 2021.

indicate that mortality among those aged 60 and older exhibits a considerable upward tendency. In terms of gender, the mortality rate of male residents between the ages of 15 and 60 is increasing, with this effect being more pronounced in rural regions, while the mortality rate of female residents in both urban and rural areas is decreasing consistently.

Figure 2 (C–H) presents the effects of age, period, and cohort on HD mortality in China. The age effects manifested as an expected exponential curve, with mortality rates increasing exponentially with age. Mortality increased rapidly in older ages, with a higher rate of increase in rural areas than in urban areas and higher in men than in women. Period effects tended to show the same patterns across gender and regions, with a strikingly descending trend from 1987–2005, suggesting significant improvements across the study period. However, since 2007, HD mortality rate in urban and rural areas has shown an increasing trend. Cohort effects tended to show similar patterns between rural and urban areas, with the most striking improvements across birth cohorts in the urban female population and a progressive improvement in mortality in those born from 1930 onward. For the male population, there was little improvement.

DISCUSSION

Previous studies have demonstrated a high burden of HD in China, but few studies have recorded urban versus rural HD mortality rates over a long period. This paper examines China's HD mortality patterns over the past 35 years, emphasizing the age-period-cohort effects from an urban-rural viewpoint. This study revealed that China's HD mortality has risen sharply in the previous 20 years, with mortality increasing at a faster rate among men than among women, particularly in rural regions compared to cities. The age-standardized mortality rate (ASMR) of HD is relatively stable, but it also showed a large increasing trend from 2005 to 2010. This finding indicates that the escalation of HD mortality in China is not solely driven by the primary factors of

TABLE 1. Joinpoint analysis of crude and age-standardized mortality rates from heart disease in urban and rural areas.

Subgroup	Mortality rate (per 100,000)		Total study period[§]		Period 1		Period 2		Period 3		Period 4		Period 5	
	1987	2021	AAPC (%)	95% *CI*	Years	APC (%)	Years	APC (%)	Years	APC (%)	Years	APC (%)	Years	APC (%)
Crude mortality														
Heart disease in urban areas														
Total	92.84	165.37	1.6*	(0.3, 3.0)	1987–1993	−1.2	1993–1999	4.3*	1999–2002	−9.0	2002–2009	6.0*	2009–2021	2.1*
Male	87.74	171.26	1.9*	(0.5, 3.3)	1987–1993	−0.5	1993–1999	4.8*	1999–2002	−8.6	2002–2010	5.9*	2010–2021	1.8*
Female	98.26	159.40	1.3*	(0.0, 2.5)	1987–1992	−2.9*	1992–1999	3.2*	1999–2002	−8.9	2002–2009	5.6*	2009–2021	2.1*
Heart disease in rural areas														
Total	89.73	188.58	2.3*	(0.9, 3.7)	1987–1992	−6.0*	1992–2001	2.5*	2001–2005	−6.0	2005–2009	15.9*	2009–2021	4.3*
Male	86.45	192.09	2.4*	(0.8, 4.1)	1987–1992	−6.0*	1992–2002	2.8*	2002–2005	−9.2	2005–2009	17.8*	2009–2021	4.1*
Female	93.13	184.93	2.2*	(0.4, 4.1)	1987–1995	−3.9*	1995–1998	9.9	1998–2005	−2.5	2005–2011	10.0*	2011–2021	4.1*
Age-standardized mortality[†]														
Heart disease in urban areas														
Total	130.89	94.19	−0.6*	(−1.1, −0.2)	1987–2007	−1.5*	2007–2021	0.7	–	–	–	–	–	–
Male	134.15	110.59	−0.5*	(−1.0, −0.1)	1987–2007	−1.4*	2007–2021	0.6	–	–	–	–	–	–
Female	126.75	78.63	−1.2*	(−2.2, −0.1)	1987–2008	−2.0*	2008–2016	2.3	2016–2021	−3.4	–	–	–	–
Heart disease in rural areas														
Total	130.34	110.60	−0.4	(−2.2, 1.6)	1987–1993	−7.5*	1993–2002	2.6*	2002–2005	−11.1	2005–2010	9.9*	2010–2021	−0.1
Male	140.20	128.48	−0.3	(−2.5, 1.9)	1987–1993	−7.0*	1993–2002	2.2*	2002–2005	−12.6	2005–2009	15.0*	2009–2021	−0.2
Female	123.29	93.54	−0.4	(−2.3, 1.4)	1987–1994	−6.8*	1994−2001	4.1*	2001−2006	−6.5	2006–2010	10.3	2010–2021	0.1

Note: "–" means no joinpoints identified.
Abbreviation: APC=annual percent change; AAPC=average annual percent change; *CI*=confidence interval.
* Significant difference from zero ($P<0.05$).
[†] Standardized to the World Health Organization world standard population.
[§] Years 1987 to 2021.

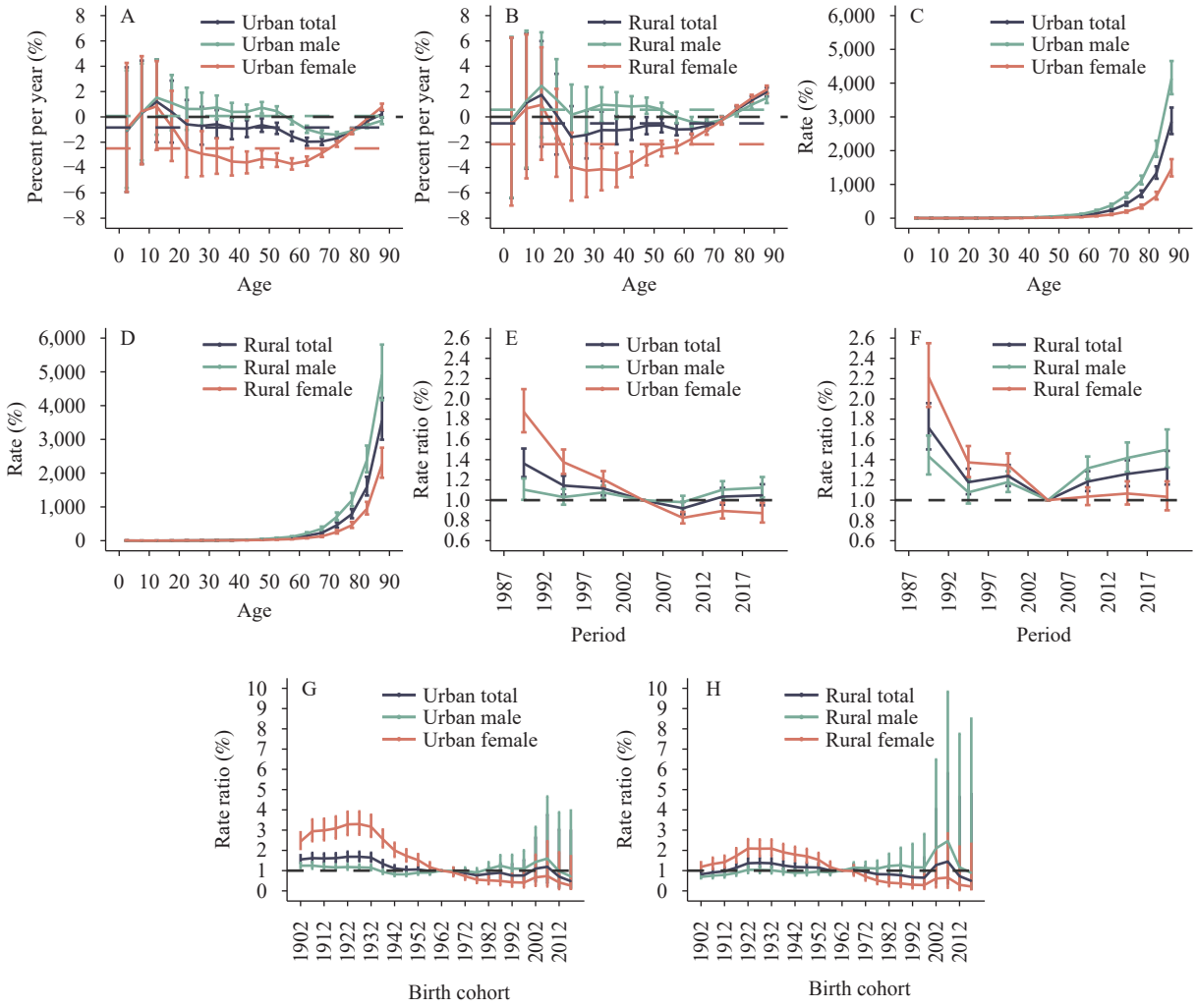

FIGURE 2. Parameter estimates of age, period, and cohort effects on heart disease mortality rates in rural and urban China from 1987 to 2021. (A) Net and local drifts in urban areas; (B) Net and local drifts in rural areas; (C) Age effects in urban areas; (D) Age effects in rural areas; (E) Period effects in urban areas; (F) Period effects in rural areas; (G) Cohort effects in urban areas; (H) Cohort effects in rural areas.

population aging, but rather that social-environmental factors also serve as significant contributing factors. Socio-economic factors, such as poverty, low education levels, and lack of access to healthcare, have been proven to be risk factors for HD, and people who live in poverty may have limited access to healthy foods, healthcare, and safe places to exercise, which can all contribute to the development of HD. Environmental factors, such as air pollution and exposure to toxins, can also contribute to HD. Air pollution can damage the blood vessels and increase the risk of heart disease, while exposure to toxins can also damage the heart and blood vessels. Multiple other factors, such as diabetes mellitus, high blood pressure, unhealthy diet, obesity, cigarette and alcohol use, and physical activity may also contribute to the development of HD (*5*).

Previous research indicates that the prevalence of HD and accompanying mortality has shifted from developed to developing countries in recent decades, and the high prevalence of obesity, hypertension, diabetes, and hyperlipidemia due to the prevalence of Western lifestyles is the leading cause of HD deaths (*6*). The period between 2002 and 2011 marked the fastest increase in HD deaths within the Chinese population. Notably, these were also the years when China experienced the fastest economic growth. Dramatic lifestyle changes, environmental pollution, and a large unmet medical need may have contributed to the rapid increase in HD deaths (*7*–*8*).

Significant regional differences in HD mortality have been observed, with urban deaths being higher than rural deaths until 2013. However, in recent years, rural areas have begun to outpace urban areas, resulting in a widening urban-rural gap. It is likely that

smoking, drinking alcohol, and other risk factors, such as high serum cholesterol and blood pressure, have contributed to the higher HD mortality in rural China (*9*).

At the same time, trends in HD deaths show significant gender and age differences. Men have always been at high risk for HD deaths, and this situation has not improved due to socioeconomic risk factors. Biological, lifestyle, and psychosocial factors may explain this gender disparity (*10*). Both period and cohort effects show that HD deaths in older groups are increasing, indicating that these populations need special attention. To conclude, the rising mortality from HD and the burden on rural and elderly populations remain a significant public health priority for the Chinese government. It is imperative for healthcare providers to prioritize their attention towards heart disease among older males living in rural areas. Such a targeted approach would contribute to the improvement of overall health and well-being in China.

This study has several limitations. First, the APC effects were estimated using cross-sectional data, not panel data. Second, in addition to urban-rural differences, there are also significant socioeconomic differences between local regions in China, so more fine-grained mortality data for HDs (e.g., provinces) need to be further analyzed. Third, the mortality data were categorized using different ICD versions, ICD-9 before 2002 and ICD-10 subsequently in China, which may affect the accuracy of the trends of CVDs due to an incomplete match. Previous publications have indicated that there is an underreporting of 2%–5% in the mortality data used in this study (*2*); however, this does not affect the research and analysis of the overall trend.

doi: 10.46234/ccdcw2023.056

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Submitted: February 13, 2023; Accepted: March 24, 2023

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Preplanned Studies

Enterohemorrhagic *Escherichia coli* O157:H7 — Xuzhou City, Jiangsu Province, China, 2001–2021

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Summary

What is already known about this topic?

The largest and longest outbreak of diarrhea, which was complicated with hemolytic uremic syndrome (HUS) caused by enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, occurred in Xuzhou City and its adjacent areas from 1999 to 2000 in China.

What is added by this report?

According to surveillance results from 2001 to 2021, there was a significant decrease in the isolation rate of O157:H7, and cattle and sheep remained the primary hosts. However, non-Shiga toxin-producing O157:H7 emerged as the dominant strain, with *stx2+stx1-* strains following closely behind.

What are the implications for public health practice?

National surveillance of O157:H7 effectively serves as an early warning system and guidance for assessing the intensity and trend of disease epidemics. It is crucial to raise awareness of the public health risks associated with Shiga toxin-producing *E. coli*.

Escherichia coli (*E. coli*) that produce Shiga toxin or verocytotoxin is commonly known as Shiga toxin-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli* (VTEC). Hemorrhagic enteritis (HC), caused by foodborne zoonotic pathogen enterohemorrhagic *E. coli* (EHEC) O157:H7, was first reported in the United States in 1982 and has since caused outbreaks of human infections in various countries such as Canada, Japan, and China. In China, the pathogen was isolated from the feces of patients with diarrhea in Xuzhou City, Jiangsu Province in 1986. It later caused an outbreak in Xuzhou and adjacent areas from 1999 to 2000 (*1*). Measures that included investigation of diarrhea patients, identification of the pathogen, and clearing epidemiological characteristics were implemented to control the spread of the disease. Subsequently, a surveillance program was reformulated in 2001 in Tongshan District, Xuzhou City based on the National O157:H7 surveillance program and the reality of Jiangsu Province. The program included 18 towns for diarrhea patients and food surveillance, and 3 major animal farming towns for animal surveillance. The program has led to changes in the detection rates and virulence genes of the strains. In this study, we analyzed O157:H7 surveillance data from 2001 to 2021 to predict possible transmission risks.

Between 2001 and 2021 (April to October), raw and cooked meat samples, as well as fecal samples from patients with diarrhea, cattle, sheep, chickens, and pigs were collected by medical workers from local township health centers under the guidance of Tongshan District Center for Disease Control and Prevention during the epidemic season. Isolation of the samples was performed using immunomagnetic bead adsorption. The isolated strains were analyzed via polymerase chain reactions (PCR) detection of virulence genes, namely *stx*, *eaeA*, and *hly*, to determine their virulence characteristics. During the outbreak period from 1999 to 2000, samples were predominantly collected from epidemic villages and towns. Correlation analysis of O157:H7 isolation rates between host animals, diarrhea patients and meat samples from 2001 to 2021 were performed via SPSS (version 19.0, IBM Corp, NY, USA). $P<0.05$ was considered statistically significant.

The 20-year surveillance period, from 2001 to 2021, revealed that the isolation rates of animal feces, meats, and diarrhea patients were 0.41% (31/7,539), 0.12% (3/2,526), and 0.07% (4/5,491), respectively. A pairwise correlation analysis was conducted on the O157:H7 isolation rates from 2001 to 2021 among the aforementioned samples, which found a significant correlation between diarrhea patients and meat, with a correlation coefficient of r=0.745 ($P<0.001$). No significant correlation was found between the isolation rate of host animals and either diarrhea patients or meat. During the O157:H7 outbreak from 1999 to 2000, the isolation rates were 13.56% (154/1,136) in the feces of host animals, 4.35% (7/161) in meat, and

3.88% (52/1,339) in the feces of diarrhea patients. Compared to the outbreak period, the isolation rates of O157:H7 from various types of samples were significantly reduced after surveillance sites were established in 2001. After 2011, no O157:H7 strain was isolated from diarrhea patients (Figure 1).

Between 1999 and 2000, there was an outbreak period, during which the isolation rates of O157:H7 were observed from cattle, sheep, chickens, and pigs. The isolation rates were found to be 19.51% (8/41), 19.06% (57/299), 10.97% (34/310), and 13.13% (39/297), respectively. Cattle and sheep were identified as the most significant carriers of O157:H7 during this outbreak.

However, surveillance results from 2009 to 2021 showed that the isolation rates of feces from cattle, sheep, and pigs were substantially reduced to 1.05% (11/1,049), 0.55% (6/1,100), and 0.21% (2/963) respectively. Notably, O157:H7 was no longer isolated from chicken feces. Even with this decrease in isolation rates, cattle and sheep remain the primary carrier hosts of O157:H7.

Shiga toxin is a crucial virulence factor for O157:H7 and is strongly linked to severe complications such as hemolytic uremic syndrome (HUS) (*2*). We isolated 22 strains of O157:H7 between 2009 and 2021, all of which tested positive for *eaeA* and *hly* genes. The gene profiles for Shiga toxin were *stx2*+ *stx1*- in 22.73% (5/22) of the strains, *stx2*+ *stx1*+ in 4.54% (1/22), and *stx2*- *stx1*- in 72.73% (16/22), indicating that non-Shiga toxin-producing EHEC O157:H7 strains have become dominant (Table 1).

DISCUSSION

Following the outbreak of O157:H7 in 1999, Xuzhou launched a comprehensive patriotic health campaign focusing on managing water, diet, feces, and eliminating flies. This led to timely control of the

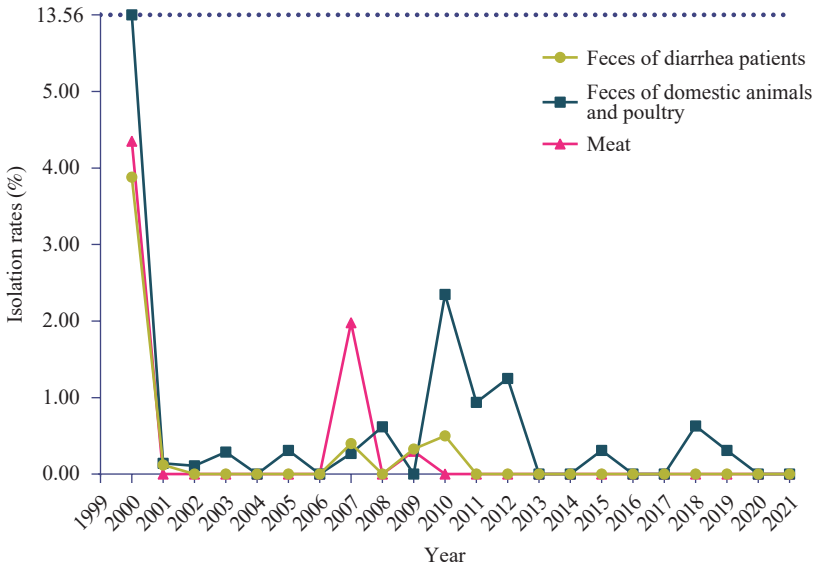

FIGURE 1. Variation curve of isolation rates of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 from various samples, 1999–2021.

TABLE 1. The distribution of virulence genes of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 from different sources of samples, 2009–2021.

Source of sampling	No.of detected samples	No.of positive samples	*stx2+ stx1- eaeA+ hly+*	*stx2+ stx1+ eaeA+ hly+*	*stx2- stx1- eaeA+ hly+*	Positive rate (%)
Diarrhea patients	3,240	2	1	0	1	0.06
Cattle	1,049	11	2	1	8	1.05
Sheep	1,100	6	1	0	5	0.55
Pigs	963	2	0	0	2	0.21
Meat	1,026	1	1	0	0	0.10
Total	7,378	22	5	1	16	0.30

disease spread, and a national surveillance site was established in Tongshan District which was the epicenter of the outbreak. From 2001 to 2021, surveillance reports show a significant reduction in the incidence of infectious diarrhea caused by O157:H7 and the host carrier rates. Since 2011, no cases of O157:H7 infection were found in diarrhea patients. However, the pathogen was still prevalent in the feces of animal hosts, with cattle and sheep being the main carriers. Therefore, it is necessary to enhance health education among farmers, especially among individual free-range households, to regulate the proper disposal of host animal fecal waste. This action is essential to prevent the spread and infection of O157:H7.

In Xuzhou, sporadic cases of human infection with O157:H7 have been reported, but there have been no reports of clusters in recent years. This may be due to improved health education and hygiene practices, as well as a decrease in the rate of O157:H7 infection among diarrheal patients, which may be related to a decrease in the carrier rate among host animals and changes in the virulence gene profiles of strains, reducing their pathogenicity. EHEC O157:H7 can produce Stx1 or Stx2 Shiga toxins, with Stx2 more often associated with human disease than Stx1 (*3*). Strains that produce only Stx2 are considered to be more virulent than strains that produce both Stx2 and Stx1, or only Stx1 (*4*). During 1999–2000, *stx2*+ *stx1*- O157:H7 strains predominated in the region, which may have been the main cause of the serious outbreak (*5*).

Surveillance from 2009–2021 found that Shiga toxin-free (*stx2- stx1-*) strains are now dominant, while *stx2*+ *stx1- eaeA*+ *hly*+ strains remain the main producers of Shiga toxins in the region. Loss of *stx*-encoding bacteriophages may occur during infection or culturing of strains, resulting in O157:H7 strains lacking *stx* virulence factors (*6*). Therefore, it is important to remain vigilant of changes in the O157:H7 virulence gene profile and improve our ability to identify outbreak risks early.

Limitations of this study include missing data on the virulence profile of strains from 2001 to 2008 due to improper preservation, and an incomplete understanding of the virulence trend.

The United States was the first country to identify O157:H7 as a foodborne pathogen that caused outbreaks. Recently, according to the US CDC (www.cdc.gov/ecli/outbreaks.html), outbreaks of O157:H7 infection have mostly been associated with the consumption of green vegetables such as spinach and lettuce, whereas in the past, the main cause was related to the consumption of beef and related products (*7*). During 1999–2000, there was an O157:H7 outbreak in Xuzhou, which was mainly caused by water and food contaminated with human and animal feces. Host animals and infected persons were the primary sources of infection (*8*). As China's national economic level improves, people's living habits are changing, and there is an increase in the number of people who eat green organic vegetables, especially raw food. Green organic vegetables are mostly irrigated with animal feces, which may become a potential risk factor for human infection with O157:H7. Therefore, we need to pay attention to the change in this transmission pattern.

In conclusion, national surveillance of EHEC O157:H7 in Xuzhou, where the earliest and largest outbreak in China occurred, has proven to be effective in providing early warning and guidance for assessing the intensity of the disease. Continuous surveillance of the baseline of O157:H7, particularly *stx* virulence trend, is critical for early warning. As a result of these efforts, the monitoring program in Jiangsu Province has been transitioning from O157 to STEC (VTEC) since 2021.

Conflicts of interest: No conflicts of interest.

Acknowledgements: We thank Charlesworth author services (Paper no.112635) for their critical editing and helpful comments regarding our manuscript.

Funding: Supported by National Science and Technology Major Project (2018ZX10713-003-002).

doi: 10.46234/ccdcw2023.057

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Submitted: February 22, 2023; Accepted: April 03, 2023

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Perspectives

Back to Science in Searching for SARS-CoV-2 Origins

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In recent decades, emerging and re-emerging human-infecting pathogens have been represented as huge threats to public health and have become a global concern (*1*). After outbreaks of two coronaviruses (CoVs), severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became the first-known pandemic hastening CoV with tremendous wrecking to the world (*2*). The origin tracing of these emerging pathogens is of great significance in infectious disease prevention and control (*3*–*4*). The origin of SARS-CoV-2 remains elusive after the more than 3-year pandemic, though scientists around the world are making great efforts. From the experience of studying many other infectious pathogens, origin tracing is systematic and time-consuming work. The supposed origins of many infectious pathogens are still in debate, including SARS-CoV and human immunodeficiency virus, etc (*5*).

The establishment of a defined origin of an emerging human-infecting pathogen requires rigorous logical relationships, sufficient causal relationships, and a solid chain of evidence. Generally speaking, to understand the concept of "origin," we believe that it can be divided into two perspectives: 1) From a macro perspective, for the global origin of coronavirus disease 2019 (COVID-19), zoonotic origin is still the most possible way. A natural host would exist, which may or may not require intermediate animals. However, based on the current global evidence available, there is no definitive conclusion for both natural and intermediate animals for SARS-CoV-2 yet. 2) In a narrower sense, for the origin (or more accurately, the "introduction" or the route of infection of the index case) in a particular geographic range (such as a country, city, school or market) or in a particular population, we believe that this origin may have multiple possibilities. For example, the initial case in a geographical area was infected by a person entering this area, by contact with the items entering the area as a vector of the pathogen, or by an animal carrying the pathogen entering the area. However, it should be noted that even if the origin within a certain area (even as the early reported outbreak site) is found, it cannot be equated with the origin of the global pandemic, that is, "the location of discovery" does not equal to "the location of origin".

To draw the scientific conclusion that a certain item is the origin of an emerging pathogen, scientific logic and evidence for research are also needed. First of all, to prove that the infection origin of item B, i.e., index case is item A (A and B refer to individuals rather than groups), which can be human, animal, or environmental medium items, the following representatives in the evidence chain are required: 1) Prior to the onset of disease, B has a history of exposure to A and the infection of A is prior to B (epidemiological evidence); 2) the pathogen is detected and/or isolated from A, in contact by B (laboratory evidence); and 3) the genome of the pathogen sequenced from the samples of A and B showed high homology or progeny relationship (molecular evidence). Considering the complexity and rigor of causality, these are necessary but not sufficient conditions to prove that A, as the origin, transmitted the pathogen to B.

On the issue of SARS-CoV-2 origin, all the studies and the related conclusions should be based on science. Chinese scientists have always maintained an open, transparent, and responsible attitude and practice and worked together with scientists from all over the world on origin tracing and contributed a lot of scientific references, with WHO-China Joint Report as one of the achievements (*6*).

During the early stage of COVID-19 outbreak in late December 2019, a certain percentage of cases were found to be linked to the Huanan Seafood Market (HSM) in Wuhan, China. Thus, the surveillance of SARS-CoV-2 in the environment and animal samples of the HSM is of great importance for investigating how the viruses were introduced into the market. The market was closed on the morning of January 1, 2020,

and at the same time, the Chinese Center for Disease Control and Prevention (China CDC) and local CDCs dispatched experts to the HSM to collect environmental samples. From January to March 2020, environmental samples from different locations within and around this market and animal samples including animal bodies, stray animals, and their feces were collected. The surveillance results reflect the profile of SARS-CoV-2 contamination by early cases in the market during the early phase of the outbreak. These results were timely and continuously informed to the public through official media (http://www.gov.cn/xinwen/gwylflkjz16/wzsl.htm). After the surveillance work in the field, the data were further analyzed and during the WHO-convened Global Study of Origins of SARS-CoV-2: China Part from July 2020 to February 2021, the results were reported to WHO experts and comprehensively discussed by a joint expert team of WHO and China. The joint team also entered the site of HSM in January 2021 and they recognized the importance of the surveillance methods, data, and results, which were written into the Joint Report (*6*). Furthermore, the Joint Report also suggested a list of more investigations and analyses to be performed in the future, including DNA barcoding analysis of the samples within the market. Meanwhile, the data on the HSM together with the barcoding analyses were summarized into a scientific paper submitted to a peer-review journal and also released as a preprint for the public (*7*–*8*) and further in-depth analyses were also performed during this process. Meanwhile, the raw data related to the study were deposited to GISAID (*9*). The data were not removed or deleted since the deposit. In accordance with the usual practice of publication, and by agreement with the journal and GISAID, the data would be released simultaneously with the formal publication, but the link to access the data for journal review has always existed. At present, all the data have been released simultaneously in four international databases, i.e. 1) GISAID; 2) Sequence Read Archive (SRA), National Center for Biotechnology Information (NCBI); 3) National Genomics Data Center (NGDC), China National Center for Bioinformation (CNCB); 4) China National Microbiology Data Center (NMDC).

The study included the SARS-CoV-2 detection results of 1,380 samples collected from the environment and the animals within the market in early 2020. By SARS-CoV-2-specific RT-qPCR, 73 environmental samples tested positive for SARS-CoV-2 and three live viruses were successfully isolated. The viruses from the market shared nucleotide identity of 99.980% to 100% with the SARS-CoV-2 isolates from early COVID-19 cases. No virus was detected in the animal swabs covering 18 species of animals in the market. The RNA-seq analysis of SARS-CoV-2 positive and negative environmental samples showed the abundance of different vertebrate species. Through the DNA barcoding analysis, the high abundance of *Homo sapiens* within the environment samples highly suggests that the SARS-CoV-2 in the environmental samples was derived from early cases in HSM. Also, the results indicated the existence of *Sus*, *Bos*, *Gallus*, *Anas*, and *Nyctereutes* and other animals through gene barcode within the market before the closure. However, these environmental samples could not prove the infection of the animals. Furthermore, even if the animals were infected, the possibility of human-to-animal transmission occurring could not be ruled out after human infection. Thus, the possible potential introduction of the virus through human or cold chain products into the market can still not be ruled out. All these data showed that the market has just acted as an early amplifier during the pandemic due to the high number of consumers every day, causing many initially identified infection clusters. The origin of the virus involving animal-to-human transmission cannot be determined based on the current data.

At the same time as this study, Chinese scientists have also published a series of papers from the perspectives of serological testing of potential SARS-CoV-2 infection using blood from blood donors in Wuhan in 2019 (*10*), animal surveillance (*11*–*13*), the cold chain (*14*), etc (*3*–*4*,*15*). These studies have provided more scientific references for origin tracing and also provided scientific perspectives for future research.

Based on the current scientific logic and the evidence available globally, the several hypotheses and the conclusions on the origins of SARS-CoV-2 in the previous WHO-China Joint Report are scientific and objective (*6*). That is, traceability is a scientific issue that requires scientific evidence and logical inference. The origin tracing of SARS-CoV-2 may still be a long way off. However, it is believed that the experience accumulated in the studies on COVID-19 will shed light on the prevention and control of other emerging and re-emerging infectious diseases in the future (*16*).

doi: 10.46234/ccdcw2023.055

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Submitted: March 29, 2023; Accepted: March 30, 2023

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Notes from the Field

Genomic Recombination of SARS-CoV-2 Subvariants BA.5.2.48 and BF.7.14 — China, 2023

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded positive RNA virus, exhibits a high rate of genome mutation and recombination (*1*–*2*). Recombinant strains of this virus could potentially affect their binding ability to the ACE2 receptor, which may in turn diminish the protection offered by vaccines and neutralizing antibodies. Notably, the Delta and Omicron recombinations have been collectively referred to as Deltacron (*3*). Furthermore, XBB.1.5, a recombinant strain resulting from the combination of the BA.2 sublineages BA.2.10.1 and BA.2.75, has demonstrated enhanced transmissibility (*4*). As such, it is imperative to monitor the genome recombination of SARS-CoV-2 in order to provide valuable insights regarding epidemic and transmission trends.

In late 2022 and early 2023, SARS-CoV-2 mutation monitoring results from China revealed that the dominant lineages included BF.7.14, BA.5.2.48, and BA.5.2.49, among others (*5*). Importantly, the Chongqing CDC recently documented a case of co-infection involving both BF.7.14 and BA.5.2.48 (*6*). Based on this evidence, we hypothesize that genomic recombination may occur between the prevalent SARS-CoV-2 strains BF.7.14 and BA.5.2 in China.

We analyzed the SARS-CoV-2 genomic sequences submitted to GISAID from China (*7*) and identified nine potential recombinant sequences from Henan Province (n=2), Anhui Province (n=3), and Sichuan Province (n=4) (GISAID: EPI_SET_230323sq). A phylogenetic tree was constructed including early sequences from the BA.2, BA.5, BA.5.2, BA.5.2.6, BA.5.2.48, BA.5.2.49, BF.7, and BF.7.14 lineages. These nine recombinant sequences were classified within the BA.5.2.1 (abbreviated as BF) clade and exhibited the closest relationship with the BF.7 and BF.7.14 lineages (Figure 1A). Notably, the topology between these recombinant sequences and the BF.7 lineage was found to be complex and poorly supported.

Relative to the BF.7.14 lineage, all recombinant sequences carried two defining mutations (C27012T and C27513T) of the BA.5.2 lineage. In addition, the A27038G mutation, a defining feature of the BF.7.14 lineage, was absent from these sequences. Consequently, we inferred that these nine sequences from the three provinces may have resulted from genomic recombination between the BF.7.14 and BA.5.2 lineages (Figure 1B). Moreover, we examined the original sequencing datasets of these sequences from Henan and Anhui provinces and validated the key mutation sites within the recombination region (Figure 1C).

The identified recombinant sequences primarily consisted of the BF.7.14 framework, with a relatively low contribution from the BA.5.2 lineage. All nine recombinant sequences contained the signature mutation G22599C (S:R346T) found in the BF.7 lineage. Six of the sequences, not including three from Anhui Province, aligned with the BA.5.2 lineage at position 25290. We suspected that the left breakpoint of these recombination events could be situated between positions 22599 and 25290, and designated this region as Left Breakpoint 1 (LB1). The G25290T mutation in the three Anhui Province sequences matched that of BF.7.14. Considering the low likelihood of multiple SARS-CoV-2 recombination events occurring within a brief period, we hypothesize that the left breakpoint of the recombination event in the Anhui sequences might be located between positions 25290 and 27012 (LB2). Intriguingly, a sequence from Sichuan province (Sichuan-4, EPI_ISL_16737882) shared the C29632T mutation with BF.7.14, suggesting that the right breakpoint of the recombination event in this particular sequence might lie between positions 27532 and 29632, which we refer to as Right Breakpoint 1 (RB1). The remaining sequences were consistent with the BA.5.2 lineage at position 29632, implying that the recombination fragment might extend towards the end of the sequences. Consequently, we provisionally define the region between 29632 and the genome's

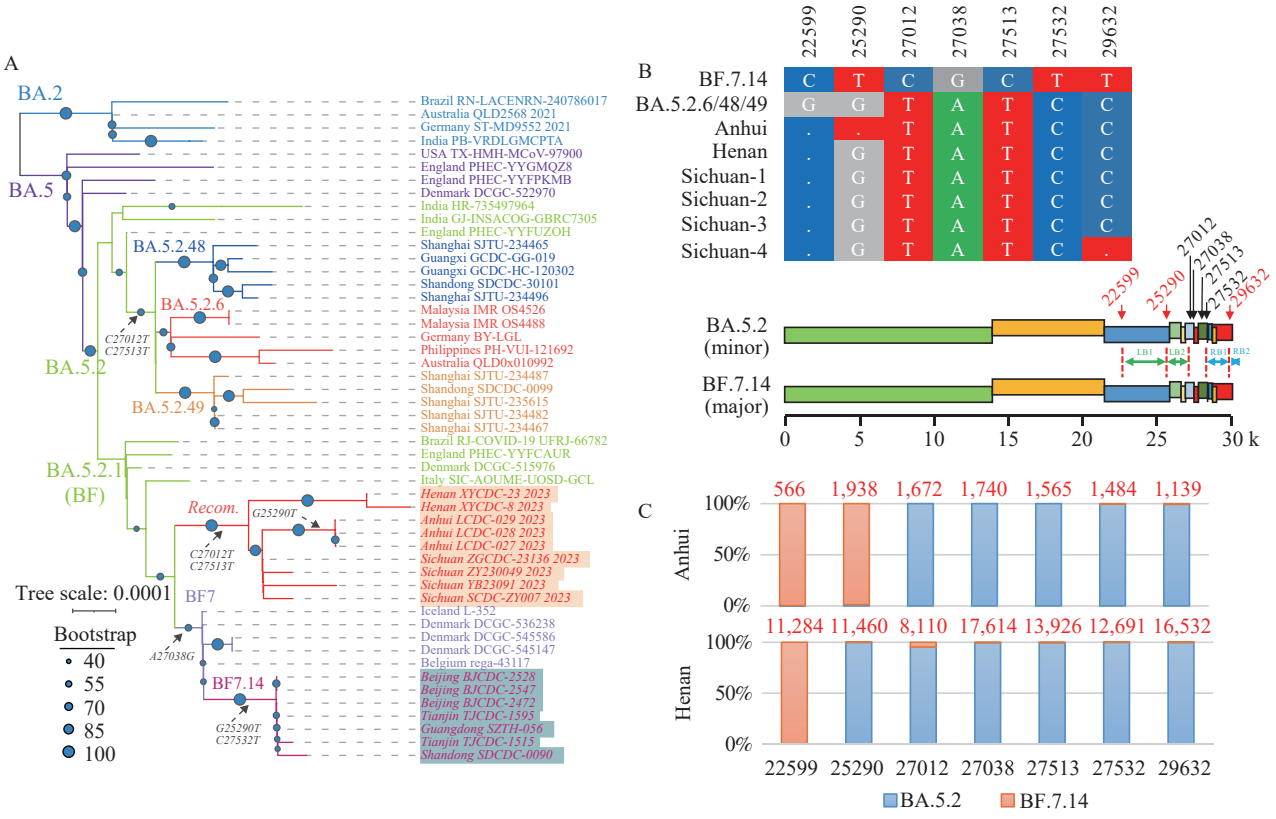

FIGURE 1. Genome recombination between SARS-CoV-2 BF.7.14 and BA.5.2 sublineages. (A) Phylogenetic tree of the recombined SARS-CoV-2 genomes; (B) The nucleotides of seven key sites; (C) Validation of the mutations of key sites using original sequencing datasets.
Note: In panel (A), the key mutations and sequences were italicized. In panel (B), positions with mutations used to determine the break points were colored in red. In panel (C), sequencing depth of the key sites were colored in red.
Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; LB=left breakpoint; RB=right breakpoint.

three prime end.

The primary recombination regions are situated from the tail of the Spike gene to the 3' end of the genome. The S protein of the recombinant sequence, particularly the S1 portion containing the receptor binding domain (RBD), retains the majority of the BF.7.14 characteristics. Consequently, we cautiously hypothesize that the effect of this recombination event on the existing transmission and entry routes of the coronavirus in China may be limited. Given the relatively low number of reported cases involving the recombinant virus, additional research is required to ascertain whether it will result in severe clinical symptoms in infected individuals.

Acknowledgments: Sichuan Provincial Center for Disease Control and Prevention for uploading the sequencing data to GISAID.

Funding: Supported by National Key R&D Program of China (2022YFC230410, 2022YFC2303401, 2021YFC2300101 and 2021YFA1201003).

doi: 10.46234/ccdcw2023.058

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Submitted: March 26, 2023; Accepted: April 04, 2023

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Indexed by Science Citation Index Expanded (SCIE), Social Sciences Citation Index (SSCI), PubMed Central (PMC), Scopus, Chinese Scientific and Technical Papers and Citations, and Chinese Science Citation Database (CSCD)

The inauguration of *China CDC Weekly* is in part supported by Project for Enhancing International Impact of China STM Journals Category D (PIIJ2-D-04-(2018)) of China Association for Science and Technology (CAST).

CCDC weekly

Vol. 5 No. 14 Apr. 7, 2023

Responsible Authority
National Health Commission of the People's Republic of China

Sponsor
Chinese Center for Disease Control and Prevention

Editing and Publishing
China CDC Weekly Editorial Office
No.155 Changbai Road, Changping District, Beijing, China
Tel: 86-10-63150501, 63150701
Email: weekly@chinacdc.cn

CSSN
ISSN 2096-7071
CN 10-1629/R1