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Authors
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Integrated molecular and risk-based assessment of microbial hazards during catastrophic urban flooding

Yue Zhang[a,b,c,d], Xiao-Ming Li[a,b,*], Mingchen Yao[a,b,c], Walter van der Meer[e]
and Gang Liu[a,b,c]

[a] Key Laboratory of Environmental Aquatic Chemistry, State Key Laboratory of Regional Environment and Sustainability, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, P.O. Box 5048, 2600 GA Delft, The Netherlands
[d] Wangcheng District Government, Changsha City 41020, Hunan Province, China
[e] Science and Technology, University of Twente, P.O. Box 217, 7500AE Enschede, The Netherlands
*Corresponding author. E-mail: xmli@rcees.ac.cn

X-ML, 0000-0002-2930-4273

ABSTRACT

Extreme rainfall and urban flooding pose escalating risks to public health by mobilizing sewage and pathogenic microorganisms. In July 2021, record-breaking rainfall in Henan Province, China, caused catastrophic flooding, yet the microbial health risks associated with such events remain poorly quantified. Here, we applied high-throughput qPCR arrays to detect 21 pathogenic bacteria in floodwater and postflood tap water, and used quantitative microbial risk assessment (QMRA) to estimate infection probabilities for exposed residents. Our results showed that in floodwater, 21 pathogenic bacteria were detected, with *Cryptosporidium* spp. (579.8 gc/L) and *Pseudomonas aeruginosa* (13,500.9 gc/L), being prominent, which were also identified in tap water. Floodwater exposure substantially increases infection risks, highlighting ingestion and inhalation as primary pathways. Simple protective measures, such as avoiding contact with contaminated water, can significantly reduce risks. This study provides the first integrated molecular and risk-based assessment of microbial hazards during an extreme flood event. The findings underscore the importance of water quality monitoring, improved sewage and drainage management, and timely public health interventions such as boil water advisories. As climate change intensifies the frequency of extreme rainfall events, proactive surveillance and international collaboration will be essential to prevent waterborne disease outbreaks and protect vulnerable populations.

Key words: high-throughput qPCR, pathogen, public health, tap water, urban floodwater

HIGHLIGHTS

- Combined high-throughput qPCR with quantitative microbial risk assessment (QMRA) to evaluate floodborne pathogens.
- Provided the first integrated molecular-risk framework for assessing urban floodwater contamination.
- Linked microbial evidence to public health protection and emergency water management.
- Offered data-driven guidance for climate adaptation and flood-resilient urban systems.

1. INTRODUCTION

Extreme rainfall events are intensifying with climate change and rapid urbanization, leading to unprecedented urban floods and widespread public health crises. In July 2021, Henan Province, China, experienced catastrophic flooding, including a record 201.9 mm of rainfall within an hour in Zhengzhou (since 1951) (Guo *et al.* 2023). The disaster affected more than 14 million people, caused over 82 billion yuan in damages, and triggered sharp increases in infectious diarrhea and respiratory illness (Liu *et al.* 2023). Such outcomes underscore the vulnerability of urban populations to floodborne pathogens.

Floodwaters in cities with combined sewer systems pose particular risks because stormwater and domestic sewage are conveyed through the same network. During heavy rainfall, these systems overflow, releasing untreated sewage into rivers and water supplies (Mengel *et al.* 2014). Previous studies have shown that exposure to fecally contaminated waters increases the risk of acute gastrointestinal illness (Fewtrell & Kay 2015; Mark *et al.* 2018). Yet, most risk assessments rely on nonspecific fecal indicators such as *Escherichia coli* and enterococci (ten Veldhuis *et al.* 2010; Dorevitch *et al.* 2015). These studies typically use concentrations of fecal indicator organisms like enterococci and *E. coli* (Dorevitch *et al.* 2015; Rodrigues *et al.* 2016), which fail to capture the diversity and pathogenic potential of human-derived microorganisms.

Quantitative microbial risk assessment (QMRA) offers a powerful framework to estimate infection risks by integrating pathogen concentrations, exposure scenarios, and dose–response relationships (Ichida *et al.* 2016; WHO 2016). Although QMRA has been widely applied in microbial water quality management, few studies have applied it to large-scale flood disasters with multiple human pathogens. This knowledge gap is critical given epidemiological evidence of excess diarrheal and respiratory disease following urban floods worldwide (Watson *et al.* 2007; McKee & Cruz 2021).

Pathogenic matter in the environment may be ingested by humans through multiple environmental pathways. High-throughput quantitative PCR (HT-qPCR) was implemented to detect the pathogenic bacteria in the water samples, which were found to be contaminated with pathogens (An *et al.* 2020), such as *Vibrio cholerae*, *Cryptosporidium* spp., *Giardia* spp., *Campylobacter* spp., and *E. coli*, which are prevalent in urban wastewater or have been frequently reported in both animal feces and human wastewater. These pathogens may cause health risks when people are exposed to floodwater.

Here, we quantify human health risks associated with the 2021 Henan flood. Using HT-qPCR, we detected 21 pathogenic bacteria in floodwaters and selected 5 with available dose–response models for QMRA. Exposure data from questionnaires were combined with pathogen concentrations to estimate infection probabilities from floodwater ingestion. By linking molecular pathogen detection with risk modeling, our study provides a comprehensive assessment of microbial health risks during extreme flooding events. These findings highlight the urgent need for improved urban drainage systems and proactive public health strategies as climate extremes intensify.

2. MATERIALS AND METHODS

2.1. Sample collection and DNA extraction

In Zhengzhou City, five sites were selected: (i) a center commercial area with high pedestrian density and prolonged stagnant water during flooding; (ii) an old residential district with an obsolete drainage system and severe waterlogging; (iii) a river confluence area where backflow and mixing of multiple pollution sources occur; and (iv and v) two peripheral sites in an industrial park and an urban green space to compare water quality across functional zones.

In Xinxiang City, four sites were chosen: (i) a major transportation hub where floodwaters carried substantial road pollutants; (ii) a newly developed residential area affected by localized flooding despite modern infrastructure; (iii) an irrigation canal in agricultural fields, to evaluate potential contamination of agricultural water; and (iv) lakes in an urban park, to assess impacts on recreational waters. The distribution of all sampling sites is shown in Figure 1(a).

At each site, ~5 L of floodwater was collected using sterilized 1 L glass bottles (Fisher, Waltham, MA) between July 22 and 25, 2021, corresponding to 1–7 days after the flood. Indoor tap water samples were also collected within 1 week following restoration of the supply. Samples were transported on ice, filtered onto 0.2 μm pore-size mixed cellulose ester filters (Millipore, Billerica, MA), and stored at −20 °C before transfer to the Research Center for Eco-Environmental Science (RCEES) for DNA extraction. DNA was extracted from fragmented filters using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH) according to manufacturer instructions.

2.2. Pathogen detection by high-throughput qPCR

Pathogen detection was performed using HT-qPCR, capable of simultaneously quantifying 68 marker genes from 33 human pathogens and 23 fecal markers from 10 hosts (An *et al.* 2020). Target organisms included *Cryptosporidium* spp., *E. coli*, *Pseudomonas aeruginosa*, *Giardia lamblia*, *Legionella pneumophila*, *Mycobacterium* spp., *Salmonella enterica*, *Helicobacter pylori*, *Klebsiella pneumoniae*, and *Clostridium difficile*. Details of the HT-qPCR process and data analysis are provided in Supplementary Figure S3.

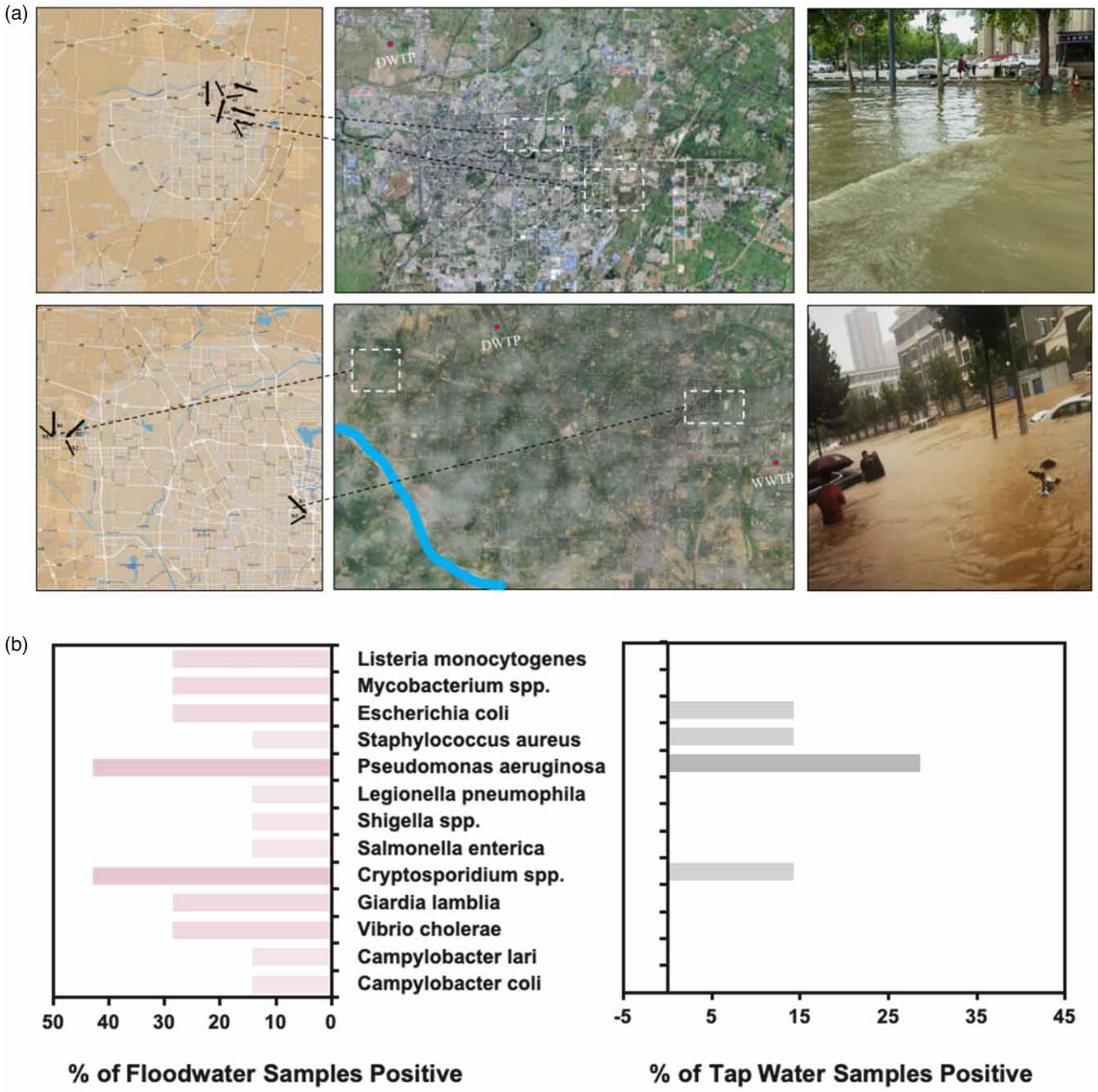

Figure 1 | Sampling sites and detected pathogens. (a) Sampling sites in Xinxiang and Zhengzhou, (b) top 8 detected pathogens with the highest detection rates in floodwater samples, and (c) top 4 detected pathogens with the highest detection rates in postflood tap water samples. The samples were collected on from July 22 to July 25 in 2021.

2.3. Questionnaire survey

Exposure data were collected using structured questionnaires (Supplementary material). Information included demographic characteristics, activities during rainstorms and flooding, estimated exposure duration, and volumes of water potentially ingested via different pathways.

2.4. Quantitative microbial risk assessment

The risk of infection was estimated following standard QMRA procedures. Exposure pathways considered were: (i) accidental ingestion of floodwater, (ii) hand-to-mouth contact, (iii) ingestion of droplets and aerosols generated during contact activities, and (iv) ingestion via utensils washed in contaminated water.

The ingested dose (*D*) was calculated as the product of pathogen concentration and ingestion volume, with parameter distributions derived from literature and questionnaire data (de Man *et al.* 2014). The probability of risk of infection for exposure to floodwater, as a function of the ingested volume per exposure event to each pathogen (*V. cholerae*, *Cryptosporidium* spp., *Giardia* spp., *Campylobacter* spp., and *E. coli*). To perform the method, the dose–response model aims to establish a mathematical relationship between the dose (any non-negative number) and the probability of an infection in the exposed population. Briefly, the risk of infection was calculated using the dose–response model, in which the ingested dose of pathogens D was calculated using the following equation:

$$Q = h \times a \times f$$

$$V_{\text{total}} = V_d + V_m + Q * t$$

$$D = C \times V_{\text{total}}$$

where h denotes the thickness of the water film on hands, a represents the skin-surface area of the hand that came into contact with the mouth, and f stands for the frequency of hand–mouth contact. As per the information provided by USEPA (2017), it was hypothesized that h had a uniform distribution within the range of 2.34×10^2 to 1.97×10^2 mm, a had a uniform distribution between 100 and 2,000 mm^2, and the value was obtained from the questionnaires. Through a straightforward analysis of the data of f, its distribution was discovered to be in line with the Poisson distribution, which accorded with Freeman *et al.*'s conclusion that the data of f were Poisson distributed with an average value of 2 times per hour for children (Freeman *et al.* 2001). However, due to the insufficiency of data regarding children in the questionnaires, the data of a, f, and t were sourced from adults. Thus, it was assumed, albeit with uncertainty, that the volume of ingestion through hand-to-mouth contact for adults was equivalent to that of children.

In the equal which V_d was the volume of ingestion of a few drops (mL), V_m was the volume of mouth ingestion of water (mL), Q was the ingestion rate (mL/min) due to hand–mouth contact with wet hands, and t was the time of the contact to floodwater according to the questionnaire (min). The volume of ingestion of droplets, V_d, was estimated to be uniformly distributed between 0.5 and 5 mL (Schijven & de Roda Husman 2006). Parameters can be estimated again with a binomial likelihood function.

It is hypothesized that exposure to floodwater from combined sewers greater than 5 mL, floodwater from storm sewers greater than 50 mL, and floodwater from surface runoff from rainfall greater than 800 mL will result in a risk of infection greater than 0.01. In the field of epidemiology, the risk of infection value of 0.01 is of significance and is considered to be a critical threshold value. This infection risk value is considered to be the threshold level at which epidemiological studies can identify health risks (Jamal *et al.* 2020).

A simple exponential model can be used to quantify the individual probability of infection, assuming that a minimum number of organisms have survived in the host to cause infection and that the microorganisms are randomly distributed (Poisson) (Armstrong & Haas 2007; Jamal *et al.* 2020). Beta-Poisson models are generally used when the probability r is not constant (ten Veldhuis *et al.* 2010), only a few cysts or virions of the pathogen can already cause infection (Haas 2015), or when the survival rate of the microorganism is influenced by different pathogens (Byappanahalli *et al.* 2012). In the case of *V. cholerae*, the probability of infection can be expressed as

$$P_{\text{event}} \cong 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}$$

The Beta-distribution parameters values of $\alpha = 0.169$ and $\beta = 2305$ are used following Mark *et al.* (2018).

The risk of infection per exposure event of *Cryptosporidium* spp. and *Giardia* spp. was calculated using the equation:

$$P_{\text{event}} = 1 - e^{-rD}$$

In the case of *Cryptosporidium* spp., the best estimate of r is 0.0040, and in the case of *Giardia* spp., the best estimate of r is 0.0199 (Teunis & Havelaar 2000; An *et al.* 2020). It was assumed that all pathogens measured are infectious, and in the case of enteroviruses, given the particular ratio of infectious to defective particles, the estimated ratio of infectious to defective particles is 1:100 based on Rutjes *et al.*'s study (Rutjes *et al.* 2009).

The risk of infection per exposure event of *Campylobacter* spp. and enterotoxigenic *E. coli* was calculated using the following equation:

$$P_{\text{event}} = 1 - F_1(\alpha, \alpha + \beta, -D)$$

where F_1 is the hypergeometric distribution, and a and b are the parameters of the Beta-distribution (Teunis & Havelaar 2000). In the case of *Campylobacter* spp., the best estimates of parameters a and b are 0.024 and 0.011, respectively (Ang *et al.* 2011). In the case of *E. coli*, the best estimates of a and b are 0.167 and 0.191, respectively (Teunis & Havelaar 2000).

According to the results of HT-qPCR, the overall risk of infection per exposure event was calculated as follows:

$$P_{\text{inf\_event}} = 1 - (1 - P_{\text{inf\_Vibrio}})(1 - P_{\text{inf\_Crypto}})(1 - P_{\text{inf\_Giardia}})(1 - P_{\text{inf\_Campylo}})(1 - P_{\text{inf\_Entero}})$$

where $P_{\text{inf\_Vibrio}}$ represented the risk of infection with *V. cholerae*, $P_{\text{inf\_Crypto}}$ represented the risk of infection with *Cryptosporidium* spp., $P_{\text{inf\_Giardia}}$ represented the risk of infection with *Giardia* spp., $P_{\text{inf\_Campylo}}$ represented the risk of infection with *Campylobacter* spp., and $P_{\text{inf\_Entero}}$ represented the risk of infection with enterotoxigenic *E. coli*.

Likelihood at each probability of infection were fit to a hypergeometric model using the method of moments; this allowed variation in parameter estimates to be nested in Monte Carlo risk simulation models. Assuming that the microorganism has r probability of causing an infection, the probability of infection (P_{inf}) after exposure to j microorganisms can be described as follows, according to Teunis *et al.* (1997):

$$P_{\text{inf}\,(j)} = \sum_{k=1}^{j} P_{\text{sur}}(k|j) = \sum_{k=1}^{j} \binom{j}{k} r^k (1-r)^{j-k}$$

where $P_{\text{sur}}(k|j)$ is the survival probability of k microorganisms after ingestion of j microorganisms.

The above equations were used to calculate the probability of infection of various pathogens detected in the floodwater (as shown in Supplementary Table S2) and thus assess the public health risks. The above pathogens were selected as the main objectives of calculation, as this model has been applied in a number of studies, such as in de Man *et al.* (2014) and WHO (2016). Human infectious potential was treated as a point estimate based on previous studies and did not contribute to variability in risk estimates (Curriero *et al.* 2001; Rutjes *et al.* 2009), although its impact would be anticipated.

2.5. Statistical and computational analyses

Differences between groups were tested using Student's t-test or, when assumptions of normality or homogeneity were not met, nonparametric tests (Wilcoxon rank-sum for two groups; Kruskal–Wallis followed by Wilcoxon with Bonferroni correction for multiple groups). Risk models were implemented in Mathematica (v13.1; Wolfram Research, Champaign, IL). Scenario analyses were conducted to assess infection risks as a function of ingestion volume and exposure frequency, allowing exploration of thresholds relevant to epidemiological detection (risk ≥ 0.01).

3. RESULTS AND DISCUSSION

3.1. Occurrence and abundance of pathogens in floodwater and tap water

The July 2021 Henan flood submerged residential areas for weeks (Figure 1(a)), raising concerns about microbial contamination of both floodwater and postflood tap water. Using HT-qPCR, we detected 21 pathogenic bacteria across samples from Zhengzhou and Xinxiang. Among floodwater samples, *Cryptosporidium* spp. and *P. aeruginosa* were most frequently detected (42.9%), followed by *V. cholerae* and *G. lamblia* (28.6%) (Figure 1(b); Table 1). Concentrations were highly variable, ranging from 36.8 gc/L (*Cryptosporidium* spp.) to >27,000 gc/L (*Acanthamoeba* spp.) in a Zhengzhou residential sample (B1). Several clinically important pathogens exceeded 500 gc/L, including *G. lamblia*, *Listeria monocytogenes*, *H. pylori*, *L. pneumophila*, *Shigella* spp., and *P. aeruginosa* (up to 13,500 gc/L).

Tap water collected within 10 h of supply restoration also showed contamination. Four of seven samples contained pathogenic bacteria, with *P. aeruginosa* (28.6%), *E. coli* (14.3%), *Cryptosporidium* spp. (14.3%), and *Staphylococcus* spp. (14.3%) detected at levels up to ∼13,000 gc/L. Notably, tap water from site b2 (paired with a highly contaminated floodwater sample B1) showed multiple pathogens, suggesting infiltration or persistence of contamination in the distribution system.

The diversity of detected pathogens points to multiple contamination sources. Combined sewer overflows during heavy rainfall likely introduced untreated sewage (Rodríguez *et al.* 2012), while agricultural runoff may

Table 1 | Concentration of waterborne pathogens detected in samples of urban floodwater and postflood tap water

	Xx City								**Zz City**					
	Floodwater				**Tap water**				**Floodwater**			**Tap water**		
Pathogens (copies/L)	**A1**	**A2**	**A3**	**A4**	**a1**	**a2**	**a3**	**a4**	**B1**	**B2**	**B3**	**b1**	**b2**	**b3**
Campylobacter coli	0	0	0	0	0	0	0	0	281.7	0	0	0	0	0
V. cholerae	0	0	0	0	0	0	0	0	466	0	0	0	0	0
G. lamblia	0	0	199.8	0	0	0	0	0	881.7	0	0	0	0	0
Cryptosporidium	0	0	0	0	0	0	0	0	36.8	128.3	579.8	0	650.3	0
C. difficile	0	0	0	0	0	0	0	0	181.9	0	0	0	0	0
L. monocytogenes	0	1,041.7	0	0	0	0	0	0	1,238.1	0	0	0	0	0
Staphylococcus aureus tufA	0	0	0	0	0	0	0	0	455.9	0	0	0	399.5	0
V. cholerae-toxigenic	0	0	142.3	0	0	0	0	0	126.8	0	0	0	0	0
S. enterica invA	0	0	0	0	0	0	0	0	343	0	0	0	0	0
H. pylori	0	0	2,136.6	0	0	0	0	0	2,100.5	0	0	0	0	0
enteroaggregative E. coli	0	0	0	0	0	0	0	0	273.5	0	0	0	0	0
enterotoxigenic E. coli elt	0	0	0	0	0	0	0	0	54.3	0	0	0	0	0
enteropathogenic E. coli	0	0	260.5	0	0	0	0	0	317	0	0	0	0	0
L. pneumophila	1,651.9	0	0	0	0	0	0	0	0	0	0	0	0	0
Mycobacterium	0	0	379.9	0	0	0	0	0	339.9	0	0	0	0	0
K. pneumoniae	0	0	381.6	0	0	0	0	0	0	0	0	0	0	0
Campylobacter lari pepT	0	0	0	0	0	0	0	0	372.6	0	0	0	0	0
Escherichia coli eaeA	0	0	0	0	0	0	0	0	266.3	0	0	9,197.8	0	0
P. aeruginosa	0	0	4,165.2	13,500.9	12,985.4	5,889.2	0	0	581.3	0	0	0	0	0
Shigella ipaH Shig1	0	0	0	0	0	0	0	0	607.3	0	0	0	0	0
Acanthamoeba spp.	0	0	340.9	0	0	0	0	0	2,7354	0	0	0	0	0

have contributed zoonotic pathogens (Kumar *et al.* 2021). Inundation of urban infrastructure could also mobilize environmental pathogens, such as *Legionella* spp., from damaged plumbing.

3.2. Exposure assessment from questionnaire survey

To quantify human exposure, a questionnaire was administered to 134 residents in September 2022. Respondents included 59 women and 75 men (average age 34.8 years for adults; 7.5 years for children; Supplementary Figure S2). Most adults (73%) reported no direct contact with floodwater, though ingestion occurred through accidental swallowing, hand-to-mouth contact, or droplet exposure during cleanup. Only one child responded, so the data were pooled with adults (Supplementary Table S1). These exposure data were integrated with pathogen concentrations to parameterize the QMRA.

3.3. Risk of infection from floodwater exposure

Riverine flooding from rainstorms coincided with elevated pathogen abundance, likely exacerbated by overloaded wastewater treatment plants (McKee & Cruz 2021). Using the de Man model, we calculated ingested doses as the product of pathogen concentration and exposure volume (Table 2).

Infection risks increased monotonically with ingestion volume across all modeled pathogens (*V. cholerae*, *Cryptosporidium* spp., *Giardia* spp., *Campylobacter* spp., and *E. coli*) (Figure 2(a)–2(e)). Figure 2(f) illustrates how risk scales with both pathogen concentration and water ingestion. While average risks remained low at typical exposure levels, the potential for outbreaks among highly exposed or immunocompromised groups remains substantial.

Interestingly, *P. aeruginosa* concentrations were higher in tap water than in floodwater. One explanation is that open floodwater was diluted, while stagnation and nutrient accumulation in indoor plumbing promoted bacterial growth (Yu *et al.* 2018). This highlights the overlooked risk of secondary contamination within distribution systems after floods.

3.4. Linking model predictions with observed health outcomes

Epidemiological data support our risk estimates. Reported diarrhea cases in Henan were significantly higher in 2021 compared with 2020 and 2022 ($p < 0.05$; Supplementary Figure S4), particularly among children and elderly populations (Health Commission of Henan Province 2021). These findings align with our modeled probabilities of infection, which emphasize heightened vulnerability among immunocompromised groups.

Table 2 | QMRA input parameters on the risk of pathogens in this study

Model parameter (unit)	**Distribution and fit parameter**	**Refs**
E. coli concentration (Log gc/L)	N; $\mu = 1.32$, $\sigma = 0.66$	This study
Campylobacter coli concentration (Log gc/L)	N; $\mu = 0.76$, $\sigma = 0.62$	This study
V. cholerae concentration (gc/L)	LN; $\mu = 6.071$, $\sigma = 2.765$	This study
Giardia spp. concentration (Log gc/L)	N; $\mu = 1.87$, $\sigma = 0.54$	This study
Cryptosporidium spp. concentration (Log gc/L)	N; $\mu = 1.98$, $\sigma = 0.85$	This study
E. coli (beta-Poisson model)		
β, α	0.191, 0.167	Teunis & Havelaar (2000)
Campylobacter coli (hypergeometric-beta model)		
β, α	0.011, 0.024	Teunis *et al.* (2005)
V. cholerae (beta model)		
β, α	2,305, 0.169	
Giardia spp. (exponential model)		
r	0.0199	Teunis *et al.* (2005)
Cryptosporidium spp. (exponential model)		
r	0.0040	Teunis *et al.* (2016)

N, normal distribution; LN, lognormal distribution.

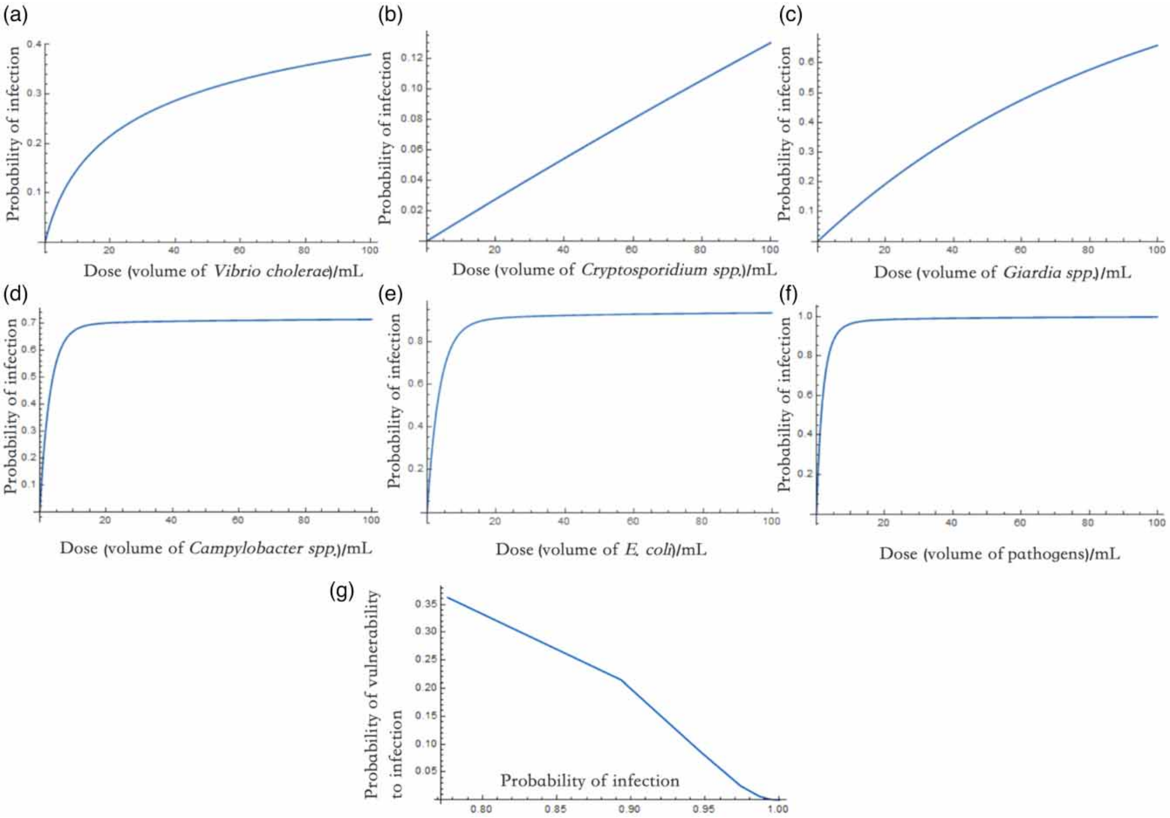

Figure 2 | Mean risk of infection for exposure to floodwater, as a function of the ingested volume of pathogens. (a) *V. cholerae*, (b) *Cryptosporidium* spp., (c) *Giardia* spp., (d) *Campylobacter* spp., (e) *E. coli*, (f) pathogens above per exposure event, and (g) likelihood of mean risk of infection for exposure to floodwater.

Previous studies reported increased illness risks of 39–75% following combined sewer overflows (McGinnis *et al.* 2022). Similarly, we observed that postflood tap water contained fecal indicators and enteropathogenic bacteria in >40% of samples, consistent with prior reports of urban floodwater contamination (Cann *et al.* 2013).

Although the absolute infection probabilities per single exposure were generally low, even small risks can translate into substantial case burdens given the large exposed population. Moreover, our models may underestimate risks for children, who were underrepresented in surveys but are known to swallow larger volumes during play (Cann *et al.* 2013; Leandro *et al.* 2022). Seasonal variability, differences in exposure routes, and individual health status further shape outcomes.

Together, our findings demonstrate that floods mobilize diverse pathogenic bacteria into both surface and tap waters, posing measurable risks to public health. Future interventions should strengthen urban drainage systems, safeguard water distribution infrastructure, and prioritize rapid monitoring of microbial contamination during and after extreme weather events.

4. CONCLUSION

This study applied high-throughput qPCR arrays to profile pathogenic bacteria in floodwater and postflood tap water, providing the first QMRA of infection risks associated with the 2021 Henan flood. Our findings demonstrate that exposure to floodwaters substantially increases the probability of infection, underscoring the urgent need for personal protection during flood events. Simple measures – such as avoiding ingestion or inhalation of floodwater – can markedly reduce health risks.

Beyond individual protection, our results highlight the need for systemic responses. Continuous monitoring of floodwater, tap water, and drinking water distribution systems is essential for early detection of pathogens and timely interventions, such as boil water advisories. Improved wastewater and sewage management, together with investments in urban drainage infrastructure, will further mitigate contamination risks.

Future research should refine exposure parameters (e.g., ingestion volumes, dose–response relationships) and expand assessments to pathogens lacking established dose–response models. Linking molecular detection with epidemiological data – particularly for vulnerable populations such as children – will strengthen risk estimates and improve public health preparedness.

Finally, floods are increasingly a global public health threat under climate change. International collaboration to share monitoring frameworks, risk assessment tools, and best practices will be critical to safeguard urban populations. By combining pathogen surveillance, infrastructure improvements, and public education, societies can reduce the spread of waterborne diseases and build resilience against future extreme rainfall events.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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