CHINA CDC WEEKLY

CCDCweekly

Vol. 3 No. 53 Dec. 31, 2021

中 国 疾 病 预 防 控 制 中 心 周 报

Happy New Year

The past has gone and static,
nothing we do will change it;
The future is before us and dynamic,
everything we do will affect it.

CHINESE CENTER FOR DISEASE CONTROL AND PREVENTION

Molecular Epidemiological Characteristics of Gastroenteritis Outbreaks Caused by Norovirus GII.4 Sydney [P31] Strains — China, October 2016–December 2020

Xi Zhu[1,&]; Yaqing He[2,&]; Xingyan Wei[1]; Xiangyu Kong[1]; Qing Zhang[1]; Jingxin Li[1]; Miao Jin[1,#]; Zhaojun Duan[1,#]

ABSTRACT

Introduction: Human noroviruses are the leading cause of acute viral gastroenteritis (AGE) worldwide in all age groups. GII.4 strains have been the predominant genotype circulating globally over the last 2 decades and since 2012. GII.4 Sydney viruses have emerged and caused the majority of AGE outbreaks worldwide.

Methods: Data from norovirus outbreaks from the laboratory-based surveillance of norovirus outbreaks in China (CaliciNet China) between October 2016–December 2020 were analyzed.

Results: During October 2016–December 2020, 1,954 norovirus outbreaks were reported, and positive fecal samples from 1,352 (69.19%) outbreaks were genotyped. GII.4 Sydney [P31] viruses accounted for 2.1% (October 2016–August 2017), 5.5% (September 2017–August 2018), 3.3% (September 2018–August 2018), 26.6% (September 2019–August 2020), and and 1.1% (September 2020–December 2020) of GII outbreaks, respectively. Compared to reference strains of GII.4 Sydney [P31] from 2012 to 2013, 7 amino acid mutations in epitopes[A (297, 372 and 373), B (333), E (414), and H (309 and 310)] and 1 in human histo-blood group antigens binding site at site II 372 were found by analyzing 9 GII.4 Sydney [P31] complete genomic sequences.

Conclusions: This report identified the genomic variation of GII.4 Sydney [P31] from CaliciNet China. Continued surveillance with prompt genotyping and genetic analysis is necessary to monitor the emergence of novel GII.4 variants.

Human noroviruses (NoVs) are the leading cause of acute viral gastroenteritis (AGE) worldwide for all age groups (*1*). NoVs are highly contagious, making them the major cause of outbreaks, especially in semi-enclosed settings such as schools, hospitals, and nursing homes (*2*). Similar to other RNA viruses, NoVs gain novel genetic properties not only by accumulating mutations that make NoVs evade the host immune response but also by recombination at the junction of the open reading frame (ORF) 1 and 2 that can result in the emergence of a novel strain (*3*).

Among the more than 30 genotypes of norovirus that cause disease in humans, GII.4 viruses have been reported to cause the majority of norovirus infections worldwide (*4*). Novel GII.4 variants have emerged every several years, displacing previous dominant strains to cause a new wave of outbreaks (*4*). The GII.4 Sydney [P31] variant (previously named GII.Pe.-GII.4 Sydney) emerged in late 2012 and became a prevalent strain worldwide (*5*). GII.4 Sydney [P16] emerged in 2015 and replaced GII.4 Sydney [P31] as the predominant strain in the US and European countries in the 2016–2017 winter season (*5*).

GII.4 Sydney [P31] emerged in 2012 and caused outbreaks during the 2012–2013 season and has remained the predominant strain in sporadic cases up to the present day in China (*6*). Although non-GII.4 genotypes GII.17[P17] and GII.2[P16] caused outbreaks and increased in prevalence during the 2014–2015 and 2016–2017 seasons, respectively, GII.4 Sydney [P31] has been continuously detected in outbreak surveillance (*7–8*). This study described the outbreaks in China caused by GII.4 Sydney [P31] from October 2016 to December 2020, as well as the genome information of GII.4 Sydney [P31], the important amino acid mutation sites of antigen epitopes and receptor binding sites, human histo-blood group antigens (HBGAs), which were assumed to be associated with the epidemic pattern of GII.4 noroviruses.

Since 2016, laboratory-based surveillance of norovirus outbreaks in China (CaliciNet China) has been conducted, which is a network of county-level, city-level, and provincial CDCs coordinated by China CDC. As a representative member of the Caliciviridae family, norovirus has cup-shaped depressions under the electron microscope, which is the origin of the network name. A detailed description of CaliciNet China has

been reported previously (*8*). By 2018, the number of network laboratories has increased to 31, 14 provinces are covered, including 11 provincial-level network laboratories (Beijing, Chongqing, Shanghai, Hebei, Shandong, Jiangsu, Zhejiang, Hubei, Hunan, Fujian, and Guangdong CDC), 11 municipal network laboratories (Shenyang, Qingdao, Jinan, Wuxi, Huai'an, Nanjing, Mianyang, Guilin, Xiamen, Guangzhou, and Shenzhen CDC); and 8 district-level network laboratories (Beijing's Fengtai, Changping, Mentougou, Shunyi, and Chaoyang Districts, Shanghai's Changning District, Guangdong's Huadu District, and Shenzhen's Nanshan and Luohu District CDC). Norovirus outbreaks were defined as >5 epidemiologically linked AGE cases within 3 days with >2 norovirus positive samples confirmed through real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) (whole fecal, rectal swab, or vomitus) (*8*). Commercial norovirus real-time RT-PCR kits (BioPerfectus Technology Co., Jiangsu, China; Aodong Technology Co., Shenzhen, China) were used by network labs. Norovirus positive samples were then amplified using the dual polymerase-capsid genotyping protocol (*5*). The near-complete genome of GII.4 Sydney [P31] strains from 4 different outbreaks were sequenced by Sanger sequencing using primers designed by primer 5.0 (version 5.0, Premier, Palo Alto, Canada) (Supplementary Table S1, available in http://weekly.chinacdc.cn/). Contig assembly was performed by DNAstar (version 7.0, DNASTAR, Madison, Wisconsin, USA) and sequence alignments with Clustal W (version 2.0, Conway Institute UCD Dublin, Dublin, Ireland) (http://www.clustal.org/clustal2). Phylogenetic analysis of these sequences was performed in the MEGA (version 6.0, Mega Limited, Auckland, New Zealand). Phylogenetic trees with bootstrap analysis from 1,000 replicates were generated by using the neighbor-joining method.

During October 2016–December 2020, 1,954 norovirus outbreaks were reported to CaliciNet China. The numbers of cases were available in 1,631 outbreaks (83.47%) and ranged from 5 to 391 people per outbreak, with a median of 15 people. Of the 1,887 outbreaks with the reported setting, 852 (45.15%) occurred in childcare centers, followed by 565 (29.94%) in primary schools, 223 (11.81%) in middle schools, and 94 (4.98%) in other schools. Of 1,833 (93.80%) with reported transmission mode, person-to-person (1,748, 95.36%) was predominant, followed by 59 (3.21%) foodborne and 26 (1.42%) waterborne transmission. The season peaks occurred from November–December and in March of the subsequent year (Figure 1).

Among the 1,352 outbreaks (69.19%) that were genotyped, 1,244 cases (92.01%) were GII positive, 63 cases (4.66%) were GI positive, and 45 cases (3.33%) were mixed genotypes. Overall, 10 GI genotypes and 16 GII genotypes were detected. Among the GII outbreaks, 69.0% (859/1,244) were GII.2 [P16], followed by GII.3 [P12] [101 (8.12%)], GII.6 [P7] [82 (6.59%)], GII.4 Sydney [P31] [77 (6.19%)], and GII.17 [P17] [73 (5.87%)]. Other genotypes accounted for less than 5%. The proportion of GII.4 Sydney[P31] in different epidemic seasons of GII outbreak was 2.1% (October 2016–August 2017), 5.5% (September 2017–August 2018), 3.3% (September 2018–August 2019), 26.6% (September 2019–August 2020), and 1.12% (September 2020–December 2020) (Figure 1).

In the GII.4 Sydney [P31] outbreak, foodborne transmission accounted for 2.60% (2/77), water-borne transmission accounted for 2.60% (2/77), and interpersonal transmission accounted for 87.01% (67/77). GII.4 Sydney [P31] outbreak sites include kindergartens (63.63%, 49/77), primary schools (13.00%, 10/77), hospitals (5.19%, 4/77), families or communities (3.90%, 3/77), nursing homes (2.60%, 2/77), junior high schools (1.30%, 1/77), universities (1.30%, 1/77), other schools (1.30%, 1/77), and companies (1.30%, 1/77) (Figure 2).

A total of 9 near-complete genomes of GII.4 Sydney [P31] strain were obtained from 4 outbreaks by Sanger sequencing during 2019–2020 (1 in 2019 and 8 in 2020). Sequence alignment showed that the strain from this study shared a pairwise identity of 95.6%–99.9% at the nucleotide level and 98.3%–100% at the amino acid level. According to the phylogenetic analysis in ORF2, the Sydney 2012 clades can be further divided into 2 subclades, according to the GII.P16 and GII.P31 polymerases. The strains from this study belonged to GII.4 Sydney 2012 [P31] subclades, which showed a general time distribution (2012–2013, 2014–2017, and 2018–2020 clusters). In addition, 2 strains from an outbreak in August 2020 formed a branch independently (Figure 3). The amino acid changes of GII.4 Sydney strain blood group antigen receptor binding site (HBS) tended to be conservative, and only amino acid mutations were found at site II 372 (Figure 4A). Through epitope analysis, the strains after 2014 have changed in epitopes A (297, 372 and 373), B (333), E (414), and H (309 and 310). A total of 4 stains (20HN253–20HN261) from 1 outbreak in December
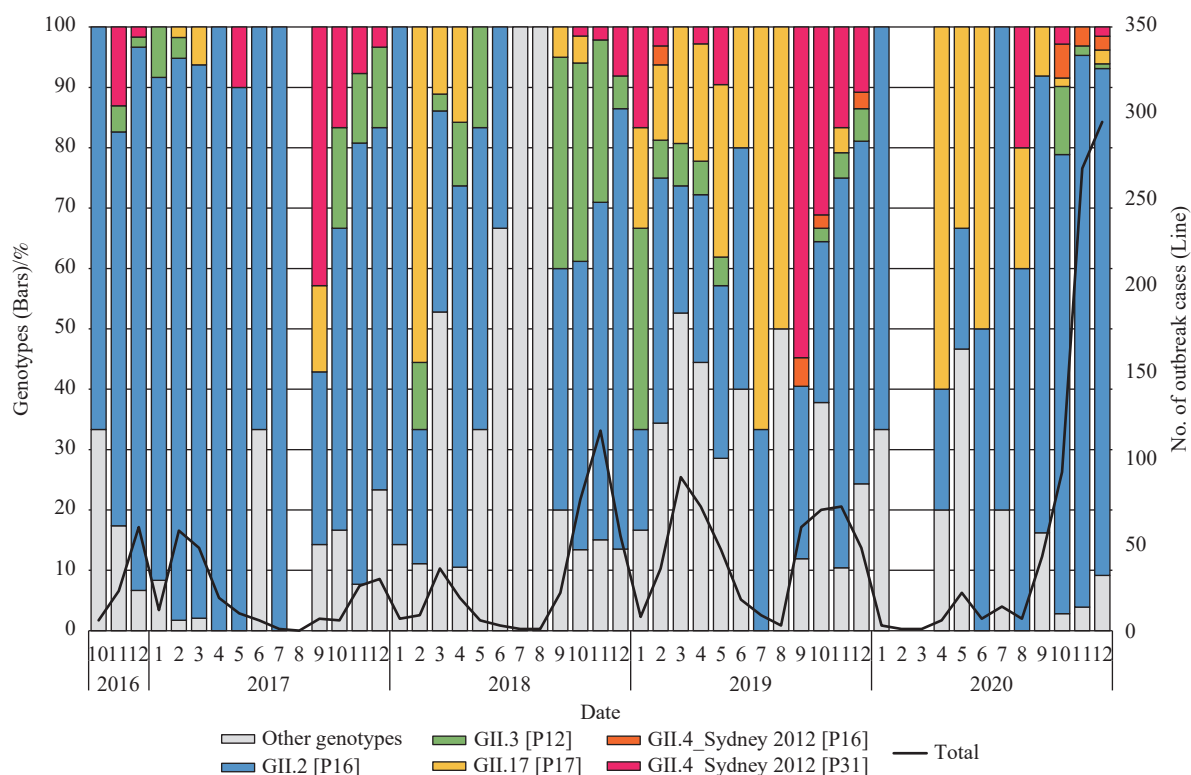
FIGURE 1. Monthly trends of norovirus genotypes for outbreak cases of acute gastroenteritis outbreak uploaded to CaliciNet China, October 2016–December 2020.
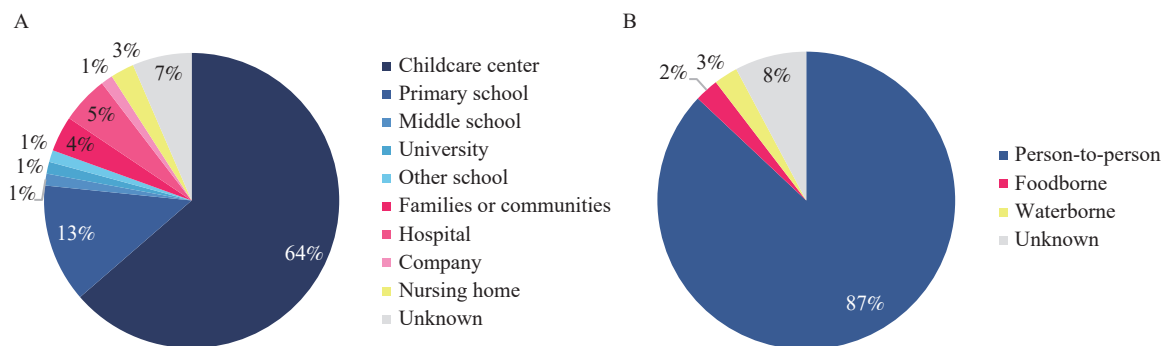
FIGURE 2. Settings and transmission routes of GII.4 Sydney[P31] norovirus outbreaks uploaded to CaliciNet China, October 2016–December 2020. (A) the outbreak settings of GII.4 Sydney [P31] norovirus outbreaks. (B) the transmission routes of GII.4 Sydney[P31] norovirus outbreaks.

2020 showed new changes in A epitope (368) and G epitope (355) compared with the previous strains (Figure 4B).

DISCUSSION

Since CaliciNet China was launched in October 2016 in China, more laboratories were included to report norovirus outbreaks data and sequence information. Therefore, prompt analysis of the genetic characteristics and variation of norovirus strains from

China was available. Moreover, the real-time monitoring of the emergence of new norovirus variants has strengthened the capacity on early warning and prediction of norovirus outbreaks in China.

Our study showed the epidemiological characters of outbreaks by noroviruses and identified GII.2 [P16] as the predominant strain in China during October 2016 and December 2020. Similar to studies from other countries in the northern hemisphere, the peak of norovirus outbreaks mainly occurred from October to March of the next year (9). Like other Asian countries
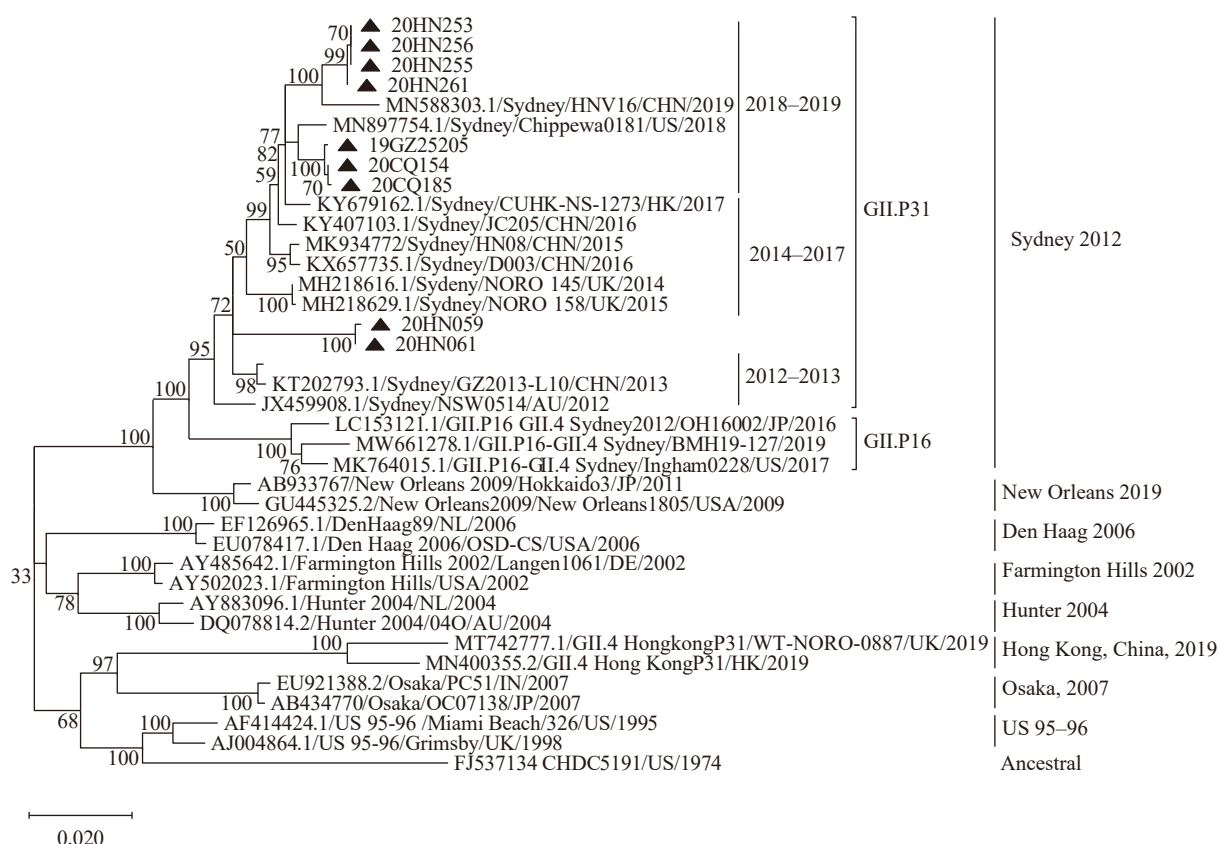
FIGURE 3. Neighbor-joining phylogeny of complete sequences of open reading frame 2 of norovirus GII.4 variants.
Note: Strains from this study were marked with a triangle.

such as Japan and the Republic of Korea, schools and kindergartens were also identified as the most common places of norovirus outbreaks in China (*10–11*). The high proportion of norovirus outbreaks in kindergartens and schools may be related to the high population density in these environments, as well as our enhanced monitoring and reporting of school outbreaks in China (*12*).

Since mid-1995, the genotype causing the global outbreak of norovirus has only been related to the GII.4 genotype (*4*). Since 2012, the GII.4 Sydney variant has been predominant all over the world. Therefore, the monitoring of GII.4 Sydney strain is important for potentially emerging GII.4 variants (*5*). This study described the epidemiological characteristic and the genome variation of GII.4 Sydney [P31]. It was consistent that GII.4 viruses have been associated with person-to-person transmission both from this study and other previous data from CaliciNet USA and NoroNet. The GII.4 Sydney [P31] outbreaks in our study mainly occurred in childcare centers and primary schools. In other parts of the world, GII.4 Sydney viruses have been reported as the dominant genotype among adults and the elderly especially in outbreaks in

long-term care facilities (*13*).

Much evidence from our study suggested that GII.4 Sydney [P31] was undergoing evolution. First, according to the phylogenetic tree, GII.4 Sydney strains with P31 polymerase were clustered in three lineages by epochal evolution (2013–2014, 2014–2017, and 2018–2019) (Figure 3). GII.4 Sydney outbreak strains in this study were mainly clustered in lineage 2018–2019. However, two GII.4 Sydney strains from an outbreak that occurred in 2020 formed an independent branch. Second, compared to reference strains from 2012 to 2013, these GII.4 Sydney strains from 2014–2020 exhibited some changes or switches that occurred in HBGAs binding sites (II) and in epitope sites (A, B, E, G, and H). Notably, GII.4 Sydney outbreak strains in 2020 from our study had unique mutated changes at sites 368 (epitope A) and 355 (epitope G) (Figure 4). Previous studies have confirmed that the amino acid composition of several antigenic epitopes (A, C, D, E, and G) was related to the epidemic pattern of GII.4 variant. A total of 3 epitopes (A, C, and G) contained amino acid residues (352, 357, 368, and 378), which were related to the diversified selection pressure of the new GII.4 variant

A

GII.4 Sydney strain	Major components of the HBGA binding sites																								
	Site I												Site II							Site III						
	338	339	340	341	342	343	344	345	346	347	348	349	370	371	372	373	374	375	376	440	441	442	443	444	445	446
JX459908.1_AU_2012	T	R	T	D	G	S	T	R	G	H	K	A	D	T	D	R	D	F	E	G	C	S	G	Y	P	N
KT202793_CHN_2013	T	R	T	D	G	S	T	R	G	H	K	A	D	T	D	R	D	F	E	G	C	S	G	Y	P	N
MH218616.1_UK_2014	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	H	D	F	E	G	C	S	G	Y	P	N
MK934772_CHN_2015	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
KY407103_CHN_2016	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
KY679162_HK_2017	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
MN897754.1_US_2018	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
19GZ25205(2019)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
20HN059(2020.8)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	H	D	F	E	G	C	S	G	Y	P	N
20HN061(2020.8)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	H	D	F	E	G	C	S	G	Y	P	N
20CQ154(2020.10)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
20CQ185(2020.10)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
20HN253(2020.12)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
20HN255(2020.12)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
20HN256(2020.12)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N
20HN261(2020.12)	T	R	T	D	G	S	T	R	G	H	K	A	D	T	N	N	D	F	E	G	C	S	G	Y	P	N

B

		Capsid protein																												
		A							B		C		D					E					G						H	
	294	295	296	297	298	368	372	373	333	389	340	376	393	394	395	396	397	407	411	412	413	414	352	355	356	357	359	364	309	310
JX459908_AU_2012	T	G	S	R	N	E	D	R	V	I	T	E	G	T	T	H	R	S	R	N	T	H	Y	S	A	D	A	R	N	D
KT202793_CHN_2013	T	G	S	R	N	E	D	R	V	I	T	E	G	T	T	H	R	S	R	N	T	H	Y	S	A	D	A	R	N	N
MH218616_UK_2014	T	G	S	H	N	E	N	H	V	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
MK934772_CHN_2015	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
KY407103_CHN_2016	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
KY679162_HK_2017	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
MN897754_US_2018	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
19GZ25205(2019)	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
20HN059(2020.8)	T	G	S	H	N	E	N	H	V	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
20HN061(2020.8)	T	G	S	H	N	E	N	H	V	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
20CQ154(2020.10)	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
20CQ185(2020.10)	T	G	S	H	N	E	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	S	A	D	A	R	S	N
20HN253(2020.12)	T	G	S	H	N	Q	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	G	A	D	A	R	S	N
20HN255(2020.12)	T	G	S	H	N	Q	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	G	A	D	A	R	S	N
20HN256(2020.12)	T	G	S	H	N	Q	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	G	A	D	A	R	S	N
20HN261(2020.12)	T	G	S	H	N	Q	N	N	M	I	T	E	G	T	T	H	R	S	R	N	T	P	Y	G	A	D	A	R	S	N

FIGURE 4. Sequence analysis of GII.4 Sydney strains. (A) The sequence analysis of histo-blood group antigen (HBGA)–binding interface of the GII.4 major capsid protein VP1 protein. (B) The sequence analysis of antigenic residues of major capsid protein of GII.4 Sydney strains.
Note: Residues mapping on previously characterized A–H epitopes. Amino acid residues that differ from those of the prototype were highlighted in blue. Sequences of this study were in bold.
Abbreviations: HBGA=histo-blood group antigen.

(*14*). These residue changes were likely to enable the norovirus to escape the pressure of population immunity and cause a global epidemic of norovirus once again.

In addition to antigenic drift, recombination resulting in polymerase switching was also an important mechanism for the evolution of noroviruses (*3*). Acquisition of the GII.P16 polymerase and/or associated nonstructural proteins appeared to be the impetus for the predominance of GII.P16-GII.4 Sydney viruses in 2015 to 2016 in the USA (*5*).

However, a limited number of studies have evaluated the evolutionary rate of the ORF1 gene. Each genotype has an evolutionary mechanism. Evolutionary analyses may vary depending on the genotype and the database used (*15*). Future studies are needed to determine what structural differences contemporary GII.P16 polymerases have gained and what the functional role of these changes is.

There were several limitations in this study. First, local CDCs participated in the network voluntarily, so these data may only represent places covered by

Calicinet China and are not generalizable. Still, China CDC is constantly making efforts to include more laboratories and build a more comprehensive surveillance system. Second, the epidemiological information collected in each outbreak was not sufficient, hindering further analysis. Third, the timeliness of the data reporting in CaliciNet China needs to be improved. Due to the increasing burden of laboratory testing caused by COVID-19, not all network members could submit data to China CDC on a monthly basis. In the future, a web-based information submission system will contribute to the near real-time surveillance network.

In conclusion, the molecular epidemiological characteristics of norovirus outbreaks and the genome variation of GII.4 Sydney [P31] from CaliciNet China were analyzed. Data suggested that GII.4 Sydney [P31] had experienced minor but important changes in HBGAs binding sites and antigenic epitope sites associated with the epidemic pattern of GII.4 variant, which should be monitored by continuous surveillance with real-time genotyping and genetic analysis for the emergence of novel GII.4 variant.

Corresponding authors: Miao Jin, jinmiao@ivdc.chinacdc.cn; Zhaojun Duan, zhaojund@126.com.

¹ Key Laboratory of Medical Virology and Viral Diseases, Ministry of Health of the People's Republic of China, Beijing, China; National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China; ² Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China.
& Joint first authors.

REFERENCES

1. Lopman BA, Steele D, Kirkwood CD, Parashar UD. The vast and varied global burden of norovirus: prospects for prevention and control. PLoS Med 2016;13(4):e1001999. http://dx.doi.org/10.1371/journal.pmed.1001999.
2. Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. Gastroenterol Clin North Am 2006;35(2):275 – 90. http://dx.doi.org/10.1016/j.gtc.2006.03.001.
3. Martella V, Medici MC, De Grazia S, Tummolo F, Calderaro A, Bonura F, et al. Evidence for recombination between pandemic GII.4 norovirus strains New Orleans 2009 and Sydney 2012. J Clin Microbiol 2013;51(11):3855–7. http://dx.doi.org/10.1128/JCM.01847-13.
4. Lindesmith LC, Donaldson EF, Baric RS. Norovirus GII.4 strain antigenic variation. J Virol 2011;85(1):231 – 42. http://dx.doi.org/10.1128/JVI.01364-10.
5. Cannon JL, Barclay L, Collins NR, Wikswo ME, Castro CJ, Magaña LC, et al. Genetic and epidemiologic trends of norovirus outbreaks in the United States from 2013 to 2016 demonstrated emergence of novel GII.4 recombinant viruses. J Clin Microbiol 2017;55(7):2208 – 21. http://dx.doi.org/10.1128/JCM.00455-17.
6. He YQ, Jin M, Chen KN, Zhang HL, Yang H, Zhuo F, et al. Gastroenteritis outbreaks associated with the emergence of the new GII.4 Sydney norovirus variant during the epidemic of 2012/13 in Shenzhen city, China. PLoS One 2016;11(11):e0165880. http://dx.doi.org/10.1371/journal.pone.0165880.
7. Jin M, Zhou YK, Xie HP, Fu JG, He YQ, Zhang S, et al. Characterization of the new GII.17 norovirus variant that emerged recently as the predominant strain in China. J Gen Virol 2016;97(10):2620 – 32. http://dx.doi.org/10.1099/jgv.0.000582.
8. Jin M, Wu SY, Kong XY, Xie HP, Fu JG, He YQ, et al. Norovirus outbreak surveillance, China, 2016-2018. Emerg Infect Dis 2020;26(3):437 – 45. http://dx.doi.org/10.3201/eid2603.191183.
9. Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinjé J. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol 2014;52(1):147 – 55. http://dx.doi.org/10.1128/JCM.02680-13.
10. Thongprachum A, Khamrin P, Maneekarn N, Hayakawa S, Ushijima H. Epidemiology of gastroenteritis viruses in Japan: prevalence, seasonality, and outbreak. J Med Virol 2016;88(4):551 – 70. http://dx.doi.org/10.1002/jmv.24387.
11. Cho HW, Chu C. Norovirus outbreaks occurred in different settings in the Republic of Korea. Osong Public Health Res Perspect 2015;6(5):281 – 2. http://dx.doi.org/10.1016/j.phrp.2015.11.001.
12. Bureau of Disease Prevention and Control, National Health Commission of the People's Republic of China. Criterion for reporting infectious diseases in schools and child-care institutions (for trial implementation). http://www.nhc.gov.cn/zhjcj/s9139/200804/b7fc75fdc7ee43afaadc76803dbbbaff.shtml. [2007-3-5]. (In Chinese).
13. Bok K, Abente EJ, Realpe-Quintero M, Mitra T, Sosnovtsev SV, Kapikian AZ, et al. Evolutionary dynamics of GII.4 noroviruses over a 34-year period. J Virol 2009;83(22):11890 – 901. http://dx.doi.org/10.1128/JVI.00864-09.
14. Bull RA, Eden JS, Rawlinson WD, White PA. Rapid evolution of pandemic noroviruses of the GII. 4 lineage. PLoS Pathog 2010;6(3):e1000831. http://dx.doi.org/10.1371/journal.ppat.1000831.
15. Ozaki K, Matsushima Y, Nagasawa K, Motoya T, Ryo A, Kuroda M, et al. Molecular evolutionary analyses of the RNA-dependent RNA polymerase region in norovirus genogroup II. Front Microbiol 2018;9:3070. http://dx.doi.org/10.3389/fmicb.2018.03070.

SUPPLEMENTARY TABLE S1. Primers designed for amplifying and sequencing GII.4 [P31] NoV genomes in this study.

Primer	Sequence (5' to 3')	Fragment length (bp)	Annealing temperature (℃)
SYD-ORF1-1-F	GCCAACAGCAACAACGAC	1,116	52.3 ℃
SYD-ORF1-1-R	TTAAGATTTCCAGTCCGTAT		
sydney-ORF1-2-F	GGGGATAGGTTTGGTGCT	1,220	53.5 ℃
sydney-ORF1-2-R	TCGCGATGTTCTTGAGTG		
sydney-ORF1-3-F	CAGCCCTCACCACTTTCA	1,292	53.8 ℃
sydney-ORF1-3-R	CATACCCATTTGCCCTCC		
sydney-ORF1-4-F	GTGACGGGCATGATTCTA	1,166	53.8 ℃
sydney-ORF1-4-R	ACTATCTGGGCCAGGTGT		
sydney-ORF1-5-F	GGATGAACTCAAGGCACA	1,382	52.4 ℃
sydney-ORF1-5-R	GGGTAAGGGAATCAACAC		
NEO_GII.4_SYD_ORF2_F	AGTGCAGGTAATTCTCGC	1,253	52.3 ℃
NEO_GII.4_SYD_ORF2_R	TGACCCAGGAATCAAACC		
II4SYDORF3-F	TTTGTGAATCCAGACAC	933	49.8 ℃
II4SYDORF3-R	CAGTCCAGGAGTCCAAAA		

Abbreviations: NoV=noroviruse.

Vital Surveillances

Changing Proportions of HIV-1 Subtypes and Transmitted Drug Resistance Among Newly Diagnosed HIV/AIDS Individuals — China, 2015 and 2018

Jingjing Hao[1]; Shan Zheng[1]; Mengze Gan[1]; Aobo Dong[1]; Ruihua Kang[1]; Miaomiao Li[1]; Shuai Zhao[1]; Jing Hu[1]; Chang Song[1]; Lingjie Liao[1]; Yi Feng[1]; Yiming Shao[1]; Yuhua Ruan[1]; Hui Xing[1,#]

ABSTRACT

Introduction: With the expansion of human immunodeficiency virus (HIV) antiretroviral therapy (ART), HIV drug resistance is becoming more and more serious. This study describes the changing prevalence of HIV-1 subtypes and transmitted drug resistance (TDR) among newly diagnosed individuals in China, 2015 and 2018.

Methods: A total of 8,980 individuals in 2015 and 2018 from 31 provincial-level administrative divisions (PLADs) were enrolled in this study. Viral RNAs were amplified and sequenced using an in-house polymerase chain reaction (PCR) protocol. The Stanford HIV Drug Resistance Database (HIVdb) was used to predict susceptibility to 12 antiretroviral drugs.

Results: The prevalence of TDR was not significantly increased over time. The prevalence of TDR was 3.8% and 4.4% in 2015 and 2018, respectively (P=0.13). The prevalence of CRF55_01B increased from 2.3% in 2015 to 3.9% in 2018 (P<0.001). The drug resistance prevalence of non-nucleoside reverse transcriptase inhibitors (NNRTI) increased from 2.4% in 2015 to 3.3% in 2018 (P<0.01). The prevalence of E138 (P<0.001), H221 (P=0.03), and V179 (P<0.001) mutations increased from 0.30%, 0.09%, and 0.70% in 2015 to 1.10%, 0.30%, and 1.70% in 2018, respectively.

Conclusions: HIV drug resistance affects the effect of antiretroviral treatment, so the monitoring of HIV TDR should be strengthened to control the transmission of HIV drug resistance.

INTRODUCTION

The four main human immunodeficiency virus (HIV)-1 subtypes in China over the past 20 years include CRF07_BC, CRF01_AE, CRF08_BC, and subtype B. The prevalence of CRF07_BC and CRF01_AE has increased since 2006 and they remain the dominant subtypes in China present in 31 provincial-level administrative divisions (PLADs) (*1*). In 2012, a study reported the HIV-1 CRF55_01B subtype, composed of CRF01_AE and subtype B, which possibly originated in men having sex with men (MSM) in Shenzhen (*2*). After its origin in Shenzhen, it spread rapidly to all cities in China (*3*). Various factors contribute to the spread and diversification of HIV-1 subtypes in China, including the emergence of more dating apps, the rapid development of the economy and means of transportation, and people moving to different parts of the country for employment.

From 2012, several first-line antiretroviral drugs were available to HIV patients for free, including TDF/AZT+3TC+EFV/NVP. In 2014, only HIV patients with a cluster of differentiation 4 (CD4) cell count of less than 500 cells/µL were eligible to receive ART. The Chinese government adjusted the standard for free ART in 2016 and recommended that all HIV patients receive ART (*4*). Adherence to ART is critical to achieving viral suppression; however, poor drug adherence or withdrawal from treatment can result in the development of drug resistance (*5*). Moreover, the problem of drug resistance can be exacerbated by the expansion of ART regimens. One possible solution would be conducting TDR surveys, which can offer an effective guide for structuring future first- and second-line ART regimens.

METHODS

The study design was conducted according to the WHO protocol for TDR in each region of China. Depending on the number of newly reported HIV/AIDS cases, China was divided into high prevalence regions, moderate prevalence regions and low prevalence regions. At least 300 patients were recruited in high prevalence regions, moderate

prevalence regions and low prevalence regions (6–7). The individuals (16 years old or older at the time of HIV-1 diagnosis) diagnosed with HIV-1 infection in 2015 and 2018 were enrolled in this study. All individuals provided written informed consent. Study variables included the following epidemiological and clinical characteristics: age, sex, education, HIV-1 transmission route, and HIV-1 subtype. Plasma from participants was collected and sent to the laboratory via cold chain transport.

Viral RNAs were extracted from 200 µL of plasma using the QIAsymphony platform. The pol fragment (HXB2 positions 2,253–3,312, 1,060 bp) was amplified and sequenced using an in-house PCR protocol. The nucleotide sequences were aligned separately using the HIV Align tool (https://mafft. cbrc.jp/alignment/server/add_fragments.html). The aligned sequences were manually adjusted using BioEdit (version 7.0.9.1, Borland, CA, USA). Sequences less than 1,000 nucleotides in length were excluded from the analysis. HIV-1 subtypes were determined based on a neighbor-joining tree with Mega [Mega 7.0: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar S, Stecher G, and Tamura K 2016)]. The Stanford HIV Drug Resistance Database (HIVdb) (https://HIV-1db.stanford.edu/HIV-1db/by-sequences) genotypic resistance interpretation system was used to predict susceptibility to 12 antiretroviral drugs recommended for use by the WHO (EFV, NVP, ABC, AZT, 3TC, TDF, FTC, D4T, DDI, LPV/r, ATV/r, and DRV/r). This system assigns a drug penalty score to each mutation. Scores for individual mutations are greater than -15: negative numbers indicate increased susceptibility compared to wild type virus; 0 indicates no change to susceptibility; susceptible (0–9), potential low-level resistance (10–14), low-level resistance (15–29), intermediate resistance (30–59), and high-level resistance (≥60). Drug resistance is defined as 15 points or more.

SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The χ^2 test was used to analyze variations of 12 types of drugs and drug resistance mutations in 2015 and 2018. Univariate and multivariate logistic regression tests were used to analyze the factors associated with drug resistance, with $P<0.05$ considered as statistically significant.

RESULTS

A total of 8,980 individuals were diagnosed with HIV-1 in 2015 and 2018, with 4,704 diagnosed in 2015 and 4,276 in 2018. The characteristics of the individuals included in this study are listed in Table 1. The proportions of HIV-1 individuals aged 26–49 and ≥50 years old were 56.8% and 24.8%, respectively. Approximately 81.9% of HIV-1 individuals were men. About 40.6% of HIV-1 individuals graduated from high school or had higher education. The main pattern of transmission was heterosexual intercourse (52.5%).

The most common HIV-1 subtype was CRF07_BC (39.3%), followed by CRF01_AE (36.2%), CRF08_BC (8.9%), B (4.3%), and CRF55_01B (3.1%) (Table 1). The prevalence of CRF55_01B increased from 2.3% in 2015 to 3.9% in 2018 ($P<0.001$) (Table 2).

The TDR prevalence was 3.80% in 2015 and 4.40% in 2018; overall TDR prevalence did not change significantly between 2015 and 2018. TDR prevalence of NNRTI, NRTI, and PI changed from 2.40%, 1.10%, and 0.20% in 2015, to 3.30%, 0.80%, and 0.07% in 2018, respectively. The increased prevalence of NNRTI drug resistance was from 2.40% in 2015 to 3.30% in 2018 ($P<0.01$), whereas no increase in drug resistance was observed for NRTI and PI. Moreover, the prevalence of certain mutations, including E138, H221, and V179, significantly increased from 0.30%, 0.09%, and 0.70% in 2015 to 1.10%, 0.30%, and 1.70% in 2018, respectively (Table 3).

Multivariate logistic regression analysis showed that CRF08_BC [adjusted odds ratio (AOR)=1.51, 95% confidence interval (CI): 1.03–2.23], CRF55_01B (AOR=4.17, 95% CI: 2.77–6.27), and URF (AOR=1.69, 95% CI: 1.14–2.49) were independent factors associated with TDR (Table 4).

DISCUSSION

In China, CRF07_BC and CRF01_AE were still the two main subtypes. The proportion of the CRF55_01B subtype increased from 2015 to 2018. As a new recombinant subtype formed by the recombination of CRF01_AE and subtype B, CRF55_01B prevalence increased exponentially from 2005 to 2009 after its origin in Shenzhen. One of the factors contributing to its rapid transmission was the spread of the virus to different PLADs in 2007, as mediated by the rapid development of the Beijing-Guangzhou and Beijing-Kowloon railways (8). Moreover, even though this particular subtype

TABLE 1. General characteristics of newly diagnosed HIV individuals in 2015 and 2018, China.

Variable	2015		2018		Total	
	Number of cases	Proportion (%)	Number of cases	Proportion (%)	Number of cases	Proportion (%)
Total	4,704	100.0	4,276	100.0	8,980	100.0
Age (years)						
16–25	862	18.3	676	15.8	1,538	17.1
26–49	2,749	58.5	2,350	55.0	5,099	56.8
≥50	1,003	21.3	1,227	28.7	2,230	24.8
Unknown	90	1.9	23	0.5	113	1.3
Sex						
Male	3,763	80.0	3,595	84.1	7,358	81.9
Female	876	18.6	681	15.9	1,557	17.4
Unknown	65	1.4	0	0.0	65	0.7
Education						
Primary school	1,338	28.5	993	23.2	2,331	26.0
Junior high school	1,412	30.0	1,205	28.2	2,617	29.1
Senior high school or above	1,769	37.6	1,877	43.9	3,646	40.6
Unknown	185	3.9	201	4.7	386	4.3
Route of HIV transmission						
HET	2,533	53.9	2,181	51.0	4,714	52.5
MSM	1,855	39.4	1,906	44.6	3,761	41.9
IDU	115	2.4	53	1.2	168	1.9
Others	201	4.3	136	3.2	337	3.7
Subtype						
CRF01_AE	1,835	39.0	1,578	36.9	3,413	38.0
CRF07_BC	1,675	35.6	1,696	39.7	3,371	37.5
CRF08_BC	418	8.9	384	9.0	802	8.9
CRF55_01B	110	2.3	168	3.9	278	3.1
B	261	5.6	183	4.3	444	5.0
Others	405	8.6	267	6.2	672	7.5

Abbreviations: HIV=human immunodeficiency virus; HET=heterosexual; MSM=men who have sex with men; IDU=injecting drug users.

TABLE 2. Changes in HIV subtypes among newly diagnosed HIV individuals in 2015 and 2018, China.

Subtype	2015		2018		P
	Number of cases	Proportion (%)	Number of cases	Proportion (%)	
CRF01_AE	1,835	39.0	1,578	36.9	0.04
CRF07_BC	1,675	35.6	1,696	39.7	<0.0001
CRF08_BC	418	8.9	384	9.0	0.88
CRF55_01B	110	2.3	168	3.9	<0.0001
B	261	5.6	183	4.3	0.006
Others	405	8.6	267	6.2	<0.0001

Abbreviation: HIV=human immunodeficiency virus.

originated in homosexual males, it was transmitted among heterosexuals. Currently, in-depth studies on CRF55_01B are lacking, especially as it relates to the underlying mechanism of drug resistance.

The TDR prevalence was 3.80% in 2015 and 4.40% in 2018, with no significant increase over time. A study

TABLE 3. Changes of HIV mutations among newly diagnosed HIV individuals in 2015 and 2018, China.

Variable	2015		2018		P
	Number of cases	Proportion (%)	Number of cases	Proportion (%)	
Total	178	3.80	189	4.40	0.13
NNRTI	113	2.40	142	3.30	0.01
A98G	5	0.10	7	0.20	0.46
E138A/G/K/Q	13	0.30	45	1.10	<0.001
G190A	13	0.30	9	0.20	0.53
H221Y	4	0.09	12	0.30	0.03
K101E	15	0.30	11	0.30	0.59
K103N/S	34	0.70	34	0.80	0.69
L100I	1	0.02	1	0.02	1.00
P225H	1	0.02	5	0.10	0.11
V106I/M	16	0.30	23	0.50	0.15
V108I	15	0.30	8	0.20	0.22
V179A/D	35	0.70	72	1.70	<0.001
Y181C	7	0.20	11	0.30	0.25
NRTI	52	1.10	33	0.80	0.10
D67N	3	0.06	4	0.09	0.72
K219Q	3	0.06	2	0.05	1.00
K65R	7	0.10	7	0.20	0.86
K70E/R	8	0.20	8	0.20	0.85
L210W	7	0.20	3	0.07	0.35
L74I	2	0.04	2	0.05	1.00
M184I/V	15	0.30	9	0.20	0.32
M41L	9	0.20	5	0.10	0.37
T69D/N	6	0.10	0	0.00	0.03
V75M/A	2	0.04	2	0.05	1.00
Y115F	1	0.02	1	0.02	1.00
PI	7	0.20	3	0.07	0.26
I50V/L	1	0.02	1	0.02	1.00
I54L/M	1	0.02	0	0.00	1.00
M46I/L	3	0.06	2	0.05	1.00
F53L	1	0.02	0	0.00	1.00
I47V	2	0.04	0	0.00	1.00
L90M	0	0.00	1	0.02	0.96
L33F	3	0.06	1	0.02	0.63

Abbreviations: HIV=human immunodeficiency virus; NNRTI=non-nucleoside reverse transcriptase inhibitor; NRTI=nucleoside reverse transcriptase inhibitor; PI=protease inhibitor.

that included TDR prevalence data from 2001 to 2017 in China estimated that TDR prevalence was 4.1% (9), which is consistent with the results of this study. Reports on drug resistance testing from the WHO revealed that between 2014 and 2019, the median rate of transmission-resistant drugs was 4.1% in Southeast Asia, 6.0% in Sub-Saharan Africa, 9.1% in Latin America, 8.5% in Europe, and 14.2% in North America (10). Conversely, the prevalence of TDR was much higher in Europe and North America, which was consistent with the longer existence and variety of antiviral treatment.

The prevalence of transmitted NNRTI resistance increased from 2015 to 2018. According to the analysis of resistance mutations, the prevalence of the E138, H221, and V179 mutations increased. V179E is a nonpolymorphic mutation weakly selected by NVP and EFV, E138G is another nonpolymorphic accessory mutation occasionally in patients receiving NVP and EFV (11). Molecular monitoring results of newly diagnosed individuals in China showed that the most common mutations in patients' resistance to NNRTI were E138G and V179E (12). Moreover, E138K was found to be the most common mutation in HIV-1 patients resistant to NNRTI in the US, according to an analysis of TDR from 2014 to 2018 (10). The impact of the combination of E138 and V179 on both NNRTI susceptibility and virologic outcome in patients deserves investigation.

CRF55_01B was associated with drug resistance. Moreover, V179E often appears in CRF55_01B together with E138G and has low resistance to EFV and NVP (13). The rapid spread of the CRF55_01B subtype across China was mediated by an increase of plasma HIV RNA load and the relatively lower CD4 count (14). Moreover, these factors may prolong the asymptomatic phase and increase the risk of HIV transmission, which may soon lead to further increases and a potential epidemic. Finally, these factors may also help explain the recent surging dominance and continued expansion of this subtype among MSM in China.

This study was subject to several limitations. First, during the onsite questionnaire survey, the information was not filled in detail, such as ID number, so there were deviations in the data analysis. The second is that the two-year sequences were obtained by the same method, but not by the same team, so there will be certain differences in comparison. The experimental methods were the same, but the Stanford drug resistance discriminant version was different. This study retested for resistance against the latest version.

Although the overall prevalence of TDR was low in China, it is necessary to remain vigilant, especially of

TABLE 4. Factors associated with HIV drug resistance among newly diagnosed HIV individuals in 2015 and 2018, China.

Factors	Total	TDR (%)	OR (95% CI)	P	AOR (95% CI)	P
Total	8,980	367 (4.1)				
Age (years)						
16–25	2,230	78 (3.5)	1.00		1.00	
26–49	5,099	209 (4.1)	1.24 (0.95–1.61)	0.12	1.21 (0.93–1.58)	0.16
≥50	1,538	72 (4.7)	1.41 (1.02–1.95)	0.04	1.39 (1.00–1.94)	0.05
Unknown	113	8 (7.1)	2.11 (1.00–4.48)	0.05	2.05 (0.96–4.37)	0.06
Sex						
Male	7,358	299 (4.1)	1.00			
Female	1,557	65 (4.2)	1.03 (0.78–1.35)	0.84		
Unknown	65	3 (4.6)	1.12 (0.35–3.60)	0.84		
Education						
Primary school	2,331	90 (3.9)	1.00			
Junior high school	2,617	120 (4.6)	1.20 (0.91–1.58)	0.21		
Senior high school or above	3,646	147 (4.0)	1.05 (0.80–1.37)	0.74		
Unknown	386	10 (2.6)	0.66 (0.34–1.28)	0.22		
Route of HIV transmission						
HET	4,714	201 (4.3)	1.00			
MSM	3,761	140 (3.7)	0.87 (0.70–1.08)	0.21		
IDU	168	11 (6.5)	1.57 (0.84–2.95)	0.16		
Others	337	15 (4.5)	1.04 (0.61–1.78)	0.89		
Subtype						
CRF01_AE	3,413	135 (4.0)	1.00		1.00	
CRF07_BC	3,371	107 (3.2)	1.26 (0.97–1.63)	0.08	1.25 (0.97–1.62)	0.09
CRF08_BC	802	37 (4.6)	1.48 (1.01–2.16)	0.05	1.51 (1.03–2.23)	0.04
CRF55_01B	278	34 (12.2)	4.25 (2.83–6.39)	<0.001	4.17 (2.77–6.27)	<0.001
B	444	19 (4.3)	1.36 (0.83–2.25)	0.22	1.36 (0.83–2.24)	0.23
Others	672	35 (5.2)	1.67 (1.13–2.47)	0.01	1.69 (1.14–2.49)	0.01
Year						
2015	4,704	178 (3.8)	1.00			
2018	4,276	189 (4.4)	1.18 (0.95–1.45)	0.13		

Abbreviations: HIV=human immunodeficiency virus; TDR=transmitted drug resistance; OR=odds ratio; CI=confidence interval; AOR=adjusted odds ratio; HET=heterosexual; MSM=men who have sex with men; IDU=injecting drug users.

CRF55_01B. Strengthened surveillance of TDR can help reduce the transmission of TDR to the key target populations, especially MSM. In 2018, TDR reached a moderate epidemic level in Xinjiang Uygur Autonomous Region and other regions. Key areas should pay attention and take measures to control the spread of drug resistance. Reducing the prevalence of TDR is important for formulating future treatment and prevention guidelines. Surveillance and prevention of drug resistance should be a critical component of China's programmatic response to HIV to ensure the long-term efficacy and sustainability of ART and achieve 95-95-95 targets.

[#] Corresponding author: Xing Hui, xingh@chinaaids.cn.

[1] State Key Laboratory of Infectious Disease Prevention and Control (SKLID), National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Control and Prevention (China CDC), Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Beijing, China.

REFERENCES

1. He X, Xing H, Ruan YH, Hong KX, Cheng CL, Hu YY, et al. A comprehensive mapping of HIV-1 genotypes in various risk groups and regions across China based on a nationwide molecular epidemiologic survey. PLoS One 2012;7(10):e47289. http://dx.doi.org/10.1371/journal.pone.0047289.

2. Han XX, An MH, Zhang WQ, Cai WP, Chen X, Takebe Y, et al. Genome sequences of a novel HIV-1 circulating recombinant form, CRF55_01B, identified in China. Genome Announc 2013;1(1):e00050 – 12. http://dx.doi.org/10.1128/genomeA.00050-12.

3. Liang BY, Wei QY, Yang Y, Yang Y, Liu J, Chu JM, et al. Identification of a novel HIV-1 CRF55_01B/B recombinant isolate in Guangxi, China. AIDS Res Hum Retroviruses 2020;36(5):434 – 9. http://dx.doi.org/10.1089/aid.2019.0222.

4. Chinese Center for Disease Control and Prevention. Notice of the General Office of the National Health and Family Planning Commission on adjusting the standard of free antiviral treatment for AIDS. 2016. http://www.chinaaids.cn/zlgh/hdjz6/201606/t20160615_131433.htm. [2016-6-15]. (In Chinese).

5. Benson C, Wang X, Dunn KJ, Li N, Mesana L, Lai J, et al. Antiretroviral adherence, drug resistance, and the impact of social determinants of health in HIV-1 patients in the US. AIDS Behav 2020;24(12):3562 – 73. http://dx.doi.org/10.1007/s10461-020-02937-8.

6. Kang RH, Liang SJ, Ma YL, Liang S, Xiao L, Zhang XH, et al. Pretreatment HIV drug resistance in adults initiating antiretroviral therapy in China, 2017. Infect Dis Poverty 2020;9(1):54. http://dx.doi.org/10.1186/s40249-020-00668-5.

7. World Health Organization. HIV drug resistance surveillance guidance, 2015 update. 2016. https://apps.who.int/iris/handle/10665/204471. [2020-2-5].

8. Gan MZ, Zheng S, Hao JJ, Ruan YH, Liao LJ, Shao YM, et al. The prevalence of CRF55_01B among HIV-1 strain and its connection with traffic development in China. Emerg Microbes Infect 2021;10(1):256 – 65. http://dx.doi.org/10.1080/22221751.2021.1884004.

9. Ye JR, Hao MQ, Xing H, Zhang FJ, Wu H, Lv W, et al. Transmitted HIV drug resistance among individuals with newly diagnosed HIV infection: a multicenter observational study. AIDS 2020;34(4):609 – 19. http://dx.doi.org/10.1097/QAD.0000000000002468.

10. McClung RP, Oster AM, Ocfemia MCB, Saduvala N, Heneine W, Johnson JA, et al. Transmitted drug resistance among HIV-1 diagnoses in the United States, 2014–2018. Clin Infect Dis, 2021ciab583. http://dx.doi.org/10.1093/cid/ciab583..

11. Liu YJ, Li HP, Wang XL, Han JW, Jia L, Li TY, et al. Natural presence of V179E and rising prevalence of E138G in HIV-1 reverse transcriptase in CRF55_01B viruses. Infect Genet Evol 2020;77:104098. http://dx.doi.org/10.1016/j.meegid.2019.104098.

12. Zhang D, Zheng CL, Li HP, Li H, Liu YJ, Wang XL, et al. Molecular surveillance of HIV-1 newly diagnosed infections in Shenzhen, China from 2011 to 2018. J Infect 2021;83(1):76 – 83. http://dx.doi.org/10.1016/j.jinf.2021.04.021.

13. Dong AB, Liu L, Xiao L, Liang S, Li K, Hu J, et al. First detection of a circulating recombinant form of HIV-1 CRF01_AE/08_BC (CRF105_0108) with drug-resistant mutations in Sichuan, China. AIDS Res Hum Retroviruses 2020;36(7):625 – 30. http://dx.doi.org/10.1089/AID.2020.0034.

14. Wei L, Li H, Lv X, Zheng CL, Li GL, Yang ZR, et al. Impact of HIV-1 CRF55_01B infection on the evolution of CD4 count and plasma HIV RNA load in men who have sex with men prior to antiretroviral therapy. Retrovirology 2021;18(1):22. http://dx.doi.org/10.1186/s12977-021-00567-z.

Preplanned Studies

Awareness Towards Rabies and Exposure Rate and Treatment of Dog-Bite Injuries Among Rural Residents — Guangxi Zhuang Autonomous Region, China, 2021

Mantong Zhu[1,&]; Di Mu[2,&]; Qiulan Chen[2,#]; Ning Chen[1]; Yanping Zhang[2]; Wenwu Yin[2]; Yu Li[2]; Yingjie Chen[3]; Yidan Deng[3]; Xianyan Tang[1]

Summary

What is already known about this topic?

Although fatal once clinical signs appear, rabies is preventable through three proven, effective interventions including mass dog vaccination, post-exposure prophylaxis (PEP), and, though sometimes neglected, awareness of rabies.

What is added by this report?

The total awareness rate of rabies-related knowledge among rural residents was only 57.9% in Guangxi Zhuang Autonomous Region, one of the provincial-level administrative divisions (PLADs) with endemic rabies in China, and the exposure rate of dog-bite injuries was as high as 7.2% in the past year. In addition, 14.8% of bite victims failed to seek PEP service.

What are the implications for public health practice?

This study can provide evidence for the targeted prevention and control program of rabies in rural areas and help realize the international goals of eliminating dog-mediated rabies by 2030.

Rabies is almost always fatal once symptoms appear, causing approximately 59,000 deaths worldwide each year (*1*). However, rabies is preventable through three proven, effective interventions. Except for mass dog vaccination and post-exposure prophylaxis (PEP), awareness of rabies is also important (*2–3*). In 2015, the World Health Organization (WHO), the World Organisation for Animal Health (OIE), and the Food and Agriculture Organization of the United Nations (FAO) in collaboration with Global Alliance for Rabies Control (GARC) proposed eliminating dog-mediated rabies globally by 2030 ("Zero by 30") (*3*). China is one of the countries in Asia that is most affected by rabies, with the vast majority of rabies cases occurring in rural areas (*4*). Among human rabies cases, more than 99% of cases were caused by bites from rabid dogs

(*5*). However, in the last five years, there have been few reports on the rabies awareness and the exposure rate of dog-bite injuries, especially in rural areas. In order to fill the gaps, this study conducted a cross-sectional survey on awareness towards rabies and exposure rate and treatment of dog-bite injuries using a uniform structural questionnaire to interview 2,544 rural residents in Guangxi Zhuang Autonomous Region in 2021. The study found that in Guangxi, the total awareness rate of rabies-related knowledge among rural residents was only 57.9%, and the exposure rate of dog-bite injuries was as high as 7.2% in the past year. However, 14.8% of those bitten did not seek PEP services. Much more effort should be made for promoting the awareness of rabies disease and the accessibility of PEP service in rural China, especially in the endemic areas. In addition, it is necessary to design targeted prevention and control program for promotion.

A multistage random sampling method was used in this study. To estimate the sample size, the sample was divided into 3 layers according to age and 2 layers according to gender for a total of 6 layers. It was assumed that the awareness rate was 50%, and the test level α was set to 0.05 and the allowable error was set to 5%. According to the simple random sampling sample size calculation formula and considering the loss-follow-up rate of 10% known in previous studies, the minimum sample size was calculated to be 2,534 people. Overall, 127 villages were randomly selected in Guangxi, and 20 interviewees were selected in each village based on systematic sampling method. Finally, a total of 2,544 people aged from 15 to 75 years old with verbal consent were investigated face to face.

There were 6 basic rabies-related questions for respondents to answer. Points were awarded as follows: 0 for incorrect or unclear answers, 1 for correct answers, and a full score was 6. If the final total score $\geq$4, the respondents were classified as having awareness. The test level α was set to 0.05 and used the chi-

squared test to analyze the awareness rate of different populations. Finally, a value was assigned to the awareness situation (0=unknown, 1=awareness), and the influencing factors were analyzed by binary logistic regression. The survey subjects were asked about whether they have been bitten by dogs in the past year, as well as the situation of treatment after being bitten. The exposure rate of dog-bite injuries among rural residents in Guangxi was calculated = (number of bitten/number of people investigated) × 100%. Excel (version 2017, Microsoft Corporation, Beijing, China) was used for data collation, and R (version 4.0.5, University of Auckland, New Zealand) was used for statistical analysis.

The survey subjects were all rural residents, including 1,246 males and 1,298 females, with an average age of 44.6±17.0 years old. The survey subjects were mainly young people (15–44 years old), followed by middle-aged people (45–59 years old) and elderly people (60 years old and above), accounting for 45.8%, 31.6%, and 22.6%, respectively. The education levels of the population were primarily junior high school, followed by primary school and

below and high school and above, accounting for 37.5%, 37.4%, and 25.1%, respectively. The total awareness rate of rabies-related knowledge was 57.9%. The awareness rate of single questions ranged from 28.8% to 92.8%. Overall, 9.4% respondents had never heard of rabies, and 55.7% of respondents did not know that rabies was incurable (Table 1).

The results of single-factor analysis showed that there were significantly statistical differences in the awareness rate among subjects in different age groups and different education levels ($P<0.05$), and there was no significantly statistical difference in awareness rate between female and male ($P>0.05$). For both men and women, the older the age, the lower the awareness rate, among which the group over 60 years old was the lowest, with 42.6% and 32.9%, respectively. Among subjects with different education levels, the awareness rate of the subjects with education level of primary school or below was the lowest at only 37.2% (Table 1). The results of multifactor analysis showed that the education level of the respondents was an influencing factor of the awareness rate. Compared to the primary school and below, the respondents with

TABLE 1. The awareness rate of rabies-related knowledge for 2,544 rural residents in Guangxi Zhuang Autonomous Region, 2021.

Variable	Point 1 n (%)	Point 2 n (%)	Point 3 n (%)	Point 4 n (%)	Point 5 n (%)	Point 6 n (%)	Total points N (%)	χ^2 value	P value
Male									
15–44 (years)	511 (95.0)	282 (52.4)	439 (81.6)	520 (96.7)	352 (65.4)	213 (39.6)	386 (71.7)		
45–59 (years)	386 (92.1)	179 (42.7)	302 (72.1)	384 (91.6)	203 (48.4)	99 (23.6)	223 (53.2)	74.040	0.000
≥60 (years)	252 (87.2)	117 (40.5)	193 (66.8)	254 (87.9)	90 (31.1)	54 (18.7)	123 (42.6)		
Total	1,149 (92.2)	578 (46.4)	934 (75.0)	1,158 (92.9)	645 (51.8)	366 (29.4)	732 (58.7)		
Female									
15–44 (years)	601 (95.9)	300 (47.8)	543 (86.6)	610 (97.3)	422 (67.3)	239 (38.1)	456 (72.7)		
45–59 (years)	330 (85.7)	152 (39.5)	267 (69.4)	356 (92.5)	171 (44.4)	88 (22.9)	192 (49.9)	139.336	0.000
≥60 (years)	225 (78.7)	96 (33.6)	167 (58.4)	237 (82.9)	86 (30.1)	39 (13.6)	94 (32.9)		
Total	1,156 (89.1)	548 (42.2)	977 (75.3)	1,203 (92.7)	679 (52.3)	366 (28.2)	742 (57.2)		
Level of education									
Primary school and below	800 (83.8)	323 (33.8)	589 (61.7)	831 (87.0)	326 (32.8)	160 (16.1)	370 (37.2)		
Junior high school	889 (93.4)	433 (45.5)	757 (79.5)	910 (95.6)	520 (54.6)	289 (30.4)	592 (62.2)	283.038	0.000
High school and above	616 (96.7)	370 (58.1)	565 (88.7)	620 (97.3)	478 (75.0)	283 (44.4)	512 (80.4)		
Total	2,305 (90.6)	1,126 (44.3)	1,911 (75.1)	2,361 (92.8)	1,324 (52.0)	732 (28.8)	1,474 (57.9)		

Note: Question 1: Have you heard of rabies (rabies)?
Question 2: Can rabies be cured?
Question 3: Do you know that rabies can be prevented by injection (vaccine)?
Question 4: What do you think should be done after being scratched or bitten by a dog (cat)?
Question 5: Do you know that dogs should also be vaccinated against rabies?
Question 6: Do you know where to vaccinate dogs against rabies?
n: Number of respondents who answered correctly; n%=(n/number of respondents) × 100%.
N: Number of the respondents who regarded as awareness; N%=(N/number of respondents) × 100%.

junior high school [odds ratio (OR)=2.04, 95% confidence interval (CI): 1.67–2.50, *P*<0.05] and high school and above (OR=4.29, 95% CI: 3.27–5.66, *P*<0.05) had higher awareness rate.

The exposure rate of dog-bite injuries among the respondents was as high as 7.2% (183/2,544) in the past year. Among these 183 victims, 162 reported the situation of their PEP. Overall, 85.2% (138/162) of dog-bite injuries victims sought PEP services, of which 40.0% were treated in township hospitals, 23.7% in county CDCs, 23.0% in county hospitals, and 13.3% in village clinics (Table 2). In addition, 7.3% (10/138) among PEP seekers had not received the vaccine against rabies; 14.8% (24/162) of the victims failed to seek PEP service after dog bites. As for the reasons, 75.0% respondents answered as "no need to go to hospital", 29.2% selected "expensive cost", 29.2% chose "long distance", and 29.2% thought there was no time to go to doctor (Table 3).

DISCUSSIONS

The survey showed that the awareness rate of rabies among rural residents in Guangxi was lower than 60%, especially the group aged above 60 years old and those with low education level. The exposure rate of dog-bite injuries in rural areas of Guangxi was relatively high, and over half of the victims preferred to seek PEP

TABLE 2. The medical institutions of 135* dog-bite injuries victims seeking PEP services in Guangxi Zhuang Autonomous Region, 2020.

Medical institution	Respondents	Proportion (n/N)
Village health room / clinic	18	13.3% (18/135)
Township hospital	54	40.0% (54/135)
County hospital	31	23.0% (31/135)
County CDC	32	23.7% (32/135)
Total	135	100.0% (135/135)

Abbreviation: PEP=post-exposure prophylaxis.
* Three victims did not answer this question.

TABLE 3. The reasons of 24 dog-bite injuries victims for not going to the hospital for treatment in Guangxi Zhuang Autonomous Region, 2020.

Reason	Respondents	Proportion (n/N)
No need to go to hospital	18	75.0% (18/24)
Expensive cost	7	29.2% (7/24)
Long distance	7	29.2% (7/24)
No time	7	29.2% (7/24)
Else	3	12.5% (3/24)

service in township hospitals or the village clinics. Unfortunately, nearly one-fifth of victims failed to seek PEP service with the main reason of being ignorant of the rabies threat. In addition, a small number of PEP seekers did not receive a rabies vaccine.

In this study, we found that the rabies awareness rate of rural residents in Guangxi in 2021 was similar to that in Guangxi in 2010 (*6*), as well as that in rural areas of Rongcheng City, Shandong Province in 2021 (*7*), but lower than the survey results in rural areas of Pingliang City, Gansu PLADs in recent years (*8*). This indicated that health education for rabies in rural areas needs to be further strengthened. Low education levels were the hindering factor of the rabies awareness rate; however more than one-third of respondents had an education level lower than primary school. To further popularize knowledge about rabies, targeted material and methods should be developed to ensure local rural residents especially the elderly and low education level population could easily accept.

The exposure rate of dog-bite injuries among rural residents in Guangxi was significantly higher than that of 2.18% in Hunan Province (*9*), even though both were rabies-endemic areas. The disparity may be attributed to differences in dog raising rates and dog densities between the two areas. After dog bites, timely and standardized PEP service is the key to preventing rabies (*10*). But nearly one-fifth of dog bites victims failed to seek PEP service and still a small number of PEP seekers did not receive a rabies vaccine. By the scaling up of the rabies vaccination demonstration zone project during the period of 2009–2016, the number of rabies cases in Guangxi has dropped significantly from 324 to 41 (*11*), and continue to decrease to only 11 in 2020, which may have led to a relaxation of people's vigilance against rabies. However, this survey showed the high exposure rate of dog-bite injuries in rural residents of Guangxi, which indicated that the risk of rabies remains high, as well as the necessity of constraining dogs to prevent dog-bite injury in rural areas.

We also found that most of the victims preferred to seek PEP service in township hospital and the village clinics, which may be due to convenience and shorter distances when compared with county hospitals. This suggests the necessity to improve the standardization and accessibility of PEP in rural areas especially in rabies endemic regions. First, professionals in township health centers and village clinics should be trained to have the knowledge and skills for PEP treatment after being bitten or scratched by a risk animal. Second,

township health centers and village clinics, even in remote areas, should be equipped with wound treatment facilities, the rabies vaccine, and rabies immunoglobulin if appropriate. These can improve the accessibility and correct implementation of PEP by the above measures, which can help to achieve the goal of eliminating rabies as soon as possible (*12*).

This study was subject to some limitations. First, only a preliminary investigation was conducted on where rural residents chose to go for medical treatment after being bitten by dogs, without further investigation into detailed reasons. Second, when investigating the exposure rate of dog-bite injuries, the number of exposures was not questioned in detail. This may lead to a low exposure rate, but when compared with the results in other regions, the exposure rate of dog-bite injuries in this study was still significantly higher, which has important significance.

Conflicts of interest: No conflicts of interest.

Acknowledgement: Animals Asia and all the college student volunteers.

Corresponding author: Qiulan Chen, chenql@chinacdc.cn.

---

1 School of Public Health, Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China; 2 Division of Infectious Disease, Key Laboratory of Surveillance and Early Warning on Infectious Disease, Chinese Center for Disease Control and Prevention, Beijing, China; 3 Animals Asia, Chengdu, Sichuan, China.
& Joint first authors.

REFERENCES

1. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, et al. Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis 2015;9(4):e0003709. http://dx.doi.org/10.1371/journal.pntd.0003709.
2. Ruan S. Modeling the transmission dynamics and control of rabies in China. Math Biosci 2017;286:65 – 93. http://dx.doi.org/10.1016/j.mbs.2017.02.005.
3. World Health Organization. Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030. https://www.who.int/publications/i/item/9789241513838. [2021-8-5].
4. Mu D, Tao ZF, Li ZJ, Li Y, Tao XY, Zhu WY, et al. Analysis of epidemic characteristics of human rabies in China in 2007-2018. Chin J Exp Clin Virol 2021;35(2):168 – 71. http://dx.doi.org/10.3760/cma.j.cn112866-20201013-00261. (In Chinese).
5. Zhou HP, Liu YJ, Zhang XJ, Zhu L, Huang BX. Global epidemic situation and elimination strategies of rabies. Chin Anim Health Inspect 2015;32(1):50 – 2. http://dx.doi.org/10.3969/j.issn.1005-944X.2015.01.019. (In Chinese).
6. Mo Y, Mo ZJ, Liu B, Tan Y, Mo JJ, Li H, et al. A cross-sectional study on kownledge, attitude and practice regarding Rabies on rural residents in Guangxi. J Appl Prev Med 2010;16(6):343 – 5. http://dx.doi.org/10.3969/j.issn.1673-758X.2010.06.010. (In Chinese).
7. Chen LF, Qiao DL, Jiang B, Xiao ZG, Guo YK, Kong QY, et al. Investigation on KAP of rabies prevention and treatment knowledge in Rongcheng, Shandong. Anim Breed Feed 2021;20(3):126 – 9. http://dx.doi.org/10.3969/j.issn.1671-427X.2021.03.057. (In Chinese).
8. Ren RF, Ren RQ, Lin ZY, Li L, Liu L. Investigation and analysis on the cognition degree of rabies among rural residents in Kongtong District of Pingliang City, Gansu Province. J Med Pest Control 2019;35(5):488 – 90,493. http://dx.doi.org/10.7629/yxdwfz201905024. (In Chinese).
9. Yang H, Li BX, Luo KW, Sun QL, Zhang HJ, Zhao SL, et al. Investigation on rabies exposure and its influencing factors in population in rural areas of Hunan Province. J Trop Dis Parasitol 2021;19(1):19 – 24. http://dx.doi.org/10.3969/j.issn.1672-2302.2021.01.005. (In Chinese).
10. Zhou H, Li Y, Chen RF, Tao XY, Yu PC, Cao SC, et al. Technical guideline for human rabies prevention and control (2016). Chin J Epidemiol 2016;37(2):139 – 63. http://dx.doi.org/10.3760/cma.j.issn.0254-6450.2016.02.001. (In Chinese).
11. Wei XK, Xiong Y, Li XN, Zheng M, Pan Y, He XX, et al. Vaccination demonstration zone successfully controls rabies in Guangxi Province, China. BMC Infect Dis 2018;18(1):386. http://dx.doi.org/10.1186/s12879-018-3301-8.
12. Chen QL, Ma XY, Rainey JJ, Li Y, Mu D, Tao XY, et al. Findings from the initial Stepwise Approach to Rabies Elimination (SARE) assessment in China, 2019. PLoS Negl Trop Dis 2021;15(3):e0009274. http://dx.doi.org/10.1371/journal.pntd.0009274.

Notes from the Field

A Total of 2,657 Reported Cases and 14 Deaths Due to Hemorrhagic Fever with Renal Syndrome — Shaanxi Province, China, January 1–December 19, 2021

Jing Wei[1,&]; Xiaoxia Huang[2,&]; Shen Li[1]; Shanshan Du[2]; Pengbo Yu[1,#]; Jiandong Li[2,#]

Hemorrhagic fever with renal syndrome (HFRS) is a group of illnesses that are caused by hantaviruses. Although increased exposure to rodents or their urine and droppings could be a decisive factor of the increasing in incidence, further research is still needed to elucidate the causes of the epidemic (1).

Since October 2021, the number of reported cases of HFRS has been increasing rapidly in Shaanxi Province. As of December 19, 2021, a total of 2,657 cases were reported with 14 deaths, 35.84% more than that in 2020 (2,051 cases and 3 deaths). Furthermore, in 2020, there were 8,121 cases and 48 deaths nationwide for morbidity and mortality rates of 0.5785 and 0.0034 per 100,000 population, and a case-fatality rate of 0.59%. In all reported cases, 7.56% were aged 1–14 years, 67.11% aged 15–59 years, and 25.29% aged over 60 years. Compared to 2020, student cases (272 cases) had increased in proportion from 9.00% in 2020 to about 10.24% in 2021, and farmer cases continued to comprise the largest proportion from about 70% in 2020 to about 66.58% in 2021.

Shaanxi Province had been recognized as an endemic area since an outbreak of HFRS was found on the north slope of Qinling Mountains in 1955 (2). In 2021, of the 2,657 cases reported in the 10 affected cities, 2,522 (94.92%) cases were from 4 cities: Xi'an (1,553, 58.45%), Weinan (475, 17.88%), Xianyang (293, 11.03%), and Baoji (200, 7.56%); and 2,062 (77.6%) cases were reported from 20 affected county-level jurisdictions and city districts. The Hantavirus carrying rate of rodents as reported by regular surveillance showed no significant changes in rodent population.

Compared with the past five years, the national HFRS prevalence remained low in China (3). As of December 18, 2021, 8,502 cases with 54 (0.63%)

deaths were reported from 29 provincial-level administrative divisions (PLADs), which were 9.10% and 17.39% higher than that (7,793 cases and 46 deaths) in 2020. The reported cases from top 10 PLADs accounting for 81.6% of the total reported cases in China. After Shaanxi (2,657 cases), the numbers of cases in Shandong (875 cases), Liaoning (501 cases), Henan (352 cases), Yunnan (295 cases), and Jilin (291 cases) increased by a range from 11.1% to 76.9%, and the reported number of cases in Heilongjiang (875 cases), Hunan (499 cases), Hubei (396 cases), and Jiangxi (354 cases) decreased from 15.6% to 31.1% compared with that in 2020. Variations of Hantavirus have also previously been found in central and southwest China.

[1] Center for Disease Control and Prevention of Shaanxi Province, Xi'an, Shaanxi, China; [2] Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China.
& Joint first authors.

REFERENCES

1. Shang C, Zhang QF, Yin QL, Li DX, Li JD. Influence factors related epidemics on hantavirus disease. Chin J Epidemiol 2020;41(6):968 – 74. http://dx.doi.org/10.3760/cma.j.cn112338-20190916-00678. (In Chinese).
2. Ministry of Health. Handbook of epidemic hemorrhagic fever prevention and control. 2nd ed. Beijing: China People's Health Publishing House. 1998. (In Chinese).
3. Shang C, Sun YW, Yin QL, Huang XX, Liu XS, Zhang QF, et al. Hemorrhagic fever with renal syndrome — Liaoning province, China, 1999–2018. China CDC Wkly 2020;2(20):350 – 4. http://dx.doi.org/10.46234/ccdcw2020.091.

A Death Due to Neurobrucellosis — Linfen City, Shanxi Province, China, May 2021

Yufeng Du[1]; Hongxia Yang[2]; Hai Jiang[3,#]; Xiufang Du[1,#]

On May 8, 2021, a death due to brucellosis was reported to the Shanxi CDC. An epidemiological investigation was conducted to determine the cause of death.

The patient, a 41-year-old man engaged in mining, was brought to the emergency room of Xinjiang Military General Hospital on March 13, 2021, with complaints of gradual-onset fever and headache. He was admitted for diagnostic workup. Two days later, he developed convulsions and altered consciousness and was transferred to the intensive care unit. After a week, the patient again developed high-grade fever and delirium, with his temperature rising to 39.5 ℃. The fever was not associated with chills. There was no history of exposure to toxic substances. The patient's wife reported that he had consumed roasted mutton. Blood culture and next-generation sequencing of cerebrospinal fluid (CSF) were both positive for *Brucella*. Based on clinical manifestations, epidemiological history, and etiological test results, the patient was diagnosed with neurobrucellosis.

He was transferred to Xinjiang Medical University Affiliated Hospital on March 25. He was treated with intravenous antibiotics (ceftriaxone, rifampicin, and doxycycline) and sodium valproate. However, fever, headache, and disturbance of consciousness persisted. Head computerized tomography (CT) revealed large brain abscesses and cerebral herniation. Surgical treatment was advised, but the patient's family refused surgery and asked for transfer back to the local hospital. On April 1, the patient was transferred to Linfen Infectious Disease Hospital, Shanxi Province. Repeat CT scan showed enlargement of the brain abscesses. Multiple large, annular low-density shadows were seen in the left temporal lobe, frontal lobe, and basal ganglia region, with a fluid plane visible in the latter. The left lateral ventricle was compressed, and the midline was displaced to the right (Figure 1). At 20:30 on April 23, physical examination showed bilateral dilated pupils, with sluggish response to light, and absent orbital pressure reflex. On April 25, the patient died.

Human brucellosis is a multisystem disease that can present with a broad spectrum of clinical manifestations and complications. Death is usually due to endocarditis or neurobrucellosis. Neurobrucellosis, which may occur at any stage of the disease, develops in <5% of patients with *Brucella* infection and presents with diverse neurological symptoms and signs (*1–3*). Standard first-line treatment is with doxycycline, rifampicin, and third-generation cephalosporins for at least 6 weeks. Prognosis varies according to clinical manifestations (*4*). The diagnosis of neurobrucellosis is based on clinical and radiological features and serological tests. Underdiagnosis of neurobrucellosis may be due to poor awareness among health workers
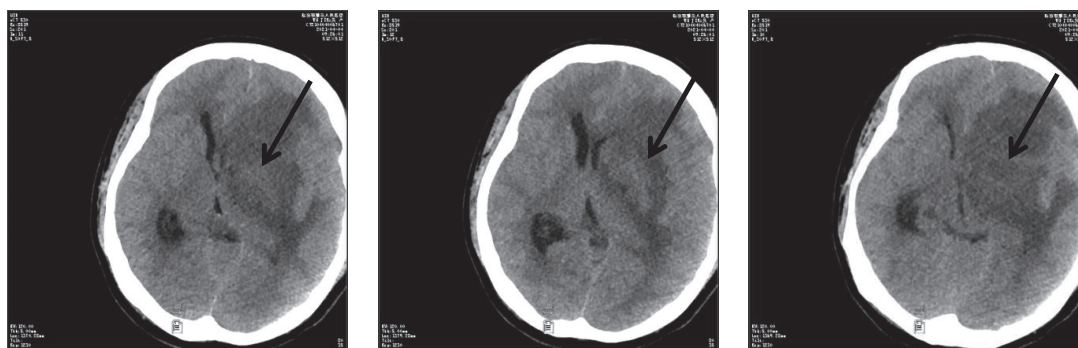

FIGURE 1. The progression of the brain abscess for the patient by computerized tomography (CT) — Linfen, Shanxi, China, 2021.

and lack of diagnostic facilities.

There are few reports of neurobrucellosis from China (*5*). In this paper, delayed diagnosis and extra referrals likely factored into the serious outcome. The present case report highlights the importance of early diagnosis of neurobrucellosis in the laboratory. We recommend the following measures: 1) establishing rapid detection methods, such as polymerase chain reaction, for early diagnosis; 2) raising the awareness of healthcare workers, especially in the department of neurology; and 3) improving multidisciplinary support for case management.

Conflicts of interest: No conflicts of interest declared.

Corresponding authors: Hai Jiang, jianghai@icdc.cn; Xiufang Du, crduxiufang@163.com.

1 Linfen Third People's Hospital, Linfen, Shanxi, China; 2 Shanxi Provincial Centre for Disease Control and Prevention, Taiyuan, Shanxi, China; 3 State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China.

REFERENCES

1. Guven T, Ugurlu K, Ergonul O, Celikbas AK, Gok SE, Comoglu S, et al. Neurobrucellosis: clinical and diagnostic features. Clin Infect Dis 2013;56(10):1407 – 12. http://dx.doi.org/10.1093/cid/cit072.
2. McLean DR, Russell N, Khan MY. Neurobrucellosis: clinical and therapeutic features. Clin Infect Dis 1992;15(4):582 – 90. http://dx.doi.org/10.1093/clind/15.4.582.
3. Zheng N, Wang W, Zhang JT, Cao Y, Shao L, Jiang JJ, et al. Neurobrucellosis. Int J Neurosci 2018;128(1):55 – 62. http://dx.doi.org/10.1080/00207454.2017.1363747.
4. Ceran N, Turkoglu R, Erdem I, Inan A, Engin D, Tireli H, et al. Neurobrucellosis: clinical, diagnostic, therapeutic features and outcome. Unusual clinical presentations in an endemic region. Braz J Infect Dis 2011;15(1):52 – 9. http://dx.doi.org/10.1016/S1413-8670(11)70140-4.
5. Wu TY. Clinicopathological report of brucellosis complicated with meningoencephalitis, diabetes insipidus and cirrhosis. Chin J Intern Med 1961;9(7):449. (In Chinese).

Indexed by PubMed Central (PMC), Emerging Sources Citation Index (ESCI), and Chinese Scientific and Technical Papers and Citations

CCDCweekly

Vol. 3 No. 53 Dec. 31, 2021