ISSN 2096-7071 (Print)
ISSN 2096-3101 (Online)
CN 10-1629/R1

CHINA CDC WEEKLY

CCDC weekly

Vol. 7 No. 16 Apr. 18, 2025

中国疾病预防控制中心周报

VECTOR-BORNE INFECTIOUS DISEASES ISSUE

ISSN 2096-7071
9 772096 707255 1 6

China CDC Weekly

Cover Image: credited from Beijing Shengyue Yongdong Education Technology Co., Ltd.

Vital Surveillances

Variations in the Bacterial Ecosystems of Mosquito Populations — Haikou and Sanya Cities, Hainan Province, China, 2019

Xun Kang[1,2,&]; Yanhong Wang[3,&]; Rui Zheng[1]; Rajaofera Mamy Jayne Nelly[1]; Lin Liu[1]; Siping Li[1]; Xiaomei Sun[3,4]; Le Kang[3,4]; Nan Zhang[1,5,#]; Zhen Zou[4,#]; Qianfeng Xia[1,#]

ABSTRACT

Introduction: This study explores the midgut microbiota of mosquitoes in Haikou and Sanya cities, regions critical for understanding vector-borne disease dynamics in Hainan Province, China. It provides baseline data on microbial composition and examines their potential role in influencing mosquito biology and vector competence, while highlighting the need for further research into their association with vector-borne viral infections.

Methods: Adult mosquitoes were collected using light traps and human bait methods. Species identification was conducted through morphological examination and DNA barcoding using the cytochrome c oxidase subunit 1 gene (cox1). The V3–V4 hypervariable regions of the microbial 16S ribosomal RNA (rRNA) gene were sequenced using high-throughput methods to investigate the midgut microbiota. Statistical analyses, including Alpha and Beta diversity assessments of the sequencing results, were performed using SPSS 21.0 and R version 3.11.

Results: The predominant mosquito species identified were *Aedes albopictus*, *Armigeres subalbatus*, and *Culex pipiens*. Microbiota analysis of 281 midguts revealed that *Proteobacteria* dominated (85.28%), with significant fractions being *Alphaproteobacteria* (52.14%), *Gammaproteobacteria* (29.90%), and *Betaproteobacteria* (3.22%). Other notable phyla included *Firmicutes* (6.24%), *Actinobacteria* (3.81%), and lesser quantities of *Thermi*, *Cyanobacteria*, and *Bacteroidetes*. Significant geographic variation in bacterial communities was observed between Haikou and Sanya ($P<0.05$), with unique taxa like *Thermi* and *Cyanobacteria* identified only in Haikou and *Chlamydiae* found solely in Sanya. The analysis revealed 204 overlapping species, with 473 unique to Haikou and 64 to Sanya.

Conclusions: This study revealed significant geographic differences in the midgut microbiota of mosquitoes from Haikou and Sanya, providing foundational data for understanding their potential impact on mosquito biology and disease transmission. While the direct relationship between these microbial variations and vector-borne disease dynamics requires further investigation, these findings underscore the importance of mosquito microbiota research as part of broader strategies to mitigate vector-borne disease risks.

Mosquitoes serve as crucial vectors for infectious diseases such as Zika and dengue, particularly affecting tropical regions like Hainan Province, China (*1*). With over 3,500 species worldwide, mosquitoes play significant roles in both public health and ecosystem dynamics (*2*). Their blood-feeding behavior directly connects them to vertebrate hosts, making them primary carriers of various pathogens (*3*). The midgut of female mosquitoes functions as a key site for both pathogen entry and blood digestion, hosting diverse microbial communities that influence disease transmission capacity (*4*). This microbiota can significantly affect mosquito development and pathogen resistance, highlighting its importance in contemporary entomological research.

Symbiotic relationships between organisms and their microbiomes are well-established phenomena that influence various biological processes. In female mosquitoes, particularly those that feed on blood, the midgut serves as both a digestion site and an entry point for pathogens including viruses, protozoa, and nematodes (*5*–*6*). Research has demonstrated that the microbiome within the midgut significantly influences vector competence — the ability to acquire, maintain, and transmit pathogens to vertebrates (*7*). Additionally, gut bacteria affect the biological development of mosquitoes and can modulate their vulnerability to pathogens. Increased diversity and abundance of gut bacteria have been linked to reduced susceptibility to pathogens, suggesting potential disease control strategies (*8*).

In Hainan, the tropical monsoon climate and extensive forest coverage provide an ideal environment for mosquito proliferation. A recent surge in dengue cases underscores the need for enhanced understanding of mosquito microbiomes to develop novel disease control strategies (*9*). Our study examines the midgut microbiota of mosquitoes from Haikou and Sanya cities, exploring how environmental factors shape microbial diversity and influence disease transmission potential. This research contributes to both our understanding of mosquito-borne disease dynamics and ongoing efforts to control these diseases through targeted microbial interventions.

METHODS

Mosquito Collection

Adult female mosquitoes were collected over an eight-day period (July 12–19, 2019) in Haikou and Sanya cities, Hainan Province, China. Collection sites were selected in tropical and subtropical areas using CO_2-augmented light traps and human landing catches to ensure diverse sampling (*10*). The collection sites and their geographic coordinates are presented in Table 1.

Identification and Processing

Mosquitoes were identified morphologically, and their identities were confirmed via DNA barcoding using the cytochrome c oxidase subunit 1 (cox1) gene (*11*). All specimens were stored at −80 °C. To minimize the influence of host blood on microbiota analysis, blood-fed mosquitoes were maintained in the laboratory for 4 days to allow for blood digestion before midgut dissection.

DNA Extraction

Following molecular identification, mosquitoes were sorted by species, and ten midguts per species were dissected in triplicate to ensure data reliability. The samples were initially stored at −80 °C, surface-sterilized in 75% ethanol for 30 seconds, and this process was repeated twice. After rinsing twice in sterile PBS to remove residual ethanol, the midguts were pooled into sterile 1.5 mL tubes and stored at −20 °C until DNA extraction (*12*). Dissections were performed under a stereomicroscope to ensure sample integrity, with the foregut, hindgut, and Malpighian tubules carefully removed. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, USA) (*13*) according to the manufacturer's instructions. The purified DNA was diluted in 50 μL ddH_2O for microbiome library construction at BGI Shenzhen.

16S rRNA Gene Library Preparation, Illumina HiSeq Sequencing

A total of 281 midguts were collected from various mosquito species in Haikou and Sanya, using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') to amplify the V3-V4 hypervariable regions of the bacterial 16S rRNA gene (*14*). The genomic DNA was sequenced using the Illumina HiSeq 4000 system.

TABLE 1. Geographic coordinates and number of mosquito samples processed for midgut bacteria.

Site	Villages /towns	Geographic coordinates	Mosquito species	Number of midgut samples	
				Initial	Final
Haikou (HK)	Xiyi (XY)	110°19'52.284"E 20°1'55.095"N	*Culex pipiens* (CPI)	42	30
			Culex gelidus (CGE)	13	11
	Xinghui (XH)	110°20'30.01"E 20°02'44.99"N	*Aedes albopictus* (AAL)	35	30
			Culex pipiens (CPI)	34	30
	Changwang (CW)	110°18'17.93"E 20°01'37.16"N.	*Aedes albopictus* (AAL)	35	30
Sanya (SY)	Shiwaitaoyuan (SW)	110°27'31.36"E 19°48'08.82"N	*Armigeres subalbatus* (ASU)	32	30
	Sanya (SY)	109°30'35.96"E 18°15'19.19"N	*Aedes albopictus* (AAL)	34	30
	Nanya (NY)	109°14'25.76"E 18°27'50.47"N	*Armigeres subalbatus* (ASU)	30	30
	Nanbin (NB)	109°11'56.00"E 18°21'37.15"N	*Aedes albopictus* (AAL)	35	30
	Chicao (CC)	109°10'4.51"E 18°24'47.56"N	*Aedes albopictus* (AAL)	34	30
				324	281

Following sequencing, we implemented quality control measures, including the removal of chimeric sequences, and identified Operational Taxonomic Units (OTUs) using the RDP Classifier (*15*). From 1,508,554 raw reads, 1,384,001 clean reads were generated (Supplementary Table S1, available athttps://weekly.chinacdc.cn/) and clustered into OTUs at 97% similarity. Only OTUs with a relative abundance above 0.5% were analyzed to focus on the predominant bacterial communities (Supplementary Table S2, available at https://weekly.chinacdc.cn/).

Statistical Analysis

Statistical evaluations were conducted using R (version 3.1.1; R Foundation, Austria) and Metastats (version 1.0; Whitehead Institute, USA) to assess differentiation among microbial communities (*16*). Rarefaction curves and box plots were generated to visualize biodiversity and analyze significant differences in microbial diversity.

Alpha and Beta Diversity Analyses

Microbial diversity within and across mosquito samples were quantified using alpha and beta diversity indices. Alpha diversity, which measures diversity within a sample, included indices such as Simpson, Shannon, ACE, and Chao1, calculated using Mothur (version 1.31.2; Michigan State University, USA) at a 97% similarity threshold for OTUs. Beta diversity, which compares differences between samples, was evaluated using Bray-Curtis, weighted UniFrac, and unweighted UniFrac indices. Significant differences in *alpha* diversity were observed between mosquito populations in Haikou and Sanya, as visualized by matrix heatmaps of diversity metrics, highlighting the presence, abundance, and phylogenetic relationships of microbial communities.

Visualization and Phylogenetic Analysis

Venn diagrams were used to illustrate shared and unique OTUs, effectively visualizing microbial overlap and distinctiveness across samples. For phylogenetic analysis, sequences were aligned using QIIME's align_seqs.py, and phylogenetic trees were constructed using the Fasttree method (*17*). These trees, representing evolutionary relationships among bacterial *species*, were visualized using R software, providing comprehensive insights into the microbial community structure across different environmental contexts.

Cluster Analysis Methodology

Cluster analysis was performed using QIIME (version 1.8.0; Knight Lab, University of Colorado, USA) with an iterative algorithm that selected 75% of sequence data from the least abundant samples. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was employed for hierarchical clustering to reveal relationships among microbial communities. The resulting clustering trees were visualized using R software, effectively illustrating the structural organization of microbial communities across the studied samples (*18*).

RESULTS

Mosquito Microbial Community Composition

This study analyzed the V3–V4 regions of the 16S rRNA gene in 281 midguts from field-collected female mosquitoes (*Aedes albopictus*, *Culex pipiens*, *Culex gelidus*, and *Armigeres subalbatus*) from Haikou and Sanya. The majority of sequences belonged to the *phylum* *Proteobacteria* (85.28%), primarily *Alphaproteobacteria* (52.14%), *Gammaproteobacteria* (29.90%), and *Betaproteobacteria* (3.22%). Other significant *phyla* included *Firmicutes* (6.24%), *Actinobacteria* (3.81%), and smaller contributions from *Thermi*, *Cyanobacteria*, *Bacteroidetes*, *Spirochaetes*, *TM7*, and *Chloroflexi*. At the *family* level, *Rickettsiaceae* dominated (46.96%), followed by *Enterobacteriaceae* and *Aeromonadaceae*. The most abundant *genera* were *Wolbachia* (46.96%), *Acinetobacter*, and *Escherichia* (Figure 1A–B). *Rickettsiaceae* prevalence varied by location, being higher in Sanya (87.36% in *Ae. albopictus* and *Ar. subalbatus*) compared to Haikou (20.02%), with a much lower abundance in *Ae. albopictus* from XH (0.32%). This demonstrates significant variations in the gut microbiomes across mosquito species and different locations.

Venn Analysis of Midgut Bacteria

OTUs were defined at 97% similarity, and shared an unique taxa were visualized with Venn diagrams to identify core microorganisms across environments. Comparative analysis revealed that mosquito species within the same *genus* in Haikou and Sanya harbored similar bacteria, with 204 overlapping *species*, 473 unique *species* in Haikou, and 64 unique *species* in Sanya (Figure 2A). *Phylum*-level analysis showed distinct bacterial communities between the two

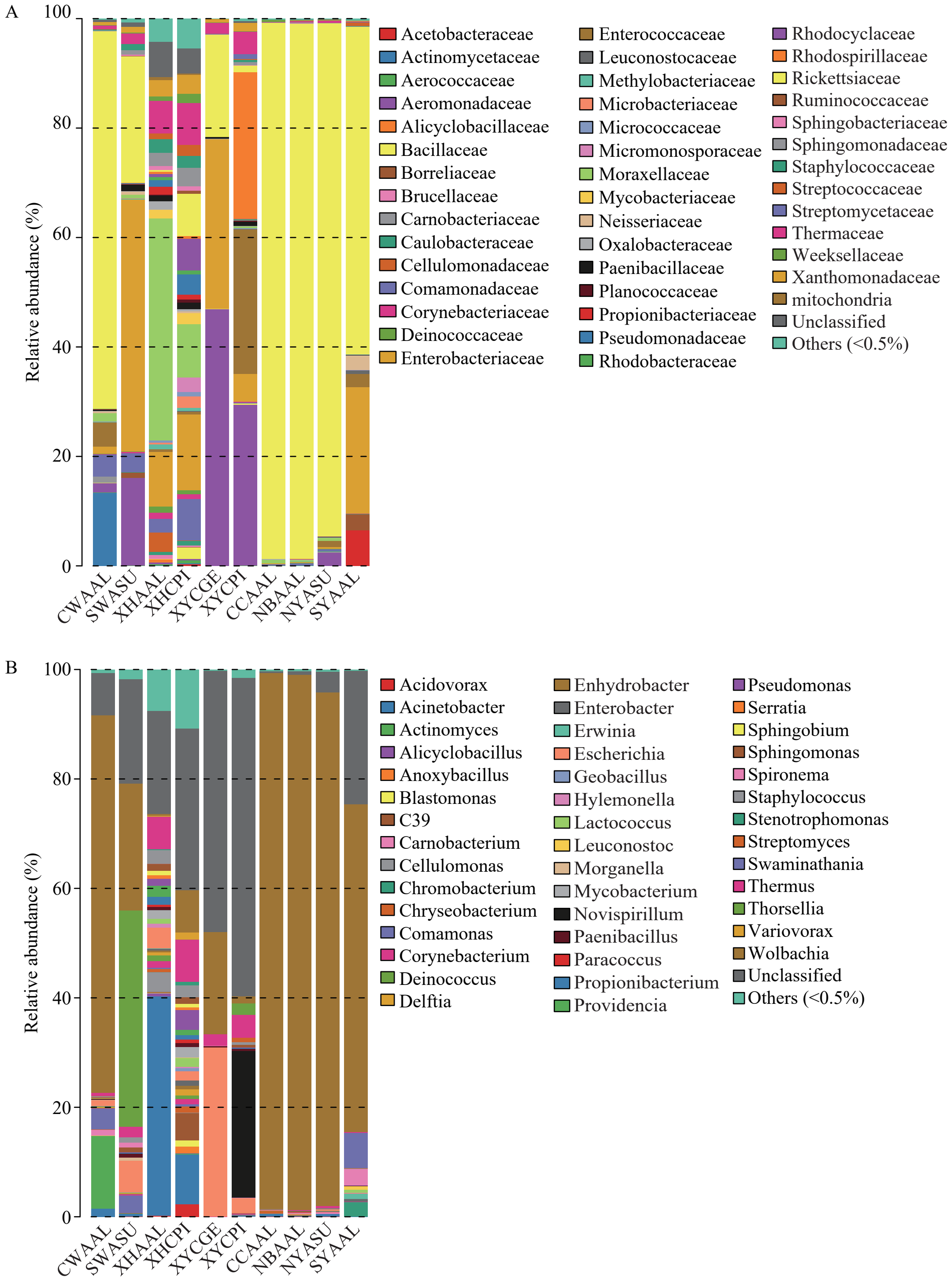


FIGURE 1. Mean relative abundances of (A) bacterial families and (B) genera associated with four mosquito species from Haikou and Sanya.

Note: Families and genera with abundance less than 0.5% were pooled together as "Other."

Abbreviation: CW=Changwang, HK; SW=Shiwaitaoyuan, HK; XH=Xinghui, HK; XY=Xiyi, HK; CC=Chicao, SY; HK=Haikou; NB=Nanbin, SY; NY=Nanya, SY; SY=Sanya; AAL=*Aedes albopictus*, ASU=*Armigeres subalbatus*, CGE=*Culex gelidus*, CPI=*Culex pipiens*.

locations. Haikou was dominated by *Firmicutes* (32.77%), followed by *Proteobacteria* (23.26%), and *Bacteroidetes* (16.28%), while Sanya had higher proportions of *Proteobacteria* (32.81%) and *Firmicutes*

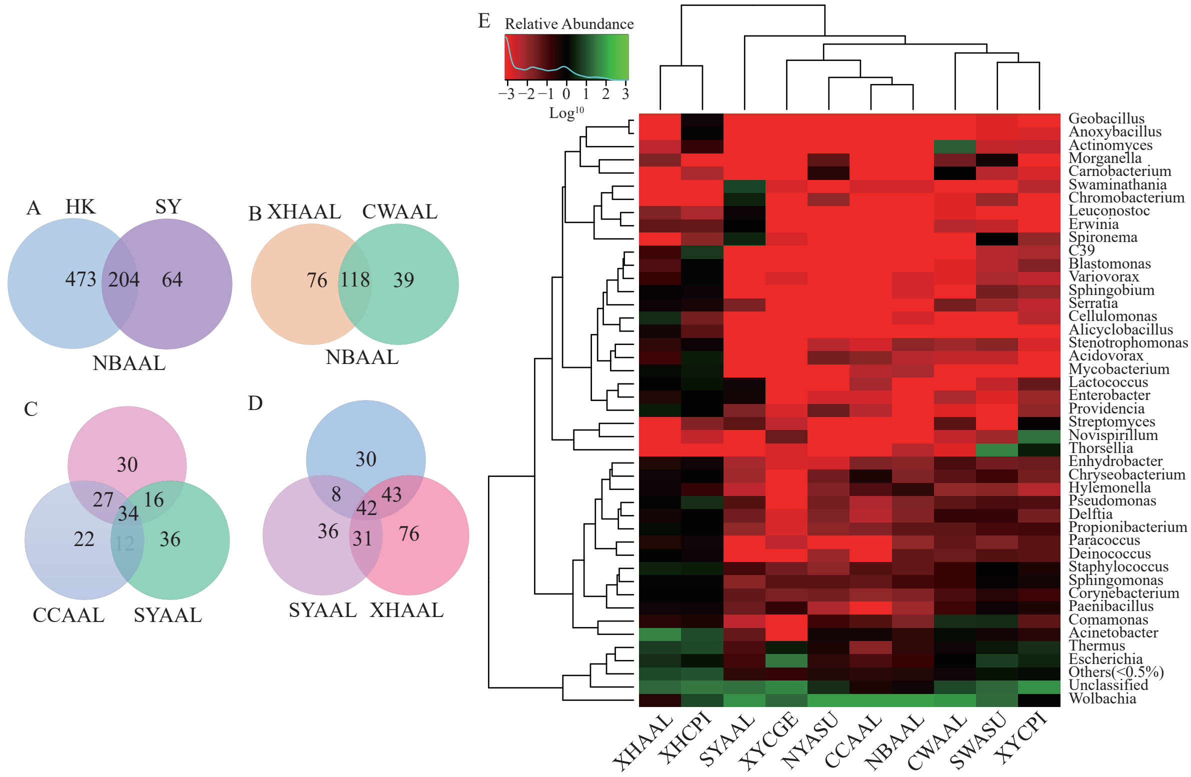


FIGURE 2. Venn diagrams and heatmap illustrating bacterial composition overlap and gut microbiota of mosquito species across habitats. (A) Venn diagram showing overlaps of OTUs at 97% similarity between the HK and SY data sets; (B) Number of bacterial taxa specific and common to *Aedes albopictus* of XH, CW in Haikou; (C) Number of bacterial taxa specific and common to *Ae. albopictus* of CC, NB, SY (street) in Sanya; (D) Number of bacterial taxa specific and common to *Aedes albopictus* of XH, NB, and SY; (E) Heatmap in log scale depicting the gut bacterial community of mosquito midguts obtained with open reference OTU picking methods at the *genus* level.
Note: For (E), Green colors represent high abundance, and red colors represent low abundance; black indicates absence.
Abbreviation: OTUs=Operational Taxonomic Units; CW=Changwang, HK; SW=Shiwaitaoyuan, HK; XH=Xinghui, HK; XY=Xiyi, HK; CC=Chicao, SY; HK=Haikou; NB=Nanbin, SY; NY=Nanya, SY; SY=Sanya.

(26.56%). Haikou also contained unique phyla, including *Thermi* and *Cyanobacteria*, while Sanya exclusively harbored *Chlamydiae*. Statistical tests confirmed significant differences between locations, such as for *Actinobacteria* (Wilcoxon test: P=0.038).

Further analysis of *Ae. albopictus* in Haikou revealed that XH and CW shared 118 species, with 76 and 39 unique species, respectively, indicating greater diversity in XH (Figure 2B). In contrast, three Sanya locations (SY, CC, NB) shared only 34 species, demonstrating lower diversity (Figure 2C). Between Haikou's XH and Sanya's SY and NB, 42 overlapping species were found (Figure 2D), with fewer unique species in Nanban farm and Sanya Street (30 and 36, respectively) compared to Haikou's Xinghui Village (76 unique *species*). Across all sites, 32 *species* were consistently identified, representing a core microbial community. Key *genera* included *Corynebacterium*, *Wolbachia* and *Cupriavidus*.

Analysis of *species* and Their Abundance

The phylogenetic tree and heatmap visually represent the composition and abundance of bacterial communities in mosquito midguts, highlighting structural similarities and differences. Clustering analyses revealed three major groups: 1) *Ae. albopictus* and *Cx. pipiens* from XH Village in Haikou; 2) *Cx. gelidus* from XY Village, along with *Ae. albopictus* from NY Farm, CC Village, and Sanya Street; and 3) mosquitoes from CW, SW, and XY Villages in Haikou. Three primary *genera* were identified: *Wolbachia*, *Escherichia*, and *Thermus*, which were widespread across locations and species. *Wolbachia* was abundant in all mosquitoes except for those from XH and XY Villages. *Acinetobacter* and *Comamonas* were found predominantly in *Ae. albopictus* and *Cx. pipiens* from XH Village and *Ae. albopictus* and *Ar. subalbatus* from CW and SW Villages. *Novispirillum* and *Thorsellia* were more abundant in *Cx. pipiens* from XY

Village and *Ar. subalbatus* from SW Village (Figure 2E).

The Diversity Analysis of Intestinal Bacteria in Regions

Alpha diversity indices (observed *species*, Chao1, ACE, Simpson, and Shannon) were used to assess bacterial diversity and richness in mosquitoes from Haikou and Sanya, revealing differences between locations. Alpha diversity showed significantly higher bacterial richness in *Cx. pipiens* from Haikou compared to *Ae. albopictus* from Sanya, with *Cx. pipiens* and *Ar. subalbatus* in Haikou exhibiting notably higher diversity (Chao1: 491.54, ACE: 487.92; Chao1: 411.25, ACE: 405.33) versus Sanya (Chao1: 220.87, ACE: 261.89) (Figure 3A).

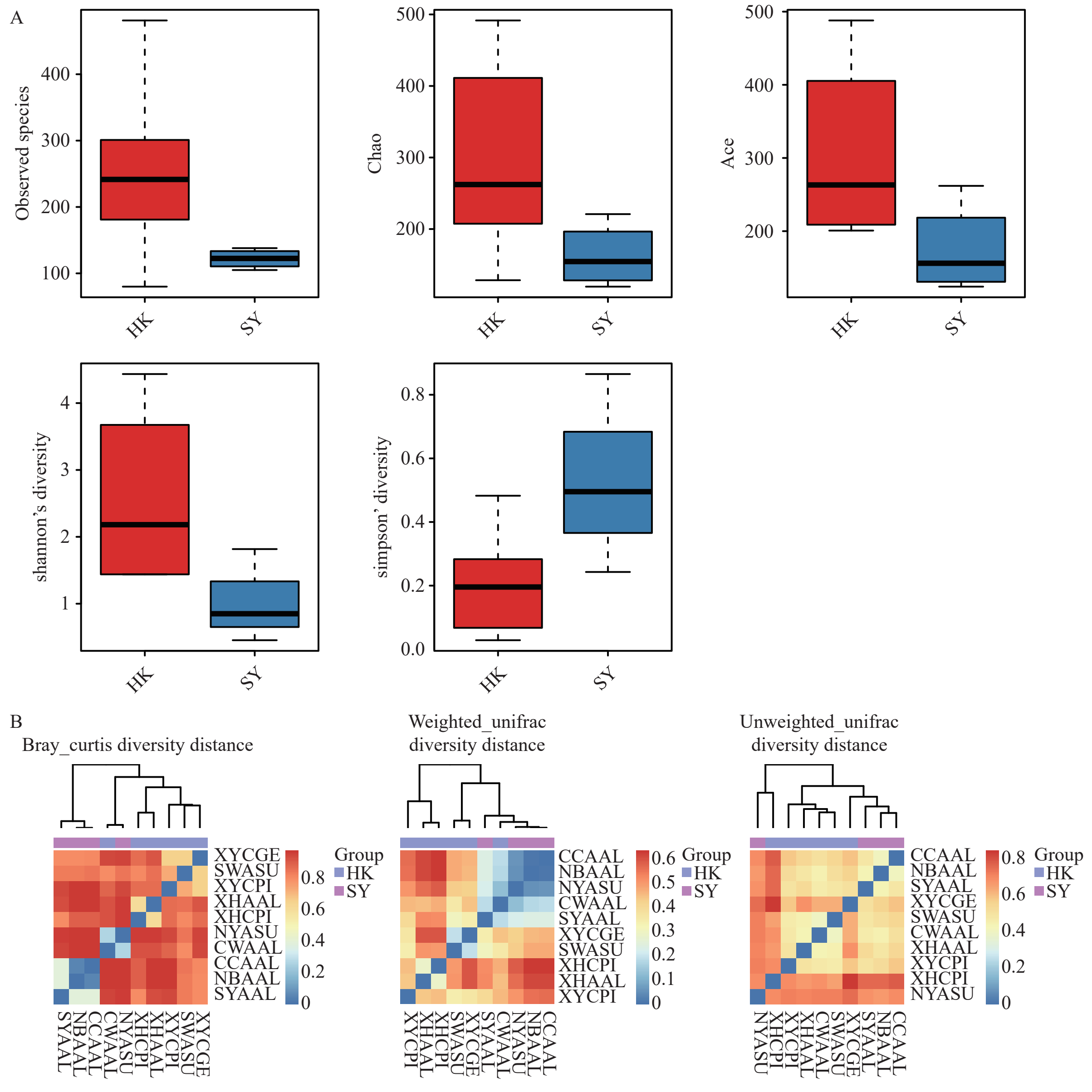


FIGURE 3. Boxplot and heatmap representations of bacterial diversity across mosquito species and habitats. (A) Boxplot representation of observed *species*, Chao1, and Shannon diversity indices. (B) Matrix heatmap of Bray-Curtis distances (left), Weighted UniFrac Beta diversity (middle), and Unweighted UniFrac Beta diversity (right) between microbial communities of four mosquito species from Haikou and Sanya.

Note: For (A), boxplots show the distribution of bacteria between mosquito samples categorized by location and species. Significant differences between the groups were investigated using pairwise comparisons of means (Dunn test; *P* value adjustment: Holm). *Species* richness is represented by the number of bands. Boxplots show median, minimum, and maximum values, with black lines indicating medians.

Beta diversity analysis revealed significant microbial differences between Haikou and Sanya populations, with Bray-Curtis, weighted UniFrac, and unweighted UniFrac metrics demonstrating distinct clustering patterns (Figure 3B). Bray-Curtis indicated dissimilarity between *Ar. subalbatus* (SY-ASU) from Sanya and *Cx. gelidus* (HK-CGE) and *Ae. albopictus* (HK-AAL) from Haikou, while *Ae. albopictus* from both regions overlapped. The unweighted UniFrac distance metric highlighted the microbial divergence between *Cx. gelidus* (HK-CGE) and *Ar. subalbatus* (SY-ASU), while *Ae. albopictus* populations showed greater similarity. The weighted UniFrac metric revealed microbial overlap between *Ae. albopictus* (SY-AAL) and *Ar. subalbatus* (SY-ASU) in Sanya, but distinctions from *Cx. pipiens* (HK-CPI) and *Cx. gelidus* (HK-CGE) in Haikou. These patterns indicate that microbial communities in Sanya were more similar within the region, while those in Haikou exhibited more variation. Overall, geographic and species-specific factors strongly influence microbial community composition.

CONCLUSIONS

This study investigated bacterial communities in the midguts of four mosquito species from Haikou and Sanya using high-throughput sequencing of the 16S rRNA gene's V3-V4 regions. *Proteobacteria*, particularly *Wolbachia*, dominated the microbial composition, with significantly higher prevalence in Sanya than in Haikou, suggesting regional differences in microbial composition that could influence disease transmission dynamics (*19*).

Blood meals substantially alter gut microbiota, increasing bacteria such as *Serratia* and *Elizabethkingia* that participate in blood digestion, while reducing symbiotic bacteria like *Wolbachia*, which may affect mosquito immunity and vector competence (*20*). To minimize blood-feeding effects, mosquitoes were allowed to digest blood for 4 days before dissection, though residual effects on bacterial diversity cannot be ruled out. Regional and species-specific variations, such as the higher prevalence of *Wolbachia* in Sanya mosquitoes, may also be influenced by dietary and environmental factors. Future studies should include controlled feeding experiments to distinguish intrinsic microbiota from transient changes due to blood meals.

Venn diagrams revealed both shared and unique microbial communities between locations. Mosquitoes from Haikou displayed richer bacterial diversity, including *Thermi* and *Cyanobacteria*, indicating regional variations. Alpha diversity was higher *Cx. pipiens* from Haikou than in *Ae. albopictus* from Sanya, highlighting the importance of understanding local mosquito ecology for disease control. Phylogenetic and heatmap analyses showed distinct microbial clusters based on geographic location and mosquito species, reflecting the complex interplay between mosquitoes and their microbiomes and suggesting the influence of environmental factors. However, the analysis was limited to four mosquito species and two regions, and 16S rRNA sequencing offers limited taxonomic resolution.

In conclusion, significant variations in microbial ecosystems were found between mosquito populations from Haikou and Sanya, with *Wolbachia*'s higher prevalence in Sanya mosquitoes, indicating potential for biologically-based control strategies to enhance public health in tropical regions (*21*).

Conflicts of interest: No conflicts of interest.

Funding: Supported by the Hainan Provincial Natural Science Foundation of China (824QN269, 822QN324), Hainan Province Science and Technology Talent Innovation Project (KJRC2023D29), Hainan Tropical Disease Research Center (Hainan Sub-Center, Chinese Center for Tropical Disease Research) (HNTDC202303), National Key Plan for Scientific Research and Development of China (2023YFA1801002).

doi: 10.46234/ccdcw2025.086

[#] Corresponding authors: Qianfeng Xia, xiaqianfeng@hainmc.edc.cn; Zhen Zou, zouzhen@ioz.ac.cn; Nan Zhang, hy0211075@hainmc.edu.cn.

[1] NHC Key Laboratory of Tropical Disease Control, School of Tropical Medicine, Hainan Medical University, Haikou City, Hainan Province, China; [2] Hainan Tropical Disease Research Center (Hainan Sub-Center, Chinese Center for Tropical Disease Research), Haikou City, Hainan Province, China; [3] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China;; [4] CAS Center for Excellence in Biotic Interactions, University of Chinese Academy of Sciences, Beijing, China; [5] School of Biomedical Engineering, Sun Yat-sen University, Shenzhen City, Guangdong Province, China.
[&] Joint first authors.

Submitted: June 13, 2024
Accepted: April 13, 2025
Issued: April 18, 2025

REFERENCES

1. Liu L, Wu T, Liu B, Nelly RMJ, Fu YM, Kang X, et al. The origin and

molecular epidemiology of dengue fever in Hainan Province, China, 2019. Front Microbiol 2021;12:657966. https://doi.org/10.3389/fmicb.2021.657966.

2. De Niz M, Kehrer J, Brancucci NMB, Moalli F, Reynaud EG, Stein JV, et al. 3D imaging of undissected optically cleared *Anopheles stephensi* mosquitoes and midguts infected with *Plasmodium parasites*. PLoS One 2020;15(9):e0238134. https://doi.org/10.1371/journal.pone.0238134.
3. Coon KL, Valzania L, McKinney DA, Vogel KJ, Brown MR, Strand MR. Bacteria-mediated hypoxia functions as a signal for mosquito development. Proc Natl Acad Sci USA 2017;114(27):E5362 – 9. https://doi.org/10.1073/pnas.1702983114.
4. Michalski ML, Erickson SM, Bartholomay LC, Christensen BM. Midgut barrier imparts selective resistance to filarial worm infection in *Culex pipiens pipiens*. PLoS Negl Trop Dis 2010;4(11):e875. https://doi.org/10.1371/journal.pntd.0000875.
5. Sun XM, Wang YH, Yuan F, Zhang YA, Kang X, Sun J, et al. Gut symbiont-derived sphingosine modulates vector competence in *Aedes* mosquitoes. Nat Commun 2024;15(1):8221. http://dx.doi.org/10.1038/s41467-024-52566-1.
6. Dada N, Jupatanakul N, Minard G, Short SM, Akorli J, Villegas LM. Considerations for mosquito microbiome research from the Mosquito Microbiome Consortium. Microbiome 2021;9(1):36. https://doi.org/10.1186/s40168-020-00987-7.
7. Muturi EJ, Bara JJ, Rooney AP, Hansen AK. Midgut fungal and bacterial microbiota of *Aedes triseriatus* and *Aedes japonicus* shift in response to La Crosse virus infection. Mol Ecol 2016;25(16):4075 – 90. https://doi.org/10.1111/mec.13741.
8. Coatsworth H, Caicedo PA, Van Rossum T, Ocampo CB, Lowenberger C. The composition of midgut bacteria in *Aedes aegypti* (diptera: culicidae) that are naturally susceptible or refractory to dengue viruses. J Insect Sci 2018;18(6):12. https://doi.org/10.1093/jisesa/iey118.
9. Liu Q, Cui F, Liu X, Fu YM, Fang WJ, Kang X, et al. Association of virome dynamics with mosquito species and environmental factors. Microbiome 2023;11(1):101. https://doi.org/10.1186/s40168-023-01556-4.
10. Kang X, Wang YH, Li SP, Sun XM, Lu XY, Rajaofera MJN, et al. Comparative analysis of the gut microbiota of adult mosquitoes from eight locations in Hainan, China. Front Cell Infect Microbiol 2020;10:596750. https://doi.org/10.3389/fcimb.2020.596750.
11. Wilke ABB, de Oliveira Christe R, Multini LC, Vidal PO, Wilk-da-Silva R, de Carvalho GC, et al. Morphometric wing characters as a tool for mosquito identification. PLoS One 2016;11(8):e0161643. https://doi.org/10.1371/journal.pone.0161643.
12. Tutagata J, Pocquet N, Trouche B, Reveillaud J. Dissection of mosquito ovaries, midgut, and salivary glands for microbiome analyses at the organ level. J Vis Exp 2024;000(212):13. http://dx.doi.org/10.3791/67128.
13. Yadav KK, Bora A, Datta S, Chandel K, Gogoi HK, Prasad GBKS, et al. Molecular characterization of midgut microbiota of *Aedes albopictus* and *Aedes aegypti* from Arunachal Pradesh, India. Parasit Vectors 2015;8:641. https://doi.org/10.1186/s13071-015-1252-0.
14. Zhang JH, Yu N, Xu XX, Liu ZW. Community structure, dispersal ability and functional profiling of microbiome existing in fat body and ovary of the brown planthopper, *Nilaparvata lugens*. Insect Sci 2019;26(4):683 – 694. https://doi.org/10.1111/1744-7917.12575.
15. Wang Y, Gilbreath III TM, Kukutla P, Yan GY, Xu JN. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. PLoS One 2011;6(9):e24767. https://doi.org/10.1371/journal.pone.0024767.
16. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, et al. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 2015;12(2):115 – 21. https://doi.org/10.1038/nmeth.3252.
17. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7(5):335 – 6. https://doi.org/10.1038/nmeth.f.303.
18. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009;75(23):7537 – 41. https://doi.org/10.1128/AEM.01541-09.
19. Hugo LE, Stassen L, La J, Gosden E, Ekwudu O, Winterford C, et al. Vector competence of Australian *Aedes aegypti* and *Aedes albopictus* for an epidemic strain of Zika virus. PLoS Negl Trop Dis 2019;13(4):e0007281. https://doi.org/10.1371/journal.pntd.0007281.
20. Flores GAM, Lopez RP, Cerrudo CS, Perotti MA, Consolo VF, Berón CM. *Wolbachia dominance* influences the *Culex quinquefasciatus* microbiota. Sci Rep 2023;13(1):18980. https://doi.org/10.1038/s41598-023-46067-2.
21. Monteiro VVS, Navegantes-Lima KC, de Lemos AB, da Silva GL, de Souza Gomes R, Reis JF, et al. *Aedes*-chikungunya virus interaction: key role of vector midguts microbiota and its saliva in the host infection. Front Microbiol 2019;10:492. https://doi.org/10.3389/fmicb.2019.00492.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Total of raw reads and clean reads (Mean±SE) of mosquito samples in Haikou and Sanya.

Total group name	Sample name	Raw reads	Clean reads
HK-AAL	CWAAL1	70,891	68,031
	CWAAL2	72,279	63,213
	XHAAL	35,568	28,714
HK-CPI	XYCPI1	56,087	52,400
	XYCPI2	72,649	68,035
	XYCPI3	75,244	67,931
	XHCPI1	35,283	32,180
	XHCPI2	51,493	47,317
	XHCPI3	35,079	28,335
HK-ASU	SWASU1	73,236	68,226
	SWASU2	72,354	66,109
	SWASU3	72,157	66,638
HK-CGE	XYCGE	72,319	67,306
SY-AAL	NBAAL1	56,890	52,681
	NBAAL2	57,483	52,904
	NBAAL3	55,997	52,694
	CCAAL1	57,124	53,717
	CCAAL2	57,182	53,787
	CCAAL3	57,690	53,796
	SYAAL1	67,396	62,515
	SYAAL2	59,038	55,249
	SYAAL3	68,797	61,006
SY-ASU	NYASU1	58,182	53,889
	NYASU2	59,052	53,825
	NYASU3	59,084	53,503
Sum		1,508,554	1,384,001
Mean		60,342.16	55,360.04
SE		11,652.34	11,422.78

SUPPLEMENTARY TABLE S2. Sequences from mosquitoes in Haikou and Sanya were clustered into 21 bacterial OTUs belonging to 21 *phyla*, 45 *families*, and 45 *genera*. Filtered tags were clustered into OUT with 97% similarity.

#OTUId Abundance	Phylum	Families	Genera
Otu787	*Acidobacteria*	*Acetobacteraceae*	*Acidovorax*
Otu306	*Actinobacteria*	*Actinomycetaceae*	*Acinetobacter*
Otu161	*Armatimonadetes*	*Aerococcaceae*	*Actinomyces*
Otu77	*Bacteroidetes*	*Aeromonadaceae*	*Alicyclobacillus*
Otu46	*Chlamydiae*	*Alicyclobacillaceae*	*Anoxybacillus*
Otu45	*Chloroflexi*	*Bacillaceae*	*Blastomonas*
Otu56	*Cyanobacteria*	*Borreliaceae*	*C39*
Otu229	*Fibrobacteres*	*Brucellaceae*	*Carnobacterium*
Otu80	*Firmicutes*	*Carnobacteriaceae*	*Cellulomonas*
Otu86	*Fusobacteria*	*Caulobacteraceae*	*Chromobacterium*
Otu8	*GN02*	*Cellulomonadaceae*	*Chryseobacterium*
Otu7	*Gemmatimonadetes*	*Comamonadaceae*	*Comamonas*
Otu99	*Planctomycetes*	*Corynebacteriaceae*	*Corynebacterium*
Otu105	*Proteobacteria*	*Deinococcaceae*	*Deinococcus*
Otu133	*Spirochaetes*	*Enterobacteriaceae*	*Delftia*
Otu28	*TM7*	*Enterococcaceae*	*Enhydrobacter*
Otu26	*Tenericutes*	*Leuconostocaceae*	*Enterobacter*
Otu20	*Thermi*	*Methylobacteriaceae*	*Erwinia*
Otu37	*Thermotogae*	*Microbacteriaceae*	*Escherichia*
Otu15	Unclassified	*Micrococcaceae*	*Geobacillus*
Otu12	*Verrucomicrobia*	*Micromonosporaceae*	*Hylemonella*
		Moraxellaceae	*Lactococcus*
		Mycobacteriaceae	*Leuconostoc*
		Neisseriaceae	*Morganella*
		Oxalobacteraceae	*Mycobacterium*
		Paenibacillaceae	*Novispirillum*
		Planococcaceae	*Paenibacillus*
		Propionibacteriaceae	*Paracoccus*
		Pseudomonadaceae	*Propionibacterium*
		Rhodobacteraceae	*Providencia*
		Rhodocyclaceae	*Pseudomonas*
		Rhodospirillaceae	*Serratia*
		Rickettsiaceae	*Sphingobium*
		Ruminococcaceae	*Sphingomonas*
		Sphingobacteriaceae	*Spironema*
		Sphingomonadaceae	*Staphylococcus*
		Staphylococcaceae	*Stenotrophomonas*
		Streptococcaceae	*Streptomyces*
		Streptomycetaceae	*Swaminathania*
		Thermaceae	*Thermus*
		Unclassified	*Thorsellia*
		Weeksellaceae	Unclassified
		Xanthomonadaceae	*Variovorax*
		mitochondria	*Wolbachia*
		Acetobacteraceae	Others (<0.5%)

Preplanned Studies

Dengue Fever Screening Awareness and Capacity in Healthcare Facilities — Guangzhou City, Guangdong Province, China, 2024

Haipeng Luo[1,2,&]; Lei Luo[2,&]; Wenhui Liu[2,&]; Pengzhe Qin[2,#]; Zhoubin Zhang[1,2,#]

Summary

What is already known about this topic?

Dengue fever represents a significant public health challenge in tropical and subtropical regions globally, including China. The effective management of dengue cases depends critically on accurate and timely clinical and laboratory diagnosis, supported by well-coordinated healthcare services.

What is added by this report?

This survey revealed that healthcare facilities still needed to enhance dengue case screening and public health education initiatives. Polymerase chain reaction testing capacity was severely insufficient and inconsistent reimbursement rates across health insurance types and institutional levels. Notably, significant variations in dengue diagnostic awareness existed across hospital levels, departments, ages, and professional titles (P<0.05). Targeted training significantly enhanced diagnostic competence [odds ratio (OR)=13.78, 95% confidence interval (CI): 2.94–64.65].

What are the implications for public health practice?

Healthcare facilities must maintain heightened vigilance during dengue fever outbreaks. Robust screening and diagnostic capabilities are essential for early case detection and management. Understanding and addressing identified deficiencies and their contributing factors can strengthen the response capabilities while offering valuable lessons for other regions.

ABSTRACT

Introduction: Dengue fever represents a significant public health challenge in tropical and subtropical regions worldwide, including China. This study aims to enhance early dengue detection and diagnosis by evaluating healthcare facilities' diagnostic capacity and clinicians' awareness.

Methods: In June 2024, surveys were conducted in 11 secondary and higher-level hospitals and 11 community health centers. Data from facilities evaluations and clinician questionnaires were analyzed using chi-square tests and logistic regression.

Results: Secondary and higher-level hospitals demonstrated more robust dengue-related institution-building but exhibited deficiencies in suspected case screening and public awareness efforts. Additionally, polymerase chain reaction (PCR) testing capacity was limited to one higher-level hospital, and nonstructural protein 1 (NS1) testing costs were high in secondary and higher-level hospitals, with varying reimbursement rates due to different insurance types and institutional levels. Significant disparities in diagnostic awareness were found across hospital levels, departments, ages, and professional titles (P<0.05). The regression analysis shows that education can significantly enhance diagnostic awareness [odds ratio (OR)=13.780, 95% confidence interval (CI): 2.937, 64.650].

Conclusions: These findings underscore the need for dynamically adjust dengue testing strategies at different epidemic stages and improve NS1 testing cost reimbursement. Also, there should be more efforts in enhancing PCR testing in healthcare facilities and promoting health education.

Dengue fever, an acute infectious disease caused by the dengue virus, poses a significant global health threat in tropical and subtropical regions. With an estimated 100 million to 400 million infections annually, approximately half of the world's population lives in at-risk areas (*1*). In China, Guangdong, Yunnan, and Hainan provinces represent high-prevalence regions, with Guangzhou City, the capital of Guangdong Province, emerging as a major dengue fever hotspot. The city experiences substantial numbers of both imported and locally transmitted cases, largely attributable to its subtropical climate, humid environment, and extensive international trade networks (*2–4*). While timely and accurate clinical and laboratory diagnosis, coupled with well-organized healthcare services, are fundamental to effective dengue management (*5*), significant disparities exist in screening and diagnostic capabilities among healthcare facilities, impacting early detection and case management. Therefore, a comprehensive assessment of diagnostic capabilities and awareness across medical institutions is essential for developing targeted improvement strategies.

A cross-sectional study was conducted in June 2024 across 11 districts in Guangzhou using stratified random sampling. From each district, 1 secondary or higher-level medical institution and 1 Community Health Service Center were selected, resulting in 22 participating medical institutions. The study focused on relevant departments (pediatrics, internal medicine, fever clinics, and general practice), with 501 doctors completing the diagnostic awareness and capability survey, achieving a 92.3% response rate. Participants were required to be doctors from the selected institutions who voluntarily participated, while those from non-dengue-related departments or those who submitted incomplete electronic questionnaires were excluded.

The survey consisted of two components. The first assessed institutional dengue prevention and control measures, including system infrastructure, epidemic reporting mechanisms, laboratory testing capabilities, treatment conditions, hospital infection control protocols, staff training, and public health education initiatives. The second component utilized a self-designed questionnaire to evaluate healthcare workers' awareness of dengue diagnosis. This questionnaire gathered demographic information (including age, gender, education, and professional title) and assessed dengue knowledge through seven questions, with proficiency defined as correctly answering six or more questions.

Statistical analysis employed categorical variables expressed as percentages and continuous variables as mean ± standard deviation. Chi-square tests were used to analyze differences in diagnostic awareness across demographic variables (age, education level, and professional title), while t-tests were applied for continuous variables. The relationship between various factors and diagnostic awareness was examined using binary logistic regression, calculating odds ratios (*OR*) and 95% confidence intervals (*CI*). All analyses were performed using IBM SPSS Statistics (version 27, IBM SPSS Inc., Chicago, USA), with statistical significance defined as $P<0.05$.

This study encompassed 22 healthcare facilities and yielded 501 valid questionnaires from dengue-related healthcare workers. The facilities were evenly distributed between secondary or higher-level medical institutions (50.0%, n=11) and Community Health Service Centers (50.0%, n=11). The healthcare worker distribution included 68 pediatricians (13.6%), 150 fever clinic doctors (29.9%), 178 internal medicine doctors (35.5%), and 105 general practitioners (21.0%). Analysis revealed that secondary or higher-level medical institutions demonstrated superior performance in dengue-related system construction, environmental control, and detection capabilities compared to Community Health Service Centers, though they showed slight deficiencies in suspected case screening and dengue-related public education (Table 1). Significant variations in diagnostic awareness were identified across different institution levels, departments, ages, and professional titles ($P<0.05$) (Table 2). Binary logistic regression analysis revealed that higher hospital levels (*OR*=2.753, 95% *CI*: 1.565, 4.868) and participation in dengue training (*OR*=13.780, 95% *CI*: 2.937, 64.650) were positively associated with enhanced diagnostic awareness. Notably, compared to fever clinics, pediatric departments demonstrated higher diagnostic awareness (*OR*=2.588, 95% *CI*: 1.257, 5.328) (Table 3).

DISCUSSION

This cross-sectional survey evaluated the dengue fever screening awareness and capacity of healthcare facilities in Guangzhou. All 22 surveyed facilities demonstrated the capability to perform nonstructural protein 1 (NS1) antigen testing, with a notable increase in NS1 screenings from January to June 2024 (271 cases) compared to the same period in 2023 (102 cases). The diagnostic awareness assessment of clinicians revealed a 60.1% pass rate, indicating an overall improvement in dengue screening awareness compared to 2023, though certain areas still require enhancement.

Notable deficiencies persist in healthcare facilities regarding the establishment of severe case and death reporting systems and the formation of dengue fever expert groups. According to the "Guangdong Province Dengue Fever and Other Mosquito-Borne Infectious Diseases Surveillance Program (2019)" Yue Wei Ban [2019] No. 10 (*6*), secondary and higher-level medical institutions are mandated to promote NS1 testing

TABLE 1. Basic survey of healthcare facilities.

Variables	Secondary and higher-level medical institutions [*N*, (%)] (*N*=11)	Community health service centers [*N*, (%)] (*N*=11)	Total[*N*, (%)] (*N*=22)
Institution-building			
Work programme	11 (100)	10 (90.9)	21(95.5)
Treatment-related procedures	10 (90.9)	11 (100.0)	21 (95.5)
Relevant expert groups	9 (81.8)	8 (72.7)	17 (77.3)
Serious illness/death reporting system	8 (72.7)	6 (54.5)	14 (63.6)
laboratory test capacity			
NS1 test capacity	11 (100.0)	11 (100.0)	22 (100)
NS1 test charge	11 (100.0)	8 (72.7)	19 (86.4)
Antibody test capacity	9 (81.8)	1 (9.1)	10 (45.5)
PCR test capacity	1 (9.1)	0 (0.0)	1 (4.5)
NS1 examination of suspected patients (*N*=226)	24/113 (21.2)	34/113 (30.1)	58/226 (25.7)
NS1 detection volume, Jan–June 2023 (*N*=102)	95/102 (93.1)	7/102 (6.9)	102/102 (100.0)
NS1 detection volume, Jan–June 2024 (*N*=271)	207/271 (76.4)	64/271 (23.6)	271/271 (100.0)
Patient admission conditions	10 (90.9)	4 (36.4)	14 (63.6)
Breeding site clean-up			
1/week	10 (90.9)	10 (90.9)	20 (90.9)
1/half month or longer	1 (9.1)	1 (9.1)	2 (9.1)
Nosocomial mosquito control			
1/week	9 (81.8)	6 (54.5)	15 (68.2)
1/half month	2 (18.2)	5 (45.5)	7 (31.8)
Dengue-related training			
1/month	1 (9.1)	3 (27.2)	4 (18.2)
1/half year or longer	10 (90.9)	8 (72.7)	18 (81.8)
knowledge publicity			
Posters	8 (72.7)	10 (90.9)	18 (81.8)
Distribution of folders	8 (72.7)	11 (100.0)	19 (86.4)
Electronic screen publicity	6 (54.5)	10 (90.9)	16 (72.7)
Broadcasting	0 (0.0)	2 (18.2)	2 (9.1)
One-on-one consultation publicity	2 (18.2)	5 (45.5)	7 (31.8)
With three or more publicity methods	4 (36.4)	10 (90.9)	14 (63.6)

Abbreviation: *N*=number; NS1=nonstructural protein 1; PCR=polymerase chain reaction.

methods and, where feasible, implement polymerase chain reaction (PCR) testing to enhance early case detection capabilities. However, this study's findings revealed that among the 11 surveyed secondary and higher-level medical institutions, only 1 had established PCR testing capabilities.

Secondary and higher-level medical institutions demonstrate comparatively lower screening rates for suspected dengue fever cases and less frequent dissemination of dengue-related knowledge. This may be attributed to more selective patient screening protocols by medical staff or other institutional factors. The reduced health education efforts could be related to the distinct operational priorities of these institutions or limitations in health communication resources, though these associations require further investigation. Additionally, dengue NS1 testing fees vary by institutional tier, as established by the Medical Insurance Bureau (*7*). Secondary and higher-level institutions charge approximately 70 CNY (Chinese Yuan) compared to 50 CNY at community health service centers. While dengue NS1 screening is covered under Guangzhou's medical insurance, reimbursement rates differ across insurance types and institutional

TABLE 2. Basic demographic characteristics of physicians by diagnostic awareness scores.

Variable	Qualified, *N* (%)	Not qualified, *N* (%)	χ^2 /*t*	*P*
Location			0.300	0.584
Central urban area	176 (61.1)	112 (38.9)		
Peripheral regions	125 (58.7)	88 (41.3)		
Hospital levels*			28.817	<0.001
Secondary and higher-level medical institutions	246 (67.2)	120 (32.8)		
Community health service centers	55 (40.7)	80 (59.3)		
Department*			27.172	<0.001
Pediatrics	56 (82.4)	12 (17.6)		
Fever clinic	91 (60.7)	59 (39.3)		
Internal medicine	109 (61.2)	69 (38.8)		
General practice	45 (42.9)	60 (57.1)		
Gender			0.033	0.855
Male	151 (59.7)	102 (40.3)		
Female	150 (60.5)	98 (39.5)		
Age, mean±SD*	38.1±8.8	40.3±8.8	2.782	0.006
Professional title*			12.468	0.002
Physician	99 (66.9)	49 (33.1)		
Attending physician	160 (61.8)	99 (38.2)		
Chief physician	42 (44.7)	52 (55.3)		
Education			2.888	0.236
Associate degree or below	26 (66.7)	13 (33.3)		
Bachelor’s degree	245 (60.8)	158 (39.2)		
Graduate degree or above	30 (50.8)	29 (49.2)		
Dengue-related training*			12.593	<0.001
Yes	299 (61.4)	188 (38.6)		
No	2 (14.3)	12 (85.7)		

Abbreviation: SD=standard deviation.
* P<0.05.

levels (*8*), resulting in variable out-of-pocket expenses for patients. Community health service centers face their own challenges, with only 36.4% meeting dengue treatment facility requirements and conducting insufficient environmental mosquito control measures. These deficiencies likely stem from limited healthcare resource availability (*9*).

This study’s survey of dengue fever diagnostic awareness among medical staff revealed higher competency levels in secondary and higher-level medical institutions, with pediatricians demonstrating superior diagnostic awareness compared to other departments and general practitioners showing the lowest levels. The enhanced awareness among pediatricians may reflect their extensive experience with childhood skin infections and associated conditions (*10*), particularly following dengue-specific training. Conversely, general practitioners, primarily working in community health centers, face substantial workloads that may contribute to their comparatively lower diagnostic awareness. Unlike Lee’s study (*11*), which found higher diagnostic competency among middle and senior-aged clinicians, this study’s findings showed enhanced dengue diagnostic awareness among younger and mid-career doctors with lower to middle-level professional titles, possibly due to their frontline roles and increased exposure to relevant training. Notably, medical staff who participated in dengue-specific training demonstrated significantly improved diagnostic awareness.

This study has several limitations. First, as the sample was restricted to Guangzhou, the findings may not be generalizable to the national population, limiting the broader applicability of the results.

TABLE 3. Binary logistic regression analysis for physicians' diagnostic awareness.

Variable	Comparison group	Reference group	β	$s_{\bar{x}}$	Wald χ^2	*P*	*OR* (95% *CI*)
Hospital levels	Secondary and higher-level medical institutions	Community health service centers	1.013	0.289	12.243	<0.001	2.753 (1.565, 4.868)
Department	Pediatrics	Fever clinic	0.951	0.368	6.661	0.010	2.588 (1.257, 5.328)
	Internal medicine		0.029	0.240	0.015	0.904	1.029 (0.644, 1.646)
	General practice		−0.022	0.335	0.004	0.948	0.978 (0.507, 1.887)
Age			−0.024	0.014	3.002	0.083	0.976 (0.950, 1.003)
Professional title	Attending physician	Physician	−0.099	0.250	0.156	0.693	0.906 (0.555, 1.478)
	Chief physician		−0.451	0.363	1.545	0.214	0.637 (0.313, 1.297)
Dengue-related training	Yes	No	2.623	0.789	11.063	0.001	13.780 (2.937, 64.650)

Abbreviation: β=regression coefficient; $s_{\bar{x}}$=Standard Error of the Coefficient; Wald χ^2=Wald Chi-Squared; *OR*=odds ratio; *CI*=confidence interval.

Second, the cross-sectional design precludes causal inference. Additionally, regarding the low screening rates observed in secondary and higher-level medical institutions, the absence of comparative data from relevant secondary and tertiary institutions necessitates further investigation for a more comprehensive understanding.

While prevention and control priorities may vary across different stages of dengue fever outbreaks, the diagnostic capabilities and screening awareness of healthcare facilities remain fundamental determinants of effective disease management. The findings of this study have significant implications for public health practice, leading to the following recommendations: 1) local Centers for Disease Control and Prevention should implement dynamic adjustments to case detection and management strategies based on epidemic status and medical resource allocation during different phases of local dengue outbreaks. During early epidemic stages, healthcare facilities in outbreak hotspots should implement a "test-upon-fever" approach, while areas without local cases should follow a "test-upon-suspicion" protocol. Hospitals should prioritize hospitalization and isolation for dengue patients to facilitate early detection and minimize transmission. As the epidemic progresses to middle and later stages with increased case numbers, the focus should shift to rational medical resource allocation to prevent healthcare system overcrowding and minimize severe and fatal cases. During these stages, dengue-specific testing should support clinical diagnosis of suspected cases, with hospitalization and isolation priorities focusing on high-risk populations, including elderly individuals, pregnant women, and children. 2) Healthcare facilities should strengthen their dengue fever testing capabilities through multiple approaches. These include expanding PCR testing capacity, implementing comprehensive IgM/IgG antibody and PCR testing protocols, maintaining adequate NS1 antigen reagent stockpiles, and enhancing early case detection systems. Additionally, facilities should intensify dengue awareness initiatives, particularly among general practitioners, through regular training on diagnostic criteria, epidemiological assessment, testing methodologies, and procedural protocols. Educational activities should be amplified during peak transmission seasons. Furthermore, healthcare facilities should implement systematic monitoring of outpatient records to ensure prompt sampling and testing of patients meeting suspected dengue case criteria. Community health centers must also improve their compliance with dengue fever admission and treatment environment standards. 3) The medical insurance department should conduct thorough feasibility studies regarding increasing the NS1 testing reimbursement rate to 60% under the "residents' medical insurance" scheme. This adjustment would be particularly beneficial in community and outpatient settings during the early dengue fever season, typically occurring from May to August annually.

Implementation of these comprehensive measures would significantly enhance early detection and accurate diagnosis of dengue fever cases, ultimately contributing to more effective control of dengue fever epidemics.

Acknowledgements: All research personnel from participating Centers for Disease Control and Prevention, hospitals, and healthcare facilities for their valuable contributions to this project.

Conflicts of interest: No conflicts of interest.

Ethical Statement: This study did not involve human subjects or any intervention in clinical practice, as it focused on healthcare facilities' diagnostic capacity and clinicians' awareness of dengue early detection and diagnosis. Additionally, no personally identifiable information was collected or analyzed throughout the study.

Funding: This work was supported by the National Key Research and Development Program of China (grant number 2024YFC2311500), The Key Project of Medicine Discipline of Guangzhou (No.2025-2027-11), and General Guidance Project for Health Science and Technology in Guangzhou (No. 20231A011069).

doi: 10.46234/ccdcw2025.087

Corresponding authors: Pengzhe Qin, gzcdc_qinpz@gz.gov.cn; Zhoubin Zhang, gzcdc_zhangzb@gz.gov.cn.

[1] School of Public Health, Sun Yat-sen University, Guangzhou City, Guangdong Province, China; [2] Department of Communicable Disease Control and Prevention, Guangzhou Center for Disease Control and Prevention, Guangzhou City, Guangdong Province, China.
[&] Joint first authors.

Submitted: July 24, 2024
Accepted: March 13, 2025
Issued: April 18, 2025

REFERENCES

1. World Health Organization. Dengue and severe dengue. 2024. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. [2024-7-14].
2. Lai SJ, Huang ZJ, Zhou H, Anders KL, Perkins TA, Yin WW, et al. The changing epidemiology of dengue in China, 1990-2014: a descriptive analysis of 25 years of nationwide surveillance data. BMC Med 2015;13:100. https://doi.org/10.1186/s12916-015-0336-1.
3. Luo L, Jiang LY, Xiao XC, Di B, Jing QL, Wang SY, et al. The dengue preface to endemic in mainland China: the historical largest outbreak by *Aedes albopictus* in Guangzhou, 2014. Infect Dis Poverty 2017;6(1):148. https://doi.org/10.1186/s40249-017-0352-9.
4. Li CX, Wang ZD, Yan Y, Qu YN, Hou LY, Li YJ, et al. Association between hydrological conditions and dengue fever incidence in coastal southeastern China From 2013 to 2019. JAMA Netw Open 2023;6(1): e2249440. https://doi.org/10.1001/jamanetworkopen.2022.49440.
5. Muller DA, Depelsenaire ACI, Young PR. Clinical and laboratory diagnosis of dengue virus infection. J Infect Dis 2017;215(S2):S89 – 95. https://doi.org/10.1093/infdis/jiw649.
6. Guangdong Provincial Health Commission. Guangdong Province dengue fever and other mosquito-borne infectious diseases surveillance program (2019). 2019. https://wsjkw.gd.gov.cn/gkmlpt/content/2/2484/%20post_2484867.html#2532. [2024-7-14]. (In Chinese).
7. Guangzhou Medical Security Bureau. Summary table of prices of basic medical service Items of public medical institutions in Guangzhou Area (September 2024). 2024. https://file.m12333.cn/upfile/download/46733976-85c6-cc7c-4d7d-bcccce4c65fa.pdf. [2024-11-25]. (In Chinese).
8. Guangzhou Medical Security Bureau, Guangzhou Finance Bureau, Guangzhou Health Commission. Notice on the standards of employee medical insurance and maternity insurance benefits in Guangzhou. 2022. https://www.gz.gov.cn/gzybj/gkmlpt/content/8/8689/post_8689749.html#14461. [2025-11-25]. (In Chinese).
9. Dong EH, Xu J, Sun XT, Xu T, Zhang LF, Wang T. Differences in regional distribution and inequality in health-resource allocation on institutions, beds, and workforce: a longitudinal study in China. Arch Public Health 2021;79(1):78. https://doi.org/10.1186/s13690-021-00597-1.
10. Tempark T, Whaidee K, Bongsebandhu-Phubhakdi C, Suteerojntrakool O. Prevalence of skin diseases in school-age children. Fam Pract 2022;39(3):340 – 5. https://doi.org/10.1093/fampra/cmab164.
11. Lee LK, Thein TL, Kurukularatne C, Gan VCH, Lye DC, Leo YS. Dengue knowledge, attitudes, and practices among primary care physicians in Singapore. Ann Acad Med Singap 2011;40(12):533 – 8. https://doi.org/10.47102/annals-acadmedsg.V40N12p533.

Preplanned Studies

Pathogenic Bacteria Detection in Parasitic Fleas — Jiangxi Province, China, 2023

Lijuan He[1,&]; Zhiyun Liu[2,&]; Like Duan[1]; Lin Zhang[1]; Xuexia Hou[1]; Jingwen Li[1]; Qin Hao[1,#]

Summary

What is already known about this topic?

Fleas are recognized as one of the most important vectors of various diseases in humans and animals. Studies have reported the distribution of fleas in different regions of China and the pathogens they carry. However, limited research exists on the detection and classification of flea-borne pathogens in Jiangxi Province. Additionally, we identified previously unreported pathogens in *Pulex irritans*.

What is added by this report?

In this study, 3 *Ctenocephalides felis* and 118 *Pulex irritans* collected from domestic dogs were tested for six pathogens.The results revealed that *Pulex irritans* carried six pathogens: *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Borrelia hermsii*, *Anaplasma phagocytophilum*, *Coxiella burnetii,* and *Rickettsia slovaca*. This is the first report of these six pathogens in *Pulex irritans.*

What are the implications for public health practice?

Both humans and animals in Suichuan County, Ji'an City, Jiangxi Province, may be at risk from flea-borne infectious agents. Therefore, there is an urgent need for public health alerts, active flea surveillance and effective screening for infections in humans and animals in Suichuan County.

ABSTRACT

Objective: Fleas are vectors for the transmission of various pathogens. The study is to understand the pathogens carried by parasitic fleas in domestic dogs and to evaluate the pathogenic potential risk to humans.

Methods: 121 fleas were collected from 6 dogs in different farmers' households in Suichuan County, Ji' an City, Jiangxi Province in July 2023. Flea species were determined through morphological identification and *CoII* gene detection. Whole genomic DNA was extracted from all 121 fleas, and six pathogens - *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Coxiella burnetii*, *spotted fever group Rickettsia,* and *Ehrlichia chaffiensis* — were detected using nested polymerase chain reaction (PCR).

Results: Positive products were sequenced, and the carrier status of each pathogen was analyzed. Of the 121 fleas identified, 118 were *Pulex irritans* and 3 were *Ctenocephalides felis*. PCR results revealed that *Borrelia burgdorferi* (5%, 6/118), *Borrelia miyamotoi* (0.8%, 1/118), *Borrelia hermsii* (9%, 11/118), *Anaplasma phagocytophilum* (0.8%, 1/118), *spotted fever group Rickettsia* (0.8%, 1/118), and *Coxiella burnetii* (0.8%, 1/118) were detected in *Pulex irritans*. Additionally, one sample showed mixed infection with both *Borrelia burgdorferi* and *Anaplasma phagocytophilum* (0.8%, 1/118).

Conclusions: This study suggests that *Pulex irritans* can carry multiple pathogens, with implications for public health needs that warrant further investigation.

Fleas are ectoparasites that infest humans and animals, capable of carrying and transmitting various pathogens through bites. Species like *Pulex irritans*, *Xenopsylla cheopis*, and *Ctenocephalides felis* are known vectors of zoonotic diseases such as plague, murine typhus, and spotted fever (*1*). Flea-borne diseases pose significant risks to human and animal health, with their prevalence influenced by environmental factors like temperature changes.

Jiangxi Province, located in Southeast China, has a warm and humid climate ideal for the breeding of medical insects, including fleas. While studies have examined flea distribution and pathogen carriage in some regions like Yunnan Province and Inner Mongolia Autonomous Region, contributing to understanding local flea-borne diseases (*2–3*), research on flea-borne pathogens in Jiangxi remains limited.

Understanding the pathogens carried by fleas in Jiangxi Province is crucial for controlling flea-borne diseases. In July 2023, 121 fleas were collected from six dogs in Suichuan County, Ji'an City, Jiangxi Province. Six pathogens, including *Borrelia burgdorferi* (*B. burgdorferi*), *Borrelia miyamotoi* (*B. miyamotoi*), *Anaplasma phagocytophilum* (*A. phagocytophilum*), *Coxiella burnetii* (*C. burnetii*), *spotted fever group Rickettsia* (*SFGR*), and *Ehrlichia chaffiensis* (*EC*) were detected via nested PCR and sequencing. These findings provide essential data for local disease surveillance and control. The CoII gene, with primers F-leu: TCTAATATGGCAGATTAGTGC and R-lys: GAGACAGTACTTGCTTTCAGTCATC (*4*), was used for flea species identification.

Six pathogens, including *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, *C. burnetii*, *SFGR,* and *EC* were detected via nested polymerase chain reaction (PCR) method. The sequences of target genes and primers for PCR detection are shown in Table 1 (*5*–*7*). Amplified products were electrophoresed on a 1.5% agarose gel, and positive samples were purified, sequenced by Beijing De Aoping Biotechnology Co., Ltd., and analyzed using NCBI BLAST for homology comparison. Reference sequences from GenBank were used to construct a phylogenetic tree with MEGA11.0. Additionally, *B. burgdorferi* and *Borrelia hermsii* (*B. hermsii*) positive samples were tested via quantitative real-time PCR (qPCR) targeting the *recA* and *fla-B* genes, respectively, using a Probe qPCR mix (Premix Ex Taq^{TM}, TaKaRa) on a LightCycler 480 System (Roche Diagnostics, United States).

The 121 collected fleas were disinfected by soaking in sodium hypochlorite bleach for 30 seconds, followed by immersion in 75% alcohol for 10 minutes, washed twice, and rinsed with ultra-pure water. Each flea was

TABLE 1. Sequences of target genes and primers for polymerase chain reaction detection.

Bacteria	Target gene	Primer name	Sequence (5'–3')	Size (bp)	Reference
Borrelia burgdorferi	*5S-23S rRNA IGS*	P1	CGACCTTCTTCGCCTTAAAGC	255	(*5*)
		P2	TAAGCTGACTAATACTAATTACCC		
		P3	TCCTAGGCATTCACCATA		
		P4	GAGTTCGCGGGAGA		
Borrelia miyamotoi	*glpQ*	Q1	CACCATTGATCATAGCTCACAG	424	
		Q2	CTGTTGGTGCTTCATTCCAGTC		
		Q3	GCTAGTGGGTATCTTCCAGAAC		
		Q4	CTTGTTCTTTATGCCAGAAGGGT		
Anaplasma phagocytophilum	16S rRNA	AP-F	GTCGAACGGATTATTCTTTATAGCTTG	389	
		AP-R	TATAGGTACCGTCATTATCTTCCCTAC		
Ehrlichia chaffiensis	16S rDNA	ECB	AGAACGAACGCTGGCGGCAAGCC	389	
		ECC	CGTATTACCGCGGCTGCTGGCA		
		H3	TATAGGTACCGTCATTATCTTCCCTAT		
		H1	CAATTGCTTATAACCTTTTGGTTATAAAT		
Spotted fever group Rickettsia	*ompA*	F	ATGGCGAATATTTCTCCAAAA	533	
		R	GTTCCGTTAATGGCAGCATCT		
		602R	AGTGCAGCATTGGCTCCCCCT		
Coxiella burnetii	*IS1111*	F1	TACTGGGTCTTGATATTGC	297	
		R1	CCGTTTCATCCGCGGTG		
		F2	GTAAAGTGATCTACACGA		
		R2	TTAACAGCGCTTGAACGT		
Borrelia burgdorferi	*recA*	F	AGGTGGGATAGCTGCTTTTATTGAT	83	(*6*)
		R	GTTCTGCAACATTAACACCTAAAGCTT		
		P	6-FAM-ACAGGATCAAGAGCATG-BHQ1		
Borrelia hermsii	*fla-B*	F	GGACATTGAGAGTACATGTGGGC	136	(*7*)
		R	CCTCTTGCTGTCCTATCTCTTGCA		
		P	AGCCTGAGCRCCTTCACCTGCAAAAAGA		

placed in a 1.5 mL grinding tube and homogenized using a high-throughput grinder. Morphological characteristics and *CoII* gene detection and sequencing identified 3 *Ctenocephalides felis* and 118 *Pulex irritans*. The sequence analysis of the flea *CoII* gene is shown in Figure 1.

Pathogen detection in 118 *Pulex irritans* revealed *B. burgdorferi* (5%, 6/118), *B. miyamotoi* (0.8%, 1/118), *B. hermsii* (9%, 11/118), *A. phagocytophilum* (0.8%, 1/118), *Rickettsia slovaca* (0.8%, 1/118), and *C. burnetii* (0.8%, 1/118). One sample showed mixed infection with *B. burgdorferi* and *A. phagocytophilum*. *E. chaffeensis* was negative, and no pathogens were detected in the 3 *Ctenocephalides felis*.

In this study, six positive samples for *B. burgdorferi* were sequenced and analyzed (Figure 2A). The results revealed that the *rrf–rrl* spacer sequences from samples Bb-3, Bb-34, and Bb-109 were identical to that of *Borrelia garinii* (*B. garinii*), while the *rrf–rrl* spacer sequence from sample Bb-5 was identical to that of *Borrelia burgdorferi sensu stricto* (*B. b. s. s.*). Additionally, the *rrf–rrl* spacer sequences from samples Bb-52 and Bb-91 were identical to that of *Borrelia valaisiana* (*B. valaisiana*). Based on the nested PCR results, the six positive samples for *B. burgdorferi* were also tested via qPCR, which confirmed that samples Bb-3, Bb-5 , and Bb-109 were positive for the *B.burgdorferi recA* gene.

A total of 11 positive samples for *B. hermsii* and 1 positive sample for *B. miyamotoi* were sequenced and analyzed (Figure 2B–C). After removing duplicate sequences, the remaining 8 unique sequences were used to construct the phylogenetic tree of *B. hermsii*. These 8 sequences were found to be closely related to the USA strain LAK-6 (KX171818*)*. The *glpQ* sequence of *B. miyamotoi* detected in sample Mi-112 was closely related to USA clone GlpQ52T4 (KY008451*)*. Based on the nested PCR results, the 11 samples positive for *B. hermsii* were also tested via qPCR, which confirmed that sample B.h-103 was positive for *B. hermsii fla-B* gene.

The phylogenetic analysis results for*A. phagocytophilum*, *C. burnetii*, and *SFGR* are shown in Figure 2D, 2F. The *16S rRNA* sequence of *A. phagocytophilum* detected in sample AP-15 was closely related to China strain TMSK (PQ459373). The *IS1111* sequence of *C. burnetii* detected in sample IS-6 was closely related to Mexico isolate INIFAP Cap04 insertion (MT459145). The *ompA* sequence of *SFGR* detected in sample SF-17 was closely related to Turkey isolate Ro-715 (MF379305), which is an isolate of *Rickettsia slovaca*.

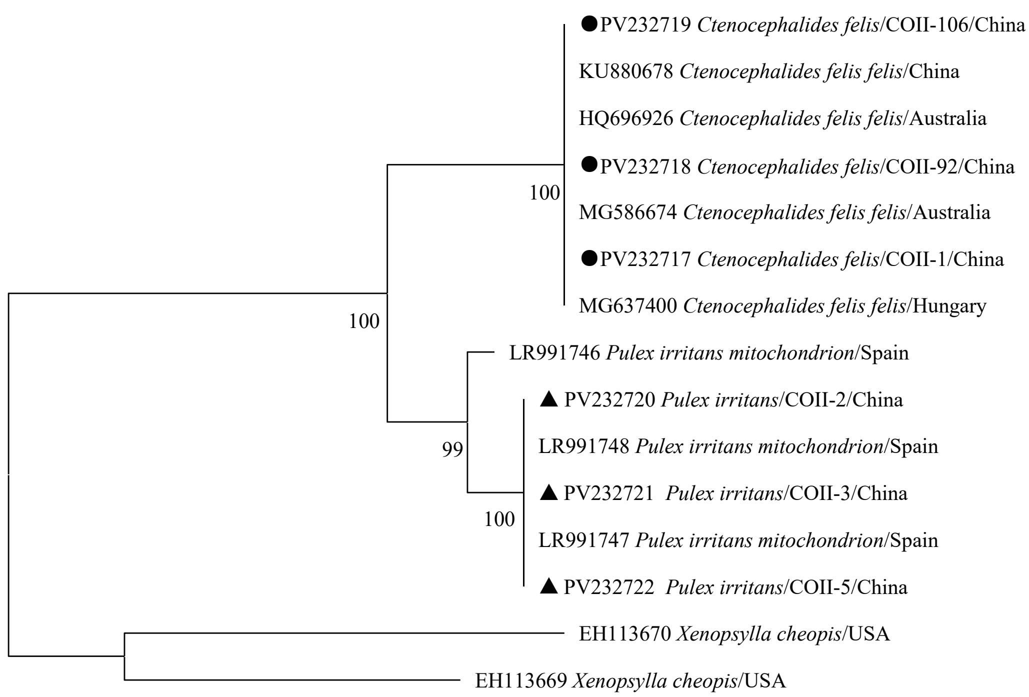
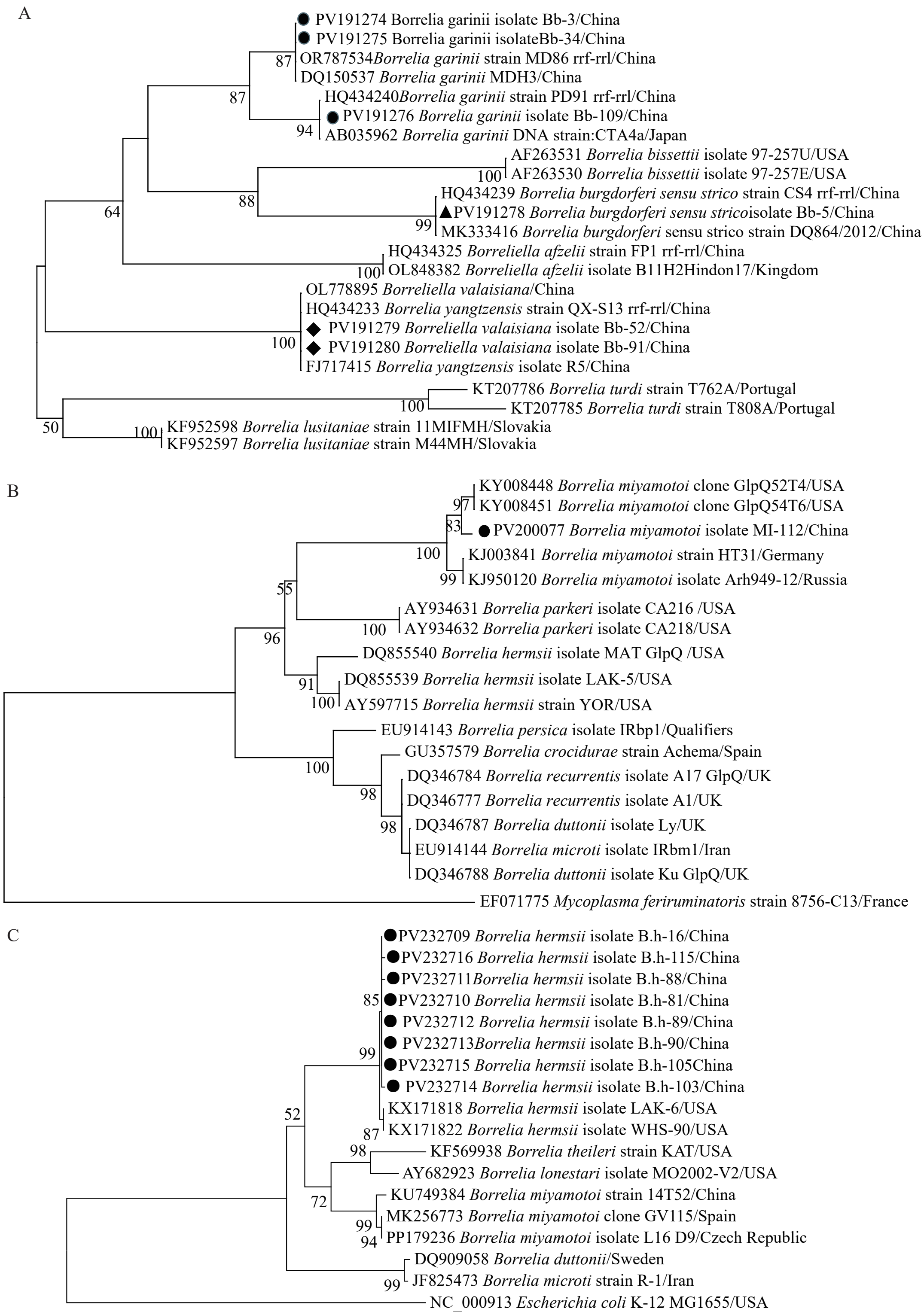


FIGURE 1. *COII* sequence analysis of fleas in Ji 'an city, Jiangxi Province, China.
Note: ● Means flea samples identified as Ctenocephalides felis in this study; ▲ Means flea samples identified as Pulex irritans in this study.

DISCUSSION

Fleas are recognized as one of the most important vectors of diseases in humans and animals, with flea-borne diseases potentially re-emerging as epidemics due to environmental and behavioral changes affecting vector-host ecology (*8*). This study is the first to report *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Borrelia hermsii*, *Anaplasma phagocytophilum*, *Rickettsia slovaca*, and *Coxiella burnetii* in *Pulex irritans*.



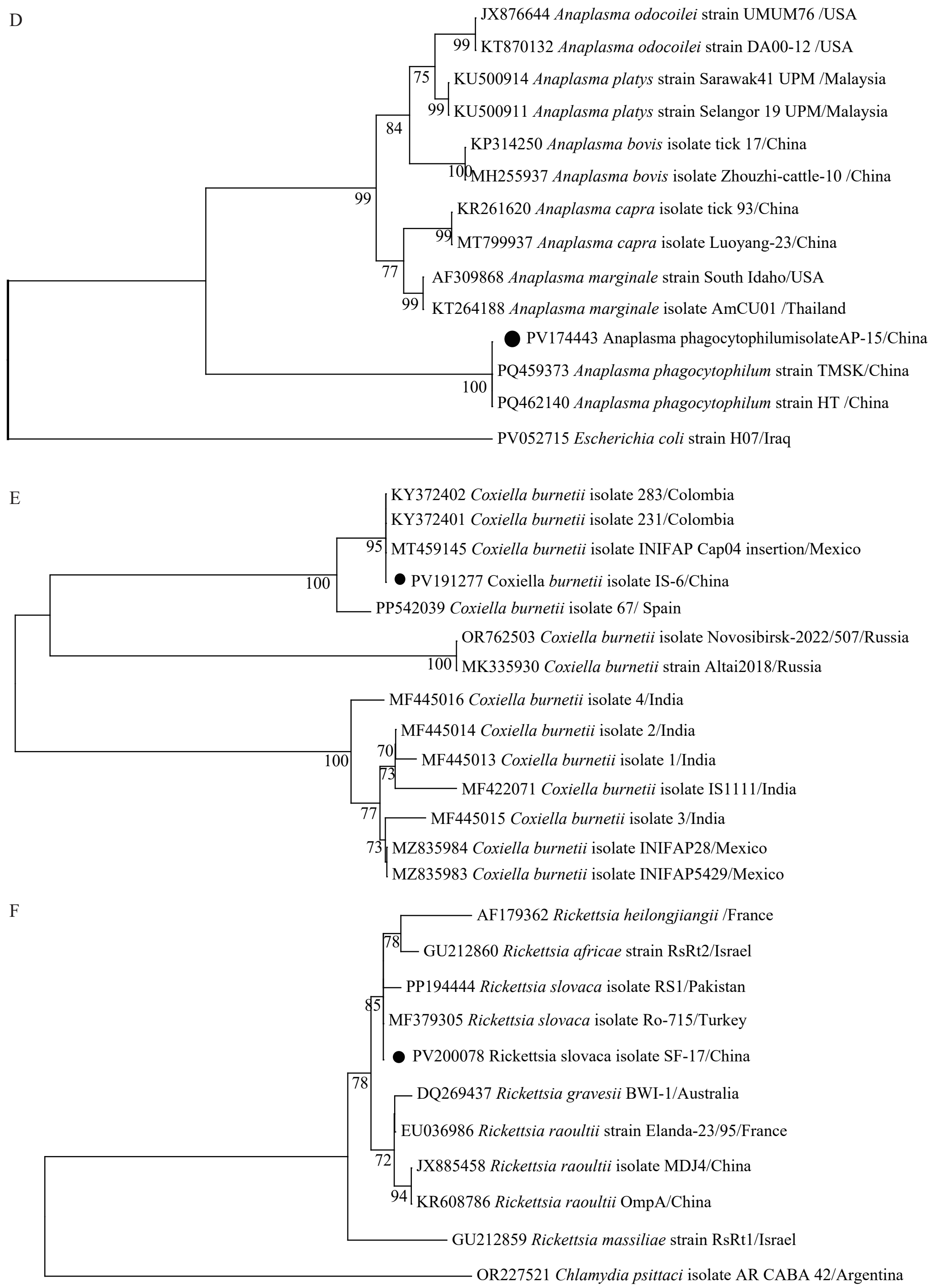


FIGURE 2. Phylogenetic analysis of six pathogens in *Pulex irritans*.(A) Phylogenetic analysis for *5S-23S rRNA* gene of *Borrelia burgdorferi*; (B) Phylogenetic analysis for *glpQ* gene of *Borrelia miyamotoi*; (C) Phylogenetic analysis for *glpQ* gene of *Borrelia hermsii*; (D) Phylogenetic analysis for *16S rRNA* gene of *Anaplasma phagocytophilum*; (E) Phylogenetic analysis for *IS1111* gene of *Coxiella burnetii*; (F) Phylogenetic analysis for *ompA* gene of *spotted fever group Rickettsia*.

Note: for (A), the sequences clustered as *Borrelia garinii* were marked as "●"; *Borrelia burgdorferi sensu stricto* were marked as "▲"; *Borrelia valaisiana* were marked as "◆"; for (B), the sequences clustered as *Borrelia miyamotoi* were marked as "●"; for (C), The sequences identified as *Borrelia hermsii* are marked with "●"; for (D), The sequence identified as *Anaplasma phagocytophilum* is marked with "●"; for (E), The sequences clustered as *Coxiella burnetii* were marked as "●"; for (F), The sequences clustered as *Rickettsia slovaca* were marked as "●".

B. burgdorferi, the causative agent of Lyme disease, and a primary pathogen typically transmitted by ticks, was detected in *Pulex irritans* in Jiangxi Province. Among 20 *Pulex irritans* positive for flea-borne bacteria, three carried *B. garinii*, two carried *B. valaisiana*, and one carried *B. b. s. s*. Additionally, three of the six positive samples were confirmedpositive results for *B. burgdorferi recA* by qPCR.These findings indicate that *Pulex irritans* in Jiangxi may harbor three *B. burgdorferi* genotypes, with *B. garinii* being the predominant pathogenic genotype in China and *B. b. s. s.* being most common in the Americas (*9*). Notably, these genotypes have also been detected in ticks from Jiangxi Province (*10*).

B. miyamotoi and *B. hermsii* can cause tick-borne *relapsing fever*. While *B. miyamotoi* is transmitted by *Ixodes* ticks, *B. hermsii* is vectored by soft ticks. Through nucleic acid detection of the *glpQ* gene, 1 *Pulex irritans* sample was positive for *B. miyamotoi*, and 11 Pulex irritans samples were positive for *B. hermsii*. Furthermore, one of the 11 *B. hermsii* positive samples was confirmed positive for *B. hermsii fla-B* by qPCR. These results demonstrate that *Pulex irritans* can carry both *B. hermsii* and *B. miyamotoi*.

Nucleic acid detection revealed one *Pulex irritans* sample positive for *A. phagocytophilum*, one positive for *SFGR*, and one positive for *C. burnetii*. Previous studies in China have reported *Ctenocephalides felis* carrying *C. burnetii* and *SFGR* (*11–12*). In northwestern Iran, *Pulex irritans* has been documented to carry *Rickettsia* sp. (*13*). However, this is the first report of *Pulex irritans* carrying *C. burnetii* and *Rickettsia slovaca*.

Among the 118 *Pulex irritans samples*, one tested positive for both *B. burgdorferi* and *A. phagocytophilum*, indicating that a single flea can harbor multiple pathogens. This finding underscores the necessity for comprehensive pathogen detection in fleas.

A previous study reported the isolation of *Borrelia yangzensis* from rodents in six counties in Jiangxi Province (*14*). Additionally, tick-borne pathogens including *B. burgdorferi*, *B. miyamotoi*, and *SFGR* have been detected in patients with arthritis or neurological symptoms (*15*), indicating that Ji'an City is a natural focus for tick-borne diseases with potential for zoonotic transmission. While ticks are the primary vectors for *B. burgdorferi*, *B. miyamotoi*, *B. hermsii*, *A. phagocytophilum*, *C. burnetii*, *SFGR*, and *E. chaffiensis*, our study identifies *Pulex irritans* as a potential carrier of these pathogens, highlighting the need for further investigation into its public health significance in pathogen transmission.

Due to the lack of methods to verify the prevalence of these six pathogens in host dogs and considering the influence of sample size, collection points, and other factors on pathogen detection rates, long-term monitoring and investigation of flea-borne pathogens are necessary. Additionally, strengthening the development of control measures for flea-borne diseases in Jiangxi Province is essential.

Conflicts of interest: No conflicts of interest.

Ethical statement: Not involving ethics.

Funding: Supported by the National Major Science and Technology Project of China (2017ZX1030 3404006003) and the Independent Research Project of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (32055).

doi: 10.46234/ccdcw2025.088

Corresponding author: Qin Hao, haoqin@icdc.cn.

[1] National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; [2] Inspection Department of Ji'an Central People's Hospital, Ji'an City, Jiangxi Province, China.
[&] Joint first authors.

Submitted: December 16, 2024
Accepted: April 14, 2025
Issued: April 18, 2025

REFERENCES

1. Brouqui P, Raoult D. Arthropod-borne diseases in homeless. Ann NY Acad Sci 2006;1078:223 – 35. https://doi.org/10.1196/annals.1374.041.
2. Yin JX, Cheng XO, Luo YY, Zhao QF, Wei ZF, Xu DD, et al. The relationship between fleas and small mammals in households of the Western Yunnan Province, China. Sci Rep. 2020;10(1):16705. https://doi: 10.1038/s41598-020-73690-0.
3. Shang M, Ji H, Li K, Wang X, Wang L, Jiang W, et al. Spatiotemporal Characteristics and Risk Zonation Analysis of Rodents and Surface-Parasitic Fleas - Inner Mongolia Autonomous Region, China, 2013-2021. China CDC Wkly. 2024;6(46):1195-1200. https://doi: 10.46234/ccdcw2024.241.
4. Whiting MF. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zool Scr 2002;31(1):93 – 104. https://doi.org/10.1046/j.0300-3256.2001.00095.x.
5. Duan LK, Zhang L, Hou XX, Bao ZH, Zeng Y, He LJ, et al. Surveillance of tick-borne Bacteria infection in ticks and forestry populations in Inner Mongolia, China. Front Public Health 2024;12:1302133. https://doi.org/10.3389/fpubh.2024.1302133.
6. Geng Z, Hou XX, Zhang L, Hao Q. Evaluation of a new real-time PCR assay for detection of *Borrelia burgdorferi* in rodents. Chin J Zoonoses 2015;31(9):812 – 6. https://doi.org/10.3969/j.issn.1002-2694.2015.09.006.

7. Modarelli JJ, Piccione J, Ferro PJ, Esteve-Gasent MD. Novel real-time PCR assays for genomic group identification of tick-borne relapsing fever species *Borrelia hermsii*. Diagn Microbiol Infect Dis 2019;93(1):24 – 9. https://doi.org/10.1016/j.diagmicrobio.2018.08.001.
8. El Hamzaoui B, Zurita A, Cutillas C, Parola P. Fleas and flea-borne diseases of North Africa. Acta Trop 2020;211:105627. https://doi.org/10.1016/j.actatropica.2020.105627.
9. Chomel B. Lyme disease. Rev Sci Tech 2015;34(2):569 – 76. https://doi.org/10.20506/rst.34.2.2380.
10. Zeng Y, Liu ZY, Duan LK, Hou XX, Zhang L, He LJ, et al. Detection and genotyping of pathogenic bacteria carried by *Rhipicephalus microplus* in Ji'an, Jiangxi Province, China. Chin J Vector Biol Control 2024;35(3):381 – 8. https://doi.org/10.11853/j.issn.1003.8280.2024.03.022.
11. Wu YL, Hu SF, Zhang XL, Wang HM, Pan HY, Liu GH, et al. Complete bacterial profile and potential pathogens of cat fleas *Ctenocephalides felis*. Acta Trop 2023;243:106923. https://doi.org/10.1016/j.actatropica.2023.106923.
12. Šlapeta J, Lawrence A, Reichel MP. Cat fleas (*Ctenocephalides felis*) carrying *Rickettsia felis* and *Bartonella* species in Hong Kong. Parasitol Int 2018;67(2):209 – 12. https://doi.org/10.1016/j.parint.2017.12.001.
13. Ghavami MB, Mirzadeh H, Mohammadi J, Fazaeli A. Molecular survey of ITS1 spacer and *Rickettsia* infection in human flea, *Pulex irritans*. Parasitol Res 2018;117(5):1433 – 1442. https://doi.org/10.1007/s00436-018-5768-z.
14. Hou XX, Xu JM, Hao Q, Xu G, Geng Z, Zhang L. Prevalence of *Borrelia burgdorferi* sensu lato in rodents from Jiangxi, southeastern China region. Int J Clin Exp Med 2014;7(12):5563-7. https://pubmed.ncbi.nlm.nih.gov/25664072/.
15. Liu ZY, Zeng Y, Duan LK, Hou XX, Zhang L, He LJ, et al. Research on suspected cases of Lyme disease in Ji'an City, Jiangxi province. Chin J Zoonoses 2024;40(4):340 – 5. https://doi.org/10.3969/j.issn.1002-2694.2024.00.058.

Outbreak Reports

First Human Case of Diphyllobothriosis Due to *Dibothriocephalus Dendriticus* Infection — China, November 2023

Jiahui Sun[1]; Jiatian Guo[1]; Yan Zhou[1]; Shaohong Chen[1]; Yan Lu[1,#]

Summary

What is already known about this topic?

Dibothriocephalus dendriticus (*D. dendriticus*) is a recognized causative agent of diphyllobothriosis, a worldwide fish-borne zoonosis affecting up to 20 million people. It is predominantly distributed in circumboreal regions, and no human infections have been previously reported in China.

What is added by this report?

This is the first human case of diphyllobothriosis caused by *D. dendriticus* in China. We report the clinical and epidemiological findings, as well as the morphological and genetic characteristics of the parasite. Retrospective investigation suggests this was an autochthonous case acquired in China.

What are the implications for public health practice?

The increasing demand for fish products and raw foods poses a growing risk of diphyllobothriosis and potential economic losses. Attention should be paid to preventing *D. dendriticus* from becoming an emerging disease in China due to the globalization of food trade and global integration.

Abstract

Objective: Human diphyllobothriosis is a global fish-borne zoonosis affecting approximately 20 million people. This study reports the first human case of *Dibothriocephalus dendriticus* (*D. dendriticus*) in China and explores its epidemiological and phylogenetic implications.

Methods: Morphological features of eggs and proglottids were examined. The mitochondrial *cox1* gene was sequenced for species identification. Phylogenetic analysis and epidemiological data were analyzed to trace the infection source.

Results: The expelled tapeworm measured 50 cm in length and 0.7 cm in width. The gravid proglottid was longer than wide, with a centrally positioned uterus. Eggs measured 63.29±1.17 × 48.31±0.94 μm (*n*=15) and had an operculum. The *cox1* gene (PQ169609) showed 99.87% homology with *D. dendriticus* (AM412738.2). Morphological and molecular analyses confirmed the parasite as *D. dendriticus*. Consumption of raw salmon in Hong Kong Special Administrative Region (May 2023) and raw trout in Beijing Municipality (August 2022) were identified as potential infection sources. Phylogenetic analysis linked the strain to one from UK fish (KY552870), suggesting a common origin.

Conclusion: This study reports the first human case of *D. dendriticus* in China. It highlights the emerging threat of *D. dendriticus* amid globalization and rising fish consumption. Strengthening food safety measures is essential to reducing infection risk.

Human diphyllobothriosis, a worldwide fish-borne zoonosis, is responsible for the most reported cestode infections in humans, with an estimated 20 million people affected globally (*1*). Infection occurs through consumption of raw or inadequately cooked fish containing plerocercoid larvae, resulting in symptoms such as diarrhea, abdominal pain, and vomiting. *Dibothriocephalus dendriticus* (*D. dendriticus*) is a prominent causative agent of human diphyllobothriosis, although human infections are considered occasional (*2*–*3*). The primary endemic regions include Northern Europe, Arctic and Subarctic North America, and Siberia, particularly the Lake Baikal region (*2*). However, no human *D. dendriticus* infections have been previously documented in China.

In November 2023, a white worm excreted by a child from Jiangsu Province, China, was submitted to the National Institute of Parasitic Diseases, China CDC for identification. Based on clinical and epidemiological findings, along with morphological and genomic analyses, the sample was identified as *D. dendriticus*.

This case highlights the importance of continued vigilance against diphyllobothriosis caused by *D. dendriticus* in China and underscores challenges to public health and food safety amid the globalization of food trade, climate change, and cultural integration (*4–5*).

INVESTIGATION AND RESULTS

A 13-year-old boy from Jiangsu Province began experiencing unexplained weight loss in October 2022, accompanied by mild symptoms including diarrhea and abdominal pain. On November 24, 2023, a white worm was observed hanging from his anus during defecation and broke when his parents attempted to pull it out. The expelled tapeworm, measuring 50 cm in length and 0.7 cm in width, was submitted to the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, for identification (Figure 1A). Morphological examination and molecular testing, including polymerase chain reaction (PCR) and sequencing, confirmed the parasite as *Dibothriocephalus dendriticus*. The patient was treated with praziquantel and a 3-day course of albendazole. Follow-up stool examination one month post-treatment revealed no parasite eggs.

A retrospective investigation revealed that the patient had experienced pyrexia of 39 °C due to influenza A virus infection for three days prior to the worm's emergence on November 21, 2023. Laboratory findings showed low direct eosinophil count (0.03×10^9/L) and percentage (0.00%), while procalcitonin (PCT, 0.07 ng/mL), C-reactive protein (CRP, 8.18 mg/L), interleukin-1 beta (IL-1β, 18.14 pg/mL), interleukin-8 (IL-8, 30.87 pg/mL), and immunoglobulin E (IgE, 377.00 IU/mL) levels were elevated. These acute immunological changes likely contributed to the parasite's expulsion.

Epidemiologically, the patient had a preference for raw fish dishes such as sushi and sashimi. Three potential infection sources were identified: sashimi consumed in Japan (February 2020), raw trout (aquacultured in a reservoir) consumed in Beijing Municipality in August 2022, and raw salmon consumed in Hong Kong Special Administrative Region (SAR), China (May 2023). The first scenario was deemed highly unlikely, as the absence of proglottid segments over a three-year period would be improbable. Therefore, this case is considered an

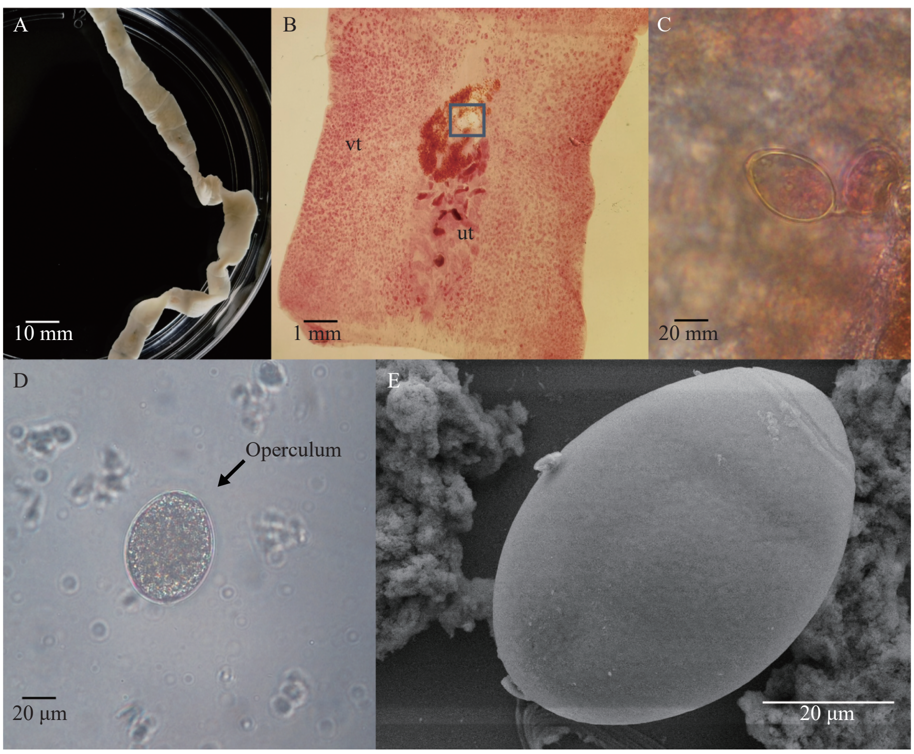


FIGURE 1. Morphological features of *D. dendriticus* from the first human case in China under different magnifications. (A) The worm; (B) Carmine-stained gravid proglottids; (C) Immature egg in the uterus (zoomed in on the square in B); (D) Eggs observed under a light microscope; (E) Eggs visualized using a SEM.
Abbreviation: ut=uterus; vt=vitellarium; *D. dendriticus=Dibothriocephalus dendriticus*; SEM=scanning electron microscope.

autochthonous infection acquired within China.

Dibothriocephalus eggs were collected and examined under 400× magnification, measuring 63.29±1.17 × 48.31±0.94 μm (*n*=15). An operculum was clearly observed (Figure 1D, 1E), although the small knob was barely visible. Proglottids were stained with alcoholic hydrochloric acid-carmine to enhance visualization of internal structures, particularly the uterus, for definitive identification (Figure 1B).

Gravid segments of *D. dendriticus* are typically wider (0.82–10.0 mm) than long (0.13–2.1 mm) and contain a centrally positioned tubular uterus (ut), which forms 6–8 coils in a rosette-like shape (*1*). However, the proglottids in our specimen were longer than wide. Although the uterus remained centrally positioned (Figure 1B), the characteristic rosette-like coiling was less pronounced, likely due to mechanical distortion during extraction.

For species confirmation, the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene was selected as a molecular marker due to the high morphological similarity among *Dibothriocephalus* spp. eggs. PCR (Figure 2) was performed using the forward primer: GTGTTTTTCATTTGATGATGACCAGTC and reverse primer: ATGATAAGGGAYAGGRG CYCA. Sequencing was conducted by BGI Tech Solution (Beijing Liuhe) Co., Ltd. The resulting sequences were analyzed against the NCBI database, confirming the worm as *D. dendriticus*.

A total of 22 related species, including all available

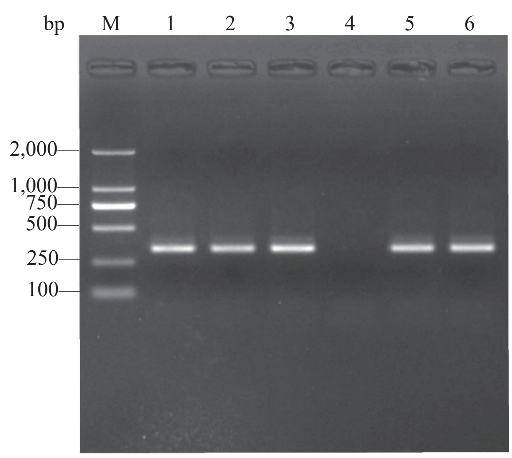


FIGURE 2. Electrophoresis analysis of *Dibothriocephalus dendriticus* using a partial *cox1* gene sequence.
Note: Lane M: molecular marker (bp); Lane 1–3: sample triplicate of *D. dendriticus*; Lane 4: NC; Lane 5–6: PC. Target bands are bright.
Abbreviation: NC=negative control; PC=positive control; *D. dendriticus*=*Dibothriocephalus dendriticus*.

human cases of *D. dendriticus* infection from the NCBI database (Table 1), were selected for phylogenetic analysis to elucidate the evolutionary relationships within the genus *Dibothriocephalus* and identify potential epidemiological connections.

A phylogenetic tree was constructed based on the *cox1* gene of *D. dendriticus*, using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Primers were designed according to Wicht et al. (*6*). Sequence alignment was performed in BioEdit 7.2.5 with *Spirometra mansoni* (LC498700) as the outgroup. For the ML analysis, the optimal model (TN+F+I) was selected based on Bayesian Information Criterion (BIC), and analysis was conducted in IQ-Tree 1.6.12 with 1,000 bootstrap replicates (*7*). For BI analysis, the best-fit model (GTR+I+G) was determined using MrModeltest 2.4, and analysis was performed in MrBayes 3.2.7 with Markov Chain Monte Carlo (MCMC) sampling using four chains over two million generations (*8*). Trees were visualized using iTOL v6 (*9*).

The phylogenetic tree (Figure 3) revealed close relationships among *D. latus*, *D. nihonkaiensis*, *D. ursi*, and *D. dendriticus*, with *D. ursi* forming a sister-group relationship with *D. dendriticus*. *D. dendriticus* was divided into two distinct clades: one consisting solely of “Group 1,” which includes strains from fish in Chile, and another containing all remaining groups.

Public Health Response

In response to this emerging public health concern, it is imperative to disseminate knowledge about diphyllobothriosis prevention. Public health efforts should promote the consumption of thoroughly cooked fish products and educate consumers about proper freezing techniques. According to U.S. FDA guidelines, freezing fish at −35 °C for at least 15 hours effectively eliminates *Dibothriocephalus* larvae (*10*).

DISCUSSION

Fish represents an essential protein source for human consumption, with demand steadily increasing in recent years. The Food and Agriculture Organization (FAO) projects that production of fish and fish products will exceed 200 million tons by 2030. This rising demand, however, corresponds with an increased risk of diphyllobothriosis. China, previously considered a non-endemic area for *D. dendriticus*, now reports its first human case of diphyllobothriosis caused by this

TABLE 1. List of human *Dibothriocephalus dendriticus* infection cases available on the NCBI site, with GenBank accession numbers.

Country	Patient (age, years)	Clinical symptom	Suspected origin of infection	Accession numbers
Netherlands	Male (31)	None	Brazil (raw fish)	KC812048
Switzerland	Female (59)	Chronically relapsing courses of diarrhea	Alaska (fish), Canada (fish), Norway (fish), Switzerland (salmon)	AB412738
Switzerland	Male (4)	Abdominal cramps, loose stools	Norway (salmon), Asia (fish), Switzerland (perch)	HQ682067
Czech	Female (28)	None	Alaska (wild salmon)	KC812047
Czech	Unknown	Unknown	Canada	MW602518
China	Male (13)	Weight lose	Japan (raw fish), China (raw fish)	PQ169609

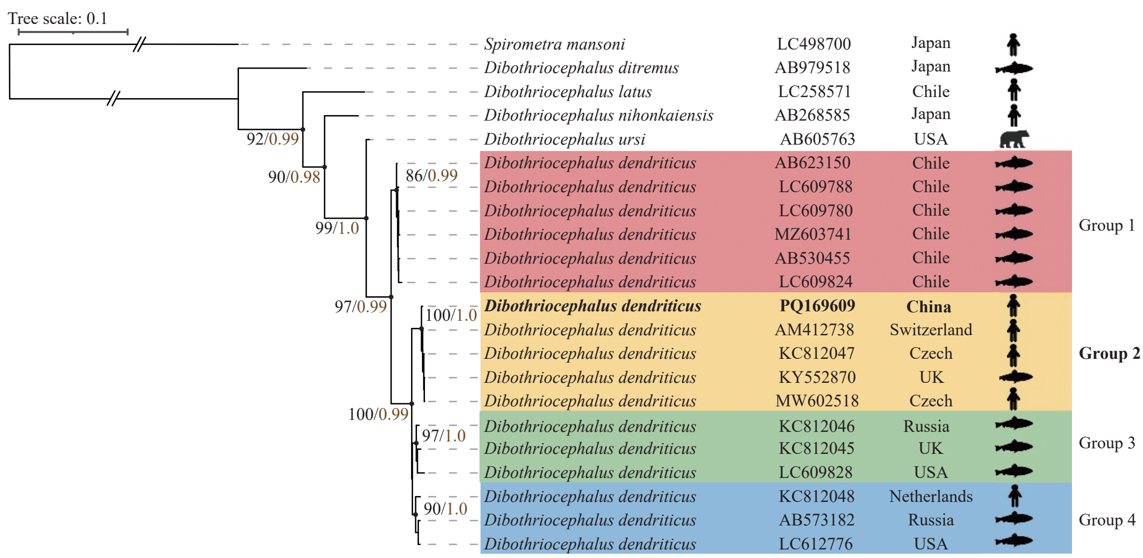


FIGURE 3. Maximum likelihood and BI phylogenetic analyses of the complete *D. dendriticus cox1* sequences (1,567 bp).
Note: *Spirometra mansoni* was selected as the outgroup. The TN+F+I model was used for ML analysis, while the GTR+I+G model was applied for the BI analysis. Branch support values are indicated on the tree, with ML=black and BI=brown. Newly obtained sequences are highlighted in bold.
Abbreviation: *D. dendriticus*=*Dibothriocephalus dendriticus*; ML=maximum Likelihood; BI=bayesian Inference.

parasite.

The precise timing and location of infection remain uncertain. *D. dendriticus* typically has a short prepatent period, with peak egg shedding occurring within one year (*11*). Based on the retrospective investigation and the parasite's life cycle, the May 2023 exposure in Hong Kong appears more likely. However, infection from raw trout consumed in Beijing in August 2022 cannot be ruled out, as the patient reported weight loss beginning in 2022, which is a common symptom of infection.

The atypical morphological features observed in this *D. dendriticus* specimen may result from post-mortem changes or mechanical distortion during extraction by the patient's parents. Similar morphological anomalies have been documented in Swiss strains. De Marval et al. (*12*) suggested that such anomalies might indicate an Asian origin of infection, while Wicht et al. (*6*) proposed they were artifacts resulting from stretching. Phylogenetic analysis reveals that the *D. dendriticus* strain from this case is closely related to strain KY552870 from the UK, suggesting a common origin.

In conclusion, this study documents the first human infection of *D. dendriticus* in China and highlights the emerging threat posed by this parasite. The increasing popularity of raw fish consumption, combined with air transportation without adequate freezing protocols, may elevate infection risk. Enhanced food safety measures and public health surveillance are essential to protect the population from *D. dendriticus* infection.

Conflicts of interest: No conflicts of interest.

Ethical statement: Authorized by the Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Ref No. 202209). Written informed consent was obtained from each patient or their proxy.

Funding: Supported by the Three-Year Initiative Plan for Strengthening Public Health System Construction in Shanghai (2023-2025) Key Discipline Project (GWVI-11.1-12) and the National Parasite Resource Center (NPRC-2019-194-30).

doi: 10.46234/ccdcw2025.089

Corresponding author: Yan Lu, luyan@nipd.chinacdc.cn.

[1] National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Chinese Center for Tropical Diseases Research, NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases, Ministry of Science and Technology, Shanghai, China.

Submitted: November 01, 2024
Accepted: April 05, 2025
Issued: April 18, 2025

REFERENCES

1. Kuchta R, Brabec J, KubáčkováP, Scholz T. Tapeworm *Diphyllobothrium dendriticum* (Cestoda) — neglected or emerging human parasite? PLoS Negl Trop Dis 2013;7(12):e2535. http://dx.doi.org/10.1371/journal.pntd.0002535.
2. Scholz T, Garcia HH, Kuchta R, Wicht B. Update on the human broad tapeworm (genus *Diphyllobothrium*), including clinical relevance. Clin Microbiol Rev 2009;22(1):146 – 60. https://doi.org/10.1128/CMR.00033-08.
3. Waeschenbach A, Brabec J, Scholz T, Littlewood DTJ, Kuchta R. The catholic taste of broad tapeworms - multiple routes to human infection. Int J Parasitol 2017;47(13):831 – 43. https://doi.org/10.1016/j.ijpara.2017.06.004.
4. Jenkins EJ, Castrodale LJ, de Rosemond SJC, Dixon BR, Elmore SA, Gesy KM, et al. Tradition and transition: parasitic zoonoses of people and animals in Alaska, northern Canada, and Greenland. Adv Parasitol 2013;82:33 – 204. https://doi.org/10.1016/B978-0-12-407706-5.00002-2.
5. Van de Vuurst P, Escobar LE. Climate change and infectious disease: a review of evidence and research trends. Infect Dis Poverty 2023;12(1):51. https://doi.org/10.1186/s40249-023-01102-2.
6. Wicht B, Yanagida T, Scholz T, Ito A, Jimenez JA, Brabec J. Multiplex PCR for differential identification of broad tapeworms (*Cestoda: Diphyllobothrium*) infecting humans. J Clin Microbiol 2010;48(9):3111 – 6. https://doi.org/10.1128/JCM.00445-10.
7. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 2015;32(1):268 – 74. https://doi.org/10.1093/molbev/msu300.
8. Didelot X, Croucher NJ, Bentley SD, Harris SR, Wilson DJ. Bayesian inference of ancestral dates on bacterial phylogenetic trees. Nucleic Acids Res 2018;46(22):e134. https://doi.org/10.1093/nar/gky783.
9. Letunic I, Bork P. Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 2021;49(W1):W293 – 6. https://doi.org/10.1093/nar/gkab301.
10. Food and Drug Administration (FDA). Parasites. In: FDA, editor. Guidance for industry on fish and fishery products hazards and controls. 4th ed. Montgomery: FDA. 2011; p. 91-98. https://www.fda.gov/files/food/published/Fish-and-Fishery-Products-Hazards-and-Controls-Guidance-Chapter-5-Download.pdf.
11. Kitamoto H, Inoue S, Yamamoto S, Okamoto K, Inokuma T. Human diphyllobothriasis - Authors' reply. Lancet 2020;396(10253):755 – 6. https://doi.org/10.1016/S0140-6736(20)31178-8.
12. de Marval F, Gottstein B, Weber M, Wicht B. Imported diphyllobothriasis in Switzerland: molecular methods to define a clinical case of *Diphyllobothrium* infection as *Diphyllobothrium dendriticum*, August 2010. Euro Surveill 2013;18(3):20355. https://pubmed.ncbi.nlm.nih.gov/23351654/.

Outbreak Reports

A *Clostridium perfringens* Related Foodborne Diarrhea Outbreak in an Elderly Care Center — Beijing Municipality, China, May 2024

Dongran Zhang[1,&]; Xinhui Guo[2,&]; Yunqi Zhang[3]; Haibo Liu[1]; Yuhang Gao[1]; Wencui Zhang[1]; Wenfeng Shi[1]; Yao Zhao[1]; Lijie Zhang[2]; Aijun Li[1,#]

Summary

What is already known about this topic?

Clostridium perfringens is a gram-positive, anaerobic, spore-forming bacillus widely distributed in the environment. Enterotoxin-producing strains of *C. perfringens* can cause foodborne diarrheal outbreaks in humans, with incubation periods typically ranging from 2 to 36 hours.

What is added by this report?

Investigation identified 98 cases aged 22–99 years. All patients experienced diarrhea. The epidemic curve suggested a point-source outbreak. Chinese hamburgers were identified as the suspected food vehicle [odds ratio (*OR*)=6.6, 95% confidence interval (*CI*): 1.7, 37.1]. A total of 23 patient samples and 1 Chinese hamburger sample tested positive for *C. perfringens*.

What are the implications for public health practice?

The slow cooling process, a common procedure during the "lu" (卤) braising technique in Chinese cuisine, could potentially allow *C. perfringens* to proliferate significantly and produce toxins. Rapid cooling through the critical temperature range of 15–55 ℃ may effectively mitigate this risk.

ABSTRACT

Introduction: On May 21, 2024, an elderly care center in Beijing reported dozens of acute diarrhea cases. The local CDC immediately initiated an epidemiological investigation to identify the outbreak's etiology and implement control measures.

Methods: Suspected cases were defined as individuals experiencing diarrhea ≥3 times or vomiting within 24 hours at the center during May 19–23, 2024. Cases were identified through review of the center's medical records. Food exposure at breakfast and lunch on May 20 was compared between 24 cases and 70 controls. We interviewed kitchen staff regarding food preparation practices and collected samples for laboratory testing.

Results: Investigation identified 77 elderly residents and 21 staff cases, ranging in age from 22 to 99 years, all of whom presented with diarrhea. The epidemic curve exhibited characteristics consistent with a point-source outbreak. Breakfast and lunch served on May 20 were implicated as suspected exposure meals, with 88% of cases having consumed Chinese hamburgers compared with 51% of controls [odds ratio (*OR*)=6.6, 95% confidence interval (*CI*): 1.7, 37.1]. The preparation process for the pork filling in Chinese hamburgers involved portioning, blanching for 20 minutes, simmering in spiced broth for 40 minutes, followed by natural cooling. Laboratory analysis confirmed the presence of *C. perfringens* in 23 patient samples and one Chinese hamburger sample.

Conclusions: The outbreak was most likely caused by Chinese hamburgers contaminated with *C. perfringens*. We recommend comprehensive training for food handlers on proper cooling procedures when preparing Chinese hamburgers.

On May 21, 2024, an elderly care center in Beijing reported to the local Center for Disease Control and Prevention (LCDC) that dozens of elderly residents and staff members had experienced acute diarrhea within the previous 48 hours. The LCDC immediately initiated an epidemiological investigation to identify the outbreak's etiology and implement control measures.

INVESTIGATION AND RESULTS

X Elderly Care Center (XECC), situated in southwestern Beijing, is a private residential facility providing comprehensive care services for elderly individuals with varying levels of independence, from self-sufficient to fully dependent. The facility encompasses 50,000 m^2 with 7 apartment buildings offering over 700 beds. At the time of the investigation, the facility housed 485 elderly residents and employed 204 staff members. A centralized kitchen and dining facility serves meals to both residents and staff.

The first case was identified as a 49-year-old female staff member who developed diarrhea at 16:00 on May 20. The patient's symptoms resolved spontaneously without medical intervention or medication by May 21.

A suspected case was defined as any individual at XECC who experienced either vomiting ≥1 time/24 hours or diarrhea ≥3 times/24 hours between May 19 and 21, 2024. A probable case was diarrhea ≥3 times/24 hours and abdominal pain. A confirmed case was a suspected or probable case who had a positive bacterial isolation for *C. perfringens*.

By May 21, a total of 98 cases met the case definition (62 suspected, 16 probable, 20 confirmed), comprising 38 males and 60 females, with ages ranging from 22 to 99 years. Cases occurred between 16:00 on May 20 and 10:00 on May 21, with a median onset time of 23:00 on May 20 (Figure 1). Clinical manifestations are detailed in Table 1. No severe cases were reported. The overall attack rate was 14.22% (98/689), with elderly residents experiencing a higher attack rate of 15.88% (77/485) compared to staff members at 10.29% (21/204) [χ^2=3.668, risk ratio (*RR*)=1.542, 95% confidence interval (*CI*): 0.980, 2.428]. Cases were distributed across all 7 apartment buildings, with attack rates varying from 8.16% to 22.09%.

The epidemic curve demonstrated a point source exposure pattern. The absence of elevated diarrheal incidence in surrounding communities sharing the same water supply with XECC strongly indicated a foodborne outbreak. Based on the established incubation period for *C. perfringens* (2–36 hours) according to the Technical Guidelines for the Identification and Management of Foodborne Illnesses by The National Health Commission of the People's Republic of China (2023), the exposure time was estimated between 22:00 on May 19 (calculated by subtracting the maximum incubation period from the last case onset) and 14:00 on May 20 (calculated by subtracting the minimum incubation period from the first case onset). This timeline implicated breakfast and lunch on May 20 as the suspected exposure meals.

A case-control study was conducted on May 23, comprising 24 confirmed/probable cases and 70 controls (asymptomatic individuals who lived or worked with the cases), maintaining a control-to-case ratio between 1∶2 and 1∶3. Face-to-face dietary history investigations focusing on breakfast and lunch consumption on May 20 were performed. Statistical analysis (Table 2) identified the Chinese Hamburger (Roujiamo) as the contaminated food item

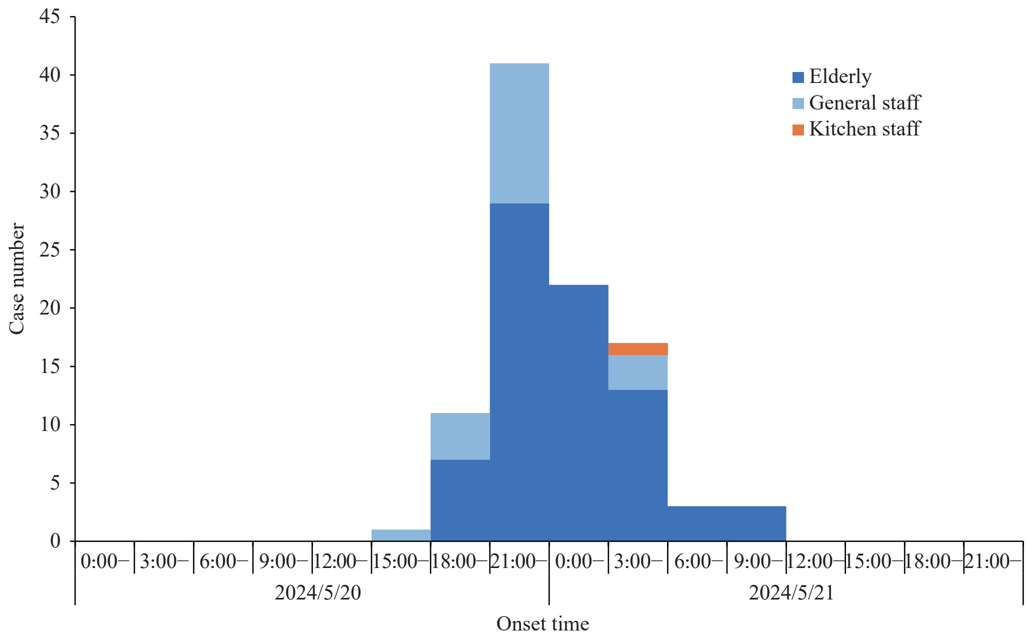


FIGURE 1. Epidemic curve of diarrhea cases in an elderly care center, Beijing, China, May 2024 (*N*=98).

TABLE 1. Clinical manifestations of diarrhea cases in an elderly care center, Beijing, China, May 2024 (N=98).

Symptoms	Elderly, n (%)	Staff, n (%)
Diarrhea*	77 (100.00)	21 (100.00)
Abdominal pain	15 (19.48)	3 (14.29)
Nausea	2 (2.60)	0 (0)
Vomiting	1 (1.30)	0 (0)
Fever†	1 (1.30)	0 (0)

* Diarrhea refers to having bowel movements ≥3 times/24 hours, accompanied by changes in fecal appearance.
† Fever refers to a body temperature ≥37.5 ℃.

(χ^2=9.743, OR=6.6111, 95% CI: 1.7089, 37.0583). The incubation period of this outbreak was 5–23 hours.

Chinese Hamburger (Roujiamo), a traditional Chinese dish consisting of a bun stuffed with chopped braised pork, was investigated for its preparation process. According to kitchen staff reports, raw pork was refrigerated upon purchase at 11:00 on May 19. Since *C. perfringens* is widely present in nature, pork might have been contaminated during production, transportation, or storage. The following day at 07:30, the pork was portioned, blanched for 20 minutes, and simmered in spiced broth for 40 minutes. However, due to the high thermal resistance of *C. perfringens*, combined with the possibility that large batches of meat may not have been thoroughly heated, the bacteria could form spores during this process, enabling survival. After cooking, the heat was discontinued, and the pork was left to cool naturally while submerged in the broth. This slow cooling process potentially allowed *C. perfringens* to proliferate significantly and produce toxins. At 11:00, the cooled pork was chopped and combined with cilantro and green peppers before being stuffed into buns.

On May 21, investigators collected 86 samples comprising 50 patient samples (42 anal swabs and 8 fecal samples), 13 anal swabs from healthy kitchen staff, 11 food samples, and 12 environmental swabs. Polymerase chain reaction (PCR) testing and bacterial isolation for *C. perfringens* were conducted on all samples. Among them, 23 patient samples and 1 food sample tested positive by PCR. By May 23, bacterial isolation confirmed *C. perfringens* presence in 23 patient samples (18 anal swabs and 5 fecal samples). The patients who tested positive by PCR were all positive in bacterial isolation. However, *C. perfringens* was not isolated from any food sample. Subsequent virulence gene detection revealed that 16 anal swabs were cpe(+)plc(+) and 2 were cpe(+), while all 5 fecal samples demonstrated cpe(+)plc(+) profiles.

PUBLIC HEALTH RESPONSE

The following public health interventions were promptly implemented: 1) Continuous monitoring of all patients' health status; 2) immediate reassignment of kitchen staff with recent history of diarrhea to non-food handling positions; 3) implementation of targeted health education programs, with emphasis on kitchen staff hygiene practices. Following the implementation of these control measures, no new cases were reported.

DISCUSSION

Clostridium perfringens (also known as *C. welchii*) is a gram-positive, anaerobic, spore-forming bacillus ubiquitously distributed in environmental reservoirs, including soil and water. This organism commonly exists as part of the normal intestinal microbiota in humans and animals. Enterotoxin-producing strains that colonize the human gastrointestinal tract can precipitate diarrheal illness with an incubation period ranging from 2 to 36 hours. Outbreaks are characteristically associated with protein-rich foods, particularly meat dishes and their accompanying broths that have been prepared in large quantities, subjected to prolonged cooling at elevated temperatures, and served without adequate reheating.

Based on the epidemiological curve, clinical presentation, incubation period, case-control analysis, and laboratory findings, this outbreak was conclusively attributed to *C. perfringens* contamination of Chinese Hamburger (Roujiamo). The outbreak affected both elderly residents and young to middle-aged staff members. Overall, diarrhea remained the predominant symptom, consistent with previous reports (*1*–*2*). However, the symptoms of elderly cases appeared more diverse compared to those of staff cases, including nausea, vomiting, and fever, indicating a broader spectrum of clinical presentations compared to their younger counterparts.

Chinese Hamburger (Roujiamo) was identified as the contaminated food vehicle in this outbreak, with the braised meat filling being the likely source of contamination. The braising process, known as "lu" (卤) in Chinese cuisine, involves simmering over low heat followed by a cooling period to enhance flavor penetration. Although most *C. perfringens* spores are inactivated within minutes at 100 ℃, certain strains possess extreme heat resistance, enabling survival after 1–6 hours of boiling (*3*). The organism's optimal

TABLE 2. Results of statistical analysis for suspected dishes of the breakfast and lunch on May 20.

Meals	Dishes	Case		Control		*OR* (95% *CI*)
		Yes	No	Yes	No	
Breakfast	Milk	16	8	55	15	0.546 (0.196, 1.517)
	Boiled egg	17	7	59	11	0.453 (0.152, 1.347)
	Pickled mustard strips	13	11	48	22	0.542 (0.210, 1.398)
	Stir-fried zucchini	21	3	53	17	2.245 (0.595, 8.467)
	Stir-fried potato shreds	22	2	57	13	2.509 (0.523, 12.034)
Lunch	**Chinese hamburger (Roujiamo)**	**21**	**3**	**36**	**34**	**6.611 (1.709, 37.058)**
	Steamed Flatfish	20	4	47	23	2.447 (0.697, 10.894)
	Stir-fried Pork and Mushrooms	19	5	52	18	1.315 (0.393, 5.157)
	Stir-fried Pork Liver	18	6	46	24	1.565 (0.505, 5.444)
	Beef Stewed with Pumpkin	15	9	51	19	0.692 (0.341, 1.406)
	Stir-fried Cauliflower	16	8	55	15	0.546 (0.178, 1.777)

Note: Dishes with statistical significance are highlighted in bold.
Abbreviation: *OR*=odds ratio; *CI*=confidence interval.

growth temperature is approximately 45 ℃ (*4*), with rapid proliferation occurring during improper cooling between 37–50 ℃ (*5*). The cooling phase of the braised pork created ideal conditions for *C. perfringens* multiplication, combining protein-rich substrate, anaerobic conditions, and favorable temperatures. To mitigate this risk, rapid cooling through the critical temperature range of 15–55 ℃ may effectively prevent bacterial proliferation (*6*).

In China, the identification and management of *C. perfringens* outbreaks are governed by two national standards: “WS/T 7-1996 Diagnostic Criteria and Principles of Management for Food Poisoning of *Clostridium perfringens*” and “GB4789.13-2012 National Food Safety Standard Microbiological Examination of Food Hygiene — Examination of *Clostridium perfringens*”. These standards mandate bacterial isolation, culture, and confirmatory testing procedures. Furthermore, enterotoxin and enterotoxigenicity assessment requires animal experimentation, resulting in a complex and time-intensive diagnostic process. Given these limitations, the incorporation of multiplex PCR technology into national standards warrants consideration to enhance diagnostic efficiency.

Conflicts of interest: No conflicts of interest.

Ethical statement: This outbreak investigation was conducted by Fangshan CDC according to the national regulations of infectious control as part of the legally authorized mandate. Ethical approval and informed consent were not necessary as the study uses routinely collected and anonymous data.

doi: 10.46234/ccdcw2025.090

Corresponding author: Aijun Li, bjfscdc@163.com.

[1] Fangshan Center for Disease Control and Prevention, Beijing, China; [2] Chinese Field Epidemiology Training Program, Chinese Center for Disease Control and Prevention, Beijing, China; [3] Fangshan District Shidu Community Healthcare Center, Beijing, China.
& Joint first authors.

Submitted: December 04, 2024
Accepted: March 28, 2025
Issued: April 18, 2025

REFERENCES

1. Mellou K, Kyritsi M, Chrysostomou A, Sideroglou T, Georgakopoulou T, Hadjichristodoulou C. *Clostridium perfringens* foodborne outbreak during an athletic event in northern Greece, June 2019. Int J Environ Res Public Health 2019;16(20):3967. https://doi.org/10.3390/ijerph16203967.
2. Saito K, Kimata K, Watahiki M, Isobe J, Kanatani JI, Ikeda K, et al. Investigation of an outbreak of *Clostridium perfringens* in Toyama, Japan, 2023 using single-nucleotide polymorphism analysis for genotyping. Jpn J Infect Dis 2025;78(1):47 – 50. https://doi.org/10.7883/yoken.JJID.2024.189.
3. Bryan FL. What the sanitarian should know about *Clostridium perfringens* foodborne illness. J Food Prot 1969;32(10):381 – 9. https://doi.org/10.4315/0022-2747-32.10.381.
4. Brynestad S, Granum PE. *Clostridium perfringens* and foodborne infections. Int J Food Microbiol 2002;74(3):195 – 202. https://doi.org/10.1016/S0168-1605(01)00680-8.
5. Labbé R, Juneja V. *Clostridium*: occurrence and detection of *Clostridium perfringens*. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of food and health. Amsterdam: Academic Press, 2016; p. 146-8. http://dx.doi.org/10.1016/B978-0-12-384947-2.00169-0.
6. Taormina PJ, Dorsa WJ. Growth potential of *Clostridium perfringens* during cooling of cooked meats. J Food Prot 2004;67(7):1537 – 47.

Review

An Alarming Public Health Problem: Ticks and Tick-Borne Pathogens in Urban Recreational Parks

Bo Yi[1,2]; Mingqiu Fan[1]; Jian Chen[1]; Junyi Yao[1]; Xin Chen[1,#]; Hongxia Liu[1,#]

ABSTRACT

Ticks function as critical vectors for a wide range of pathogens that pose significant risks to both human and animal health. In recent years, the number and diversity of tick-borne pathogens have increased at an unprecedented rate, elevating tick-borne diseases (TBDs) to a major public health concern on a global scale. TBDs present a dual challenge, not only affecting human populations but also causing substantial economic losses in livestock industries across the world. The geographic distribution of many TBDs is shifting, with emerging, re-emerging, and resurging cases influenced by environmental factors such as deforestation and climate change. In China, rapid urbanization and concurrent improvements in urban ecological conditions have contributed to the expansion of tick habitats and increased human exposure to tick populations. Recent research warns that ticks and their associated pathogens present significant risks in urban environments, particularly in locations such as parks, playgrounds, and zoos. Despite these threats, public awareness of tick-borne diseases remains critically low. This review consolidates current knowledge on tick species and tick-borne pathogens found in urban parks and proposes strategic control measures to inform effective tick management policies both in China and globally.

Ticks are hematophagous arthropods that parasitize humans and animals (*1*). Second only to mosquitoes in their epidemiological significance, ticks serve as crucial vectors for numerous infectious pathogens (*2*). Currently, China possesses approximately 125 tick species (*3*). The global prevalence of tick-borne pathogens and their associated diseases is continuously rising, posing significant threats to human health, labor productivity, livestock industry profitability, and biodiversity (*3*).

While often found in forested, mountainous, and hilly regions, environmental improvements have expanded the suitable habitat of ticks into urban areas, particularly recreational parks. The growing presence of ticks and tick-borne pathogens in these environments presents an emerging public health concern that demands attention (*4*–*5*). Multiple studies investigating urban tick populations have demonstrated their widespread presence in urban landscapes (*6*–*7*). Notably, the diversity and prevalence of tick-borne diseases (TBDs) in urban environments now rival those observed in non-urban settings. Despite increasing reports of tick infestations in urban parks, comprehensive reviews addressing this significant public health concern remain limited. This review first examines the tick species and tick-borne pathogens present in urban recreational parks, followed by an analysis of the potential prevention measures as well as control strategies.

TICK SPECIES AND TICK INFESTATION IN URBAN RECREATIONAL PARKS

Ixodes ricinus L. (Acari: Ixodidae), commonly known as the castor bean tick, is a significant vector of several pathogens of medical and veterinary importance. This species progresses through four main developmental stages: eggs, larvae, nymphs, and adults (male or female) (*8*). It predominantly inhabits deciduous and mixed forests, as well as woodlands, moorlands, and scrublands, where its survival and reproduction depend on suitable microclimatic conditions and host availability. Urban hedgehog populations can effectively maintain stable *I. ricinus* populations in metropolitan areas (*9*–*10*). Studies in the UK have documented tick presence across various life stages in 7.2% of transects in Bushy Park and 37.6% in Richmond Park. *Ixodes scapularis* Say and *I. pacificus* Cooley & Kohls (Acari: Ixodidae), known respectively as the deer tick and western black-legged tick, are principal vectors of human pathogens in the United

States. Following egg hatching, these species undergo three developmental stages — larva, nymph, and adult — with a typical lifespan of around 2 years (*11*). *I. scapularis* primarily inhabits unmaintained herbaceous vegetation, maintained lawns, and leaf litter in urban parks, with adults showing higher density in edge ecotones and nymphs predominantly occupying the leaf layer (*12*). In contrast, *I. pacificus* primarily associates with grassy areas within urban park environments (*13*).

Ixodes persulcatus Schulze (Acari: Ixodidae), the taiga tick, represents one of the most significant disease vectors affecting humans and animals across the Northern Hemisphere, with a distribution spanning the entirety of the Eurasian continent (*14*). As a characteristic forest tick, *I. persulcatus* dominates coniferous and broadleaf mixed forests. Its breeding habitats in urban recreational parks encompass coniferous forests, broadleaf forests, mixed forests, shrublands, and grasslands (*15*).

Ixodes hexagonus Leach (Acari: Ixodidae), the hedgehog tick, represents one of the most prevalent tick species in Central Europe. While *I. hexagonus* parasitizes various carnivorous mammals in suburban environments, all developmental stages most frequently occur on hedgehogs (*16*). This species-host association remains present in urban recreational parks across Europe, where hedgehogs serve as the primary hosts (*17–19*).

Haemaphysalis longicornis Neumann (Acari: Ixodidae) commonly known as the long-horned tick, is native to East Asia. Its life cycle comprises four developmental stages: egg, larva, nymph, and adult (*20*). This species inhabits diverse ecological niches within parks, including shrubland, grassland, deciduous forests, mixed forests, and coniferous forests. Among these habitats, these four major biomes support significantly higher tick populations compared to other environments: broadleaf forests, coniferous forests, shrublands, and grasslands (*21–22*).

Hemaphysalis flava Neumann (Acari: Ixodidae) is widely distributed throughout East Asia and progresses through four developmental stages: egg, larva, nymph, and adult (*23–26*). Studies have demonstrated that *H. flava* exhibits a strong association with woodland habitats in urban parks, with peak collection rates from domestic dogs and cats occurring in October and notably minor prevalence during the summer months of July and August (*24*,*27*).

Amblyomma americanum (Acari: Ixodidae), the lone star tick, is an aggressive three-host tick predominantly found in eastern North America, with particular prevalence in the south of the United States. This species maintains its population through feeding on white-tailed deer, ground-nesting birds, and various other wildlife hosts (*28*). Surveillance studies have documented substantial *Am. americanus* populations in residential parks featuring paved walking trails, golf putting greens, and recreational playgrounds in the state of Oklahoma, USA (*29*).

Dermacentor reticulatus Fabricius (Acari: Ixodidae), the ornate cow tick, belongs to the Metastriata group of ixodid ticks (*30*). The highest density of *D. reticulata* was recorded in a suburban park in northern Italy. Mixed forest areas dominated by oak trees and characterized by the presence of ponded waters are the main habitats of this tick species (*31–32*).

Dermacentor occidentalis Marx (Acari: Ixodidae) is distributed throughout California, except for the arid regions of the Central Valley and southeastern desert (*33*). The species has also been documented in neighboring US states such as Oregon and Baja California in Mexico (*34*). Its life cycle exhibits stage-specific host preferences: immature stages primarily parasitize rodents, particularly squirrels, while adults preferentially feed on larger mammals including cattle, horses, deer, and humans. Adult ticks remain active year-round, with peak activity observed during the months of April and May, while nymphal stages predominate during spring and summer months. While adults commonly parasitize cattle, horses, deer, and humans, they are rarely found on dogs and bears. The species is frequently encountered in urban parks throughout southern California, USA (*13*).

Dermacentor variabilis Say (Acari: Ixodidae), commonly known as the American dog tick or wood tick, is a widespread three-host tick species in North America that parasitizes a diverse array of hosts, including humans (*35*). Studies have demonstrated that this species predominantly inhabits grasslands, shrublands, savannahs, and woodlands in urban areas, with native encroaching tree species potentially contributing to increased tick populations (*36*). The species is frequently encountered in urban parks throughout the United States (*37–38*).

Rhipicephalus sanguineus Latreille (Acari: Ixodidae), the brown dog tick, exhibits a strong host preference for dogs but occasionally parasitizes other hosts, including humans (*39*). This species is commonly associated with stray dogs in urban parks. Host infestation can result in severe clinical manifestations, including anemia, weight loss, developmental stunting, and in extreme cases, can induce mortality (*40–42*).

Ornithodoros spheniscus (Acari: Argasidae), a human-aggressive tick species, primarily parasitizes seabirds in Chile (*43*). The saliva of ticks within the genus *Ornithodoros* contains multiple toxic compounds (*44*). *O. spheniscus* has been documented parasitizing seabirds and causing toxicosis in humans who were bitten in a Chilean national park (*45*).

Ornithodoros turicata (Acari: Argasidae) is distributed throughout several regions of North America (*46*). This species demonstrates promiscuous feeding behavior, parasitizing hosts such as ground pigs, squirrels, prairie dogs, snakes, and gopher tortoises (*47*). *O. turicata* ticks have been collected in public parks containing rodent waste (*48*).

PATHOGENS CARRIED BY TICKS IN URBAN RECREATIONAL PARKS

Tick-borne encephalitis, a significant public health concern, is caused by tick-borne encephalitis virus (TBEV). The virus comprises 5 distinct genotypes, with the European, Siberian, and Far Eastern variants being predominant, each characterized by unique epidemiological patterns and clinical manifestations (*49*). In urban parks across Europe, TBEV transmission primarily occurs through bites from *Ixodes* ticks, particularly *I. ricinus* (*50*).

Severe fever with thrombocytopenia syndrome (SFTS), an emerging infectious disease, is caused by the SFTS virus (SFTSV), a novel member of the order Bunyavirals in the family Phenuiviridae (*51–52*). This syndrome has been documented throughout East Asian countries, including the Republic of Korea (ROK) (*53–54*). SFTSV maintains its circulation through an enzootic cycle involving ticks and vertebrate hosts. *Haemaphysalis longicornis* ticks, which serve as vectors for SFTSV, are widely distributed throughout China (*55*).

Rickettsiae are obligate intracellular Gram-negative bacteria belonging to the genus Rickettsia within the Rickettsiaceae family, order Rickettsiales. These pathogens cause human diseases primarily through vector-borne transmission (via ticks, lice, mites, and fleas) and occasionally through airborne routes (*56*). *Rickettsiae* are classified into two main groups: the typhus group and the spotted fever group (SFG). SFG rickettsiae are predominantly associated with hard ticks (Ixodidae), with exceptions being *R. akari* (mite-borne) and *R. felis* (flea-borne). *Ixodes* ticks can maintain and propagate SFG *Rickettsia* (SFGR) through both transovarian and transovarial transmission (*57–58*). Recent studies have identified *R. sanguineus* as a crucial vector for SFGR transmission between domestic dogs and humans (*59*). SFGR exhibits a global distribution pattern with potential for further geographic expansion through vector ticks. Research has confirmed SFGR presence in urban forest park tick populations (*35*,*60*). Notable examples include the detection of two SFG rickettsiae — *R. rhipicephali* and *Rickettsia* sp. 364D (now *R. philipii*) (*61*) — in *D. occidentalis* in southern California, United States. In Ukraine, researchers documented *Rickettsia raoultii* presence in ticks across three different parks, with infection rates varying from 5% to 68%.

Anaplasma phagocytophilum is an obligate intracellular bacterium that causes human granulocytic anaplasmosis (HGA), an acute febrile illness prevalent throughout the Northern Hemisphere (*62*). The clinical manifestations of HGA range from mild to severe, with subclinical symptoms including fever, cough, headache, diarrhea, and vomiting, while critical cases may progress to sepsis, multiple organ failure syndrome, and acute nephritis (*63*). Studies have demonstrated significantly higher prevalence of *A. phagocytophilum* in ticks collected from urban parks (*64–65*). For instance, an ecoepidemiologic investigation conducted during 2009-2011 revealed that *A. phagocytophilum* was detected in 67 (76.1%) of 88 urban hedgehogs sampled from Margaret Island in Budapest, Hungary (*66*).

The causative agent of Lyme disease, *Borrelia burgdorferi* sensu lato (BBSL), relies on *Ixodes* ticks for transmission to vertebrate hosts. These spirochetes have evolved complex interactions with their tick vectors to maintain basic metabolic functions and optimize their colonization, persistence, and transmission cycles (*67*). BBSL infections are particularly prevalent in *I. ricinus* populations across European urban parks, though infection rates show considerable spatial variation (*10*,*12*,*68*). In the United States, studies from New York have documented high BBSL infection rates in *I. scapularis* collected from urban parks (*69*). Similarly in China, research by Cao et al. revealed a 13.1% positivity rate for *B. burgdorferi* in ticks sampled from urban parks in Quzhou, Zhejiang province.

Piroplasmas (class: Aconoidasida, order: Piroplasmida), comprising parasites in the families Babesiidae and Theileriidae, are the etiological agents of piroplasmosis (*70*). These parasites can be transmitted to mammals, including humans, during blood feeding by all tick life stages through transovarian transmission (*71*). *Babesia* species are intraerythrocytic protozoan parasites with complex life cycles involving multiple developmental stages and morphological forms, maintained in nature through transmission between *Ixodes* ticks and various mammalian hosts. While over 100 *Babesia* species have been documented, only a select few - notably *B. microti*, *B. divergens*, and *B. duncani* - are confirmed human pathogens (*70*,*72*). *I. ricinus* serves as the primary vector for piroplasma transmission across Europe, while this role is predominantly fulfilled by *I. persulcatus* in China (*8*,*73*).

TECHNIQUE AND STRATEGIES FOR TICK CONTROLS

From a macro perspective, the One Health concept provides a comprehensive framework for managing health crises by integrating human, animal, and ecosystem health, with TBD management as its integral component (*74*).

The cornerstone of TBD management lies in effective tick control. While chemical control remains a common approach for tick mitigation, there are currently no registered insecticides specifically approved for environmental tick control. For parasitic ticks, acaricide application involves direct treatment of tick-prone hosts through spraying, water-based acaricide baths, or topical "pour-on" preparations (*75*). However, prolonged acaricide use presents two significant challenges: the development of tick resistance and adverse environmental impacts on non-target organisms, particularly birds and beneficial insects. For free-ranging ticks, recent advances in pheromone-aided management techniques have shown considerable promise, as highlighted by Sonenshine (*76*). These innovative approaches include: 1) pheromone-enhanced matrices for vegetation application, 2) tick decoys, 3) bont tick (*Am. hebraeum*) decoys, and 4) pheromone confusants. The pheromone-enhanced matrix system targets nymphal and adult deer ticks by incorporating specific attractant components such as guanine, xanthine, and hematin. These components can be combined with acaricides such as permethrin in oily droplets or microfibers for field application (*77–78*). Additionally, silver nanoparticles (Ag NPs) have emerged as a promising avenue for biomedical applications, particularly in managing free-ranging tick populations (*79*). However, the implementation of chemical control methods for free-ranging ticks remains an incremental process, with limited registered pesticides available for environmental application (*80*). Research in this area has been relatively sparse, with only a few studies exploring alternatives such as plant-derived extracts for free-ranging tick control (*81–83*).

Two key interventions have been identified for effective tick control in urban parks. First, reducing potential tick host populations is essential. For example, in Basel, Switzerland, pigeon populations were halved as a result of implementing feeding bans (*84*). Additionally, implementing systematic management strategies for urban rodents and stray dogs and cats has proven effective for tick control (*85–86*). Second, maintaining park infrastructure through regular garbage collection and vegetation management, particularly along human pathways and trails, is essential (*87*). Beyond these population control measures, raising public awareness about tick bite risks, potential tick habitats, and fundamental personal protection practices is paramount (*88–89*). While regional variations exist in tick-borne disease management strategies — including environmental control, chemical interventions, personal protection measures, and health education — any adopted strategy must adhere to scientific principles and demonstrate both reasonability and feasibility to ensure effective control of tick-borne diseases.

Regular evaluation of control measures and strengthened tick surveillance are essential for assessing intervention effectiveness. China has established comprehensive monitoring networks for both parasitic and free-living ticks. In the United States, the CDC provides guidance and financial support to states for implementing tick surveillance initiatives, incorporating tick data collection within ArboNET, their existing arthropod-borne disease surveillance framework (*90*). In Europe, Italy maintains tick-borne disease surveillance as a crucial component of their human health program, emphasizing human data and expertise (*91*). These diverse national approaches to tick and tick-borne disease surveillance and control demonstrate global commitment to addressing this public health challenge. The effectiveness of control measures can be evaluated through monitoring changes in tick density and tick-borne disease infection rates.

CONCLUSION

Tick infestation in urban parks represents a significant and escalating public health concern. The documented tick species belong to two families - Ixodidae and Argasidae — with hard ticks (Ixodidae) comprising the majority of species and showing particularly high prevalence rates.

The detection of diverse pathogens in urban park ticks, including tick-borne encephalitis virus, Bunyavirus, *Rickettsia*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and Piroplasmas, appears increasingly common. Over recent decades, both the geographic distribution of tick populations in urban recreational parks and the prevalence of tick-borne diseases have demonstrated a marked expansion. To mitigate disease transmission risk, there is an urgent need to enhance public awareness and education regarding personal protection measures among urban residents.

Several critical knowledge gaps currently limit our ability to conduct precise risk assessments, particularly the lack of quantitative ecological, epidemiological, and socioecological data. There is a pressing need for comprehensive eco-epidemiological research and surveillance addressing key factors such as tick occurrence patterns, pathogen prevalence rates, vertebrate host dynamics, and human exposure patterns within urban recreational environments. From a broader perspective, understanding the complex factors influencing urban park tick distribution - including vegetation composition, climatic parameters (temperature and humidity), and host animal populations — is crucial. Additionally, further research is needed to elucidate the intricate relationships between tick microbiomes and their effects on tick development, pathogen transmission dynamics, and environmental pesticide efficacy. These challenges require interdisciplinary collaboration among medical practitioners, public health scientists, geographers, meteorologists, and urban park management stakeholders to effectively assess and reduce tick infestation risks and associated disease burden.

Conflicts of interest: No conflicts of interest.

Acknowledgments: Professor Tianyun Steven Su of Shandong University/EcoZone International LLC for his valuable editorial guidance and recommendations.

Funding: Supported by the Clinical Research Special Project in Health Profession of Shanghai (202240331) and The Sixth Round of Three-Year Public Health Action Plan of Shanghai (No. GWⅥ-11.1-13).

doi: 10.46234/ccdcw2025.091

Corresponding authors: Xin Chen, chenxin@scdc.sh.cn; Hongxia Liu, liuhongxia@scdc.sh.cn.

[1] Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China; [2] School of Public Health, Fudan University Shanghai Medical College, Shanghai, China.

Submitted: June 12, 2024
Accepted: December 29, 2024
Issued: April 18, 2025

REFERENCES

1. Parola P. Tick-borne rickettsial diseases: emerging risks in Europe. Comp Immunol Microbiol Infect Dis 2004;27(5):297 – 304. https://doi.org/10.1016/j.cimid.2004.03.006.
2. Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol 2012;28(10):437 – 46. https://doi.org/10.1016/j.pt.2012.07.003.
3. Zhang YK, Zhang XY, Liu JZ. Ticks (Acari: Ixodoidea) in China: geographical distribution, host diversity, and specificity. Arch Insect Biochem Physiol 2019;102(3):e21544. https://doi.org/10.1002/arch.21544.
4. Coutts C, Hahn M. Green infrastructure, ecosystem services, and human health. Int J Environ Res Public Health 2015;12(8):9768 – 98. https://doi.org/10.3390/ijerph120809768.
5. Kolomiiets V, Rakowska P, Rymaszewska A. New problems of environmental ecology: ticks and tick-borne pathogens in city parks of Ukraine. Environ Microbiol Rep 2022;14(4):591 – 4. https://doi.org/10.1111/1758-2229.13075.
6. Combs MA, Kache PA, VanAcker MC, Gregory N, Plimpton LD, Tufts DM, et al. Socio-ecological drivers of multiple zoonotic hazards in highly urbanized cities. Glob Chang Biol 2022;28(5):1705 – 24. https://doi.org/10.1111/gcb.16033.
7. Mackenstedt U, Jenkins D, Romig T. The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. Int J Parasitol Parasites Wildl 2015;4(1):71 – 9. https://doi.org/10.1016/j.ijppaw.2015.01.006.
8. Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Peña A, George JC, et al. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit Vectors 2013;6:1. https://doi.org/10.1186/1756-3305-6-1.
9. Hansford KM, Wheeler BW, Tschirren B, Medlock JM. Questing *Ixodes ricinus* ticks and *Borrelia* spp. in urban green space across Europe: a review. Zoonoses Public Health 2022;69(3):153 – 66. https://doi.org/10.1111/zph.12913.
10. Skuballa J, Petney T, Pfäffle M, Oehme R, Hartelt K, Fingerle V, et al. Occurrence of different *Borrelia burgdorferi* sensu lato genospecies including *B. afzelii*, *B. bavariensis*, and *B. spielmanii* in hedgehogs (*Erinaceus* spp.) in Europe. Ticks Tick Borne Dis 2012;3(1):8 – 13. https://doi.org/10.1016/j.ttbdis.2011.09.008.
11. Stewart PE, Bloom ME. Sharing the ride: *Ixodes scapularis* symbionts and their interactions. Front Cell Infect Microbiol 2020;10:142. https://doi.org/10.3389/fcimb.2020.00142.
12. Mathews-Martin L, Namèche M, Vourc'h G, Gasser S, Lebert I, Poux V, et al. Questing tick abundance in urban and peri-urban parks in the French city of Lyon. Parasit Vectors 2020;13(1):576. https://doi.org/10.1186/s13071-020-04451-1.
13. Cheng ML, Su T, Eremeeva M, Hu R. Species composition, seasonal abundance, pathogen detection in ticks collected in southwestern San Bernardino County, California. In: Proceedings of the 79th annual

meeting of mosquito and vector control association of California. Indian Wells, California, USA. 2011.

14. Pakanen VM, Sormunen JJ, Sippola E, Blomqvist D, Kallio ER. Questing abundance of adult taiga ticks *Ixodes persulcatus* and their *Borrelia* prevalence at the north-western part of their distribution. Parasit Vectors 2020;13(1):384. https://doi.org/10.1186/s13071-020-04259-z.
15. Romanenko V, Leonovich S. Long-term monitoring and population dynamics of ixodid ticks in Tomsk city (Western Siberia). Exp Appl Acarol 2015;66(1):103 – 18. https://doi.org/10.1007/s10493-015-9879-2.
16. Matuschka FR, Richter D, Fischer P, Spielman A. Nocturnal detachment of the tick *Ixodes hexagonus* from nocturnally active hosts. Med Vet Entomol 1990;4(4):415 – 20. https://doi.org/10.1111/j.1365-2915.1990.tb00459.x.
17. Dziemian S, Michalik J, Pi Łacińska B, Bialik S, Sikora B, Zwolak R. Infestation of urban populations of the Northern white-breasted hedgehog, *Erinaceus roumanicus*, by *Ixodes* spp. ticks in Poland. Med Vet Entomol 2014;28(4):465 – 9. https://doi.org/10.1111/mve.12065.
18. Jahfari S, Ruyts SC, Frazer-Mendelewska E, Jaarsma R, Verheyen K, Sprong H. Melting pot of tick-borne zoonoses: the European hedgehog contributes to the maintenance of various tick-borne diseases in natural cycles urban and suburban areas. Parasit Vectors 2017;10(1):134. https://doi.org/10.1186/s13071-017-2065-0.
19. Rubel F, Dautel H, Nijhof AM, Kahl O. Ticks in the metropolitan area of Berlin, Germany. Ticks Tick-Borne Dis 2022;13(6):102029. https://doi.org/10.1016/j.ttbdis.2022.102029.
20. Nwanade CF, Wang M, Li SS, Yu ZJ, Liu JZ. The current strategies and underlying mechanisms in the control of the vector tick, *Haemaphysalis longicornis*: implications for future integrated management. Ticks Tick-Borne Dis 2022;13(2):101905. https://doi.org/10.1016/j.ttbdis.2022.101905.
21. Chong ST, Kim HC, Lee IY, Kollars TM Jr, Sancho AR, Sames WJ, et al. Comparison of dragging and sweeping methods for collecting ticks and determining their seasonal distributions for various habitats, Gyeonggi Province, Republic of Korea. J Med Entomol 2013;50(3):611 – 8. https://doi.org/10.1603/ME12032.
22. Zheng HY, Yu ZJ, Zhou LF, Yang XL, Liu JZ. Seasonal abundance and activity of the hard tick *Haemaphysalis longicornis* (Acari: Ixodidae) in North China. Exp Appl Acarol 2012;56(2):133 – 41. https://doi.org/10.1007/s10493-011-9505-x.
23. Qi Y, Ai LL, Jiao J, Wang JH, Wu DP, Wang PC, et al. High prevalence of *Rickettsia* spp. in ticks from wild hedgehogs rather than domestic bovine in Jiangsu province, Eastern China. Front Cell Infect Microbiol 2022;12:954785. https://doi.org/10.3389/fcimb.2022.954785.
24. Iwakami S, Ichikawa Y, Inokuma H. A nationwide survey of ixodid tick species recovered from domestic dogs and cats in Japan in 2011. Ticks Tick-Borne Dis 2014;5(6):771 – 9. https://doi.org/10.1016/j.ttbdis.2014.05.008.
25. Kim HG, Jung M, Lee DH. Seasonal activity of *Haemaphysalis longicornis* and *Haemaphysalis flava* (Acari: Ixodida), vectors of severe fever with thrombocytopenia syndrome (SFTS) virus, and their SFTS virus harboring rates in Gyeonggi Province, South Korea. Exp Appl Acarol 2022;87(1):97 – 108. https://doi.org/10.1007/s10493-022-00722-x.
26. Zeng WB, Li ZQ, Jiang TG, Cheng DH, Yang LM, Hang T, et al. Identification of bacterial communities and tick-borne pathogens in *Haemaphysalis* spp. collected from Shanghai, China. Trop Med Infect Dis 2022;7(12):413. https://doi.org/10.3390/tropicalmed7120413.
27. Shimada Y, Beppu T, Inokuma H, Okuda M, Onishi T. Ixodid tick species recovered from domestic dogs in Japan. Med Vet Entomol 2003;17(1):38 – 45. https://doi.org/10.1046/j.1365-2915.2003.00403.x.
28. McClung KL, Little SE. *Amblyomma americanum* (Lone star tick). Trends Parasitol 2023;39(1):70 – 1. https://doi.org/10.1016/j.pt.2022.10.005.
29. Small M, Brennan RE. Detection of *Rickettsia amblyommatis* and *Ehrlichia chaffeensis* in *Amblyomma americanum* inhabiting two urban parks in Oklahoma. Vector-Borne Zoonotic Dis 2021;21(5):385 – 7. https://doi.org/10.1089/vbz.2020.2755.
30. Zając Z, Bartosik K, Woźniak A. Monitoring *Dermacentor reticulatus* host-seeking activity in natural conditions. Insects 2020;11(5):264. https://doi.org/10.3390/insects11050264.
31. Duscher GG, Feiler A, Leschnik M, Joachim A. Seasonal and spatial distribution of ixodid tick species feeding on naturally infested dogs from Eastern Austria and the influence of acaricides/repellents on these parameters. Parasit Vectors 2013;6:76. https://doi.org/10.1186/1756-3305-6-76.
32. Olivieri E, Gazzonis AL, Zanzani SA, Veronesi F, Manfredi MT. Seasonal dynamics of adult *Dermacentor reticulatus* in a peri-urban park in southern Europe. Ticks Tick-Borne Dis 2017;8(5):772 – 9. https://doi.org/10.1016/j.ttbdis.2017.06.002.
33. MacDonald AJ. Abiotic and habitat drivers of tick vector abundance, diversity, phenology and human encounter risk in southern California. PLoS One 2018;13(7):e0201665. https://doi.org/10.1371/journal.pone.0201665.
34. Paddock CD, Zambrano ML, Clover JR, Ladd-Wilson S, Dykstra EA, Salamone A, et al. *Rickettsia* species identified in adult, host-seeking *Dermacentor occidentalis* (Acari: Ixodidae) from Baja California, Mexico, and Oregon and Washington, United States. J Med Entomol 2024;61(3):781 – 90. https://doi.org/10.1093/jme/tjae023.
35. Myers S, Duncan K. *Dermacentor variabilis* (American dog tick). Trends Parasitol 2024;40(3):273 – 4. https://doi.org/10.1016/j.pt.2024.01.001.
36. Noden BH, Roselli MA, Loss SR. Factors influencing abundance of 3 tick species across a gradient of urban development intensity in the US Great Plains. J Med Entomol 2024;61(1):233 – 44. https://doi.org/10.1093/jme/tjad132.
37. Blanton LS, Walker DH, Bouyer DH. Rickettsiae and ehrlichiae within a city park: is the urban dweller at risk? Vector Borne Zoonotic Dis 2014;14(2):168-70. http://dx.doi.org/10.1089/vbz.2013.1473.
38. Noden BH, Loss SR, Maichak C, Williams F. Risk of encountering ticks and tick-borne pathogens in a rapidly growing metropolitan area in the U. S. Great Plains. Ticks Tick-Borne Dis 2017;8(1):119 – 24. https://doi.org/10.1016/j.ttbdis.2016.10.007.
39. Dantas-Torres F, Otranto D. *Rhipicephalus sanguineus* (Brown dog tick). Trends Parasitol 2022;38(11):993 – 4. https://doi.org/10.1016/j.pt.2022.08.011.
40. Szabó MP, Pinter A, Labruna MB. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front Cell Infect Microbiol 2013;3:27. https://doi.org/10.3389/fcimb.2013.00027.
41. Zazueta OE, Armstrong PA, Márquez-Elguea A, Hernández Milán NS, Peterson AE, Ovalle-Marroquín DF, et al. Rocky mountain spotted fever in a large metropolitan center, Mexico-United States border, 2009-2019. Emerg Infect Dis 2021;27(6):1567 – 76. https://doi.org/10.3201/eid2706.191662.
42. van Wyk CL, Mtshali K, Taioe MO, Terera S, Bakkes D, Ramatla T, et al. Detection of ticks and tick-borne pathogens of urban stray dogs in South Africa. Pathogens 2022;11(8):862. https://doi.org/10.3390/pathogens11080862.
43. Muñoz-Leal S, Lopes MG, Marcili A, Martins TF, González-Acuña D, Labruna MB. Anaplasmataceae, *Borrelia* and *Hepatozoon* agents in ticks (Acari: Argasidae, Ixodidae) from Chile. Acta Trop 2019;192:91 – 103. https://doi.org/10.1016/j.actatropica.2019.02.002.
44. Mans BJ, Gothe R, Neitz AWH. Biochemical perspectives on paralysis and other forms of toxicoses caused by ticks. Parasitology 2004;129 Suppl:S95-111. http://dx.doi.org/10.1017/s0031182003004670.
45. Llanos-Soto S, Muñoz-Leal S, Gatica JL, Misad C, González-Acuña D. Human toxicosis caused by the tick *Ornithodoros spheniscus* in a Chilean national park. Travel Med Infect Dis 2020;37:101811. https://doi.org/10.1016/j.tmaid.2020.101811.
46. Donaldson TG, Pèrez de León AA, Li AI, Castro-Arellano I, Wozniak E, Boyle WK, et al. Assessment of the geographic distribution of *Ornithodoros turicata* (argasidae): climate variation and host diversity. PLoS Negl Trop Dis 2016;10(2):e0004383. https://doi.org/10.1371/

journal.pntd.0004383.
47. Barraza-Guerrero SI, Meza-Herrera CA, García-De la Peña C, González-Álvarez VH, Vaca-Paniagua F, Díaz-Velásquez CE, et al. General microbiota of the soft tick *Ornithodoros turicata* parasitizing the bolson tortoise (*Gopherus flavomarginatus*) in the Mapimi biosphere reserve, Mexico. Biology (Basel) 2020;9(9):275. https://doi.org/10.3390/biology9090275.
48. Bissett JD, Ledet S, Krishnavajhala A, Armstrong BA, Klioueva A, Sexton C, et al. Detection of tickborne relapsing fever spirochete, Austin, Texas, USA. Emerg Infect Dis 2018;24(11):2003 – 9. https://doi.org/10.3201/eid2411.172033.
49. Gaffuri A, Sassera D, Calzolari M, Gibelli L, Lelli D, Tebaldi A, et al. Tick-borne encephalitis, Lombardy, Italy. Emerg Infect Dis 2024;30(2):341 – 4. https://doi.org/10.3201/eid3002.231016.
50. Kahl O, Bulling I, Chitimia-Dobler L. Some new findings on the endophilic vector tick *Ixodes hexagonus* in Germany. Ticks Tick-Borne Dis 2022;13(4):101954. https://doi.org/10.1016/j.ttbdis.2022.101954.
51. Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. N Engl J Med 2011;364(16):1523 – 32. https://doi.org/10.1056/NEJMoa1010095.
52. Yun SM, Lee WG, Ryou J, Yang SC, Park SW, Roh JY, et al. Severe fever with thrombocytopenia syndrome virus in ticks collected from humans, South Korea, 2013. Emerg Infect Dis 2014;20(8):1358 – 61. https://doi.org/10.3201/eid2008.131857.
53. Park SW, Ryou J, Choi WY, Han MG, Lee WJ. Epidemiological and clinical features of severe fever with thrombocytopenia syndrome during an outbreak in South Korea, 2013-2015. Am J Trop Med Hyg 2016;95(6):1358 – 61. https://doi.org/10.4269/ajtmh.16-0251.
54. Park SW, Song BG, Shin EH, Yun SM, Han MG, Park MY, et al. Prevalence of severe fever with thrombocytopenia syndrome virus in *Haemaphysalis longicornis* ticks in South Korea. Ticks Tick-Borne Dis 2014;5(6):975 – 7. https://doi.org/10.1016/j.ttbdis.2014.07.020.
55. Liu Q, He B, Huang SY, Wei F, Zhu XQ. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. Lancet Infect Dis 2014;14(8):763 – 72. https://doi.org/10.1016/S1473-3099(14)70718-2.
56. Azad AF, Beard CB. Rickettsial pathogens and their arthropod vectors. Emerg Infect Dis 1998;4(2):179 – 86. https://doi.org/10.3201/eid0402.980205.
57. Karbowiak G, Biernat B, Stańczak J, Szewczyk T, Werszko J. The role of particular tick developmental stages in the circulation of tick-borne pathogens affecting humans in Central Europe. 3. Rickettsiae. Ann Parasitol 2016;62(2):89 – 100. https://doi.org/10.17420/ap6202.38.
58. Whitworth T, Popov V, Han V, Bouyer D, Stenos J, Graves S, et al. Ultrastructural and genetic evidence of a reptilian tick, *Aponomma hydrosauri*, as a host of *Rickettsia honei* in Australia: possible transovarial transmission. Ann N Y Acad Sci 2003;990(1):67 – 74. https://doi.org/10.1111/j.1749-6632.2003.tb07339.x.
59. Salomon J, Fernandez Santos NA, Zecca IB, Estrada-Franco JG, Davila E, Hamer GL, et al. Brown dog tick (*Rhipicephalus sanguineus* sensu lato) infection with endosymbiont and human pathogenic *Rickettsia* spp. , in northeastern México. Int J Environ Res Public Health 2022;19(10):6249. https://doi.org/10.3390/ijerph19106249.
60. Vaculová T, Derdáková M, Špitalská E, Václav R, Chvostáč M, Rusňáková Tarageľová V. Simultaneous occurrence of *Borrelia miyamotoi, Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Rickettsia helvetica* in *Ixodes ricinus* ticks in urban foci in Bratislava, Slovakia. Acta Parasitol 2019;64(1):19 – 30. https://doi.org/10.2478/s11686-018-00004-w.
61. Padgett KA, Bonilla D, Eremeeva ME, Glaser C, Lane RS, Porse CC, et al. The eco-epidemiology of pacific coast tick fever in California. PLoS Negl Trop Dis 2016;10(10):e0005020. https://doi.org/10.1371/journal.pntd.0005020.
62. Prusinski M, O'Connor C, Russell A, Sommer J, White J, Rose L, et al. Associations of *Anaplasma phagocytophilum* bacteria variants in *Ixodes scapularis* ticks and humans, New York, USA. Emerg Infect Dis 2023;29(3):540 – 50. https://doi.org/10.3201/eid2903.220320.
63. Zhuo M, Calev H, Saunders SJ, Li JH, Stillman IE, Danziger J. Acute kidney injury associated with human granulocytic anaplasmosis: a case report. Am J Kidney Dis 2019;74(5):696 – 9. https://doi.org/10.1053/j.ajkd.2019.03.428.
64. Didyk YM, Blaňárová L, Pogrebnyak S, Akimov I, Peťko B, Víchová B. Emergence of tick-borne pathogens (*Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum, Ricketsia raoultii* and *Babesia microti*) in the Kyiv urban parks, Ukraine. Ticks Tick-Borne Dis 2017;8(2):219 – 25. https://doi.org/10.1016/j.ttbdis.2016.10.002.
65. Hamel D, Silaghi C, Zapadynska S, Kudrin A, Pfister K. Vector-borne pathogens in ticks and EDTA-blood samples collected from client-owned dogs, Kiev, Ukraine. Ticks Tick-Borne Dis 2013;4(1-2):152 – 5. https://doi.org/10.1016/j.ttbdis.2012.08.005.
66. Földvári G, Jahfari S, Rigó K, Jablonszky M, Szekeres S, Majoros G, et al. Candidatus neoehrlichia mikurensis and *Anaplasma phagocytophilum* in urban hedgehogs. Emerg Infect Dis 2014;20(3):496 – 8. https://doi.org/10.3201/eid2003.130935.
67. Kurokawa C, Lynn GE, Pedra JHF, Pal U, Narasimhan S, Fikrig E. Interactions between *Borrelia burgdorferi* and ticks. Nat Rev Microbiol 2020;18(10):587 – 600. https://doi.org/10.1038/s41579-020-0400-5.
68. Sormunen JJ, Kulha N, Klemola T, Mäkelä S, Vesilahti EM, Vesterinen EJ. Enhanced threat of tick-borne infections within cities? Assessing public health risks due to ticks in urban green spaces in Helsinki, Finland. Zoonoses Public Health 2020;67(7):823 – 39. https://doi.org/10.1111/zph.12767.
69. Piedmonte NP, Shaw SB, Prusinski MA, Fierke MK. Landscape features associated with blacklegged tick (Acari: Ixodidae) density and tick-borne pathogen prevalence at multiple spatial scales in central New York state. J Med Entomol 2018;55(6):1496 – 508. https://doi.org/10.1093/jme/tjy111.
70. Gray JS, Estrada-Peña A, Zintl A. Vectors of babesiosis. Annu Rev Entomol 2019;64:149 – 65. https://doi.org/10.1146/annurev-ento-011118-111932.
71. Lemieux JE, Tran AD, Freimark L, Schaffner SF, Goethert H, Andersen KG, et al. A global map of genetic diversity in *Babesia microti* reveals strong population structure and identifies variants associated with clinical relapse. Nat Microbiol 2016;1(7):16079. https://doi.org/10.1038/nmicrobiol.2016.79.
72. Yabsley MJ, Shock BC. Natural history of Zoonotic *Babesia*: role of wildlife reservoirs. Int J Parasitol Parasites Wildl 2012;2:18 – 31. https://doi.org/10.1016/j.ijppaw.2012.11.003.
73. Wang SS, Liu JY, Wang BY, Wang WJ, Cui XM, Jiang JF, et al. Geographical distribution of *Ixodes persulcatus* and associated pathogens: analysis of integrated data from a China field survey and global published data. One Health 2023;16:100508. https://doi.org/10.1016/j.onehlt.2023.100508.
74. Gebreyes WA, Dupouy-Camet J, Newport MJ, Oliveira CJB, Schlesinger LS, Saif YM, et al. The global one health paradigm: challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. PLoS Negl Trop Dis 2014;8(11):e3257. https://doi.org/10.1371/journal.pntd.0003257.
75. Ub GR, Narladkar BW. Role of entomopathogenic fungi in tick control: a Review. J Entomol Zool Stud 2018;6(1):1265-9. https://www.entomoljournal.com/archives/2018/vol6issue1/PartR/6-1-112-205.pdf.
76. Sonenshine DE. Tick pheromones and their use in tick control. Annu Rev Entomol 2006;51:557 – 80. https://doi.org/10.1146/annurev.ento.51.110104.151150.
77. Allan SA, inventor; Sonenshine DE, inventor; Burridge MJ, inventor. Tick pheromones and uses thereof. United States patent US 6331297. 2001 Dec 18. https://www.ars.usda.gov/research/publications/publication/?seqNo115=131819.
78. Benelli G, Pavela R, Canale A, Mehlhorn H. Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? Parasitol Res 2016;115(7):2545-60. http://dx.doi.org/10.1007/s00436-016-5095-1.
79. Araújo PS, Caixeta MB, Canedo A, da Silva Nunes E, Monteiro C,

Rocha TL. Toxicity of plant-based silver nanoparticles to vectors and intermediate hosts: historical review and trends. Sci Total Environ 2022;834:155299. https://doi.org/10.1016/j.scitotenv.2022.155299.

80. Wu YY, Ling F, Chen ZP, Lin JF, Shang XP, Hou J, et al. Lethal activity of propylene glycol alginate against *Haemaphysalis longicornis* larvae. Chin J Vector Biol Control 2017;28(1):16 – 9. https://doi.org/10.11853/j.issn.1003.8280.2017.01.005.
81. Brianti E, Falsone L, Napoli E, Prudente C, Gaglio G, Giannetto S. Efficacy of a combination of 10% imidacloprid and 4. 5% flumethrin (Seresto®) in slow release collars to control ticks and fleas in highly infested dog communities. Parasit Vectors 2013;6:210. https://doi.org/10.1186/1756-3305-6-210.
82. Brianti E, Pennisi MG, Brucato G, Risitano AL, Gaglio G, Lombardo G, et al. Efficacy of the fipronil 10%+(*S*)-methoprene 9% combination against *Rhipicephalus sanguineus* in naturally infested dogs: speed of kill, persistent efficacy on immature and adult stages and effect of water. Vet Parasitol 2010;170(1-2):96 – 103. https://doi.org/10.1016/j.vetpar.2010.01.033.
83. Borges LMF, de Sousa LAD, da Silva Barbosa C. Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. Rev Bras Parasitol Vet 2011;20(2):89 – 96. https://doi.org/10.1590/s1984-29612011000200001.
84. Haag-Wackernagel D. Regulation of the street pigeon in Basel. Wildl Soc Bull 1995;23(2):256-60. https://www.jstor.org/stable/3782800.
85. Drelich A, Andreassen Å, Vainio K, Kruszyński P, Wąsik TJ. Prevalence of tick-borne encephalitis virus in a highly urbanized and low risk area in Southern Poland. Ticks Tick-Borne Dis 2014;5(6):663 – 7. https://doi.org/10.1016/j.ttbdis.2014.04.020.
86. Bayles BR, Evans G, Allan BF. Knowledge and prevention of tick-borne diseases vary across an urban-to-rural human land-use gradient. Ticks Tick-Borne Dis 2013;4(4):352 – 8. https://doi.org/10.1016/j.ttbdis.2013.01.001.
87. Uspensky I. Tick pests and vectors (Acari: Ixodoidea) in European towns: introduction, persistence and management. Ticks Tick-Borne Dis 2014;5(1):41 – 7. https://doi.org/10.1016/j.ttbdis.2013.07.011.
88. Ge B, Li XC, Zhang Y, Zhang HB, Hu XD, Zhou YB, et al. Investigation on the knowledge of tick in those with high frequency contact poultry and livestock in Fengxian district of Shanghai. Chin J Hyg Insect Equip 2020;26(4):379 – 82. https://doi.org/10.19821/j.1671-2781.2020.04.024.
89. Cao GP, Zhan BD, Zhong JY, Yu ZY, Zhang JM, Chen ZB, et al. Status of tick distribution and tick-borne pathogens in urban parks of Quzhou, Zhejiang, 2017-2019. Dis Surveill 2021;36(9):879 – 83. https://doi.org/10.3784/jbjc.202106010314.
90. Mader EM, Ganser C, Geiger A, Harrington LC, Foley J, Smith RL, et al. A survey of tick surveillance and control practices in the United States. J Med Entomol 2021;58(4):1503 – 12. https://doi.org/10.1093/jme/tjaa094.
91. Machtinger ET, Poh KC, Pesapane R, Tufts DM. An integrative framework for tick management: the need to connect wildlife science, One Health, and interdisciplinary perspectives. Curr Opin Insect Sci 2024;61:101131. https://doi.org/10.1016/j.cois.2023.101131.

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

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

CCDC weekly

Vol. 7 No. 16 Apr. 18, 2025

Responsible Authority

National Disease Control and Prevention Administration

Sponsor

Chinese Center for Disease Control and Prevention

Editing and Publishing

China CDC Weekly Editorial Office

No.155 Changbai Road, Changping District, Beijing, China

Tel: 86-10-63150501, 63150701

Email: weekly@chinacdc.cn

CSSN

ISSN 2096-7071 (Print)

ISSN 2096-3101 (Online)

CN 10-1629/R1