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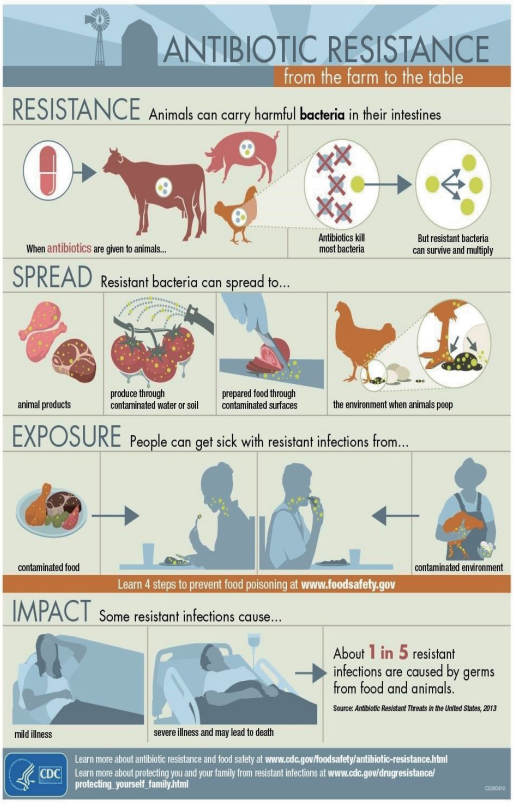

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Preplanned Studies

Prevalence of Antimicrobial Resistant of *Vibrio parahaemolyticus* Isolated from Diarrheal Patients — Six PLADs, China, 2016–2020

Haihong Han[1]; Sara M. Pires[2]; Johanne Ellis-Iversen[3]; Zhen Tang[4]; Xiaoai Zhang[5]; Jikai Liu[1]; Weiwei Li[1]; Qingpo Cui[6]; Jing Zou[6]; Ping Fu[1,#]; Yunchang Guo[1,#]

Summary

What is already known on this topic?

Vibrio parahaemolyticus (*V. parahaemolyticus*) is frequently resistant to common antimicrobials such as ampicillin and generally highly susceptible to most clinically used antimicrobials.

What is added by this report?

V. parahaemolyticus were highly resistant to cefazolin and ampicillin: 94.4% and 37.0%, respectively. However, it was below 3% resistance to all 10 other antimicrobials including clinically relevant agents and even imipenem. The overall levels of antimicrobial resistance and multidrug resistance were 95.1% and 3.3%, respectively. The distribution of antimicrobial resistance and the multidrug resistance had regional, temporal, sexual, and isolated source strain variation.

What are the implications for public health practice?

This study provides data on drug resistance of *V. parahaemolyticus* in Chinese clinical settings, which will help develop a public health strategy.

The antimicrobial resistance characteristics of *Vibrio parahaemolyticus* (*V. parahaemolyticus*) was studied by isolating the bacterium from diarrheal patients in 6 provincial-level administrative divisions (PLADs) from 2016 to 2020 reported to National Foodborne Disease Molecular Tracing Network (TraNet). A total of 2,871 clinical *V. parahaemolyticus* isolates were examined in this study, and the levels of resistance and multidrug resistance were compared within several epidemiological dimensions. The results demonstrated that *V. parahaemolyticus* isolates had high levels of resistance to cefazolin and ampicillin, and the other 10 tested antimicrobial agents including imipenem had similar levels of resistance, which were all below 3%. The distribution of antimicrobial resistance and/or the multidrug-resistance level had regional and temporal characteristics, with significant differences in sex and isolated source strain dimensions. These results demonstrated that *V. parahaemolyticus* isolates had high levels of drug resistance to some drugs and emerging resistance to many more, including carbapenem, and thus constitute a serious health risk. This study added to the knowledge of resistance in clinical *V. parahaemolyticus* isolates in China, which would help provide scientific epidemiological data, guide healthcare treatment of the disease, and develop a public health strategy accordingly.

V. parahaemolyticus can cause sporadic infection in milder forms of gastroenteritis or large-scale food-related outbreaks (*1–3*). Antimicrobial resistance as well as other information of *V. parahaemolyticus* is required to be submitted to TraNet for analysis based on the existing laboratory-based foodborne disease surveillance system in China. *V. parahaemolyticus* were isolated by network laboratories from stool samples or rectal swabs collected from diarrheal patients who were either linked to outbreaks or were sporadic cases, seek medical care in hospitals in Beijing, Guangdong, Jiangsu, Shanxi, Tianjin, and Zhejiang from 2016 to 2020. To ensure the quality and comparability of the submitted surveillance data, they were processed in local laboratories following procedures to isolate *V. parahaemolyticus* strains and perform antimicrobial susceptibility testing in accordance with the standard operation procedures regulated by the national laboratory manual for foodborne disease surveillance system and TraNet. The analysis also included temporal and regional patterns, serotype distribution, and the correlation of susceptibility status with epidemiological dimensions that may contribute to develop a public health strategy. A panel of 12 antimicrobials belonging to 9 classes were selected for resistance analysis: gentamicin, cefazolin, cefotaxime, ceftazidime, cefoxitin, ciprofloxacin, imipenem, ampicillin, ampicillin/sulbactam, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline. Isolates that were resistant to at least 1 of these 12 agents were considered resistant (R), and multiple drug resistance (MDR) was defined as "acquired resistance

to three or more classes of antimicrobials." Descriptive statistics were used to analyze the resistance prevalence to different antimicrobial agents. Pearson chi-square, continuous correction chi-square, or the likelihood ratio test were applied to test whether observed resistance level differences were statistically significant (*P*-values ≤0.05).

The temporal and spatial distribution of the strains isolated were shown in Figure 1. The incidence of *V. parahaemolyticus* isolated from diarrheal patients peaked in the warmer months from May to September in each of the six PLADs. Of the total 2,871 isolates, 95.1% (2,731/2,871) were resistant to at least 1 agent among the 12 tested antimicrobials; 3.3% of strains (94/2,871) were resistant to more than 3, and up to 8 classes, of antimicrobials, demonstrating a variety of 47 resistance patterns, and were defined as MDR strains in this study accordingly. A significant difference among different years was observed in the resistance percentage (R%), with 2017 showing the lowest percent (91.1%) and 2016 showing the highest (99.0%). Although differences by year were not significant, 2017 showed the highest MDR level (4.8%). Although also not significant, the R% and MDR percentage (MDR%) of *V. parahaemolyticus* by month showed that the drug resistance percentages isolated in July were relatively lower than those in the other months, especially in 2016 and 2020, and that higher percentages of MDR% were observed in July and August.

The levels of resistance to the 12 antimicrobials of the 2,871 examined *V. parahaemolyticus* isolated from diarrheal patients differed by region, as shown in Table 1. The majority of isolates tested were resistant to cefazolin, found in 94.4% (2,711/2,871) of the strains. Isolates were also commonly resistant to ampicillin (37.0%). Only few strains were resistant to the clinically relevant agents of cefotaxime, ceftazidime, and tetracycline (1.3%, 0.8%, and 2.6%). There is an emerging resistance to imipenem showed by 16 strains resistant and 8 strains intermediate. Low resistance levels (from 0.6% to 1.9%) were found to the 6 remaining antimicrobials: ampicillin/sulbactam, ciprofloxacin, gentamicin, cefoxitin, chloramphenicol, and trimethoprim-sulfamethoxazole. In addition, 94.6% (2,717/2,871) of strains exhibited resistance to at least one of the drugs classified as highly important antimicrobials, while 38.3% (1,100/2,871) of strains exhibited resistance to drugs classified as critically important antimicrobials by the World Health Organization (WHO). There was a significant difference in resistance to the drug agents ampicillin, cefotaxime, ceftazidime, cefazolin, cefoxitin, tetracycline, and trimethoprim-sulfamethoxazole by different PLADs.

Table 2 shows the distribution of characteristics of antimicrobial resistance levels of *V. parahaemolyticus* in terms of epidemiological dimensions. A significant difference in R% and MDR% of *V. parahaemolyticus* by sample locations was observed. The highest R%

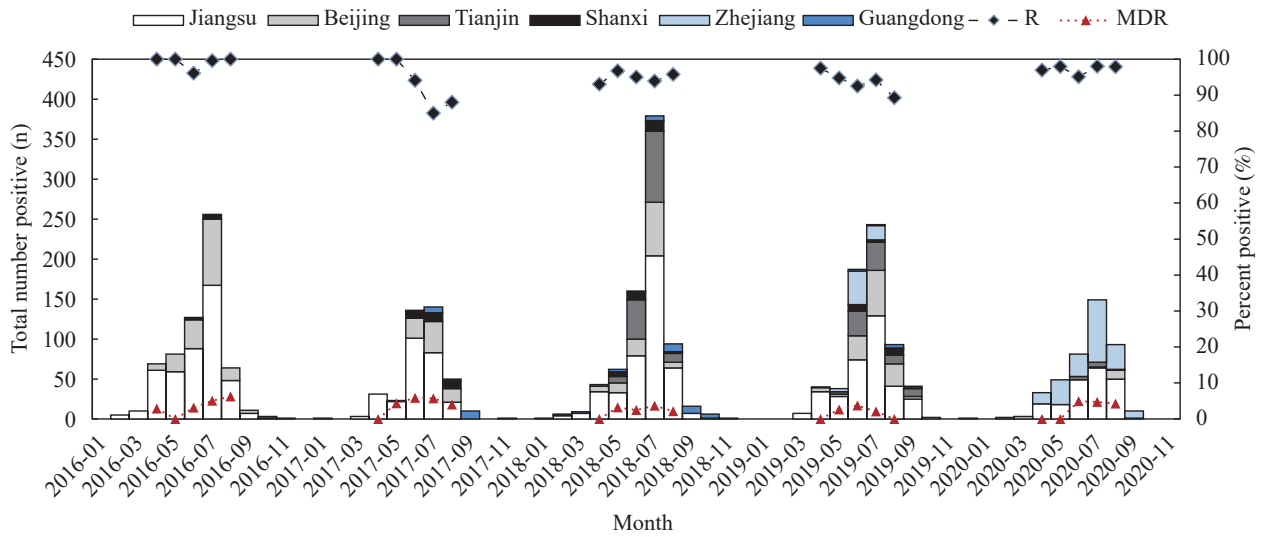

FIGURE 1. The temporal and spatial distribution of *V. parahaemolyticus* positive tests, and R% and MDR% in 6 PLADs, China, 2016–2020.
R% is the percent of isolates that were resistant to at least one of 12 agents, and MDR% is the percent of resistance to 3 or more classes of antimicrobials.

TABLE 1. Resistance of *V. parahaemolyticus* isolates to 12 antimicrobials tested differed by region.

Antimicrobial classes categorized by WHO*	Antimicrobials	MIC interpretive standard of resistance (μg/mL)	R%† differed by PLADs						*P*	Overall R%†
			Beijing	Guangdong	Jiangsu	Shanxi	Tianjin	Zhejiang		
Critically important antimicrobials	Ampicillin (AMP)	≥32	22.5	54.6	50.9	11.5	8.9	7.6	<0.05	37.0
	Ampicillin/sulbactam (AMS)	≥32/16	2.2	0.0	0.9	0.0	0.8	0.4		1.0
	Cefotaxime (CTX)	≥4	2.4	0.0	0.7	5.8	1.9	0.8	<0.05	1.3
	Ceftazidime (CAZ)	≥16	2.2	0.0	0.5	0.0	0.4	1.1	<0.05	0.8
	Ciprofloxacin (CIP)	≥4	1.0	0.0	1.2	2.3	0.8	0.0		1.0
	Gentamicin (GEN)	≥16	1.0	0.0	0.5	2.3	0.0	0.0		0.6
	Imipenem (IPM)	≥4	1.2	0.0	0.8	0.0	0.4	0.4		0.7
Highly important antimicrobials	Cefazolin (CFZ)	≥4	88.3	96.1	96.6	93.1	89.2	97.7	<0.05	94.4
	Cefoxitin (CFX)	≥32	0.8	0.0	0.7	0.0	3.1	1.1	<0.05	0.9
	Chloramphenicol (CHL)§	≥32	2.4	0.0	1.1	1.2	0.8	0.4		1.2
	Tetracycline (TET)	≥16	4.0	0.0	2.0	6.9	4.3	1.5	<0.05	2.6
	Trimethoprim-sulfamethoxazole (TMP-SMX)	≥4/76	2.6	0.0	1.7	5.8	2.7	0.8	<0.05	1.9

Abbreviations: PLADs=provincial-level administrative divisions; MIC=minimum inhibitory concentration.
* Seven antimicrobials were classified by the WHO as critically important antimicrobials and five as highly-important antimicrobials.
† R% means the percent of isolates that were resistant to at least one of 12 agents.
§ No CLSI break point for *V. parahaemolyticus* applied MIC ≥32 μg/mL as for *Vibrio cholerae*.

TABLE 2. Association between epidemiological dimensions, resistance, and multidrug-resistance in 2,871 *V. parahaemolyticus* strains from patients.

Variable	No. of tested strains	R%	*P*	MDR%	*P*
PLADs			<0.05		<0.05
Beijing	503	89.3		4.8	
Guangdong	77	96.1		0.0	
Jiangsu	1,680	97.2		3.2	
Shanxi	87	94.3		5.8	
Tianjin	259	90.4		3.1	
Zhejiang	265	97.7		1.1	
Gender*			>0.05		<0.05
Men	1,127	95.4		3.1	
Women	995	95.8		5.0	
Strain source†			<0.05		<0.05
Outbreak	362	99.2		0.8	
Patient	2,086	95.0		3.7	
Serotype§			>0.05		>0.05
O3:K6	421	91.2		2.6	
O4:K8	59	94.9		5.1	

Note: R% is the percent of isolates that were resistant to at least one of 12 agents, and MDR% is the percent of resistance to 3 or more classes of antimicrobials.
Abbreviation: PLADs=provincial-level administrative divisions.
* There were some missing values (749) for gender.
† In order to balance the difference between different regions, the strains included here were from the same PLADs.
§ Only a limited number of strains had available information on serotype and among which 480 isolates were identified as either O3:K6 or O4:K8.

were observed in Zhejiang and the lowest in Beijing. The highest level of MDR was observed in Shanxi, and in Guangdong no MDR was found. It seemed that the northern PLADs (Beijing, Tianjin, and Shanxi) had relative lower R% and higher MDR%, and the southern PLADs (Guangdong, Jiangsu, and Zhejiang) had higher R% and lower MDR%. There were significant differences in MDR between male and female patients, with strains originating from females exhibiting higher levels, while no significant differences in drug resistance. Strains isolated from outbreak related-cases and from sporadic patients had significant differences in both the drug and multidrug-resistant level, with outbreak related-isolates having higher R% and lower MDR%. Although there was no significant difference, *V. parahaemolyticus* identified as O4:K8 serotypes had higher D% and MDR% compared with serotype O3:K6 strains.

DISCUSSION

The results of this study showed that *V. parahaemolyticus* isolated from patients in 6 PLADs of China have a R% of 95.1%, and it also showed a 3.3% MDR% and multiple antimicrobial resistances of up to 8 classes of antimicrobials. Our findings placed cefazolin at the top of the clinical *V. parahaemolyticus* resistance (94.4%), which was much higher than

previous results (*4*–*5*). Especially alarming was the emergence of imipenem intermediate and resistant *V. parahaemolyticus* strains, which had not previously been reported in China. Imipenem belongs to the carbapenem class and has always been regarded as the last treatment option for the treatment of bacterial infection (*6*). These results highlight the negative impact of carbapenem misuse, which may cause failure of the clinical treatment of *Vibrio* and other infections in the future.

Resistance was found in *V. parahaemolyticus* to all 12 antimicrobials tested, which was in contradiction to other studies on *V. parahaemolyticus* showing that it remained highly susceptible to many antimicrobial agents except for ampicillin and streptomycin (*4*–*5*, *7*–*9*). Tetracycline, ciprofloxacin, cefotaxime, ceftazidime, gentamicin, and trimethoprim-sulfamethoxazole were the antimicrobial agents that were recommended for use in the treatment of *Vibrio* spp. infections (*10*). Although the resistance level was relatively low, those six clinically-applied antimicrobials were considered as constituting the defense against *V. parahaemolyticus* infections, so even a small percentage of resistance isolates could be cause for concern. In this context, the current treatment guidelines should be re-assessed, and surveillance and monitoring of antimicrobial usage and resistance should be enhanced to make sure the drug is employed only with great caution.

This study demonstrated a difference in the drug resistance levels and multiple drug resistance levels of *V. parahaemolyticus* according to several external factors. The drug resistance levels of *V. parahaemolyticus* seems related to the month of isolation, which may be related to the survival adaptability of *V. parahaemolyticus* strains with different resistant capabilities in different meteorological conditions. In terms of regional distribution, the highest drug resistance levels were found in Zhejiang and MDR in Shanxi. The MDR level of female patients was higher than that of male patients. These differences may result from different drug use habits and food habits especially towards aquatic products and other unknown factors.

This study was subject to at least some limitations. First, the top six PLADs that isolated the most *V. parahaemolyticus* were included in this study and were therefore not representative of the country. Second, antimicrobial susceptibility testing was processed in local laboratories and may bring some variation in the results.

In summary, the study showed that the clinical protocol to treat *V. parahaemolyticus* infection should be selected on the basis of a drug sensitivity testing since clinically relevant agents recommended were no longer completely effective. Both imipenem-resistant and multiple antimicrobial-resistant *V. parahaemolyticus* strains pose a great challenge to the treatment of the disease and public health problems caused by *V. parahaemolyticus*, which needs to be of wide concern to governmental departments and health institutions worldwide. The differences between external factors may give clues to the formulation of policies to prevent infection and to design related research to unveil the underlying mechanisms.

Conflicts of interest: No conflicts of interest.

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[#] Corresponding authors: Ping Fu, fuping@cfsa.net.cn; Yunchang Guo, gych@cfsa.net.cn.

[1] National Health Commission Key Laboratory of Food Safety Risk Assessment, Chinese Academy of Medical Science Research Unit (No.2019RU014), China National Center for Food Safety Risk Assessment, Beijing, China; [2] Risk Benefit Research Group, Division of Diet, Disease Prevention and Toxicology, National Food Institute, Technical University of Denmark, Lyngby, Denmark; [3] Global Capacity Building, Division of Global Surveillance, Lyngby, Denmark; [4] Jiangsu Center for Disease Prevention and Control, Nanjing, Jiangsu, China; [5] Beijing Center for Disease Prevention and Control, Institute for Nutrition and Hygiene, Beijing, China; [6] Beijing Zoom Tech Science and Technology Co., Ltd, Beijing, China.

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Preplanned Studies

Weather Variability, Socioeconomic Factors, and Pneumonia in Children Under Five-Years Old — Bangladesh, 2012–2016

Mohammad Zahid Hossain[1,2]; Shilu Tong[1,3,4,5]; Hilary Bambrick[1]; Md Alfazal Khan[6]; Wenbiao Hu[1,#]

Summary

What is already known on this topic?

Different socioecological factors were associated with childhood pneumonia in Bangladesh. However, previous studies did not assess spatial patterns, and socioecological factors and spatial variation have the potential to improve the accuracy and predictive ability of existing models.

What is added by this report?

The spatial random effects were present at the district level and were heterogeneous. Average temperature, temperature variation, and population density may influence the spatial pattern of childhood pneumonia in Bangladesh.

What are the implications for public health practice?

The study results will help policymakers and health managers to identify the vulnerable districts, plan further investigations, help to improve proper resource allocation, and improve health interventions.

Pneumonia is one of the leading causes of mortality and morbidity in children aged under five years in Bangladesh. This study aimed to identify the association between weather, social factors and childhood pneumonia and identify the spatial variation of the disease. A Bayesian spatial Poisson regression model with a conditional autoregressive prior structure was developed to quantify the association between childhood pneumonia and socioecological factors and identify the spatial variation. The study results suggested that a 1 °C increase in monthly temperature and monthly temperature variation may increase the monthly associated log relative risk (RR) of childhood pneumonia by 1.161 [95% credible interval (CrI): 1.013–1.429] and 1.463 (95% CrI: 1.170–1.839), respectively. However, the population density was inversely related with pneumonia risk (RR: 0.996, CrI: 0.994–0.998). Socioecological factors may influence the spatial pattern of childhood pneumonia, and the spatial random effects were heterogeneous.

The study was conducted in Bangladesh, which is located in the northeastern part of South Asia. Bangladesh is divided into 8 administrative divisions and 64 districts. Monthly data on under-5-years pneumonia were extracted from the District Health Information System Version 2 of the Directorate General of Health Services (DGHS) under the Ministry of Health and Family Welfare of Bangladesh from January 2012 to December 2016 (*1*). The pneumonia cases were diagnosed according to the World Health Organization pneumonia guidelines (*2*). The under-five-years population data at the district level were collected from the latest national population and household census (*3*). The sociodemographic data (percentage of education and internet use) at the district level were collected from socioeconomic and demographic reports (national series, volume-4) from the same census. The poverty data for each district was obtained from the Household Income and Expenditure Survey 2016 (*4*).

Climate data (temperature and rainfall) were obtained from the National Environmental Satellite, Data and Information service (https://www7.ncdc.noaa.gov/CDO/cdoselect.cmd?datasetabbv=GSOD), which is publicly available and widely used in previous studies (*5*–*6*). Poisson regression models in a Bayesian framework were developed for pneumonia cases at the district level. These models assume that the observed counts of childhood pneumonia cases (O_k) for the kth district (k=1…64) follow a Poisson distribution with mean μ_k:

$$O_k \sim \text{Poisson}(\mu_k) \quad (1)$$

$$\log(\mu_k) = \log(E_k) + \theta_k \quad (2)$$

where E_k (the expected number of cases in District$_k$) is an offset to control population size and θ_k is the associated log RR.

Prior to this analysis, we examined multicollinearity among the different covariates but did not find sufficiently strong associations to warrant exclusion or other treatment of any variables (Supplementary Table S1, available in http://weekly.chinacdc.cn). As a consequence, a total of 6 models were developed

(Supplementary Material, available in http://weekly.chinacdc.cn). The model which incorporated all socioecological covariates with both structured and unstructured random effects were selected for the final analysis.

The expected log relative risk θ_k was represented as follows:

$$\theta_k = \alpha + (\mathrm{Temp}_k)\beta_1 + (\mathrm{Tempva}_k)\beta_2 + (\mathrm{Rain}_k)\beta_3 + (\mathrm{Edu}_k)\beta_4 + (\mathrm{Int}_k)\beta_5 + (\mathrm{povi}_k)\beta_6 + (\mathrm{pop}_k)\beta_7 + u_k + v_k$$

where α is a constant; β_1 is the coefficient for temperature, β_2 is the coefficient for temperature variation, β_3 is the coefficient for rainfall, β_4 is the coefficient for percentage of education at the district level, β_5 is the coefficient for percentage of internet user at the district level, β_6 is percentage of poverty at the district level, and β_7 is the population density per square kilometer; v_k is a spatially unstructured random effect that is assumed to be normally distributed with mean zero and variance σ_v^2 and u_k is the spatially structured random effect that was modeled using a conditional autoregressive (CAR) prior $u_k \sim N\left(\bar{u}_{\sim k}, \sigma_u^2/n_k\right)$, where ~k denotes the neighbors of the *k*th district based on a simple adjacency matrix and n_k is the corresponding number of neighbors (*7*). WinBUGS software (version 1.4.3, MRC Biostatistics Unit, Cambridge, and Imperial College School of Medicine, London) was used to fit the Bayesian Poisson regression models. In the Markov chain Monte Carlo analysis, a 30,000 iteration "burn-in" was followed by 100,000 iteration sample collection. In every case, the Monte Carlo error was <5% of the overall standard deviation, indicating sufficient iterations of the model had been run after convergence. Model comparison was performed using the Deviance Information Criterion (DIC). Best fitted models were indicated by smaller DIC values (*8*).

The mean monthly number of pneumonia cases in children <5 years was 747.82. The mean monthly temperature, temperature variation, and rainfall were 30.97 °C, 3.63 °C, and 164.54 mm, respectively. Among the social factors, the mean percentages of education, internet use, poverty (per 100 population) and <5 years children density (per square kilometer) were 54.66%, 0.62%, 24.45%, and 117.72, respectively, at the district level (Supplementary Table S2, available in http://weekly.chinacdc.cn). The average monthly temperature was higher in the western region, while the monthly temperature variation was higher in most of the hilly areas located in the southern part of the country and two districts (Bhola and Pirojpur) of the coastal region of Bangladesh. The distributions of higher monthly average rainfall were scattered in different regions. Inclusion of spatial autocorrelation in the model was important. The model which included both structured and unstructured random effects had the smallest DIC (665.47 and 13,773.00 for models with and without random effects, respectively) (Supplementary Table S3, available in http://weekly.chinacdc.cn). The highest RRs were observed in the southeastern part (Rangamati district) and southern part (Pirojpur district) of the country (Figure 1). Supplementary Table S4 (available in http://weekly.chinacdc.cn) shows the list of districts with the higher RR.

Figure 2 depicts the distribution of spatial random effects (structured heterogeneity) of pneumonia in Bangladesh. The districts with darker color (red color) had relatively high spatial variation. These districts with high spatial variation might have some unknown factors that may have had effects on the incidence of childhood pneumonia but that we did not consider in the models (e.g., incomplete measurement of variables, lack of geocoding, and generalization of geographic features).

Our study results suggested that a rise of 1 °C average monthly temperature and temperature

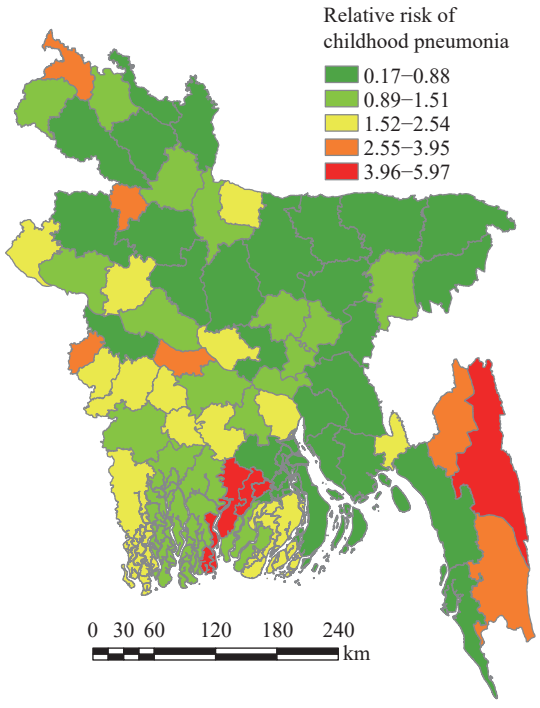

FIGURE 1. Posterior estimated Relative Risk of childhood pneumonia at the district level of Bangladesh from 2012 to 2016.

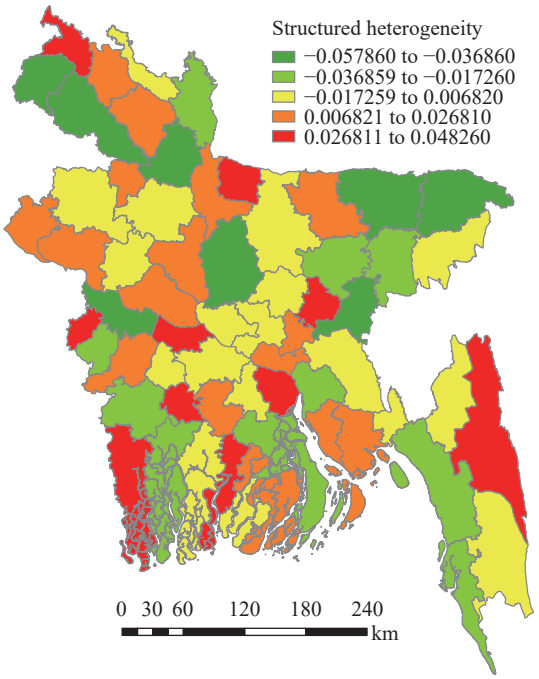

FIGURE 2. Spatial random effects of childhood pneumonia — Bangladesh, 2012−2016.

variation was associated with RR estimates of childhood pneumonia of 1.161 (95% CrI: 1.012–1.428) and 1.463 (95% CrI: 1.169–1.838), respectively. The density of children under five years in population was negatively associated with pneumonia (RR: 0.996, 95% CrI: 0.994–0.998) (Table 1). Additionally, no significant associations were found between childhood pneumonia and rainfall, education, internet use, or poverty since the corresponding 95% CrIs for the RR of each factor included 1.

DISCUSSION

In young children, the thermoregulation system is not yet matured and makes the children more vulnerable to temperature variation. This study describes the spatial pattern of childhood pneumonia and their socioecological factors in Bangladesh. Identifying the spatial variation of childhood pneumonia and important socioecological determinants can help target high-risk communities with evidence-based effective preventative measures.

Mapping of the spatially structured random effects indicated the spatial variation after controlling socioecological factors and spatial autocorrelation in the model. The Bayesian CAR model included unknown parameters as random effects, which incorporated the spatially correlated random effects (*9*). This approach can account for the residual variability resulting from spatial variation in effects that were not included in the models. The districts containing higher spatial random effects or variation may have some other risk factors remaining after adjustment of socioecological factors and spatial correlation.

TABLE 1. Crude and adjusted RR of different socioecological factors in Children Under Five-Years Old — Bangladesh, 2012−2016.

Variables	Crude RR (95% CrI)	Adjusted RR (95% CrI)
Temperature*	1.146 (0.929−1.432)	1.161 (1.013−1.429)
Temperature†	1.730 (1.694−1.763)	1.529 (1.503−1.555)
Temperature variability*	1.821 (1.376−2.491)	1.463 (1.170−1.839)
Temperature variability†	1.623 (1.596−1.649)	1.421 (1.395−1.447)
Rainfall*	1.000 (0.999−1.002)	1.000 (0.999−1.002)
Rainfall†	1.0007 (1.0006−1.0007)	1.0001 (1.0000−1.0002)
Population density*	0.995 (0.994−0.997)	0.996 (0.994−0.998)
Population density†	0.9943 (0.9942−0.9945)	0.9959 (0.9958−0.9961)
Education*	0.979 (0.956−1.004)	0.986 (0.969−1.005)
Education†	0.978 (0.977−0.979)	0.9853 (0.984−0.986)
Poverty*	1.008 (0.996−1.020)	1.000 (0.988−1.010)
Poverty†	1.009 (1.009−1.010)	1.002 (1.002−1.003)
Internet*	0.844 (0.661−1.070)	0.916 (0.743−1.126)
Internet†	0.874 (0.862−0.885)	0.929 (0.916−0.942)

Abbreviations: RR=relative risk; CrI=credible interval.
* with heterogeneity (u and v).
† without heterogeneity (u and v).

This study was subject to some limitations. First, in this study, we used data from monthly reports of the DGHS. This represented the number of patients that attended different levels of health facilities in Bangladesh for pneumonia treatment. However, there might be some patients in the community who did not attend any health facilities and who received treatment from village doctors or spiritual healers, especially in the rural areas. Therefore, there was a chance of measurement and information biases. Second, the unit of analysis was at the group level rather than at the individual level, so the results may be prone to the ecological fallacy.

The findings of this study could help policymakers better understand that childhood pneumonia has a heterogeneous spatial pattern and that socioecological factors may play a significant role in describing this pattern.

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Corresponding author: Wenbiao Hu, w2.hu@qut.edu.au.

[1] School of Public Health and Social Work, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia; [2] Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka, Bangladesh; [3] Shanghai Children's Medical Centre, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; [4] School of Public Health, Institute of Environment and Population Health, Anhui Medical University, Hefei, Anhui, China; [5] Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu, China; [6] Health System and Population Studies Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka, Bangladesh.

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Supplementary Material

Statistical models

As a consequence, a total of 6 models were developed. Model I included only ecological measures (temperature, temperature variation, and rainfall) as explanatory variables; Model II included only social factors (education, internet use, population density, and poverty) as covariates; Model III included both ecological and social factors as explanatory variables; Model IV incorporated spatially structured random effects with all socioecological covariates; Model V incorporated spatially unstructured random effects with all socioecological covariates; Model VI incorporated both structured and unstructured random effects with all socioecological covariates.

The expected log relative risk θ_k, for these models thus represented as follows:

$\theta_k = \alpha + (Temp_k)\beta_1 + (Tempva_k)\beta_2 + (Rain_k)\beta_3$ .....Model I

$\theta_k = \alpha + (Edu_k)\beta_1 + (Int_k)\beta_2 + (povi_k)\beta_3 + (pop_k)\beta_4$ .....Model II

$\theta_k = \alpha + (Temp_k)\beta_1 + (Tempva_k)\beta_2 + (Rain_k)\beta_3 + (Edu_k)\beta_4 + (Int_k)\beta_5 + (povi_k)\beta_6 + (pop_k)\beta_7$ .....Model III

$\theta_k = \alpha + (Temp_k)\beta_1 + (Tempva_k)\beta_2 + (Rain_k)\beta_3 + (Edu_k)\beta_4 + (Int_k)\beta_5 + (povi_k)\beta_6 + (pop_k)\beta_7 + u_k$ .....Model IV

$\theta_k = \alpha + (Temp_k)\beta_1 + (Tempva_k)\beta_2 + (Rain_k)\beta_3 + (Edu_k)\beta_4 + (Int_k)\beta_5 + (povi_k)\beta_6 + (pop_k)\beta_7 + v_k$ .....Model V

$\theta_k = \alpha + (Temp_k)\beta_1 + (Tempva_k)\beta_2 + (Rain_k)\beta_3 + (Edu_k)\beta_4 + (Int_k)\beta_5 + (povi_k)\beta_6 + (pop_k)\beta_7 + u_k + v_k$ .....Model VI

where α is a constant, β_1 is the coefficient for temperature, β_2 is the coefficient for temperature variation, β_3 is the coefficient for rainfall, β_4 is the coefficient for percentage of education at district level, β_5 is the coefficient for percentage of internet user at district level, β_6 is percentage of poverty at district level, and β_7 is the population density per square kilometer, v_k is a spatially unstructured random effect that is assumed to be normally distributed with mean zero and variance σ_v^2 and u_k is spatially structured random effect that was modeled using a conditional autoregressive (CAR) prior $u_k \sim N\left(\bar{u}_{\sim k}, \sigma_u^2/n_k\right)$, where ~k denotes the neighbors of the kth district based on a simple adjacency matrix and n_k is the corresponding number of neighbors.

SUPPLEMENTARY TABLE S1. Spearman correlation between pneumonia and socioecological covariates in Children Under Five-Years Old — Bangladesh, 2012−2016.

	Variables	1	2	3	4	5	6	7	8
1	Pneumonia	−							
2	Temperature	0.094							
3	Temperature variation	0.161	0.235						
4	Rainfall	−0.019	0.268*	−0.146					
5	Education	0.008	−0.011	−0.129	0.068				
6	Internet use	0.126	0.063	0.223	0.017	−0.066			
7	Population density	−0.148	−0.25*	−0.276*	0.028	0.075	0.162		
8	Poverty	0.069	−0.14	0.163	−0.077	0.095	−0.209	−0.336*	−

Note: − represnt its pneumonia itself, there will be no number.
* P<0.05.

SUPPLEMENTARY TABLE S2. Descriptive statistics of childhood pneumonia and different socioecological factors — Bangladesh, 2012−2016.

Variables	Mean ± SD	Range
Pneumonia	747.82 ± 245.32	355.53−1612.10
Temperature (°C)	30.97 ± 0.48	29.50−31.98
Temperature variation (°C)	3.63 ± 0.55	2.00−4.99
Rain (mm)	164.54 ± 101.37	3.13−386.96
Education (%)	54.66 ± 7.75	37.50−73.70
Internet use (%)	0.62 ± 0.76	0.14−6.03
Poverty incidence (%)	27.45 ± 15.31	2.60−70.80
Under five years population density (per square km)	117.72 ± 87.05	10.81−656.54

SUPPLEMENTARY TABLE S3. Model comparison for relative risk of monthly childhood pneumonia, underlying socioecological factors, and different random effects — Bangladesh, 2012−2016.

Model	Random effect	Deviance Information Criterion (DIC)	Effective number of parameters (pD)
Model I	*No*	19954.20	30.146
Model II	*No*	17382.41	5.001
Model III	*No*	13773.00	11.134
Model IV	*u*	665.98	63.940
Model V	*v*	665.67	63.812
Model VI	*u and v*	665.47	63.719

SUPPLEMENTARY TABLE S4. List of high-risk districts of Bangladesh for childhood pneumonia from 2012 to 2016.

Name of the district	Relative Risk (95% Credible interval)	Location
Rangamati	5.97 (5.63−6.31)	South-eastern
Pirojpur	4.71 (4.48−4.93)	South-western
Jhalkathi	4.38 (4.09−4.66)	South-western
Jaipurhut	3.95 (3.70−4.19)	North-eastern
Bandarbon	3.77 (3.48−4.07)	South-eastern
Meherpur	3.50 (3.23−3.78)	South-western
Rajbari	3.31(3.11−3.50)	Central
Khagrachari	3.25 (3.02−3.49)	South-eastern
Panchagarh	2.96 (2.78−3.14)	Northern

Preplanned Studies

Monitoring the Effective Sterilization of Low-Temperature Hydrogen Peroxide Gas Plasma Sterilizers in 58 Hospitals — 22 PLADs, China, June 2015–December 2019

Jiaqi Wang[1]; Baoying Zhang[1]; Huihui Sun[1]; Jian Zhang[1]; Hongyang Duan[1]; Haiqun Ban[1]; Jin Shen[1,#]; Liubo Zhang[1]

Summary

What is already known on this topic?

Hydrogen peroxide sterilizeation is widely used for luminal devices. However, the low penetrability of the sterilant is of major concern.

What is added by this report?

This report investigated the effective sterilization of low-temperature hydrogen peroxide gas plasma sterilizers and compared the applicability of different biological monitoring methods based on medical luminal devices.

What are the implications for public health practice?

It is recommended to use a biological process challenge device for monitoring the sterilization of luminal devices with low-temperature hydrogen peroxide gas plasma sterilizers.

With minimally invasive techniques improving, various types of medical luminal devices are widely used in surgeries. Low-temperature hydrogen peroxide gas plasma sterilizers significantly shorten the sterilization cycle and greatly improve the turnover rate of endoscopic instruments. This study aimed to investigate the effective sterilization of low-temperature hydrogen peroxide gas plasma sterilizers and to explore the applicability of different biological monitoring methods based on medical luminal devices. Basic information of low-temperature hydrogen peroxide gas plasma sterilizers in 58 hospitals across 22 provincial-level administrative divisions (PLADs) was investigated, and these sterilizers were tested with biological process challenge devices (PCD) and normal biological indicators (BI) at the same time to test the effectiveness of sterilization and to compare the monitoring results. The qualification rate, i.e., that the sterilizing process was effective, of biological PCD monitoring was lower than that of common biological monitoring. Therefore, more effective personnel training should be employed for the use of hydrogen peroxide gas plasma sterilizers. In addition, use of biological PCD monitoring when sterilizing tube-type/luminal devices is recommended.

Central sterile supply departments, operating rooms, and endoscopy centers in most of the hospitals are equipped with low-temperature hydrogen peroxide gas plasma sterilizers. However, due to the long lumen and the often-complex structures of luminal devices, they are often improperly sterilized. To understand the effective sterilization of luminal devices sterilized by low-temperature hydrogen peroxide gas plasma sterilizers, a survey was carried out as part of "National Surveillance Program for Hospital Disinfection and Sterilization Effects and Nosocomial Infection" conducted by China CDC. This survey included 58 sentinel hospitals across 22 PLADs of China.

A questionnaire was designed to investigate the low-temperature hydrogen peroxide gas plasma sterilizers, including items needing to be sterilized, and biological monitoring methods, etc.

PCD biological indicators used in this study, which contain a self-contained bacterial tablet with recovery medium and 2 thin plastic tubes, the inner diameter of 1 millimeter and length of 1,000 millimeters, and common self-contained biological indicators were issued to the sentinel hospitals, where the indicating strain was *Bacillus stearothermophilus* ATCC7953. From June 2015 to December 2019, the investigators from provincial and municipal-level CDCs conducted the examination of sterilizers in the surveyed hospitals.

The following biological monitoring methods were applied: 1) the common biological indicator and PCD biological indicator were placed close to the cabinet door near the lower shelf inside the sterilization chamber and were sterilized with other objects; 2) after a complete sterilization cycle, the two kinds of biological indicators were inoculated in a 56 °C incubator with positive controls and cultured for 48 hours. A positive control culture being observed with a

negative test group culture was judged as qualified sterilization, while a positive control culture with a positive test group culture was judged as unqualified sterilization. Statistical analysis was performed using SPSS for Windows (version 19, SPSS Inc., Chicago, Il, USA). The chi-squared test was used to compare the results of biological PCD monitoring with those of general biological monitoring. A *P*-value of <0.05 was considered to be statistically significant.

A total of 114 questionnaires were collected from 58 sentinel hospitals. Survey results showed that sterilizer use life ranged from 0.5 to 14 years, while about 24.6% of the sterilizers were over 10 years old. In this survey, laparoscopy, cystoscope, arthroscopy, choledochoscope, hysteroscope, and similar luminal devices were included, which implied sterilization by hydrogen peroxide. The tubes for these luminal devices have the smallest diameter of only 1 millimeter and a length of 50–130 centimeters. Low-temperature hydrogen peroxide gas plasma sterilizers are primarily used in central sterile supply departments (63.2%, 72/114), operating rooms (34.2%, 39/114), and endoscopy centers (2.6%, 3/114) (Table 1). All sterilizers are used for sterilization of luminal devices, such as endoscopies. In the survey, 38 sterilizers (33.3%) used biological PCD in daily monitoring, and the remaining 76 sterilizers still used common biological indicators for luminal devices.

Biological PCD monitoring and common biological monitoring were simultaneously conducted 338 times. The qualification rate of biological PCD monitoring was 95.6% (323/338), while common biological monitoring was 99.1% (335/338); the difference was statistically significant (*P*<0.001, Fisher exact probability test) (Table 2).

TABLE 1. Biological monitoring of low-temperature hydrogen peroxide sterilizers in 58 sentinel hospitals across 22 PLADs from June 2015 to December 2019.

Hospital grade	Department	Biological PCD	Common BI	Total
Secondary	CSSD	3	11	14
	Operating room	3	8	11
Tertiary	CSSD	23	35	58
	Endoscopic Center	1	2	3
	Operating room	8	20	28
Total	–	38	76	114

Note: Biological PCD, the number of sterilizers using biological PCD in daily monitoring for luminal devices. Common BI, the number of sterilizers using common biological indicators in daily monitoring for luminal devices.
Abbreviations: PLADs=provincial-level administrative divisions; PCD=process challenge devices; BI=biological indicators; CSSD=central sterile supply department.

TABLE 2. Comparison of 338 times biological PCD monitoring and common biological monitoring of low-temperature hydrogen peroxide sterilizers

Common BI	Biological PCD		Total
	Qualified	Unqualified	
Qualified	323	12	335
Unqualified	0	3	3
Total	323	15	338

Note: Biological PCD, the number of sterilizers using biological PCD in daily monitoring for luminal devices. Common BI, the number of sterilizers using common biological indicators in daily monitoring for luminal devices.
Abbreviations: PCD=process challenge devices; BI=biological indicators.

DISCUSSION

According to survey results, low-temperature hydrogen peroxide gas plasma sterilizers are widely used for various types of lumen devices. Common biological indicators are designed to monitor effective sterilization in non-lumen medical devices but cannot effectively monitor effective sterilization in devices with long lumens. In addition, the lumen's length and the material to be sterilized also affect the sterilization effect (*1*). The PCD is defined according to American national standard AAMI TIR31 as an item designed to simulate a product to be sterilized, to constitute a determined challenge to the sterilization process, and to assess the adequate performance of the process (*2*–*4*). According to the standard EN867, PCD is defined as an object, which simulates the worst case of conditions for the attainment of the specified sterilization conditions within the items to be sterilized (*5*). In our survey, the PCD contained a biological indicator and was placed in a position in which the sterilizer may have difficulty affecting. It should be noted that the PCD depends on the nature of the items to be sterilized.

The qualification rate of biological PCD was much lower compared to the common biological indicator, which can be interpreted as a biological luminal PCD device being more sensitive to the sterilization process. In this survey, 33.3% of the hospitals used biological PCD when sterilizing lumen devices, while the remaining hospitals were not aware of this problem.

When using hydrogen peroxide plasma sterilization, the items to be sterilized should be placed in the correct order and should not be covered (*6*–*7*).

However, in only 39.5% of the surveyed sterilizers, items to be sterilized were arranged in a single layer.

Hydrogen peroxide gas, which has a poor penetrating power, has a major role in sterilization progress. The sterilizing effect may be affected by the length, diameter, and material of the luminal devices (*8–10*), while common biological monitoring only reflects the sterilizing effects of device surfaces. In consideration of complex structures, the resistance of luminal devices to sterilization is much higher than in non-lumen devices. For these reasons, the application of luminal PCD can reflect relatively true sterilization effects. When sterilizing luminal devices, the routine use of biological luminal PCD is recommended so as to detect probable sterilization failures.

In this study standard biological PCD was used. However, under ideal conditions, the material, lumen length and inner diameter of biological PCDs should be consistent with the lumen devices which would more exactly reflect effective sterilization of low-temperature hydrogen peroxide gas plasma sterilizers. The staff awareness of disinfection process monitoring needs to be included in further investigations.

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[#] Corresponding author: Jin Shen, shenjin@nieh.chinacdc.cn.

[1] National Institute of Environmental Health, Chinese Center for Disease Control and Prevention, Beijing, China.

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Vital Surveillances

Dietary Exposure to Fumonisins and Health Risk Assessment in the Sixth China Total Diet Study — China, 2015–2020

Shuo Zhang[1]; Shuang Zhou[1,#]; Bing Lyu[1]; Nannan Qiu[1]; Jingguang Li[1]; Yunfeng Zhao[1]; Yongning Wu[1]

ABSTRACT

Introduction: Fumonisins are a group of widespread mycotoxins mainly existing in staple foods. Their toxicological effects on humans cause worldwide public health threat. During 2015–2020, the 6th China Total Diet Study (TDS) was conducted to study the dietary exposure to fumonisins in the Chinese adult population.

Methods: Fumonisins were analyzed by LC-MS/MS in 288 composite dietary samples collected from 24 provincial-level administrative divisions. After combining the national consumption data with analytical results, estimated daily intakes (EDIs) were assessed and compared with health-based guide values (HBGV).

Results: In the 6th China TDS, the highest fumonisin B (FBs) levels were found in staple foods/cereals among the 12 food categories. EDI of FBs was 104.9 ng/kg of body weight (bw)/day at the upper bound accounting 5.25% of the provisional maximum tolerable daily intake set by Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives. Among the 12 food categories, cereals and cereal products were the greatest contributor to FB exposure at 95%.

Conclusion: Although the estimated exposure to FBs in the 6th China TDS were well below the HBGV for FBs in general, it was 2 times higher than the exposure in the 5th China TDS. Furthermore, the exposure to FB3 has increased remarkable and is worth further attention in China.

INTRODUCTION

Fumonisins are secondary metabolites of *Fusarium* and *Aspergillus* species, which commonly infected crops and can contaminate the whole food chain. Fumonisin B (FB) is a group of fumonisin analogues. FBs as a group are clearly the most relevant toxin among fumonisin analogues and include fumonisin B1 (FB1), fumonisin B2 (FB2), and fumonisin B3 (FB3). FB1 is the most abundant and potent of these. As being possibly carcinogenic in humans (Group 2B) (*1*), FB1 has been shown to cause a variety of diseases in animals, including hepatotoxic, nephrotoxic, hepatocarcinogenic, and cytotoxic effects in mammals (*2*) with high potential impact on human health (*3*). To protect human health from the risk of FBs, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additive (JECFA) has set a provisional maximum tolerable daily intake (PMTDI) for the group of fumonisins (B1 and its analogues B2 and B3), at 2 μg/kg of body weight (bw)/day (*4*). Numerous countries have issued maximum levels for fumonisins in food and animal feed (*5–6*). In order to assess the risk of FB dietary intake in China, we applied a total diet study (TDS) approach. The TDS is an effective method that has been recommended by the WHO to estimate the dietary intakes of certain food chemicals (*7*). Unlike surveillance based on raw food commodities, TDS uses representative samples prepared as ready-to-eat dishes for the general population and combines consumption data to achieve a more accurate assessment (*8*). As a useful strategy, TDS has been conducted in several countries and regions for mycotoxin exposure assessment (*9*).

METHODS

China National Center for Food Safety Risk Assessment conducted the 6th China TDS in 2015–2020. This article aims to present the results of the exposure to fumonisins of the general Chinese population and evaluate the risk with regards to the international health-based guide values.

The protocol of the 6th TDS followed a similar procedure to the previous 4th and 5th TDSs in China. Collection of consumption data and food sampling were described in previous work (*10–11*). In the 6th China TDS, the number of provincial-level

administrative divisions (PLADs) were increased to 24 (Supplementary Table S1, available in weekly.chinacdc.cn). Each PLAD comprised of 3 or 6 survey sites according to population size (6 survey sites for PLADs with more than 50 million population, 3 survey sites for PLADs with less than 50 million population). Since approximately two-thirds of the Chinese population reside in rural areas, we randomly selected rural counties and urban cites with a ratio of 2∶1 for each PLAD.

The dietary survey adopted multiple survey methods. For the survey for households, the measuring of weight plus a three-day accounting method was applied. For the survey for individuals, verbal interviews were conducted every 24 hours over 3 days. Samples of various food items were purchased at local markets, grocery stores, and local farms of each survey site. Thirteen dietary sample categories, such as cereals, legumes, potatoes, meats, eggs, aquatic products, dairy products, vegetables, fruits, sugars, beverages and water, alcohols, and condiments, were included in the TDS. For each PLAD, various food cooked according to local customs and condiments were added into the other 12 sample categories at the calculated amount during cooking procedure. Thus, in total, 288 dietary samples were prepared in the 6th China TDS.

FB1, FB2, and FB3 in food samples were analyzed via an isotope dilution UPLC-MS/MS method (*11*). Briefly, this analysis involved an extraction using acetonitrile/water solvent mixture for food samples, followed by purification with MultiSep 211 Fum solid phase extraction column. The chromatographic separation and mass spectrometry parameters are described Supplementary Table S2 (available in weekly.chinacdc.cn). The method validation was also well described (*11*).

The exposure of the Chinese adult populations was assessed by combining consumption data with analytical results. When calculating the estimated daily intakes (EDI), the management for results below the limit of detection (LOD) and/or limit of quantification (LOQ), so called left-censored data, was applied based on the Global Environment Monitoring System/Food Contamination Monitoring Assessment Programme (GEMS/Food) guidelines for low-level contamination of food (*12*). In this study, the proportion of left-censored data exceeded 60%. Thus, scenarios for lower bound (LB) and upper bound (UB) were applied. To estimate the lowest (LB scenario) possible EDI, a value of zero was assigned for results below the LOD, and the LOD was assigned for results below the LOQ. To estimate the highest (UB scenario) possible EDI, a value of the LOD were assigned for results below LOD, and LOQ were assigned for results below LOQ.

The EDI (in ng/kg bw/day) of each fumonisin was calculated as follows:

$$\mathrm{EDI} = \frac{\sum_{i=1}^{p} T_i \times F_i}{bw}$$

where T_i represents the concentration of each mycotoxin in a dietary sample from each food category i (i = 1, …, p) (ng/g), F_i is the consumption of each food category i in a day (g/d), and bw is the standard body weight (kg) of 63 kg. IBM SPSS Statistics (version 22.0, IBM Corp., New York, US) was used for data processing and analysis.

RESULTS

Among the total 288 dietary samples, the occurrence data of individual toxins and total of FBs (FB1+FB2+FB3) are shown in Table 1, respectively. The concentrations and distribution of FBs in participant PLADs are demonstrated in Figure 1.

The frequency of detection for FBs was 32.6% (Table 1). Among 12 food categories, cereals had the highest incidence of 95.8%, with an average FBs level of 7.59 μg/kg. Shandong, Hebei, Sichuan, Jiangsu, and Shanxi had the highest level of FBs (Figure 1).

The average food consumption level was 2,439 g/day in the 6th TDS. Among the 12 food categories, beverages and water contributed most to the total consumption (40.7%), followed by cereals and vegetables making up 29.3% and 14.2%, respectively.

The EDI of each individual fumonisin was calculated according to the formula in Methods section. The EDI of total fumonisins was the sum of EDI of FB1, FB2, and FB3 (FBs). In the 6th TDS, the average EDI of total FBs was 102.78–104.91 ng/kg bw/day (Table 2). Cereals were the predominant contributor, making up 97.8%–98.6% of the overall EDI. Shangdong had the highest total EDI of FBs at 597.40–605.27 ng/kg bw/day (LB-UB) and the highest EDI of FB1 at 516.0–516.4 ng/kg bw/day (LB-UB) (Figure 2). However, the highest EDI of FB2 and FB3 were in Hebei at 87.18–87.62 ng/kg bw/day and 183.49–183.93 ng/kg bw/day (LB-UB), respectively.

DISCUSSION

Regarding to the contamination level of fumonisins,

TABLE 1. Contamination levels of fumonisins (μg/kg) and the positive rate of detection in the 6th China TDS, 2015−2020.

Food category	FB1	FB2	FB3	FBs
Cereals				
Positive, %	95.8	25.0	20.8	95.8
Mean	5.33	0.58	1.68	7.59
Median	2.57	0.02	0.02	2.74
Legumes				
Positive, %	58.3	0.0	12.5	58.3
Mean	0.78	0.01	0.10	0.90
Median	0.16	0.01	0.01	0.23
Potatoes				
Positive, %	66.7	4.2	16.7	66.7
Mean	0.57	0.02	0.13	0.72
Median	0.09	0.01	0.01	0.14
Meats				
Positive, %	62.5	4.2	4.2	62.5
Mean	1.08	0.02	0.03	1.13
Median	0.25	0.01	0.01	0.27
Eggs				
Positive, %	16.7	8.3	0.0	16.7
Mean	0.02	0.06	0.01	0.09
Median	0.01	0.01	0.01	0.03
Aquatic products				
Positive, %	8.3	20.8	0.0	25.0
Mean	0.03	0.75	0.01	0.79
Median	0.01	0.01	0.01	0.03
Dairy products				
Positive, %	4.2	0.0	0.0	4.2
Mean	0.02	0.01	0.01	0.04
Median	0.01	0.01	0.01	0.03
Vegetables				
Positive, %	20.8	0.0	0.0	20.8
Mean	0.17	0.01	0.01	0.19
Median	0.01	0.01	0.01	0.03
Fruits				
Positive, %	0.0	0.0	0.0	0.0
Mean	0.01	0.01	0.01	0.03
Median	0.01	0.01	0.01	0.03
Sugars				
Positive, %	0.0	0.0	0.0	0.0
Mean	0.01	0.01	0.01	0.03
Median	0.01	0.01	0.01	0.03
Beverages and water				
Positive, %	0.0	4.2	0.0	4.2
Mean	0.01	0.01	0.01	0.03
Median	0.01	0.01	0.010	0.03
Alcohols				
Positive, %	33.3	4.2	4.2	33.3
Mean	0.52	0.16	0.50	1.17
Median	0.01	0.01	0.01	0.03
Total samples (N=288)				
Positive, %	30.6	5.9	4.9	32.6

Note: for samples in which toxins were not detected, values were assumed to be half the LOD and for samples in which toxin levels were below the LOQ, values were assumed to be half the LOQ. There are 24 samples for each food category.
Abbreviations: FB1=fumonisin B1; FB2=fumonisin B2; FB1= fumonisin B1; TDS=total diet study; LOD=limit of detection; LOQ=limit of quantification.

China has not yet set a maximum limit for fumonisins. Codex Alimentarius Commission (CAC) has set the maximum level for FB1+FB2 in raw maize grain (4,000 μg/kg) and maize flour and maize meal (2,000 μg/kg) (*13*). In our study, the average contamination level of fumonisins (FB1+FB2+FB3) in cereals were 7.59 μg/kg, much lower than CAC's regulation level. Even in Shandong Province, the highest aggregated FB levels (41.56 μg/kg) were still much lower than CAC's limit. Relatively high contamination levels of FBs were found sporadically, such as in alcohol in Sichuan (16.55 μg/kg) and in meats in Shanxi (10.30 μg/kg). Among the three types of FBs, FB1 was most frequently detected and abundant. FB2 and FB3 shared similar incidence. Commonly believed, FB3 often co-exists with FB1 and FB2, and its concentration usually does not exceed that of FB1 and FB2, usually accounting for an additional 10%–15% to FB1 levels (*14*). Thus, FB3 was usually considered as a minor important mycotoxin. However, in our study, the relative level of FB3 was 21%–25% in cereals, legumes and potatoes, and even higher than FB2. The relative amount of FB1 compared to FB2 and FB3, is related to climatic factors, such as water activity and temperature (*14*). Therefore, these results provide some information for food safety surveillance and establishing China's maximum limit for fumonisins in the future. First, in addition to FB1 and FB2, FB3 should be included. Second, besides cereals and their products, potatoes and meats need to be considered as candidate food categories in surveillance

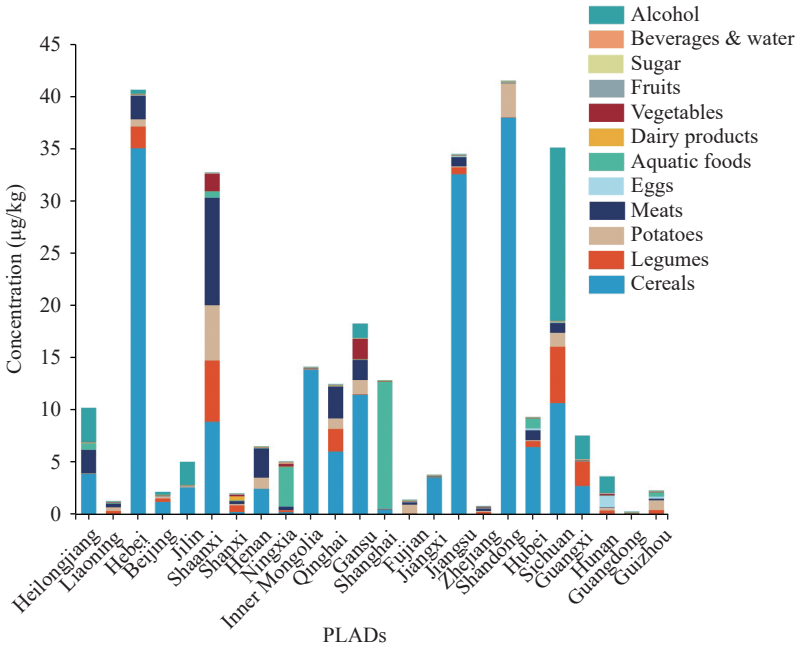

FIGURE 1. Contamination levels of FBs (FB1+FB2+FB3) in participating provinces from the 6th China TDS.
Abbreviations: FB=fumonisin B; TDS=total diet study; PLADs=provincial-level administrative divisions.

TABLE 2. Estimated dietary intake (μg/kg bw/day) of FBs in food categories with their percentage of PMTDI from the 6th TDS of general Chinese population.

Food category	EDI (ng/kg bw/day)		Percentage of PMTDI	
	LB	UB	LB	UB
Cereals	97.78	98.55	4.89	4.93
Legumes	0.85	0.90	0.04	0.04
Potatoes	0.96	1.01	0.05	0.05
Meats	1.61	1.67	0.08	0.08
Eggs	0.02	0.04	0.00	0.00
Aquatic Foods	0.59	0.61	0.03	0.03
Dairy Products	0.00	0.03	0.00	0.00
Vegetables	0.74	1.02	0.04	0.05
Fruits	0.00	0.04	0.00	0.00
Sugar	0.00	0.00	0.00	0.00
Beverages & water	0.04	0.82	0.00	0.04
Alcohol	0.19	0.20	0.01	0.01
Total	102.78	104.91	5.14	5.25

Note: Provisional Maximum Tolerable Daily Intake (PMTDI) set by JECFA is 2 μg/kg bw/day for FBs (FB1+FB2+FB3). % of PMTDI= EDI/PMTDI×100%.
Abbreviations: FB=fumonisin B; TDS=total diet study; EDI= estimated dietary intake; LB=lower bound; UB=upper bound.

plan or maximum limit scope.

The dietary exposure to FBs at 104.91 ng/kg bw/day (upper bound) accounted 5% of the PMTDI set by JECFA. It indicated the risk of dietary exposure to

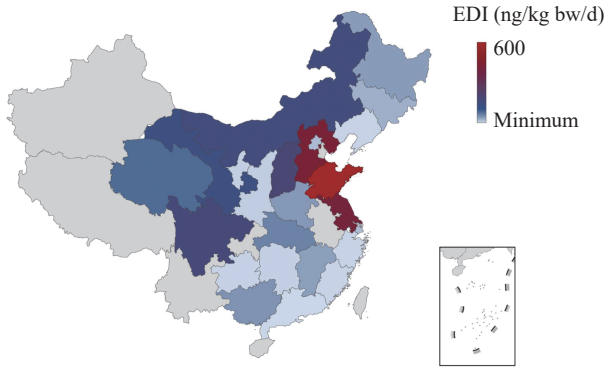

FIGURE 2. Regional distribution of EDI of FBs (FB1+FB2+FB3) in the 6th China TDS. The blue and red regions represent the participating PLADs. In the 24 participating provinces, their color intensity represents their levels of EDI respectively.
Abbreviations: EDI=estimated dietary intake; FB=fumonisin B; TDS=total diet study; PLADs=provincial-level administrative divisions.

fumonisins in China was at a safe level. The highest EDI of FBs was found in Shandong Province, accounting for approximately 30% of the PMTDI. Together with Hebei and Jiangsu, these three provinces with highest EDIs for FBs are located in the east of North China Plain (Figure 2). Whereas, comparing to the 5th China TDS (EDI, 50 ng/kg bw/day) (*2*), the exposure level doubled, indicating a trend of dramatic increase. Among the 3 types of FBs, FB1, FB2, and FB3 contributed 70.6%, 7.6%, and

21.8% of the overall dietary exposure, respectively. The contribution of FB3 to EDI of total fumonisins has also exceeded FB2 and should not be overlooked. This is the first result revealed dietary exposure to FB3 from TDS. FB1 and FB2 were investigated in most TDSs, but FB3, as a considered least important fumonisin among the three, was seldom included. The Netherlands TDS (*15*) included FB3, but the sensitivity of the method (LOD=3.3 μg/kg) was not enough to detect the existence of fumonisins in ready-to-eat dishes.

This study was subject to some limitations. As for TDS in the study, as well as other TDSs, uncertainties were existed in exposure assessment, such as analytical methods, consumption statistics, and especially sample representativeness. Mycotoxin contamination occurred sporadically and could be affected by temperature, humidity, geographic location and storage duration. For such a large-scale study, big uncertainty could be caused by limited sample numbers and heterogeneous distribution of toxins.

In the 6th China TDS, exposure estimates for FBs were generally out of concern with 5.25% of PMTDI for the general population. However, it still needs to be noted that the population in relative high exposure regions or the high consumers of certain food categories may be associated with higher risk. Cereals were the predominant source and contributed over 90% to the dietary exposure to fumonisins. The remarkable increase of EDI of fumonisins and considerable contribution from FB3 in the 6th China TDS were well worth further attention.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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[#] Corresponding author: Shuang Zhou, szhoupku@gmail.com.

[1] NHC Key Laboratory of Food Safety Risk Assessment, Food Safety Research Unit (2019RU014) of Chinese Academy of Medical Science, China National Center for Food Safety Risk Assessment, Beijing, China.

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SUPPLEMENTARY TABLE S1. Participating provincial-level administrative divisions in the 6th China TDS.

North 1 (N1)	North 2 (N2)	South 1 (S1)	South 2 (S2)
Heilongjiang (HLJ)	Shaanxi (SX)	Shanghai (SH)	Hubei (HuB)
Hebei (HeB)	Henan (HeN)	Fujian (FJ)	Sichuan (SC)
Liaoning (LN)	Ningxia (NX)	Jiangxi (JX)	Guangxi (GX)
	Inner Mongolia (IM)	Jiangsu (JS)	Hunan (HuN)
Jilin (JL)	Qinghai (QH)	Zhejiang (ZJ)	Guangdong (GD)
Shanxi (ShX)	Gansu (GS)	Shandong (SD)	Guizhou (GZ)

SUPPLEMENTARY TABLE S2. MRM parameters of fumonisins in the 6th China TDS.

Mycotoxin	Precursor ion	Precursor ion (m/z)	Daughter ion (m/z)	Collision energy (eV)	Cone voltage (V)
FB1	[M+H]+	722.3	704.2*	41	40
			334.3	55	40
FB2	[M+H]+	707.2	689.3*	40	50
			337.4	52	50
FB3	[M+H]+	707.2	337.3*	50	50
			355.3	46	50
13C-FB1	[M+H]+	756.3	738.5*	56	50
			356.4	43	50
13C-FB2	[M+H]+	740.4	358.4*	53	50
			722.4	42	50
13C-FB3	[M+H]+	740.4	358.3*	53	75
			376.4	47	75

Note: For chromatographic separation, the sample was separated on an ACQUITY UPLC BEH T3 (100 mm×2.1 mm, 1.7 μm) column at 40 ℃. A gradient elution was performed using water containing 0.2% formic acid (A) acetonitrile (B). The elution program was performed as follows: 0–2 min, 30%–60% B; 2–4 min, 60%–80% B; 4.1 min, 100% B; washing at 4.1–4.6 min, 100% B; 4.6–4.8 min, 100%–30% B; 4.8–7 min, pre-equilibration using 30% B. The flow rate was 0.3 mL/min, and a 5 μL sample was injected at 10 ℃.

Abbreviations: MRM=multiple reaction monitoring; EDI=estimated dietary intake; TDS=total diet study; FB1=fumonisin B1; FB2=fumonisin B2; FB3=fumonisin B3.

* Quantification ion

Notes from the Field

First Human Infection Case of Monkey B Virus Identified in China, 2021

Wenling Wang[1,&]; Wenjie Qi[2,&]; Jingyuan Liu[3,&]; Haijun Du[4,&]; Li Zhao[1]; Yang Zheng[5]; Guoxing Wang[2]; Yang Pan[5]; Baoying Huang[1]; Zhaomin Feng[5]; Daitao Zhang[5]; Peng Yang[5]; Jun Han[4,#]; Quanyi Wang[5,#]; Wenjie Tan[1,#]

Monkey B virus (BV), initially isolated in 1932, is currently designated as *Macacine alphaherpesvirus 1* by the International Committee on Taxonomy of Viruses (*1*). BV is an alphaherpesvirus enzootic in macaques of the genus *Macaca*, normally transmitted horizontally via direct contact and exchange of bodily secretions, just like herpes simplex virus (HSV) in humans. BV is not evident in its natural macaque hosts, but about 60 additional cases of pathogenic zoonotic BV infection have occurred sporadically and the fatality rate of zoonotic BV infections is 70%–80%. Although the risk for secondary transmission appears to be minimal, one case of human-to-human transmission of herpes B virus has previously been documented (*2*). Zoonotic BV infections have mainly involved primate veterinarians, animal care personnel, or laboratory researchers in North America. However, there were no fatal or even clinically evident BV infections in China before 2021. Here, we reported the first human infection case with BV identified in China.

This case of BV occurred in a veterinary surgeon (53 years old, male) who worked in an institute specialized in nonhuman primate breeding and experimental research in Beijing. He dissected two dead monkeys on March 4 and 6, 2021 and experienced nausea and vomiting followed by fever with neurological symptoms one month later. As a result, the patient visited doctor in several hospitals but eventually died on May 27.

On April 17, cerebrospinal fluid (CSF) was collected from this patient for next generation sequencing (NGS), 285 reads obtained suggesting possible alphaherpesvirus infection. To further identify the etiological agent, several specimens (including CSF, blister fluid, blood, airway aspirates, nasal swab, throat swab, and plasma) were collected from this patient and 2 close contacts (1 doctor, 47 years old, male; 1 nurse, 25 years old, female), then sent to National Institute for Viral Disease Control and Prevention (IVDC) of China CDC on April 19. Four sets of real-time polymerase chain reaction (rtPCR) were performed to detect BV (*3*), varicella zoster virus (VZV) (*4*), monkeypox virus and orthopoxvirus (*5*). For BV detection, the forward primer was 5′-TGGCCTACTACCGCGTGG-3′, the reverse primer was 5′-TGGTACGTGTGGGAGTAGCG-3′; and the TaqMan probe was 5′-FAM-CCGCCCTCTCCGAGCACGTG-TAMRA-3′ . The rtPCR results showed that only BV genome was detected as positive (Cycle of threshold: 34) in the CSF specimen of the patient, while BV negative in other specimens from the patient and all specimens from close contacts; moreover, all tests were negative for VZV, monkeypox virus, and orthopoxvirus (Figure 1). This result confirmed the first human infection case with BV in China.

The first human infection case with BV was identified by NGS and rtPCR in China, 2021. This implied that BV in monkeys might pose a potential zoonotic threat to the occupational workers. It is necessary to eliminate BV during the development of specific pathogen-free rhesus colonies and to strengthen surveillance in laboratory macaques and occupational workers in China.

Conflicts of interest: No conflicts of interest.

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[#] Corresponding authors: Jun Han, hanjun@ivdc.chinacdc.cn; Quanyi Wang, bjcdcxm@126.com; Wenjie Tan, tanwj@ivdc.chinacdc.cn.

[1] Key Laboratory of Biosafety, National Health and Family Planning Commission, National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China; [2] Beijing Friendship Hospital, Capital Medical University, Beijing, China; [3] Intensive Care Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China; [4] Viral Center of National Pathogen Resource Center, China CDC, Beijing, China; [5] Beijing Center for Disease Prevention and Control, Beijing, China.

[&] Joint first authors.

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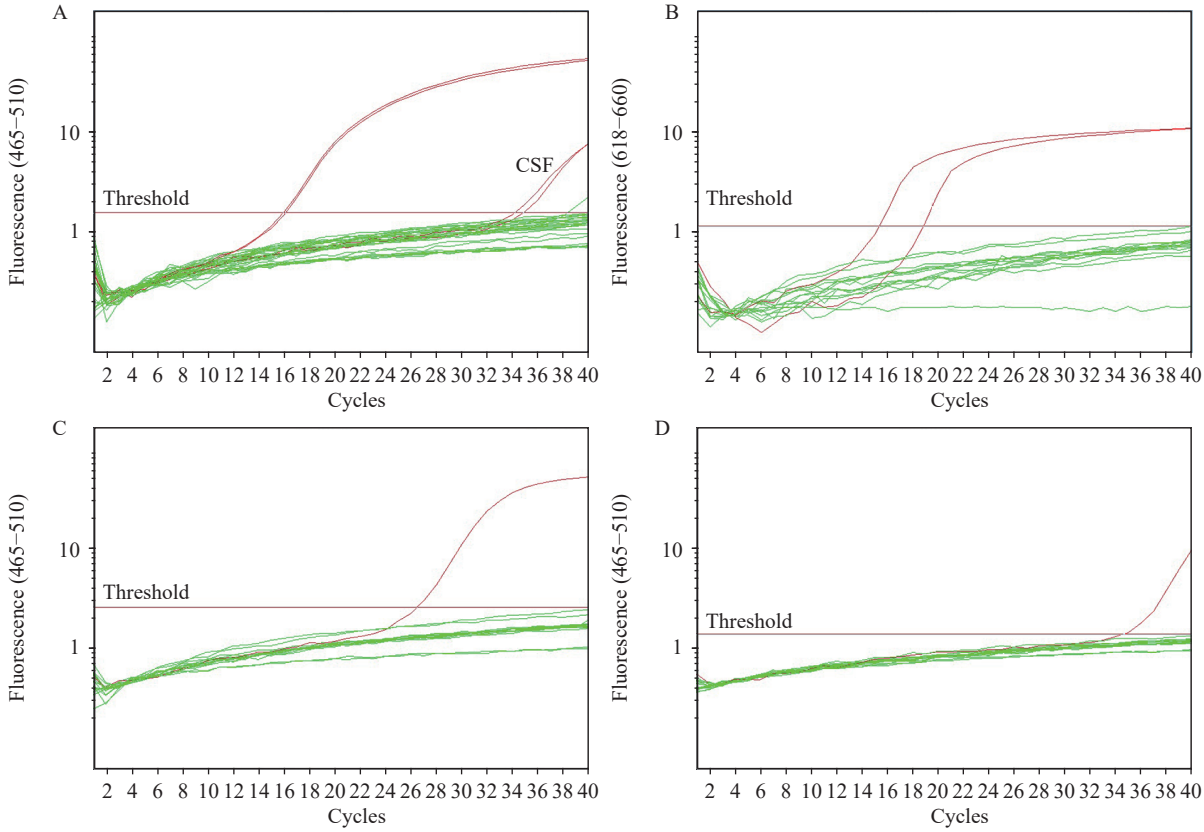

FIGURE 1. Identification of the first human infection case with monkey B virus by rtPCR. (A) The rtPCR test result for BV; (B) The rtPCR test result for VZV; (C) The rtPCR test result for monkeypox virus; (D) The rtPCR test result for orthopoxvirus. Note: For (A)–(D), rtPCR detections targeting to various virus in the 11 specimens collected from the patient (CSF, blister fluid, blood, airway aspirates, nasal swab) and 2 close contacts (throat swab, blister fluid and plasma). Each line represents a specimen, red lines represent positive controls or positive specimens, and green lines represent negative specimens. Abbrevations: CSF=cerebrospinal fluid, rtPCR=real-time polymerase chain reaction, BV=B virus, VZV=varicella zoster virus.

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Notifiable Infectious Diseases Reports

Reported Cases and Deaths of National Notifiable Infectious Diseases — China, May, 2021

Diseases	Cases	Deaths
Plague	0	0
Cholera	0	0
SARS-CoV	0	0
Acquired immune deficiency syndrome	5,047	1,352
Hepatitis	131,135	49
Hepatitis A	1,095	0
Hepatitis B	105,393	38
Hepatitis C	21,254	8
Hepatitis D	22	0
Hepatitis E	2,509	3
Other hepatitis	862	0
Poliomyelitis	0	0
Human infection with H5N1 virus	0	0
Measles	80	0
Epidemic hemorrhagic fever	685	8
Rabies	11	9
Japanese encephalitis	2	0
Dengue	5	0
Anthrax	14	0
Dysentery	5,011	2
Tuberculosis	75,243	102
Typhoid fever and paratyphoid fever	649	0
Meningococcal meningitis	11	0
Pertussis	427	1
Diphtheria	0	0
Neonatal tetanus	3	0
Scarlet fever	4,124	0
Brucellosis	8,096	1
Gonorrhea	10,773	0
Syphilis	47,999	5
Leptospirosis	9	0
Schistosomiasis	6	0
Malaria	97	0
Human infection with H7N9 virus	0	0
COVID-19*	451	0
Influenza	41,516	0
Mumps	11,970	0

Continued

Diseases	Cases	Deaths
Rubella	187	0
Acute hemorrhagic conjunctivitis	2,548	0
Leprosy	46	0
Typhus	111	0
Kala azar	32	0
Echinococcosis	265	0
Filariasis	0	0
Infectious diarrhea[†]	112,075	0
Hand, foot, and mouth disease	240,838	2
Total	**699,466**	**1,531**

* The data were extracted from the website of the National Health Commission of the People's Republic of China.
† Infectious diarrhea excludes cholera, dysentery, typhoid fever and paratyphoid fever.
The number of cases and cause-specific deaths referred to data recorded in National Notifiable Disease Reporting System (NNDRS) in China, which includes both clinically-diagnosed cases and laboratory-confirmed cases. Only reported cases of the 31 provincial-level administrative divisions in the mainland of China are included in the table, whereas data of Hong Kong Special Administrative Region, Macau Special Administrative Region, and Taiwan, China are not included. Monthly statistics were calculated without annual verification, which is usually conducted in February of the next year for de-duplication and verification of reported cases in annual statistics. Therefore, 12-month cases could not be added together directly to calculate the cumulative cases because the individual information might be verified via NNDRS according to information verification or field investigations by local CDCs.

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