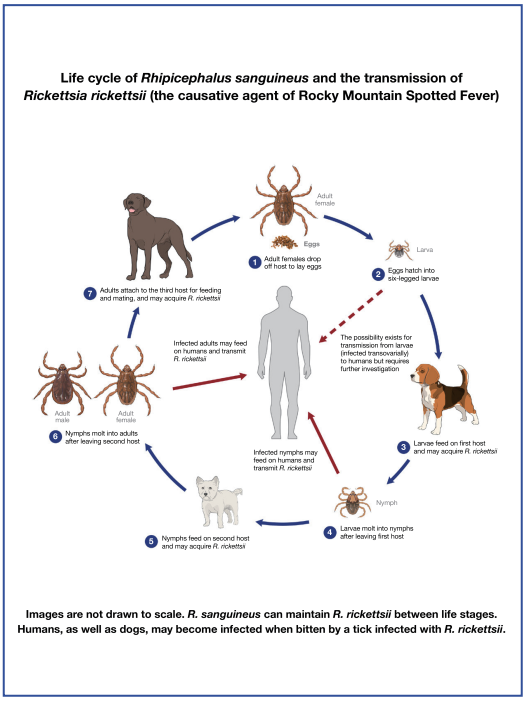
ISSN 2096-7071 (Print)
ISSN 2096-3101 (Online)
CN 10-1629/R1

CHINA CDC WEEKLY

Vol. 5 No. 37 Sept. 15, 2023

中国疾病预防控制中心周报



ISSN 2096-7071

China CDC Weekly

Cover Image: adapted from the US Centers for Disease Control and Prevention, https://www.cdc.gov/ticks/pdfs/Rhipicephalus-sanguineus-LifeCycle.pdf.

Preplanned Studies

Molecular Identification and Genetic Characterization of Public Health Threatening Ticks — Chongming Island, China, 2021–2022

Siwei Fei[1]; Hanqing Zhao[1]; Jingxian Yin[1]; Li Wang[2]; Zhishan Sun[1]; Wenge Zhang[2]; Yan Zhang[1]; Ke Dong[1]; Shan Lyu[1,3]; Xiaokui Guo[1]; Xiao-nong Zhou[1,3,#]; Kokouvi Kassegne[1,#]

Summary

What is already known about this topic?

Although ticks and tick-borne diseases are prevalent throughout China, there remains a knowledge gap regarding their biology and potential risk of distribution to human and animal populations on Chongming Island. The island, being China's third largest and a crucial component in the ecological preservation of the Yangtze Delta region, has yet to be comprehensively studied in this context.

What is added by this report?

In this study, employing molecular methodologies, a significant prevalence of *Haemaphysalis* (*H.*) *longicornis* and *H. flava* ticks — widely recognized for their high pathogenicity — is reported from Chongming Island. Additionally, the identification of two previously unreported species on the island, namely, *H. doenitzi* and *H. japonica*, expands our understanding of both the range and evolution of tick species.

What are the implications for public health practice?

The populations of humans and animals in nearly all 18 towns on Chongming Island are potentially at risk for transmission of tick-borne infectious agents. As a result, there is a pressing necessity for public health alerts, proactive tick surveillance, and effective screening of suspected clinical cases of tick-borne diseases within the Chongming population.

Ticks pose significant risks to human and animal health due to their capability of transmitting various pathogens, such as viruses, bacteria, and protozoans (*1*–*2*). To address this global health issue, the Chongming-based Center for One Health research was established on Chongming Island, China's third largest island and a crucial component in the ecological preservation of the Yangtze Delta region (*3*). The presence of ticks, including *Haemaphysalis* (*H.*) *longicornis* and *H. flava*, was previously confirmed in Dongping Forest Park and Dongtan Park on Chongming Island (*4*). Nonetheless, the potential risk ticks pose to human and animal populations on the island, along with their evolutionary impacts, remains unexplored.

In the course of 2021 to 2022, we conducted and identified ticks from Chongming Island utilizing 12S rRNA and co1 genes. We not only discovered an elevated prevalence of *H. longicornis* and *H. flava* on the island but also identified for the first time two additional tick species — *H. doenitzi* and *H. japonica*, both of which have been recognized for pathogenic properties and pose a threat to public health. Genetic diversity and neutrality tests suggested that the tick population was expanding or experiencing genetic hitchhiking. Consequently, there is an urgent need for sustained tick surveillance and targeted research on screening tick-borne pathogens and potential clinical cases to inform public health policies and actions.

In this study, ticks were collected from 18 towns and four protected regions on Chongming Island. Within each town, two to three sites were chosen for sample gathering, which was conducted bimonthly over two consecutive days between the hours of 10:00 AM and 4:00 PM from April 2021 to October 2022 (Figure 1). Each sample collection was performed by three collectors and lasted approximately 60±5 minutes.

Ticks were selected from two sources: those parasitizing domestic dogs and wild rabbits and free-living ticks collected from vegetation in parks and grasslands. The latter were sourced from areas in proximity to those parasitizing animals or from other external environments using a flag-dragging method.

After collection, ticks were classified as *Haemaphysalis* species based on their morphological characteristics as determined by microscopic examination. These characteristics include the shape of their prosthetic base, color, capitulum, conscutum, alloscutum, genital aperture, anal groove, anus, and arrangement on the posterior plate (*5*–*6*).

However, this study only distinguished between *H. flava* and *H. longicornis*, which were the most

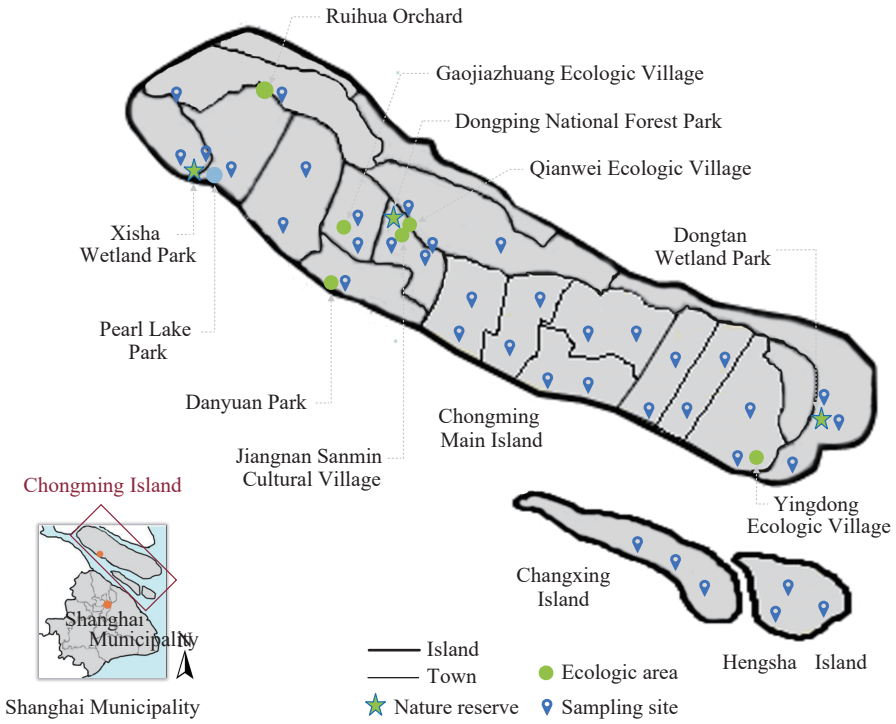


FIGURE 1. Locations on Chongming Island where ticks were collected, 2021–2022.

significantly represented species. *Haemaphysalis* species generally have eyeless, ciliated palisade, and rectangular basis capitula. *H. longicornis* is characterized by an abdomen of palpal segment 3 with a long conical spine and coxa II to IV internal spur, slightly larger and extending beyond the posterior (*5*). *H. flava* species possess thick and short abdominal spines on the palpal segment 3. Their coxa II to IV are short and triangular, and the scutum is marked with fine, shallow, evenly distributed engraved points (*6*). In females, the scutum is suborbicular, while in males, it is ovoid.

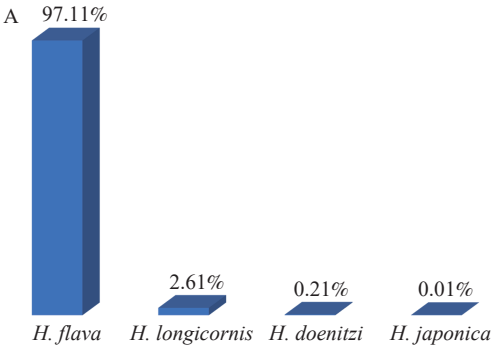
Microscopic examinations of ticks are depicted in Supplementary Figure S1 (available in https://weekly.chinacdc.cn/). Following identification, ticks were each individually preserved at −20 °C in 1.5 mL microcentrifuge tubes until further molecular analysis could be conducted.

Molecular identification and characterization were completed using polymerase chain reaction (PCR) amplification and sequencing of the produced amplicons. Genomic DNAs from a collection of 1,417 ticks were subjected to PCR amplification targeting 12S rRNA and col genes and were subsequently sequenced (*7*) with primer pairs T1B and T2A and CO1-F and CO1-R (Supplementary Table S1, Supplementary Figure S2, available in https://weekly.chinacdc.cn/). Amplification targeting the 12S rRNA locus proved more successful than that targeting the col locus, with success rates of 1,320/1,417 (93.15%) and 1,085/1,417 (76.57%), respectively. This disparity might be attributed to the limitations encountered in obtaining good-quality sequence reads from the col amplicons.

Analysis of 12S rRNA and co1 amplicons sequenced and verified in GenBank revealed that they predominantly belonged to the *Haemaphysalis* species, with *H. flava* constituting the majority at 97.11% (1,376/1,417), followed by *H. longicornis* at 2.61% (37/1,417), *H. doenitzi* at 0.21% (3/1,417), and *H. japonica* at 0.07% (1/1,417) (Figure 2A). When an intraspecific identity comparison based on the 12S rRNA gene was conducted, it was found that the ticks exhibited a high degree of identity with homologs from China and globally, with the exception of *H. japonica*, which presented an 83.48% degree of homology (Figure 2B).

Phylogenetic relationships were ascertained using both the neighbor-joining (NJ) and maximum likelihood (ML) methods. These relationships were established based on the correlation with publicly accessible homologous and orthologous sequences of the 12S rRNA and col genetic loci (Supplementary Table S2, available in https://weekly.chinacdc.cn/). From this examination, a dendrogram for 12S rRNA

A



B

Species	Marker	Confirmation/ total amplicons	Identity (%)
H. flava	12S rRNA	1,280/1,376	96.17
H. longicornis	12S rRNA	36/37	100
H. doenitzi	12S rRNA	3/3	99.11
H. japonica	12S rRNA	1/1	83.48

FIGURE 2. Molecular identification of ticks from Chongming Island (2021–2022) based on the 12S rRNA gene. (A) Distribution of species derived from Chongming Island. (B) Species confirmed through comprehensive sequence analysis, coupled with intraspecies validation, utilizing multiple alignments of the 12S rRNA gene amplicons.
Abbreviation: *H.=Haemaphysalis*.

suggested the existence of four main groups. These included *H. japonica* and *H. concinna* in the second cluster, *H. doenitzi* and *H. cornigera* in the third, and *H. longicornis* and *H. hystricis* in the fourth. Conversely, the first cluster distinctly comprised *H. flava* (Figure 3A). A comparison of the topological structure between the col and 12S rRNA phylogenetic trees yielded similar results (Figure 3B). Phylogenetic relationships derived from the ML method are available in Supplementary Figure S3 (available in https://weekly.chinacdc.cn/).

Genetic divergences were assessed at various taxonomic levels among tick species, utilizing Kimura's 2-parameter (K2P) distances based on the 12S rRNA locus (Supplementary Table S2). The highest within-species K2P distance was exhibited by *H. japonica* (0.0946), while the intraspecific distances for both *H. cornigera* and *H. hystricis* were zero. The zero intraspecific distance in these cases infers identical genetic sequences among populations within each species, offering potential insights into population history and overall biodiversity origins. The maximum interspecific K2P distance was identified between *H. hystricis* and *H. japonica* (0.1960). Conversely, the smallest interspecific distance of 0.0723 was observed between *H. cornigera* and *H. doenitzi*, indicating a potential genetic similarity between these species, as also depicted in the phylogenetic tree. An intermediate genetic distance was found between *H. hystricis* and *H. longicornis* (0.0900), comparable to the distance observed between *H. flava* and *H. japonica* (0.0903), both of which are in alignment with the phylogenetic topology.

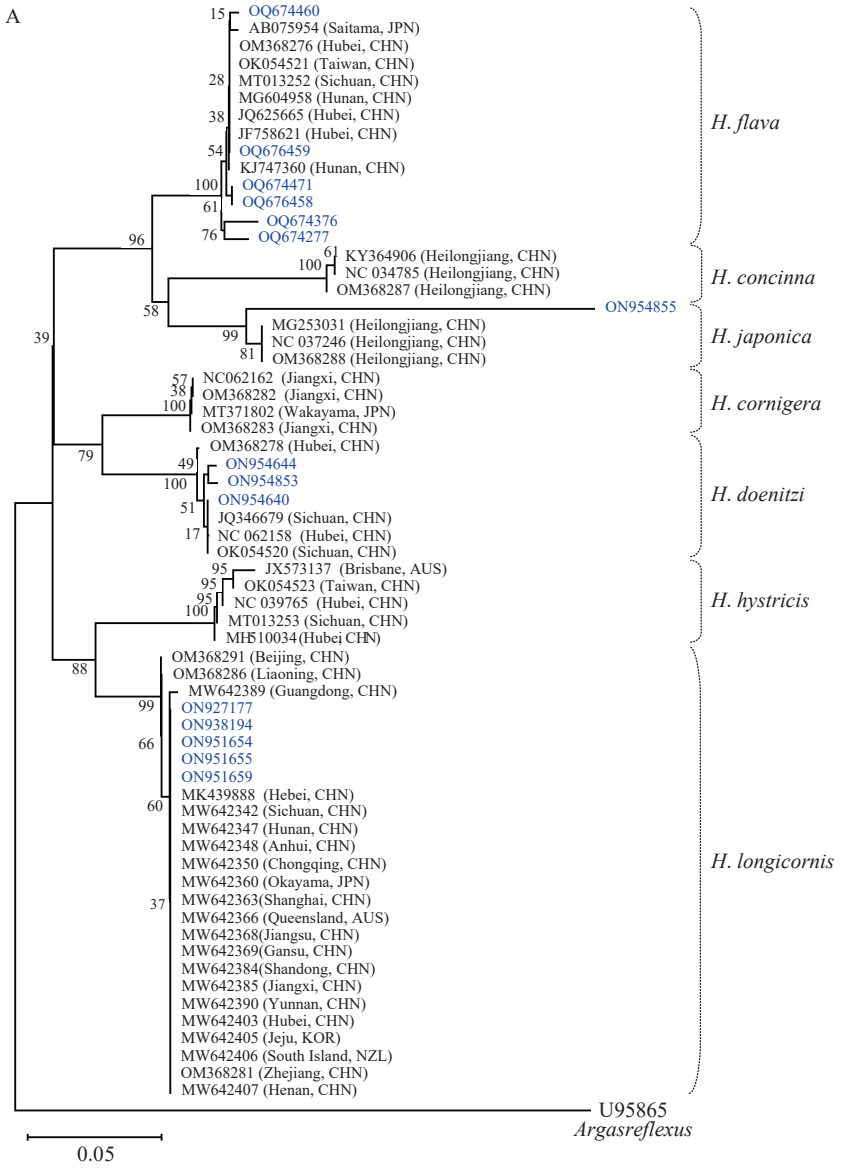
The mapping of K2P genetic distances revealed that *H. japonica* and *H. longicornis* maintained notable genetic differentiation compared to other tick species on Chongming Island. In particular, *H. japonica* presented the highest intraspecific and interspecific K2P distances. Its intraspecific distance, notably, exceeded the lowest interspecific distance (between *H. doenitzi* and *H. cornigera*) by 0.0946 (Figure 4A). Collectively, distinct interspecific boundaries were evident in more than 96% of the 12S rRNA fragments examined. We conducted genetic diversity indices and neutrality tests for these tick species using the 12S rRNA locus as a reference (Figure 4B). Among the species examined, the greatest number of polymorphic loci was found in *H. japonica* (n=56), while *H. flava* reported the lowest number (n=1). In terms of nucleic acid diversity (Pi), *H. japonica* was the most diverse (0.08333), while *H. doenitzi* exhibited the highest haplotype diversity (0.714). Neutral tests signified consistent negative values for Fu and Li's D and Tajima's D for the four species, yet they reported positive values for *H. concinna*. These findings suggest that there has been a recent expansion in global populations of *H.* longicornis, *H. doenitzi*, *H.* flava, and *H. japonica* ticks, including those based in Chongming.

DISCUSSION

The current study identified four tick species on Chongming Island, notably *H. flava* (97.11%), *H. longicornis* (2.61%), *H. doenitzi* (0.21%), and *H. japonica* (0.07%). This contrasts with a previous study that indicated *Rhipicephalus sanguineus* and *H. longicornis* as the main tick species in Shanghai (*8*). However, our findings indicate that *H. flava* and *H. longicornis* represent the majority of ticks on Chongming Island, corroborating recent research conducted in Dongping Forest Park and Dongtan Park

on the island (*4*). Notably, our research newly reports the presence of *H. doenitzi* and *H. japonica* from Dongtan Wetland Park and Dongping Forest Park, respectively, a fact not previously documented on the island. *H. doenitzi*, known as an avian tick, infests birds; thus, its presence on Chongming Island suggests potential for pathogen spillover. The low prevalence rates of *H. doenitzi* (3/1,417) and *H. japonica* (1/1,417) could imply their random occurrence and indicate that the likelihood of their detection in previous studies was relatively low. Furthermore, we posit that *H. japonica*, often referred to as "the northern tick", may have been introduced to the island via migratory birds breeding annually or increasingly common northern-to-southern trading transportation (*3*).

H. flava, a tick species, holds significant importance in public health, medical, and veterinary arenas due to its potential to cause lesions, blood loss, weight loss, and, in some cases, death. This species acts as a vector for various pathogens, including *Borrelia burgdorferi* (*9*–*10*), severe fever with thrombocytopenia syndrome virus (*11*), and tick-borne encephalitis virus (*12*). An epidemiological investigation on ticks and associated pathogens in pet dogs revealed that *H. longicornis* (*9*) was the predominant tick species across 1,140 counties in eastern and northeastern China, exposing over 40% of the population. Notably, a high incidence of *H. flava* and *H. longicornis* in 18 towns across Chongming, China, suggests a significant public health threat due to potential transmission risks of tick-borne infectious agents. *H. japonica* and *H. doenitzi* are



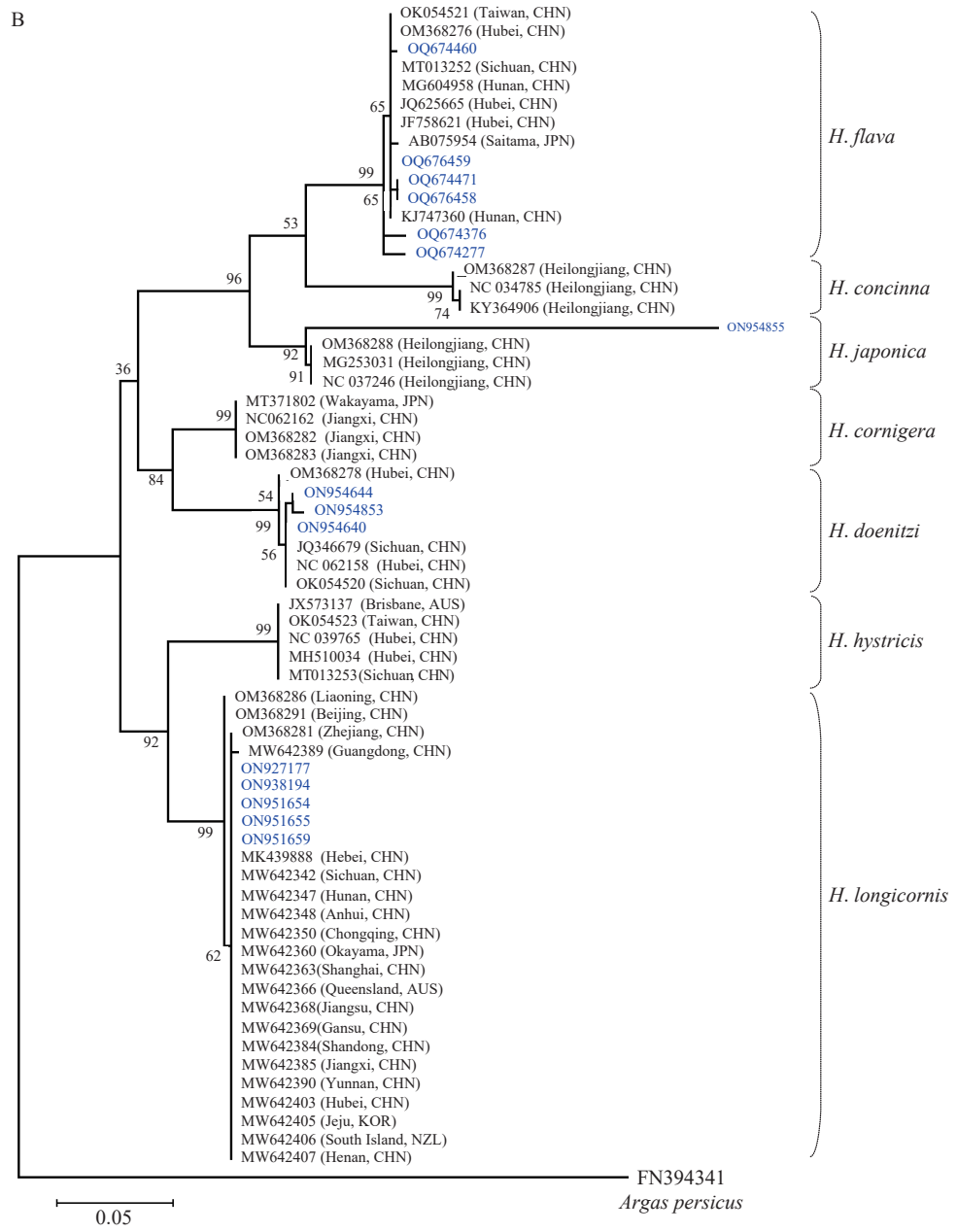


FIGURE 3. Comparison of genetic and geographical relationships between *Haemaphysalis* species from Chongming Island, 2021–2022, and those on record in GenBank. (A) Neighbor-joining tree based on 12S rRNA sequences. (B) Neighbor-joining tree based on col sequences.

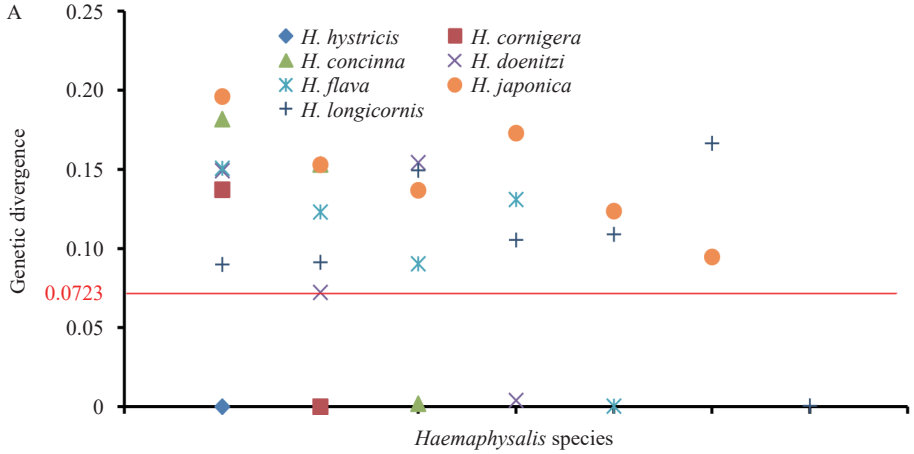
Note: Percentage values on the tree branches denote bootstrapping values from 1,000 replicates. The identification process for each *Haemaphysalis* species sequence involved its accession number and geographical location (country). The gene sequences relevant to the species identified in this study are highlighted in blue. For the 12S rRNA and col phylogenetic trees, *Argas reflexus* and *Argas persicus* were used as outgroups, respectively. The scale bar signifies nucleotide substitutions per site.

Abbreviation: *H.=Haemaphysalis*.

prevalent across several regions in China, including Fujian Province, Yunnan Province, Gansu Province, Hebei Province, and the Inner Mongolia Autonomous Region and Taiwan, China (*9*), and are known to transmit a range of pathogens. These cause infections such as Lyme borreliosis (*10*) and babesiosis in humans (*13*). Despite their low prevalence on Chongming Island, these species also pose a public health threat.

Our study revealed a significant overlap between the intraspecific and interspecific K2P distances in *H.*

A



B

Species	*n*	S	Pi	h	Hd	Fu and Li's D	Tajima's D
H. flava	14	1	0.000,44	2	0.143	−1.397,49	−1.155,24
H. longicornis	26	2	0.000,66	3	0.218	−0.689,07	−1.223,94
H. doenitzi	7	4	0.003,98	4	0.714	−0.789,27	−0.876,42
H. japonica	4	56	0.083,33	2	0.500	−0.868,31	−0.868,31
H. cornigera	4	0	0	1	0	0	NA
H. hystricis	4	0	0	1	0	0	NA
H. concinna	3	1	0.001,97	2	0.667	1.632,99	1.632,99

FIGURE 4. Analysis of Kimura's two-parameter distance, genetic diversity, and neutrality tests for *Haemaphysalis* ticks collected from Chongming Island, 2021–2022. (A) Plot of the K2P distance of the *Haemaphysalis* species using NJ-K2P distances. (B) Genetic diversity indices and neutrality tests based on the 12S rRNA gene in the *Haemaphysalis* species.
Note: The red solid line in panel A represents the minimum interspecific distance between *H. cornigera* and *H. doenitzi*.
Abbreviation: *n*=the number of species; S=the number of polymorphism loci; Pi=nucleic acid diversity; h=haplotype number; Hd=haplotype diversity; *H.*=*Haemaphysalis*.

japonica. However, clear boundaries for the barcoding gap (ranging from 0.0040 to 0.0723) were observed in other species under observation. The overlap in *H. japonica* might be attributable to a high count of polymorphic loci (*n*=56) and considerable diversity (Pi=0.0833) in its 12S rRNA. Furthermore, the 12S rRNA topology indicates that the *H. japonica* species clusters separately from the other three species, implying that 12S rRNA may not serve as an effective biomarker for its identification. All observed tick species exhibited negative neutrality tests, as evidenced by Fu and Li's D and Tajima's D values, which might suggest that the four identified tick species in Chongming may have undergone population expansion or global genetic hitchhiking (*14*). This could enhance the adaptability of the ticks to environmental variations, extend their distribution, and potentially harbor a greater number of pathogens, escalating their capacity to cross-transfer diseases and intensifying their public health risks.

This study revealed a high prevalence of *Haemaphysalis* tick distribution on Chongming Island. These findings suggest potential transmission risks of tick-borne infectious agents to both the human and animal populations on the island. Consequently, it is crucial to urgently issue public health warnings, implement active tick surveillance, and efficiently screen suspected tick-borne disease cases within the Chongming population. To address intricate ecological and health challenges, we recommend future research into the epidemiological distribution of tick-borne pathogens on Chongming Island. Such research could expedite the successful application of the One Health approach to public health threats in China.

Conflicts of interest: Xiao-nong Zhou is a editorial board member of the journal China CDC Weekly. He was not involved in the peer-review or handling of the manuscript. The authors have no other competing interests to disclose.

Acknowledgements: The authors express their gratitude to the staff at the Shanghai Chongming Centre for Disease Control and Prevention for their assistance in facilitating the collection and identification of ticks.

Supporting information: The manuscript and accompanying supplementary file contain all pertinent data. The sequences of both the 12S rRNA and col genes, central to the phylogenetic analysis, have been

lodged with the National Center for Biotechnology Information (NCBI). The GenBank accession numbers for these sequences are as follows: for 12S RNA genes — *H. flava* [OQ674277, OQ674376, OQ674460, OQ676458, OQ676459, OQ674471]; *H. longicornis* [ON927177, ON938194, ON951654, ON951655, and ON951659]; *H. doenitzi* [ON954640, ON954644, and ON954853]; *H. japonica* [ON954855]. For col genes — *H. flava* [ON954774, ON954780, ON959178, and ON959193-ON959195]; *H. longicornis* [ON954776].

Funding: Supported by the financial backing of the Science and Technology Innovation Project Fund from the School of Global Health, Shanghai Jiao Tong University School of Medicine (SGHKJCX2021-05, SGHKJCX2021-04) and the International Joint Laboratory on Tropical Diseases Control in Greater Mekong Subregion (21410750200).

doi: 10.46234/ccdcw2023.156

[#] Corresponding authors: Xiao-nong Zhou, xiaonong.zhou@sjtu.edu.cn; Kokouvi Kassegne, kassegnek@sjtu.edu.cn.

[1] School of Global Health, Chinese Centre for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2] Shanghai Chongming Centre for Disease Control and Prevention, Shanghai, China; [3] National Health Commission of the People's Republic of China (NHC) Key Laboratory of Parasite and Vector Biology, National Institute of Parasitic Diseases at Chinese Centre for Disease Control and Prevention, Chinese Centre for Tropical Diseases Research, WHO Collaborating Centre for Tropical Diseases, National Centre for International Research on Tropical Diseases of the Chinese Ministry of Science and Technology, Shanghai, China.

Submitted: June 01, 2023; Accepted: September 12, 2023

REFERENCES

1. Zhao GP, Wang YX, Fan ZW, Ji Y, Liu MJ, Zhang WH, et al. Mapping ticks and tick-borne pathogens in China. Nat Commun 2021;12(1):1075. https://pubmed.ncbi.nlm.nih.gov/33597544/.
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://pubmed.ncbi.nlm.nih.gov/22902521/.
3. Tan J, Wang M, Su JH, Ruan JJ, Wang Q. Ecological risk assessment of Chongming Eco-Island based on relative risk model. Yangtze River 2022;53(11):27 – 34. http://dx.doi.org/10.16232/j.cnki.1001-4179.2022.11.005. (In Chinese).
4. Zeng WB, Li ZQ, Wei ZX, Cheng DH, Yang LM, Fang Y, et al. An investigation of questing ticks collected from different habitats in Shanghai, China. Chin J Vector Biol Control 2022;33(6):879-87. http://www.bmsw.net.cn/CN/10.11853/j.issn.1003.8280.2022.06.022. (In Chinese).
5. Barker SC, Walker AR. Ticks of Australia. The species that infest domestic animals and humans. Zootaxa 2014;3816(1):1 – 144. http://dx.doi.org/10.11646/zootaxa.3816.1.1.
6. Walker AR, Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA, et al. Ticks of domestic animals in africa: a guide to identification of species. Edinburgh: Bioscience Reports. 2003; pp. 1-227. https://lib.ugent.be/en/catalog/rug01:000802470.
7. Abdullah HHAM, El-Shanawany EE, Abdel-Shafy S, Abou-Zeina HAA, Abdel-Rahman EH. Molecular and immunological characterization of *Hyalomma dromedarii* and *Hyalomma excavatum* (Acari: Ixodidae) vectors of Q fever in camels. Vet World 2018;11(8):1109-19. https://pubmed.ncbi.nlm.nih.gov/30250371/.
8. Meignan M. VI. FDG-PET as a biomarker in lymphoma: from qualitative to quantitative analysis. Hematol Oncol 2015;33 Suppl 1:38-41. https://pubmed.ncbi.nlm.nih.gov/26062052/.
9. Cao J, Yang QQ, Zhang JW, Zhou YZ, Zhang HS, Gong HY, et al. Seroprevalence survey of *Babesia gibsoni* infection and tick species in dogs in East China. Vet Parasitol 2015;214(1-2):12-5. https://pubmed.ncbi.nlm.nih.gov/26483164/.
10. Zhang YZ, Xu JG. The emergence and cross species transmission of newly discovered tick-borne Bunyavirus in China. Curr Opin Virol 2016;16:126-31. https://pubmed.ncbi.nlm.nih.gov/26949898/.
11. Chen XJ, Yu ZJ, Guo LD, Li LX, Meng H, Wang D, et al. Life cycle of *Haemaphysalis doenitzi* (Acari: Ixodidae) under laboratory conditions and its phylogeny based on mitochondrial 16S rDNA. Exp Appl Acarol 2012;56(2):143-50. https://pubmed.ncbi.nlm.nih.gov/22113780/.
12. Allen T, Murray KA, Zambrana-Torrelio C, Morse SS, Rondinini C, Di Marco M, et al. Global hotspots and correlates of emerging zoonotic diseases. Nat Commun 2017;8(1):1124. https://pubmed.ncbi.nlm.nih.gov/29066781/.
13. 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://pubmed.ncbi.nlm.nih.gov/24837566/.
14. Shen HM, Chen SB, Cui YB, Xu B, Kassegne K, Abe EM, et al. Whole-genome sequencing and analysis of *Plasmodium falciparum* isolates from China-Myanmar border area. Infect Dis Poverty 2018;7(1):118. https://pubmed.ncbi.nlm.nih.gov/30445995/.

SUPPLEMENTARY MATERIAL

A

H. flava (female, abdomen)

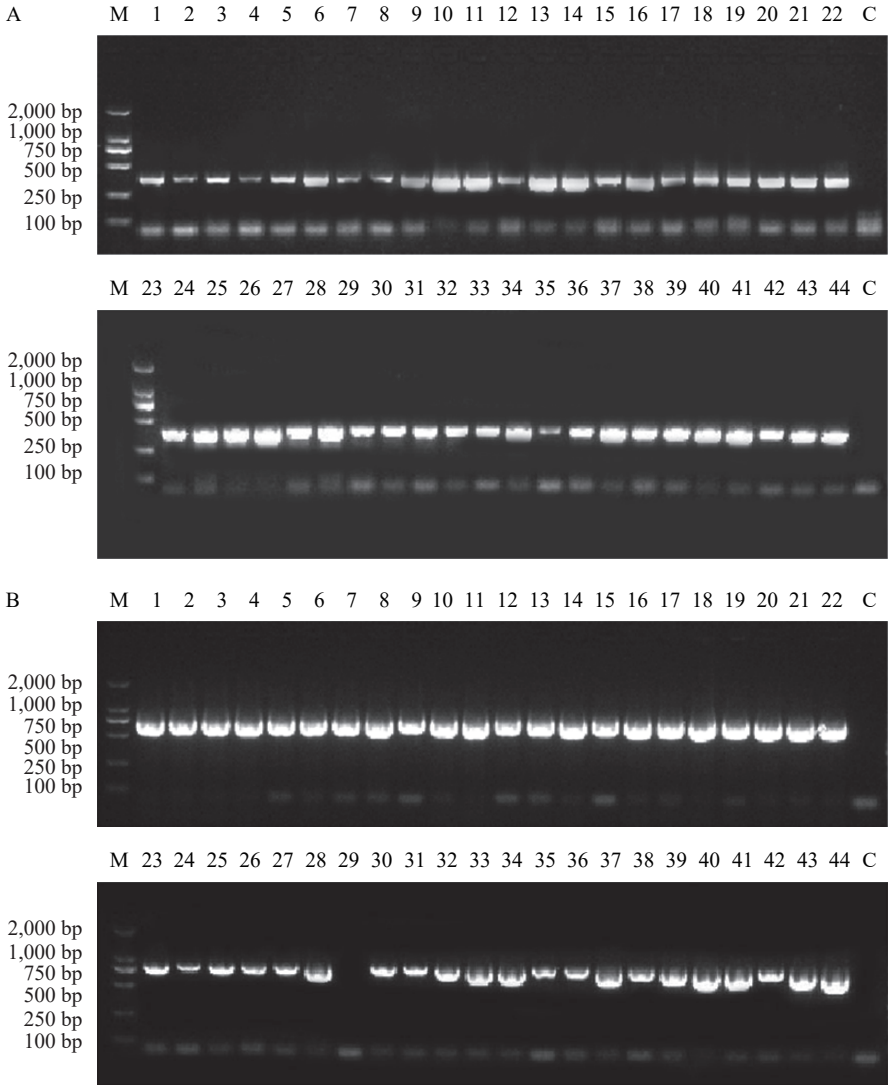
H. longicornis (female, dorsum)

B

H. longicornis (female, dorsum)

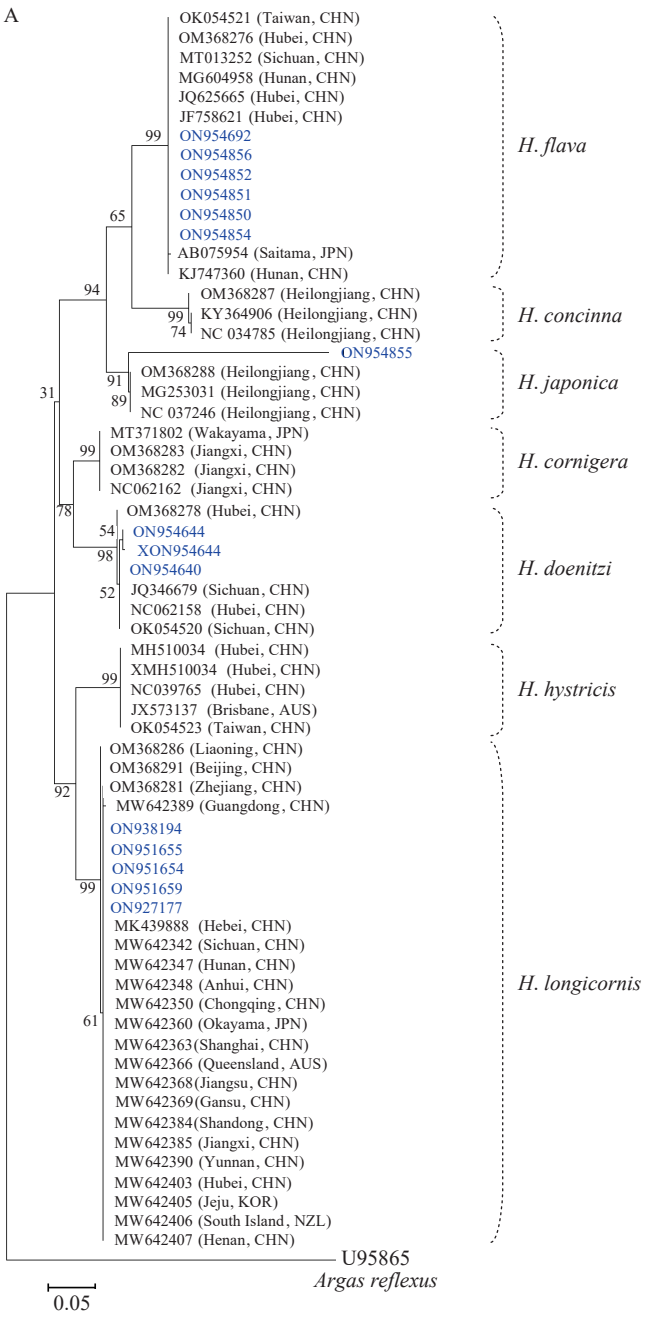
H. longicornis (female, abdomen)

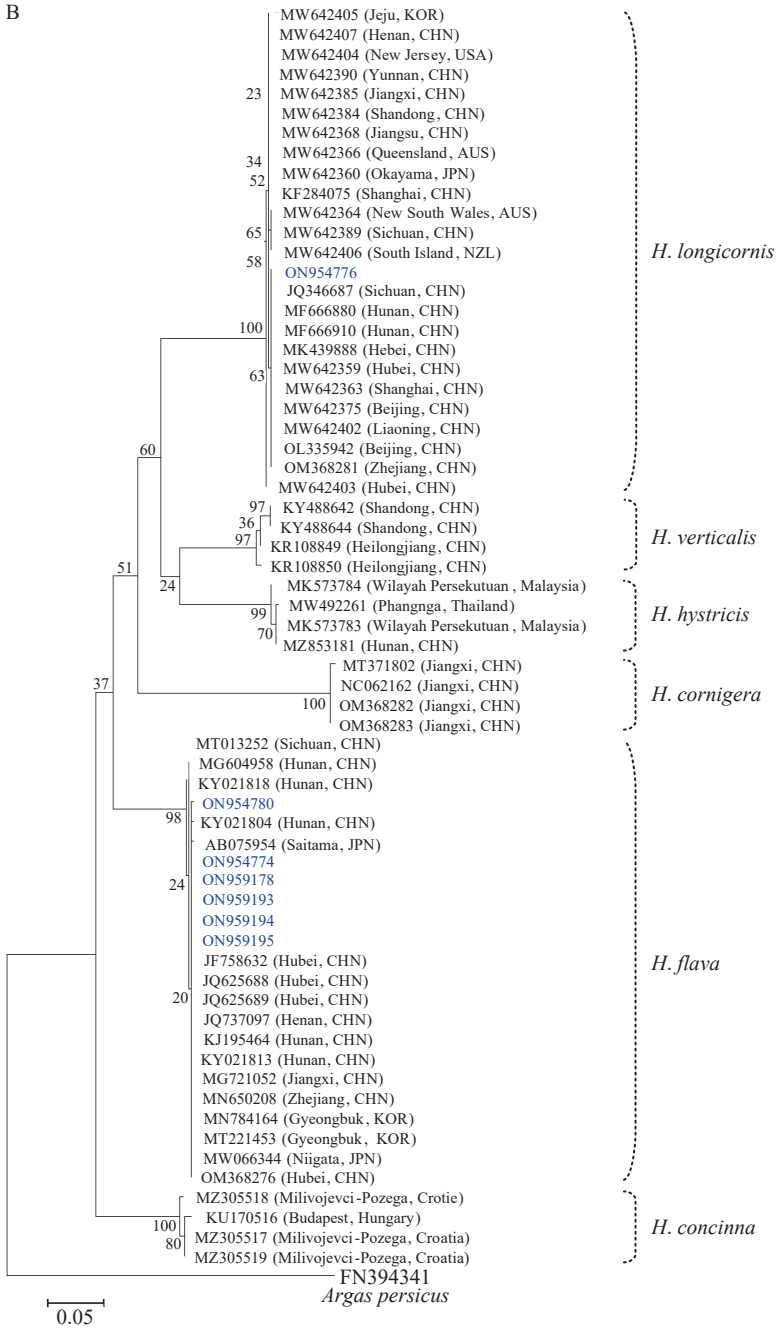
SUPPLEMENTARY FIGURE S1. Identification of primary ticks (A) *H. flava* and (B) *H.* longicornis on Chongming Island, 2021–2022.
Abbreviation: *H.=Haemaphysalis.*



SUPPLEMENTARY FIGURE S2. Specific bands of amplified mitochondrial genes were identified through agarose gel electrophoresis. (A) Amplicons of the 12S rRNA gene at approximately 380 base pairs. (B) Amplicons of the co1 gene at approximately 650 base pairs.

Note: In panel A, “M” denotes DNA Marker (DL2000, Takara); “numbered” amplicons of the 12S rRNA gene; “C” denotes negative template control. In panel B, “M” denotes DNA Marker (DL2000, Takara); “numbered” denotes amplicons of co1 gene; “C” denotes negative template control.


A
OK054521 (Taiwan, CHN)
OM368276 (Hubei, CHN)
MT013252 (Sichuan, CHN)
MG604958 (Hunan, CHN)
JQ625665 (Hubei, CHN)
JF758621 (Hubei, CHN)
ON954692
ON954856
ON954852
ON954851
ON954850
ON954854
AB075954 (Saitama, JPN)
KJ747360 (Hunan, CHN)
H. flava
OM368287 (Heilongjiang, CHN)
KY364906 (Heilongjiang, CHN)
NC 034785 (Heilongjiang, CHN)
H. concinna
ON954855
OM368288 (Heilongjiang, CHN)
MG253031 (Heilongjiang, CHN)
NC 037246 (Heilongjiang, CHN)
H. japonica
MT371802 (Wakayama, JPN)
OM368283 (Jiangxi, CHN)
OM368282 (Jiangxi, CHN)
NC062162 (Jiangxi, CHN)
H. cornigera
OM368278 (Hubei, CHN)
ON954644
XON954644
ON954640
JQ346679 (Sichuan, CHN)
NC062158 (Hubei, CHN)
OK054520 (Sichuan, CHN)
H. doenitzi
MH510034 (Hubei, CHN)
XMH510034 (Hubei, CHN)
NC039765 (Hubei, CHN)
JX573137 (Brisbane, AUS)
OK054523 (Taiwan, CHN)
H. hystricis
OM368286 (Liaoning, CHN)
OM368291 (Beijing, CHN)
OM368281 (Zhejiang, CHN)
MW642389 (Guangdong, CHN)
ON938194
ON951655
ON951654
ON951659
ON927177
MK439888 (Hebei, CHN)
MW642342 (Sichuan, CHN)
MW642347 (Hunan, CHN)
MW642348 (Anhui, CHN)
MW642350 (Chongqing, CHN)
MW642360 (Okayama, JPN)
MW642363(Shanghai, CHN)
MW642366 (Queensland, AUS)
MW642368(Jiangsu, CHN)
MW642369(Gansu, CHN)
MW642384(Shandong, CHN)
MW642385 (Jiangxi, CHN)
MW642390 (Yunnan, CHN)
MW642403 (Hubei, CHN)
MW642405 (Jeju, KOR)
MW642406 (South Island, NZL)
MW642407 (Henan, CHN)
H. longicornis
U95865
Argas reflexus
0.05



SUPPLEMENTARY FIGURE S3. Comparison of the genetic and geographical relationships between *Haemaphysalis* species from Chongming Island, 2021–2022, and those deposited in GenBank. (A) Maximum likelihood tree based on 12S rRNA sequences. (B) Maximum likelihood tree based on co1 sequences.

Note: The relationships among *Haemaphysalis* species were discerned via analysis of partial 12S rRNA and col gene sequences. The percentages on the branches represent the bootstrap values from 1,000 replications. Each *Haemaphysalis* species sequence was identified based on its accession number and geographical location (country). Sequences representing the species identified herein are highlighted in blue. *Argas reflexus* and *Argas persicus* were utilized as outgroups for 12S rRNA and col phylogenetic trees, respectively. The scale bar symbolizes the rate of nucleotide substitutions per site.

Abbreviation: *H.=Haemaphysalis.*

SUPPLEMENTARY TABLE S1. Primer sequences employed for the PCR amplification of mitochondrial genes in ticks.

Amplification fragment	Primers	Primer sequences	Annealing temperature (°C)	Amplicon size (bp)
12S rRNA	T1B T2A	AAACTAGGATTAGATACCCT AATGAGAGCGACGGGCGATGT	51/ 53	380
co1	CO1-F CO1-R	GGAACAATATATTTAATTTTTGG ATCTATCCCTACTGTAAATATATG	55	650–820

Abbreviation: bp=base pairs.

SUPPLEMENTARY TABLE S2-1. Data on the mitochondrial gene sequences (12S rRNA) utilized for phylogenetic and genetic diversity analysis.

Species	Geographic localities	GenBank accession No.
H. hystricis	Brisbane, Australia	JX573137
	Hubei, China	MH510034
	Sichuan, China	MT013253
	Hubei, China	NC_039765
	Taiwan, China	OK054523
H. cornigera	Wakayama, Japan	MT371802
	Gangzhou, China	NC062162
	Gangzhou, China	OM368282
	Guangzhou, China	OM368283
H. concinna	Heilongjiang, China	KY364906
	Heilongjiang, China	NC_034785
	Heilongjiang, China	OM368287
H. doenitzi	Sichuan, China	JQ346679
	Hubei, China	NC_062158
	Sichuan, China	OK054520
	Hubei, China	OM368278
	Shanghai, China	ON954640
	Shanghai, China	ON954644
	Shanghai, China	ON954853
H. flava	Saitama, Japan	AB075954
	Hubei, China	JF758621
	Hubei, China	JQ625665
	Hunan, China	KJ747360
	Hunan, China	MG604958
	Sichuan, China	MT013252
	Taiwan, China	OK054521
	Hubei, China	OM368276
	Shanghai, China	OQ676427
	Shanghai, China	OQ676437
	Shanghai, China	OQ674460
	Shanghai, China	OQ674471
	Shanghai, China	OQ676458
	Shanghai, China	OQ676459
H. japonica	Heilongjiang, China	MG253031
	Heilongjiang, China	NC_037246
	Heilongjiang, China	OM368288
	Shanghai, China	ON954855

Continued

Species	Geographic localities	GenBank accession No.
H. longicornis	Hebei, China	MK439888
	Sichuan, China	MW642342
	Hunan, China	MW642347
	Anhui, China	MW642348
	Chongqing, China	MW642350
	Okayama, Japan	MW642360
	Shanghai, China	MW642363
	Queensland, Australia	MW642366
	Jiangsu, China	MW642368
	Gansu, China	MW642369
	Shandong, China	MW642384
	Jiangxi, China	MW642385
H. longicornis	Guangdong, China	MW642389
	Yunnan, China	MW642390
	Hubei, China	MW642403
	Jeju, Republic of Korea	MW642405
	South Island, New Zealand	MW642406
	Henan, China	MW642407
	Zhejiang, China	OM368281
	Liaoning, China	OM368286
	Beijing, China	OM368291
Argas reflexus		U95865.1

Abbreviation: *H.=Haemaphysalis*.

SUPPLEMENTARY TABLE S2-2. Data on the mitochondrial gene sequences (co1) utilized for phylogenetic and genetic diversity analysis.

Species	Geographic localities	GenBank accession No.
H. hystricis	Wilayah Persekutuan, Malaysia	MK573783
	Wilayah Persekutuan, Malaysia	MK573784
	Phangnga, Thailand	MW492261
	Hunan, China	MZ853181
H. cornigera	Jiangxi, China	MT371802
	Jiangxi, China	NC_062162
	Jiangxi, China	OM368282
	Jiangxi, China	OM368283
H. concinna	Budapest, Hungary	KU170516
	Milivojevci-Pozega, Croatia	MZ305517
	Milivojevci-Pozega, Croatia	MZ305518
	Milivojevci-Pozega, Croatia	MZ305519
H. doenitzi	Hubei, China	JF758632
	Hubei, China	JQ625688
	Hubei, China	JQ625689
	Henan, China	JQ737097
	Hunan, China	KJ195464

Continued

Species	Geographic localities	GenBank accession No.
	Hunan, China	KY021804
	Hunan, China	KY021813
	Hunan, China	KY021818
	Hunan, China	MG604958
	Jiangxi, China	MG721052
	Zhejiang, China	MN650208
	Gyeongbuk, Republic of Korea	MN784164
	Sichuan, China	MT013252
	Gyeongbuk, Republic of Korea	MT221453
	Niigata, Japan	MW066344
	Hubei, China	OM368276
	Shanghai, China	KF284075
	Shanghai, China	ON954774
	Shanghai, China	ON954780
	Shanghai, China	ON959178
	Shanghai, China	ON959193
	Shanghai, China	ON959194
	Shanghai, China	ON959195
H. longicornis	Sichuan, China	JQ346687
	Hunan, China	MF666880
	Hunan, China	MF666910
	Hebei, China	MK439888
	Hubei, China	MW642359
	Okayama, Japan	MW642360
	Shanghai, China	MW642363
	New South Wales, Australia	MW642364
H. longicornis	Queensland, Australia	MW642366
	Jiangsu, China	MW642368
	Beijing, China	MW642375
	Shandong, China	MW642384
	Jiangxi, China	MW642385
	Sichuan, China	MW642389
	Yunnan, China	MW642390
	Dalian, China	MW642402
	Hubei, China	MW642403
	New Jersey, the United States	MW642404
	Jeju, Republic of Korea	MW642405
	South Island, New Zealand	MW642406
	Henan, China	MW642407
	Beijing, China	OL335942
	Zhejiang, China	OM368281
	Shanghai, China	ON927177
	Shanghai, China	ON938194

Continued

Species	Geographic localities	GenBank accession No.
H. longicornis	Shanghai, China	ON951654
(continued)	Shanghai, China	ON951655
	Shanghai, China	ON951659
	Shanghai, China	ON954776
H. verticalis	Heilongjiang, China	KR108849
	Heilongjiang, China	KR108850
	Shandong, China	KY488642
	Shandong, China	KY488644
Argas persicus	Southwestern Romania	FN394341

SUPPLEMENTARY TABLE S3. Average intraspecific and interspecific K2P distances predicated on the 12S rRNA gene in *Haemaphysalis* species.

Species	*N*	*H. hystricis*	*H. cornigera*	*H. concinna*	*H. doenitzi*	*H. flava*	*H. japonica*	*H. longicornis*
H. hystricis	5	0						
H. cornigera	4	0.1371328831	0					
H. concinna	3	0.1817426049	0.1532318083	0.0019841330				
H. doenitzi	4	0.1491843529	0.0723418465	0.1542762170	0.0039905820			
H. flava	14	0.1507102241	0.1230000555	0.0903495504	0.1309744074	0.0004275540		
H. japonica	4	0.196004581*	0.1529088927	0.1368672590	0.1728339139	0.1235672509	0.0945783130	
H. longicornis	26	0.0899835374	0.0913522722	0.1493494257	0.1054751596	0.1089099103	0.1665811766	0.0006666250

Note: Intraspecific distance data are shown in boldface for clarity. The underlined data indicate the highest intraspecific and lowest interspecific distances.
Abbreviation: *N*=number of sequences. *H.=Haemaphysalis.*
* the highest interspecific distance.

Recollections

Assessment of Tick-Borne Diseases in Hainan Province, China

Weiqing Zheng[1]; Guangyuan Zhao[1]; Qianfeng Xia[1,#]

ABSTRACT

China's six tropical regions include Guangdong Province, Yunnan Province, Hainan Province, Hong Kong Special Administrative Region (SAR), Macau SAR, and Taiwan, China. Hainan, seated in the southernmost tropical region of China, is home to ticks that remain active throughout all four seasons. This heightens their potential to transmit tick-borne diseases to both animals and humans. This study provides a succinct overview of the prevailing tick species' spatial distribution and offers an outline of the range and dispersion of emerging tick-borne infections in tick vectors, animal hosts, and human populations within Hainan, China.

INTRODUCTION

China encompasses six tropical regions, namely Guangdong Province, Yunnan Province, Hainan Province, Hong Kong Special Administrative Region (SAR), Macau SAR, and Taiwan, China. Of these, Hainan occupies the southernmost tropical region of China, notable for active vectors year-round which enhances their capacity to transmit vector-borne diseases (VBDs) to both animals and humans (*1*–*2*). Emerging and reemerging tick-borne diseases (TBDs) play a significant role in VBDs and present new health challenges to both humans and animals on this tropical island. The first recording of ticks on Hainan Island dates to 1981 with the documentation of a novel tick species, *Amblyomma hainanensis*, by the distinguished Chinese acarologist Kuofan Teng (commonly known as Guofan Deng in contemporary Chinese pronunciation) (*3*). Further, in 1985, the initial tick-borne pathogen (TBP), indicated by seropositivity to spotted fever group *Rickettsia* (SFGR), was identified in six of 402 healthy volunteers from the island (*4*). Since then, Professor Bihu Li has been recognized for significantly contributing to the reporting and research of *Rickettsia* infections in Hainan (*5*–*7*). The discovery of severe fever with thrombocytopenia syndrome (SFTS) in Chinese mainland led to an increase in research on ticks and TBDs on Hainan Island. This study presents a succinct overview of the spatial distribution of tick species and illuminates patterns in the types and distribution of emerging tick-borne infections in tick vectors, animal hosts, and humans.

The Prevalence of Ixodid Tick Species on Hainan Island, China

In conducting a systematic review of current citations on ticks in Hainan (methods detailed in the Supplementary Material, available in https://weekly.chinacdc.cn/), 21 species of hard ticks were identified. These include ten *Haemaphysalis* species, five *Amblyomma* species, three *Rhipicephalus* species, one *Ixodes* species, one *Hyalomma* species and one *Dermacentor* species. These were discovered on the island, representing approximately 17% of the 124 tick species in China (*8*). As of yet, there's no record of soft ticks from Hainan Island.

The *Rhipicephalus* genus is the most common tick genus found on Hainan Island, albeit consisting only of three species — *Rhipicephalus sanguineus* sensu lato (s. l.), *Rhipicephalus haemaphysaloides*, and *Rhipicephalus microplus*. *Rhipicephalus* ticks have been recorded almost everywhere on the island except for the southwestern county of Dongfang, the eastern county of Qionghai and the southeastern county of Linshui (*9*–*17*) (Figure 1).

Haemaphysalis ticks are plentiful in Chengmai, Qiongzhong, and Sanya City but much less common in Wanning, Haikou City, and Changjiang. They have not been recorded in the rest of the island's counties or cities (*9*–*17*) (Figure 1). *Amblyomma* ticks have been reported in only five locations, inclusive of Chengmai, Lingao, Dingan, Qiongzhong, and Sanya (*9*,*11*,*17*–*18*) (Figure 1).

Ixodes granulatus has been documented in Haikou, Chengmai, Baisha, Qiongzhong, Wanning, and Sanya counties, with three incidences in Chengmai and Qiongzhong counties (*10*–*17*,*19*–*20*). The remaining species, *Dermacentor auratus* and *Hyalomma isaaci*,

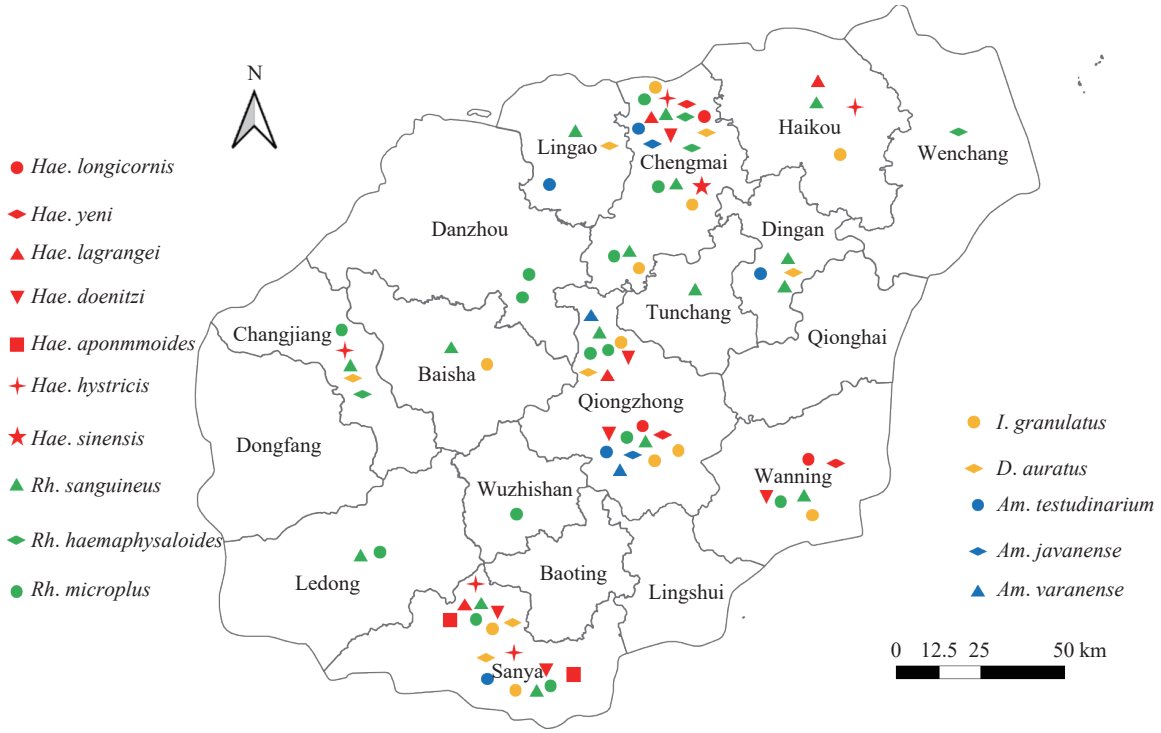


FIGURE 1. Geographical distribution of hard ticks on Hainan Island in China, 1980–2023.
Haemaphysalis formosensis, Hae. cornigera, *Hae. mageshinaensis*, *Am. hainanense*, *Am. helvolum*, and *Hy. isaaci* were not drawn on the map due to the unspecified place recorded in the references.

have likewise been recorded (*9–11*,*13*,*15*,*17*,*18*,*21–22*) (Figure 1). The host of these hard ticks on the island varies and ranges from birds and reptiles to mammals (Supplementary Table S1, available in https://weekly.chinadc.cn).

The Emergence of Tick-borne Infections on Hainan Island, China.

A comprehensive review of existing literature on TBPs in Hainan was undertaken (detailed methodology is provided in the Supplementary Material). The agents infecting ticks, animals, and humans can be classified into three categories: bacteria, protozoans, and viruses. Bacteria constitute the principal category of TBPs, represented by twenty species, including six *Borrelia*, four *Anaplasma*, four *Rickettsia*, four *Ehrlichia*, and two *Coxiella* species. Protozoans form the second largest group with ten species, including four *Babesia*, five *Theileria*, and one *Hepatozoon* species. There are only two known viral pathogens: a novel Alphavirus and the Crimean-Congo hemorrhagic fever virus (CCHFV). In the eighteen administrative divisions, TBPs are predominantly reported in the central and northern regions, with fewer instances cited in the southern parts of Hainan Island (Figures 2–5).

Rickettsia species have emerged as the primary pathological agents causing TBDs in Hainan Island, China, with documented human infections present throughout the region. Cases have been noted in Chengmai and Haikou to the north, Qionghai and Wanning to the east, Qiongzhong in the center, Danzhou City to the west, and Sanya to the south (*6*,*12*,*23–25*). As of mid-2023, the identified species of *Rickettsia* on Hainan Island include *Rickettsia siberica*, *Rickettsia heilongjiangiensis*, and an unidentified member of the SFGR group (*6*,*12*,*23–25*) (Figures 2 and 5, Supplementary Tables 2–3, available in https://weekly.chinacdc.cn/).

Species of *Anaplasma* reported in the region include *Anaplasma bovis*, *Anaplasma marginale*, *Anaplasma phagocytophilum*, and *Anaplasma platys*. *Anaplasma*, predominantly located in the northeastern areas of Hainan, like Haikou and Qionghai, but with instances of *A. platys* infections in the southwestern and southern zones (*11*,*26–30*). Reservoir hosts for *A. phagocytophilum* on the island may include cattle, goats, and dogs, with *Rh. sanguineus* and *Rh. microplus* reported as potential vector species for agent

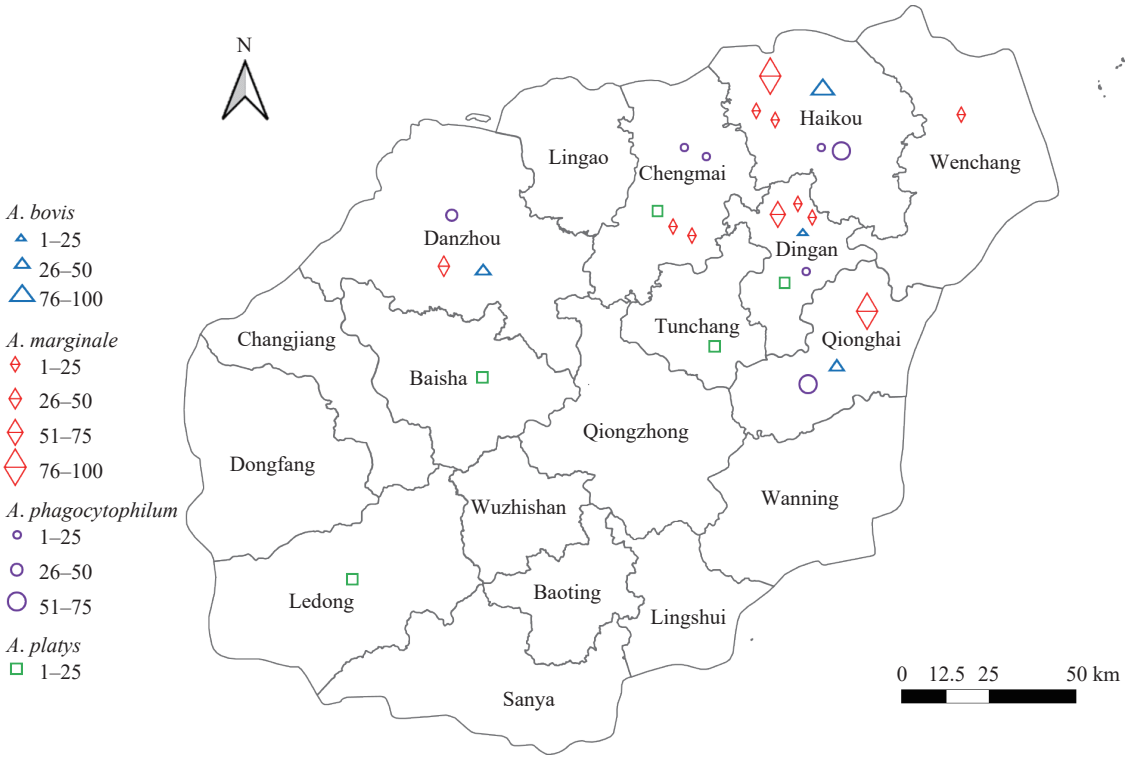


FIGURE 2. Geographic distribution of emerging infections caused by agents in the *Anaplasma* genus in ticks and animals in Hainan, China.
Note: In an uncharted area of the region, infection with *Anaplasma marginale* was detected, expanding its known presence to Haikou, the region's capital. Samples collected from Baisha, Ledong, Dingan, and Tunchang tested positive for *A. platys* with an infection rate of 1.1%. For the purposes of this study, specific values were assigned to each site: Baisha, Ledong, Dingan, and Tunchang.

circulation (*26*,*28*–*29*,*31*) (Figures 3 and 5, Supplementary Tables S2–S3).

Genospecies of *Borrelia burgdorferi* s. l., including *Borrelia garinii*, which cause human Lyme borreliosis, have been detected in human cases in Haikou (*23*), and other uncharacterized genospecies of *Bo. burgdorferi* s. l. have infected individuals in Wenchang, Danzhou, Dongfang, Qiongzhong, Haikou, and Sanya, with prevalence rates between 1.99% and 9.96% (*32*–*33*). Four spirochetes of the *Bo. burgdorferi* s. l. group, including *Borrelia afzelii*, *Bo. garinii*, *Borrelia valaisiana*, and *Borrelia yangtzensis*, were found in *Rh. microplus* ticks (Figures 3 and 5, Supplementary Tables S2–S3).

Tick-borne protozoan infections, significant threats to animal health, have been frequently reported in the northern part of Hainan Island, especially in Dingan, Haikou, Danzhou, and Chengmai (*11*,*30*,*34*–*37*) (Figure 4, Supplementary Table S2). Also, two tick-borne viruses carry potential implications for bovine health; a novel Alphavirus was identified in ticks from cattle in Danzhou (*38*), and anti-CCHFV IgG was confirmed in bovine serum samples from Haikou, Chengmai, Dongfang, and Sanya (*39*).

DISCUSSION AND CONCLUSION

The tick species *Haemaphysalis longicornis* is among the most extensively distributed across Chinese mainland, yet it has been identified in merely three out of 18 administrative regions on Hainan Island (*8*,*11*,*17*,*40*–*41*). This uneven distribution may be attributed to the potential limited exploration of hosts and associated vegetation types within other regions, which may consequently introduce a bias in its perceived distribution.

Serological methods have detected instances of human infections with SFGR on Hainan Island, an indication of the natural circulation of SFGR in this region. As such, there is a pressing need to intensify efforts to monitor infection rates and assess the risk of pathogen spillover from ticks and animals to humans in urban areas or counties where no human infection has been reported to date.

Recent years have seen an increased involvement of

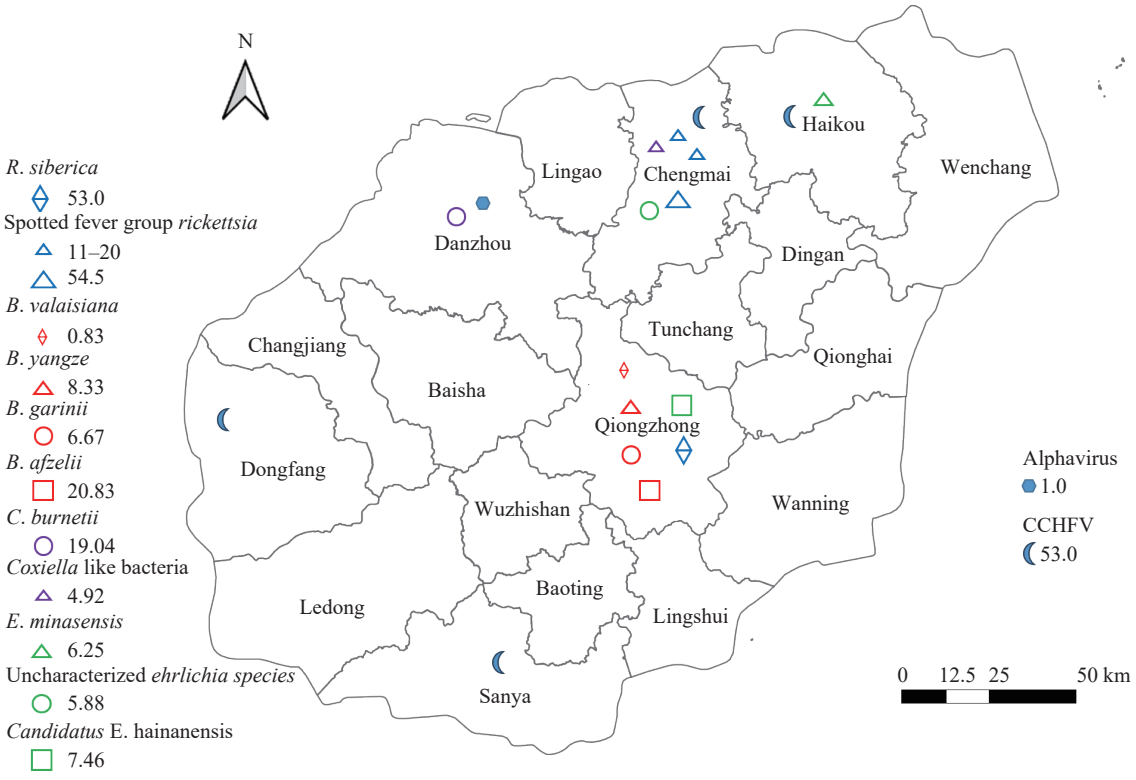


FIGURE 3. Geographic distribution of emerging infections from viruses and bacteria, excluding the *Anaplama* genus, in ticks and animals in Hainan, China.
Abbreviation: CCHFV=Crimean-Congo haemorrhagic fever virus.

scientists from diverse academic backgrounds in tick and TBD research. This has undoubtedly broadened the scope of research topics and enriched academic discourse. However, this growth also introduces potential issues concerning data reliability, particularly regarding potential misidentification errors due to minor morphological differences amongst tick species, lack of researcher expertise, or inaccurate description of new species. As an illustration, the *Hae. longicornis* species was often confused with *Haemaphysalis bispinosa* in South China until a clarification was made based on dental formula differences and distinctly pointed spurs on coxae II–IV, a morphological feature specific to *Hae. bispinosa* (*42*). Inexperienced researchers, particularly in regions where *Haemaphysalis hystricis* and *Hae. longicornis* coexist, may encounter difficulties correctly distinguishing them. However, the two species can be readily identified by noting differences in dental formula and body size: *Hae. longicornis* has a 5/5 dental formula and a smaller body size compared to *Hae. hystricis*, which possesses a 4/4 dental formula (*43*). To ensure data reliability, crucial in assessing tick and TBD risks, it is suggested that future researchers in tick identification collaborate with acarologists and employ molecular methods as means of verification and validation.

Ticks have been observed to be concentrated in specific regions of Hainan, China such as Chengmai, Qiongzhong, and Sanya, but appear less common in areas like Wenchang, Baisha, and Wuzhishan. Remarkably, several regions including Qionghai, Dongfang, Baoting, and Lingshui have yet to record any tick species. However, this distribution does not align with the clustering of TBPs which predominantly occur in northern Hainan, specifically in Dingan and Haikou.

It appears that investigations into ticks and TBPs are incomplete in several areas, evidenced by absent or inadequate reports from locales such as Dingan, Haikou, and Sanya. To rectify this, the implementation of thorough surveillance for both ticks and TBPs is strongly advised under the following conditions: 1) where surveys have not yet been undertaken; 2) where ticks and TBPs are pervasive and surrounding areas have reported none or minimal occurrences; 3) where TBPs are rife, though ticks are sparsely identified; 4) where tick sightings are frequent, necessitating intensive TBP detection; 5) where ticks

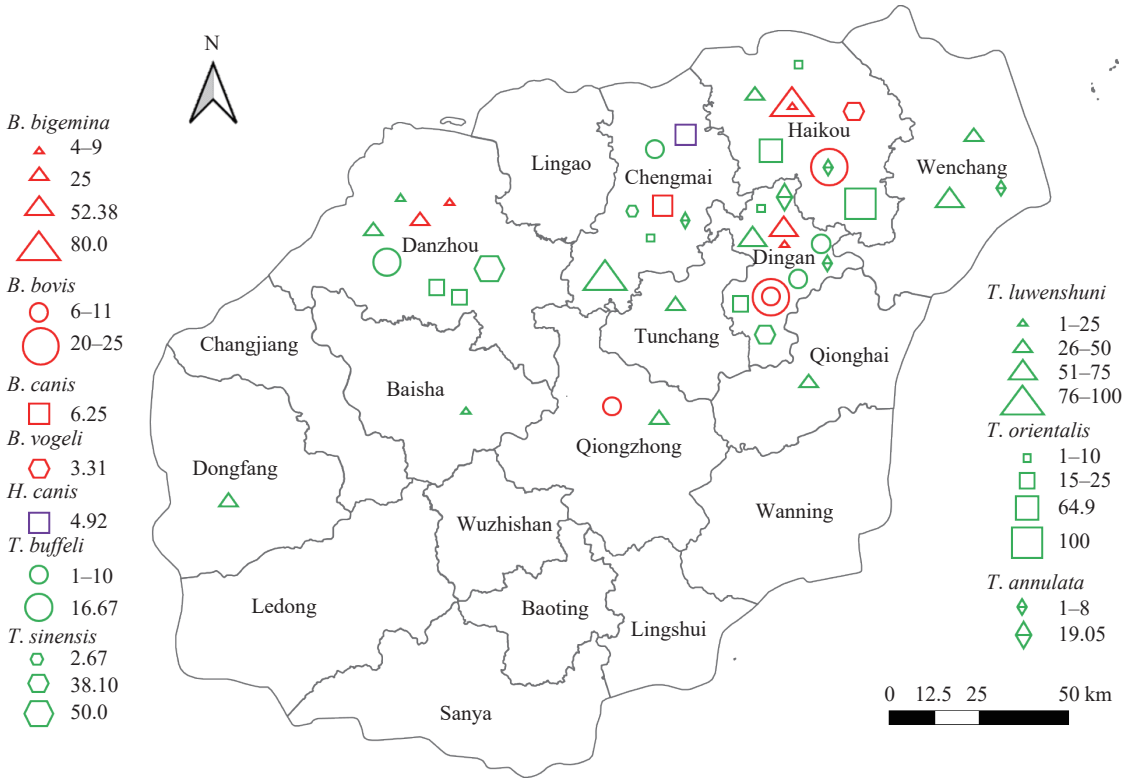


FIGURE 4. Geographic distribution of emerging protozoan infections in ticks and animals in Hainan, China.
Note: A documented case of *Babesia vogeli* infection was identified in an unspecified area of the region, extending its known occurrence to Haikou, the capital of this region. Furthermore, a case of *Babesia bovis* infection, previously found in Central Hainan, has now been registered within the location of Qiongzhong. The term "Qiongzhong" in Chinese denotes "Central part of Hainan," accurately portraying its geographical siting in the central part of the island.

and TBPs demonstrate high infection rates in humans and animals, necessitating strict monitoring of TBDs; and 6) if TBDs become epidemic, causing significant morbidity or mortality, and existing in similar latitudinal, biogeographical, or microclimatic conditions.

Finally, to streamline and boost the effectiveness of these efforts, the establishment of a comprehensive surveillance system for ticks and TBDs is critical.

The present study exhibits certain limitations. Initially, the non-random nature of the chosen locations for surveying ticks and TBPs could have potentially introduced bias into the determination of the distribution of ticks and TBPs. Furthermore, the majority of the ticks and TBPs were extracted from domestic animals, raising the likelihood of excluding ticks and TBPs associated with wild animals within the surveyed locations. Moreover, some ticks and TBP detection records used in the study date back more than four decades; given this substantial period, significant changes should be anticipated in the habits of ticks and TBPs. Despite these constraints, the study still offers valuable insights into understanding ticks and TBDs in Hainan, China.

Acknowledgements: Professor Guojing Yang and Mr. Mingfa Li for illustrating the geographical distributions of ticks and TBPs.

Funding: Supported by the Major Science and Technology Program of Hainan Province (grants ZDKJ202003, ZDKJ2021036, and SKJ10006). Additionally, financial support was received from the National Natural Science Foundation of China (grant 81960002) and the Science and Technology Plan of Hainan Province (specifically, the Hainan Province Clinical Medical Center, SRC220002). The research benefited from a grant awarded by the Hainan Provincial Natural Science Foundation of High-Level Talent Project (821RC1152).

doi: 10.46234/ccdcw2023.157

[#] Corresponding author: Qianfeng Xia, xiaqianfeng@hainmc.edu.cn.

[1] Key Laboratory of Tropical Translational Medicine of Ministry of Education, NHC Key Laboratory of Tropical Disease Control, School of Tropical Medicine, Hainan Medical University, Haikou City, Hainan Province, China.

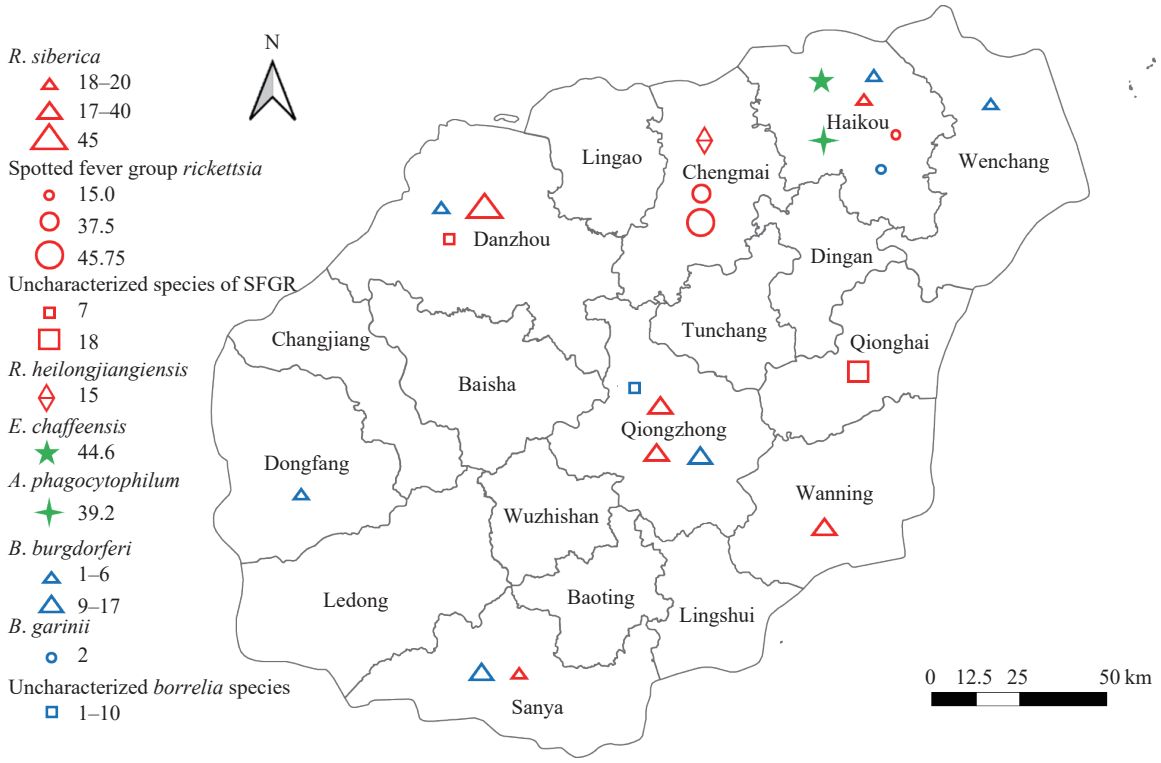


FIGURE 5. Documented distributions of human tick-borne pathogen infection at the county level in Hainan, China.
Note: The documentation of infections from the spotted fever group *Rickettsia*, *Anaplasma phagocytophilum*, and *Ehrlichia chaffeensis* has been extended to include an unidentified region within Haikou. Similarly, a case of infection with an uncharacterized species of *Borrelia* in the central area of Hainan has been reported in Qiongzhong.

Submitted: August 07, 2023; Accepted: September 08, 2023

REFERENCES

1. Li YJ, Zhou GF, Zhong SF, Wang XM, Zhong DB, Hemming-Schroeder E, et al. Spatial heterogeneity and temporal dynamics of mosquito population density and community structure in Hainan Island, China. Parasit Vectors 2020;13(1):444. http://dx.doi.org/10.1186/s13071-020-04326-5.
2. Sun DW, Wang SQ, Zhao W, Wang GR, Wang YY, Zeng LH, et al. Investigation of seasonal fluctuation of ticks on bulls and dogs in Chengmai County in Hainan Province. China Trop Med 2011;11(9):1081 – 2. http://dx.doi.org/10.13604/j.cnki.46-1064/r.2011.09.005. (In Chinese).
3. Teng KF. A new species of *Amblyomma* from Hainan island, China (Acarina: Ixodidae). Acta Zootaxon Sin 1981;6(4):399 – 401. https://kns.cnki.net/kcms2/article/abstract?v=Nk4cZQau9qG4sQaGHFMpoBUzgAZOlmtBX5tIrgel6KRCuiicwJ9jP6gVWXLBvFuG4wm0JC77YFkRKyCbnJGfPY3fyZsWdjI0P1uT1fgWDMr6ZdKhXviR9g==&uniplatform=NZKPT. (In Chinese).
4. Feng HM, Yi Q, Pang HL, Tu YY, Lin BH, Zhou SY, et al. Investigation on spotted fever Rickettsia seropositive individuals in healthy volunteers from Hainan Island. Chin J Epidemiol 1988;9(2):83. http://chinaepi.icdc.cn/zhlxbxen/ch/reader/view_abstract.aspx?file_no=19880219&flag=1. (In Chinese).
5. Zhang JZ, Fan MY, Bi DZ, Sun FX, Cheng ZX, Lan FC, et al. The application of PCR to epidemiological study on spotted fever group rickettsiae. Chin J Epidemiol 1995;16(1):25 – 8. http://dx.doi.org/10.3760/cma.j.issn.0254-6450.1995.01.112. (In Chinese).
6. Lin BH, Sun XJ, Zhan ZN, Lin YZ, Li WG. Serological and etiological studies on undefined rickettsiosis on Hainan island. J Hainan Med Univ 1997;3(3):97 – 102. https://kns.cnki.net/kns8/Detail?sfield=fn&QueryID=0&CurRec=1&recid=&FileName=HNYY703.000&DbName=CJFD9697&DbCode=CJFD&yx=&pr=&URLID=. (In Chinese).
7. Lin BH. Determination of species specific antibodies in Rickettsia by DEB-ELISA. J Hainan Med Coll 1996;2(2):49 – 51. http://dx.doi.org/10.13210/j.cnki.jhmu.1996.02.011.
8. Zhao GP, Wang YX, Fan ZW, Ji Y, Liu MJ, Zhang WH, et al. Mapping ticks and tick-borne pathogens in China. Nat Commun 2021;12(1):1075. http://dx.doi.org/10.1038/s41467-021-21375-1.
9. Deng GF, Jiang ZJ. Economic insect fauna of China.Fasc 39. Acarina: Ixodidae. Beijing: Science Press. 1991. http://find.nlc.cn/search/showDocDetails?docId=-1480479448559231182&dataSource=ucs01&query=%E4%B8%AD%E5%9B%BD%E7%BB%8F%E6%B5%8E%E6%98%86%E8%99%AB%E5%BF%97. (In Chinese).
10. Huang JL, Long ZM, Dong QZ, Liu JH, Zhou PS, Yu XL. Investigaton of Murine-like animal and their external parasites in Mountain area of Southern Hainan province. Chin J Vector Biol Control 1995;6(1):37 – 41. https://kns.cnki.net/kns8/Detail?sfield=fn&QueryID=0&CurRec=1&recid=&FileName=ZMSK501.018&DbName=CJFD9495&DbCode=CJFD&yx=&pr=&URLID=. (In Chinese).
11. Lu M, Tang GP, Bai XS, Qin XC, Wang W, Guo WP, et al. Molecular detection of tick-borne pathogens in ticks collected from Hainan Island, China. Biomed Environ Sci 2021;34(7):581 – 6. http://dx.doi.org/10.3967/bes2021.081.
12. Liu JH, Yu XL, Long ZM, Yang FR, Chen WJ, Tian XD, et al. Spotted fever foci in Hainan island. J Prev Med Chin People's Liber Army 1996;14(5):317 – 9. https://kns.cnki.net/kns8/Detail?sfield=fn&QueryID=0&CurRec=1&recid=&FileName=JYYX605.001&DbName=CJFD9697&DbCode=CJFD&yx=&pr=&URLID=. (In Chinese).

13. Huang JL, Zhou PS, Long ZM. The population structure and spatial distribution of ticks, mites and fleas on rodents. Entomol Knwl 1997;34(6):337-9. https://d.wanfangdata.com.cn/periodical/QK199700781761. (In Chinese).
14. Liu JH, Huang JL. Community structure of ticks parasitic on small mammals from Hainan, China. Zool Res 1994;15(4):45-8. https://www.zoores.ac.cn/cn/article/id/2243. (In Chinese).
15. Tian XD, Chen WJ, Lu ZZ, Zhang JZ, Liu JH, Yu XL. Investigation of the spotted fever vectors and host animals in Dafeng farm of Qiongzhong in Hainan province. Chin J Zoonoses 1999;15(2):94. https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKjkpgKvIT9Nkm5tS6uBYqSHTdS3ItQuTWgH5apOtHs3IXAhskFkBME5-aSkUmdSXAR4-1uEVe_Rs&uniplatform=NZKPT. (In Chinese).
16. Lin LH, Bai ZJ, Cai J, Fang MY. Investigation of the ticks parasitized on the domestic animals in Qiongzhong area of Hainan provice. Chin J Vector Biol Control 2008;19(6):544 – 5. http://dx.doi.org/10.3969/j.issn.1003-4692.2008.06.016. (In Chinese).
17. Sun DW, Zhao W, Zeng LH, Li SG. Investigation of species of Ixodidae and a new record species of Ixodidae in Hainan province, China. Chin J Vector Biol Control 2013;24(5):442 – 3. http://dx.doi.org/10.11853/j.issn.1003.4692.2013.05.018. (In Chinese).
18. Zhao W, Lao SJ, Wang ZG, Jia PB, Zeng LH, Li SG, et al. Investigation of rodents and their ectoparasites in Hainan province. Chin J Vector Biol Control 2007;18(6):519 – 21. http://dx.doi.org/10.3969/j.issn.1003-4692.2007.06.031. (In Chinese).
19. Li JJ, Liu XX, Mu JQ, Yu XB, Fei YD, Chang J, et al. Emergence of a novel *Ehrlichia minasensis* strain, harboring the major immunogenic glycoprotein trp36 with unique tandem repeat and C-terminal region sequences, in Haemaphysalis hystricis ticks removed from free-ranging sheep in Hainan province, China . Microorganisms 2019;7(9):369. http://dx.doi.org/10.3390/microorganisms7090369.
20. Sun DW, Wang SQ, Zhao W, Wang GR, Wang YR, Zeng LH, et al. Seasonal succession of ticks in small wild animals in Chengmai county of Hainan province. Chin J Public Health 2012;28(12):1620 – 2. http://dx.doi.org/10.11847/zgggws-2012-28-12-25. (In Chinese).
21. Chen Z, Yang XJ. Tick systematics. Beijing: Science Press. 2021. http://find.nlc.cn/search/showDocDetails?docId=5310202273693347 21&dataSource=ucs01&query=%E8%9C%B1%E7%9A%84%E7%B3%BB%E7%BB%9F%E5%88%86%E7%B1%BB%E5%AD%A6. (In Chinese).
22. Chen Z, Yang XJ, Bu FJ, Yang XH, Yang XL, Liu JZ. Ticks (acari: ixodoidea: argasidae, ixodidae) of China. Exp Appl Acarol 2010;51(4):393 – 404. http://dx.doi.org/10.1007/s10493-010-9335-2.
23. Fang LQ, Liu K, Li XL, Liang S, Yang Y, Yao HW, et al. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis 2015;15(12):1467 – 79. http://dx.doi.org/10.1016/S1473-3099(15)00177-2.
24. Wu J, Wang SL, Jin YM, Ma Y, Zeng XJ, Jia PB, et al. Detection of groEL gene in spotted fever group Rickettsia of patients with fever in Hainan province. Mod Prev Med 2014;41(13):2417-9. https://d.wanfangdata.com.cn/periodical/xdyfyx201413037. (In Chinese).
25. Jin YM, Zhang LJ, Sun LY, Lao SJ, Han J, Huang CH, et al. Epidemiologyical investigation of emerging spotted fever in Chengmai county, Hainan province. Dis Surveill 2011;26(1):18 – 22. http://dx.doi.org/10.3784/j.issn.1003-9961.2011.01.007. (In Chinese).
26. Huang LY, Zhou S, Liao HY, Wang JH. Molecular epidemiological investigation and genetic diversity analysis of Anaplasmas cattle in Hainan. Trop Agric Eng 2021;45(6):9-15. https://d.wanfangdata.com.cn/periodical/rdnygc202106003. (In Chinese).
27. Zhou S, Lin Y, Zu HY, Wang JH. Identification of tick species and investigation of *Anaplasma* spp . in some areas of Hainan. J Trop Biol 2023;14(2):159 – 65. http://dx.doi.org/10.15886/j.cnki.rdswxb.2023.02.004. (In Chinese).
28. Zhang LJ, Liu H, Xu BL, Lu QY, Li L, Chang LT, et al. *Anaplasma phagocytophilum* infection in domestic animals in ten provinces/cities of China . Am J Trop Med Hyg 2012;87(1):185 – 9. http://dx.doi.org/10.4269/ajtmh.2012.12-0005.
29. Wang SW, He J, Zhang LJ. Serological investigation of vector-borne disease in dogs from rural areas of China. Asian Pac J Trop Biomed 2012;2(2):102 – 3. http://dx.doi.org/10.1016/S2221-1691(11)60201-3.
30. Bi TL. Molecular epidemiological investigation study on cattle infected with *Anaplasma*, *Babesia* and *Theileria* in Qiongbei area [dissertation]. Haikou: Hainan University; 2021. http://dx.doi.org/10.27073/d.cnki.ghadu.2021.000933. (In Chinese).
31. Zhang LJ, Liu H, Xu BL, Zhang ZL, Jin YM, Li WM, et al. Rural residents in China are at increased risk of exposure to tick-borne pathogens *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* . BioMed Res Int 2014;2014:313867. http://dx.doi.org/10.1155/2014/313867.
32. Zhang L, Zhu X, Hou XX, Li H, Yang XN, Chen T, et al. Prevalence and prediction of Lyme disease in Hainan province. PLoS Negl Trop Dis 2021;15(3):e0009158. http://dx.doi.org/10.1371/journal.pntd.0009158.
33. Zhu X, Zhang L, Hou XX, Geng Z, Chen H, Chen T, et al. Investigation on Lyme disease of patients with arthritis or neurological disorders in Hainan Province, China. Chin J Zoonoses 2015;31(4):353 – 6. http://dx.doi.org/10.3969/cjz.j.issn.1002-2694.2015.04.013. (In Chinese).
34. Wei FR, Lan QX, Zhu D, Ye JH, Liu Q, Zhang Y. Investigation on *Babesia* in ticks infested on police dogs in selected areas of China. Chin J Parasitol Parasit Dis 2012;30(5):390-2. http://qikan.cqvip.com/Qikan/Article/Detail?id=43679584. (In Chinese).
35. Wang JM, Yang JF, Gao SD, Wang XX, Sun H, Lv ZY, et al. Genetic diversity of *Babesia* bovis MSA-1, MSA-2b and MSA-2c in China. Pathogens 2020;9(6):473. http://dx.doi.org/10.3390/pathogens9060473.
36. Wang JM, Yang JF, Liu JL, Wang XX, Xu JL, Liu AH, et al. Molecular detection and genetic diversity of *Theileria orientalis* in cattle in China . Parasitol Res 2018;117(12):3689 – 94. http://dx.doi.org/10.1007/s00436-018-6023-3.
37. Yang L, Wang JH, Upadhyay A, Zhao JG, Huang LY, Liao CH, et al. Identification of *Theileria* spp . and investigation of hematological profiles of their infections in goats in Hainan Island, China. Parasite 2022;29:13. http://dx.doi.org/10.1051/parasite/2022013.
38. Chen WZ, Qiu FX, Calisher CH, Liu JC, Chen HX, Li XD, et al. Twenty-eight alphavirus strains isolated from mosquitoes and ticks captured from Hainan Island, China. Chin J Exp Cin Virol 1997;11(2):144 – 6. http://dx.doi.org/10.3760/cma.j.issn.1003-9279.1997.02.117. (In Chinese).
39. Qi JS, Chen YB, Wu JJ, Wang M, Chen WZ, Feng CH, et al. IgG antibodies against Crimean-Congo hemorrhagic fever virus were firstly found in bovine serum in Hainan province. Chin J Vector Biol Control 1995;6(2):158. http://www.bmsw.net.cn/CN/Y1995/V06/I2/158. (In Chinese).
40. Zheng WQ, Xuan XN, Fu RL, Tao HY, Xu RM, Liu YQ, et al. Preliminary investigation of ixodid ticks in Jiangxi Province of Eastern China. Exp Appl Acarol 2019;77(1):93 – 104. http://dx.doi.org/10.1007/s10493-018-0324-1.
41. Zhao L, Li J, Cui XM, Jia N, Wei JT, Xia LY, et al. Distribution of *Haemaphysalis longicornis* and associated pathogens: analysis of pooled data from a China field survey and global published data . Lancet Planet Health 2020;4(8):e320 – 9. http://dx.doi.org/10.1016/S2542-5196(20)30145-5.
42. Chen Z, Li YQ, Ren QY, Liu ZJ, Luo J, Li K, et al. Does *Haemaphysalis bispinosa* (Acari: Ixodidae) really occur in China? . Exp Appl Acarol 2015;65(2):249 – 57. http://dx.doi.org/10.1007/s10493-014-9854-3.
43. Zheng WQ, Hu HJ, Tian JH, Liang SJ, Wang YY. Differentiation of *Haemaphysalis hystricis* from *Haemaphysalis longicornis* based on the QIAxcel Connect automated RNA/DNA analysis system . Acta Parasitol Med Entomol Sin 2023;30(1):18 – 24. http://dx.doi.org/10.3969/j.issn.1005-0507.2023.01.004. (In Chinese).

SUPPLEMENTARY MATERIAL

Search Strategy

This systematic review was devised and conducted in alignment with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Institute et al., 2020). The search for relevant literature primarily employed English language databases including Web of Science, PubMed, and Mendeley. In addition, Chinese language databases such as CNKI (China National Knowledge Infrastructure) and the Chinese Medical Journal Full-Text Database (CMJFTD) were utilized. We also incorporated other pertinent literature, including select Chinese professional texts about ticks and tick-borne diseases. Our keyword strategy incorporated terms such as "ticks of domestic animals," "ticks of livestock," "ectoparasite of domestic animals," "ectoparasite of livestock," "ticks of rodents," "ticks of cattle or cows," "ticks of cats and dogs," "ticks from vegetation," "ticks from breeding site," "tick and tick-borne disease of livestock," "the tropical island of China," "the southernmost region of China," and "Hainan." We included all publications ranging from the 1980s to May 30, 2023. Literature was systematically screened by evaluating the title, abstract, and the results and conclusions of the manuscripts.

Criteria for Inclusion and Exclusion

This review incorporated articles from the aforementioned databases that referenced ticks or ticks in conjunction with other ectoparasites and TBPs in ticks or rodents and domestic animals (inclusive of ruminants, goats, cattle, dogs, and cats) in Hainan Island. Criteria for inclusion specified the tick or TBP's collection location as Hainan Island or the tropical island of China, publication in English or Chinese, taxonomic identification of ticks to the species level, classification of tick-borne viruses to the family level, and analysis of other TBPs such as bacteria and protozoans to the genus level and then to the species level. The term "tick" also needed to be present in the title, abstract, results, or conclusion section of the text.

References were excluded from this review if they contained these characteristics: the depiction of ectoparasites other than ticks, the term "tick" or "tick-borne pathogen" only located in the introduction and discussion section, the term "tick" located in the results section but used within a phylogenetic analysis of ticks from different regions, the term "tick" linked with migratory birds or imported animals or goods on the tropical island, and tick or TBP study relying solely on laboratory data. Studies focusing on the insecticide chlorpyrifos, the Chinese term "毒死蜱" with the Chinese term "蜱", along with studies where ticks were not identified to the species level also were excluded.

Selection of Studies and Evaluation of Quality

Duplicate articles found in multiple databases were removed, leaving only one copy for further analysis. Initial screening of articles was conducted by examining the titles and abstracts. Articles that did not meet the stipulated inclusion and exclusion criteria were then disregarded. Moreover, a comprehensive review of the full articles was performed, with unqualified ones being excluded based on the established criteria. The review process was independently executed by two reviewers (WQZ and GYZ), and, in cases of disagreement, a third reviewer was brought in.

To assess the quality of articles, a checklist was generated that included categories such as the title, abstract, introduction, methods, results, and discussion. If the terms "Hainan", "South China", or "the tropical region of China" and "tick" or "TBP" concurrently appeared in the title, abstract, results, or concluding sections of an article, the article was screened as a potential candidate for further analysis.

Articles pinpointing sample locale to the county level and identifying specific tick species or specific TBP genus were primarily included in this study. Furthermore, articles providing more detailed information such as hosts of ticks or TBPs, or that specified sample locations to the township level were considered high-quality articles.

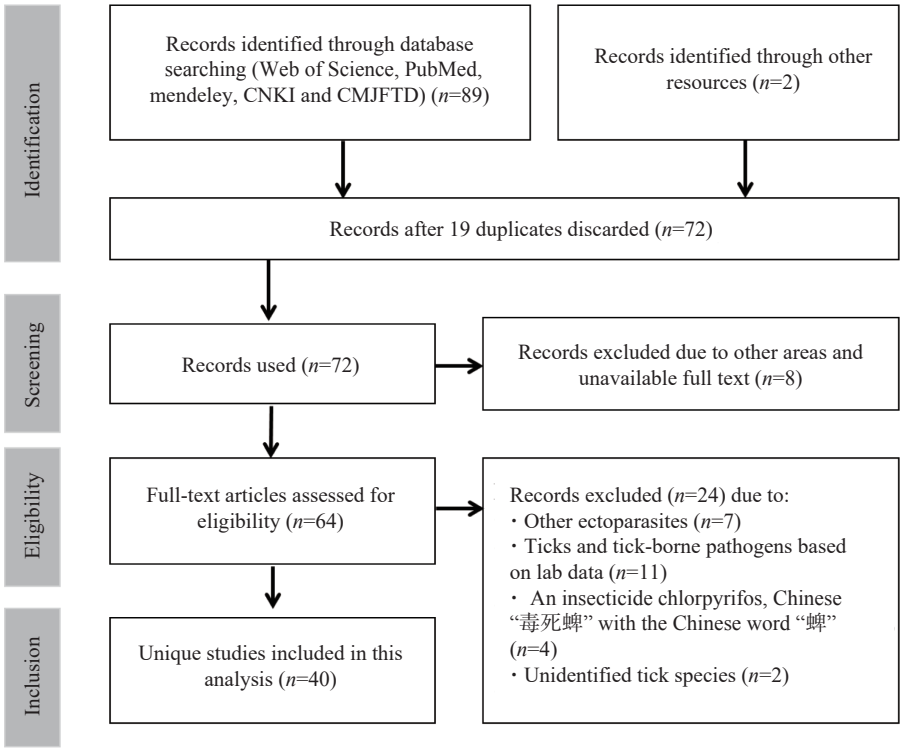
Data Extraction

The final studies included in this analysis provided the following data: 1) tick genus and species; 2) any available tick identification methods; 3) the specific county or city where the ticks were collected; and 4) details of the collection method and information on the host or environment, where applicable. The process of data extraction

was similarly implemented for tick-borne pathogens according to the aforementioned criteria. Subsequent data visualization and analysis were conducted using tables and maps.

Qualified Studies Screening

A comprehensive review was undertaken from five databases, yielding 89 pertinent publications: 28 from PubMed and CNKI each, 18 from Mendeley, 14 from Web of Science, and one from CMJFTD. An additional two publications were identified in a manual search, aligning with the eligibility requirements and were thus included. Upon subtracting 19 duplicates from this pool of 91, 72 publications were left, which then underwent vetting based on title, abstract, and full-text availability. This process resulted in the exclusion of eight additional publications. The remaining 64 underwent a comprehensive eligibility evaluation, resulting in the exclusion of seven publications that pertained to other ectoparasites, and the removal of 11 that used lab-reared ticks or lab-cultured pathogens. Four other papers that included references to chlorpyrifos, or the Chinese term "毒死蜱", were also omitted. Additionally, two more were removed due to a lack of specificity regarding the species of ticks, viral families, or other genera of the tick-borne pathogens. After two screening cycles, 40 unique studies met the selection criteria and were included in the final analysis (Supplementary Figure S1).



SUPPLEMENTARY FIGURE S1. Illustration of the study selection process for inclusion in this systematic review from 1980 to 2023, utilizing the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.
Abbreviation: CNKI=China National Knowledge Infrastructure; CMJFTD=the Chinese Medical Journal Full-Text Database.

SUPPLEMENTARY TABLE S1. Species of ticks found on the tropical island of China.

Number	Tick species	Ecological environment or zooparasite	Reference
1	*Hae. longicornis*	Wild animals and livestock, *Bubalus bubalis*, *Bos Taurus*, *Capra aegagrus hircus*, and *Canis lupus familiaris*	(*1–2*)
2	*Hae. formosensis*	*Sus scrofa*, dog and muntiacus	(*3–4*)
3	*Hae. yeni*	*Cervus unicolor*, *Cuon alpinus*, dog, wild and domestic animals, and *Capra aegagrus hircus*	(*1–3*)
4	*Hae. lagrangei*	*Muntiacus manijak* and other *Muntiacus* species, *Niviventer confucianus*, *Rattus fulvescents*, wild and domestic animals, rodent, *Bos Taurus*, and *Capra aegagrus hircus*,	(*1*, *3*, *5–9*)
5	*Hae. cornigera*	*Bubalus bubalis*, *Bos taurus* and rodent	(*3–4*)
6	*Hae. doenitzi*	*Francolinus pintadeanus*, *Centropus* spp., *Rycnonotus sinensis*, *Bambusicola thoracica*, *Rattus rattus hainanicus*, *Rattus rattus, Niviventer confucianus*, *Rattus fulvescents*, *Rattus coxingi*, *Rattus cremoriventer*, *Niviventer confucianus lotipes*, *Neohylomys hainanensis*, rodent, *Capra aegagrus hircus*, and *Gallus gallus domesticus*	(*1–3*, *5*, *7–11*)
7	*Hae. aponommoides*	*Rattus rattus hainanicus*, *Niviventer confucianus*, *Rattus coxingi*, *Rattus cremoriventer*, *Rattus rattus*, *Niviventer confucianus lotipes*, and *Neohylomys hainanensis*	(*5*, *7–8*)
8	*Hae. hystricis*	*Niviventer confucianus*, *Rattus coxingi*, *Dremomys pernyi*, wild and domestic animals, rodent, *Sus scrofa*, *Ovis aries*,	(*1*, *2*, *5–8*)
9	*Hae. sinensis*	*Bubalus bubalis*, *Canis lupus familiaris*, and farm's house	(*12*)
10	*Hae. mageshinaensis*	Wild and domestic animals	(*6*)
11	*Am. testudinarium*	*Bubalus bubalis*, *Bos Taurus*, *Sus scrofa*, *Cervus unicolor*, horse, goat, dog, pig, wild and domestic animals, and rodent	(*1–3*, *13*)
12	*Am. javanense*	*Manis pentadactyla*, *Python molurus*, *Varanus salvator*, *Geoemyda tricarinata*, wild and domestic animals, and snake	(*1–3*, *6*)
13	*Am. hainanense*	Snake	(*3–4*)
14	*Am. varanense*	*Varanus salvator*, *Python molurus*, *Naja,* and rodent	(*3*, *9–10*)
15	*Am. helvolum*	Snake	(*4*, *14*)
16	*Rh. sanguineus*	Stray dogs, *Canis lupus familiaris*, *Niviventer confucianus*, wild and domestic animals, *Canis lupus familiaris*, rodent, *Bubalus bubalis*, *Bos Taurus*, *Capra aegagrus hircus*, rodent, and *Lepus sinensis*	(*1–3*, *5–7*, *9*, *11*, *13*, *15–17*)
17	*Rh. haemaphysaloides*	*Bubalus bubalis*, *Bos Taurus*, *Equus asinus*, *Canis lupus familiaris*, *Capra aegagrus hircus*, *Ovis aries*, *Lepus sinensis*, *Sus scrofa*, *Cervus unicolor*, pig, farm's house, and wild and domestic animals	(*1–3*, *12*)
18	*Rh. microplus*	*Bos Taurus*, *Capra aegagrus hircus*, *Bubalus bubalis*, *Rattus rattus hainanicus*, *Rattus norvegicus*, farm's house, wild and domestic animals, and rodent	(*1–3*, *5*, *7*, *9*, *11*, *16*, *18–19*)
19	*I. granulatus*	*Rattus rattus hainanicus*, *Rattus rattus*, *Niviventer confucianus*, *Rattus norvegicus*, *Rattus rattoides*, *Rattus fulvescents*, *Rattus coxingi*, *Rattus cremoriventer*, *Rattus losea*, *Rattus flavipectus*, *Rattus edwardsi*, *Rattus norvegicus*, *Niviventer confucianus lotipes*, *Dremomys pernyi*, *Neohylomys hainanensis*, *Tupaia glis*, *Tamiops swinhoei maritimus*, wild and domestic animals, rodents, *Bos Taurus*, and *Capra aegagrus hircus*	(*1*, *2*, *5–11*, *20*)
20	*D. auratus*	*Sus scrofa*, *Bubalus bubalis*, *Arctonyx collaris*, *Selenarctos thibetanus*, *Canis lupus familiaris* and pig, *Rattus rattus hainanicus*, *Niviventer confucianus*, *Rattus cremoriventer*, wild and domestic animals, and rodent	(*1–3*, *5*, *7*, *9*, *13*)
21	*Hy. isaaci*	Bird, small mammals, *Bos Taurus*, and *Capra aegagrus hircus*	(*4*, *14*)

SUPPLEMENTARY TABLE S2. Incidence of ticks and their hosts for tick-borne pathogens.

Group	Genus	Species	Hosts/vector	Detection location	Detection rate (%)	Reference
Bacteria	*Anaplasma*	*A. bovis* (Ab), *A. marginale* (Am), *A. phagocytophilum* (Ap), Ab+Ap, Ab+Am, Ap+Am, Ap+Am+Ab	Cattle	Dingan	Ab, 6.8%; Am, 1.2%; Ap, 1.2%.	(*21*)
				Haikou	Ab, 100.0%; Am, 25.0%; Ap, 75.0%.	
				Qionghai	Ab, 40.0%; Am, 80.0%; Ap, 60.0%.	
				Danzhou	Ab, 50.0%; Am, 50.0%; Ap, 50.0%.	
		A. platys	*Rh. Sanguineus* from dogs	Baisha, Ledong, Dingan, Tunchang	1.1%	(*15*)
		A. phagocytophilum	RSH (*Rh. sanguineus* from farmer's house); RMGC (*Rh. microplus* from goats and cattle), RSD (*Rh. sanguineus* from dogs)	Chengmai	3.6 (RSH), 7.5 (RMGC and RSD)	(*22*)
		A. phagocytophilum	Dogs	Unknown place of Hainan	25	(*23*)
		A. marginale	Tick and cattle	Haikou	7.69 (Tick), 100.0 (cattle)	(*24*)
			Cattle and buffalo	Dingan	57.14 (cattle), 2.17 (buffalo)	
			Wagyu cattle	Chengmai	2.67	
			Tick	Wenchang	8.7	
		A. marginale	*Rh. microplus*	Chengmai	8.82	(*1*)
		A. platys	*Rh. sanguineus*	Chengmai	3.28	
	Rickettsia	*R. siberica*	Rodent	Qiongzhong	53.0	(*10*)
		Spotted fever group *Rickettsia*	Rodent, dog, and tick (*Rh. Haemaphysaloides* and *Rh. microplus*)	Chengmai	15.9 (Rodent), 16.7 (dog), 54.5 (tick)	(*12*)
	Borrelia	*Bo. afzelii*	*Rh. microplus*	Qiongzhong	20.83	(*25*)
		Bo. yangze,			8.33	
		Bo. valaisiana			0.83	
		Bo. garinii			6.67	
	Coxiella	*C. burnetii*	*Rh. microplus*	Danzhou	19.04	(*19*)
		Coxiella like bacteria	*Rh. sanguineus*	Chengmai	4.92	(*1*)
	Ehrlichia	*Candidatus* E. hainanensis	Rodents	Qiongzhong	7.46	(*26*)
		E. minasensis	*Hae. hystricis*	Haikou	6.25	(*27*)
		Uncharacterized *Ehrlichia* species	*Rh. microplus*	Chengmai	5.88	(*1*)

Continued

Group	Genus	Species	Hosts/vector	Detection location	Detection rate (%)	Reference
Protozoans	*Babesia*	*Ba. vogeli*	Ticks from dogs	Unknown place	3.31	(*28*)
		Ba. bovis	Cattle	Central part of Hainan	10.2	(*29*)
			Cattle	Haikou	20	(*24*)
			Cattle and buffalo	Dingan	23.81 (cattle), 6.52 (buffalo)	(*24*)
		Ba. bigemina (Bb), *Ba. bovis* (Bbo), Am+Bbo, Am+Bb, Bbo+Bb, Am+Bbo+Bb	Tick and cattle	Haikou	Bb: 7.69 (tick), 80 (cattle)	(*24*)
			Tick and cattle	Danzhou	Bb: 25 (cattle), 8.3 (tick)	
			Cattle and buffalo	Dingan	Bb: 52.38 (cattle), 4.35 (buffalo)	
		Ba. canis	*Rh. sanguineus*	Chengmai	6.25	(*1*)
	Theileria	*T. orientalis*	Cattle	Haikou	64.9	(*30*)
		T. luwenshuni	Goat	Baisha	20.7	(*31*)
				Qiongzhong	33.3	
				Dongfang	26.1	
				Chengmai	100	
				Qionghai	27.6	
				Dingan	62.2	
				Wenchang	65.1	
				Danzhou	31.0	
				Haikou	27.1	
				Tunchang	30.8	
		T. annulata	Tick	Haikou	7.69	(*24*)
		T. orientalis	Tick		7.69	
		T. orientalis	Cattle		100	
		T. sinensis	Cattle	Danzhou	50	
		T. orientalis	Cattle		25	
		T. orientalis	Tick		16.67	
		T. luwenshuni	Tick		8.33	
		T. buffeli	Tick		16.67	
		T. sinensis	Cattle	Dingan	38.10	
		T. orientalis	Cattle		23.81	
		T. buffeli	Cattle		9.52	
		T. annulata	Cattle		19.05	
		T. orientalis	Buffalo		2.17	
		T. buffeli	Buffalo		8.70	
		T. annulata	Buffalo		4.35	
		T. sinensis	Wagyu cattle	Chengmai	2.67	
		T. orientalis	Wagyu cattle		8.00	
		T. buffeli	Wagyu cattle		2.67	
		T. annulata	Wagyu cattle		2.67	
		T. luwenshuni	Tick	Wenchang	47.83	
		T. annulata	Tick		4.35	
	Hepatozoon	*Hepatozoon canis*	*Rh. sanguineus*	Chengmai	4.92	(*1*)
Virus	Alphavirus	NA	tick	Danzhou	1	(*32*)

Note: NA indicates data not available.

SUPPLEMENTARY TABLE S3. Incidence of tick-borne pathogens in humans.

Group	Genus	Species	Detection location	Detection rate (%) or persons	Reference
Bacteria	*Rickettsia*	*R. siberica*	Qiongzhong	38.3	(*10*)
		R. heilongjiangiensis	Chengmai	15*	(*33*)
		Uncharacterized species of SFGR	Danzhou, Qionghai	7* 18*	(*33*)
		SFGR	UP	15.0	(*34*)
		R. siberica	Wanning Sanya Danzhou Haikou Qiongzhong	27.62 18.56 45.00 19.15 39.71	(*35*)
		SFGR	Chengmai	37.5 or 45.75	(*12*)
	Borrelia	*Bo. garinii*	Haikou	2*	(*33*)
		Uncharacterized species of *Bo. burgdorferi* sensu lato	Central part of Hainan	1–10*	(*33*)
		Bo. burgdorferi s. l.	Wenchang Danzhou Dongfang Qiongzhong Haikou	1.99 3.36 4.62 16.67 5.88	(*36*)
		Bo. burgdorferi s. l.	Sanya	9.96	(*37*)
	Anaplasma	*A. phagocytophilum*	UP	39.2	(*38*)
	Ehrlichia	*E. chaffeensis*	UP	44.6	(*38*)
	Coxiella	*C. burneti*	UP	NA	(*39*)
Viruses	*Coltivirus*	Colorado tick fever virus	UP	NA	(*39*)
	Nairovirus	Crimean-Congo haemorrhagic fever virus	UP	NA	(*39*)

Note: UP stands for unspecified locations, which were not named in the relevant documents. NA indicates data not available.
* Denotes individuals with pathogen infection, rather than detection rate. SFGR refers to spotted fever group *Rickettsia*.

REFERENCES

1. Lu M, Tang GP, Bai XS, Qin XC, Wang W, Guo WP, et al. Molecular detection of tick-borne pathogens in ticks collected from Hainan Island, China. Biomed Environ Sci 2021;34(7):581 – 6. http://dx.doi.org/10.3967/bes2021.081.
2. Sun DW, Zhao W, Zeng LH, Li SG. Investigation of species of Ixodidae and a new record species of Ixodidae in Hainan province, China. Chin J Vector Biol Control 2013;24(5):442 – 3. http://dx.doi.org/10.11853/j.issn.1003.4692.2013.05.018. (In Chinese).
3. Deng GF, Jiang ZJ. Economic insect fauna of China.Fasc 39. Acarina: Ixodidae. Beijing: Science Press. 1991. http://find.nlc.cn/search/showDocDetails?docId=-1480479448559231182&dataSource=ucs01&query=%E4%B8%AD%E5%9B%BD%E7%BB%8F%E6%B5%8E%E6%98%86%E8%99%AB%E5%BF%97. (In Chinese).
4. Chen Z, Yang XJ. Tick systematics. Beijing: Science Press. 2021. http://find.nlc.cn/search/showDocDetails?docId=5310202273693347 21&dataSource=ucs01&query=%E8%9C%B1%E7%9A%84%E7%B3%BB%E7%BB%9F%E5%88%86%E7%B1%BB%E5%AD%A6. (In Chinese).
5. Huang JL, Long ZM, Dong QZ, Liu JH, Zhou PS, Yu XL. Investigaton of Murine-like animal and their external parasites in Mountain area of Southern Hainan province. Chin J Vector Biol Control 1995;6(1):37-41. https://kns.cnki.net/kns8/Detail?sfield=fn&QueryID=0&CurRec=1&recid=&FileName=ZMSK501.018&DbName=CJFD9495&DbCode=CJFD&yx=&pr=&URLID=. (In Chinese).
6. Li JJ, Liu XX, Mu JQ, Yu XB, Fei YD, Chang J, et al. Emergence of a novel *Ehrlichia minasensis* strain, harboring the major immunogenic glycoprotein trp36 with unique tandem repeat and C-terminal region sequences, in Haemaphysalis hystricis ticks removed from free-ranging sheep in Hainan province, China. Microorganisms 2019;7(9):369. http://dx.doi.org/10.3390/microorganisms7090369.
7. Huang JL, Zhou PS, Long ZM. The population structure and spatial distribution of ticks, mites and fleas on rodents. Entomol Knwl 1997;34(6):337-9. https://d.wanfangdata.com.cn/periodical/QK199700781761. (In Chinese).
8. Liu JH, Huang JL. Community structure of ticks parasitic on small mammals from Hainan, China. Zool Res 1994;15(4):45-8. https://www.zoores.ac.cn/cn/article/id/2243. (In Chinese).
9. Tian XD, Chen WJ, Lu ZZ, Zhang JZ, Liu JH, Yu XL. Investigation of the spotted fever vectors and host animals in Dafeng farm of Qiongzhong in Hainan province. Chin J Zoonoses 1999;15(2):94. https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKjkpgKvIT9Nkm5tS6uBYqSHTdS3ItQuTWgH5apOtHs3IXAhskFkBME5-aSkUmdSXAR4-1uEVe_Rs&uniplatform=NZKPT. (In Chinese).
10. Liu JH, Yu XL, Long ZM, Yang FR, Chen WJ, Tian XD, et al. Spotted Fever foci in Hainan island. J Prev Med Chin People's Liber Army 1996;14(5):317-9. https://kns.cnki.net/kns8/Detail?sfield=fn&QueryID=0&CurRec=1&recid=&FileName=JYYX605.001&DbName=CJFD9697&DbCode=CJFD&yx=&pr=&URLID=. (In Chinese).
11. Lin LH, Bai ZJ, Cai J, Fang MY. Investigation of the ticks parasitized on the domestic animals in Qiongzhong area of Hainan provice. Chin J Vector Biol Control 2008;19(6):544 – 5. http://dx.doi.org/10.3969/j.issn.1003-4692.2008.06.016. (In Chinese).
12. Jin YM, Zhang LJ, Sun LY, Lao SJ, Han J, Huang CH, et al. Epidemiologyical investigation of emerging spotted fever in Chengmai county, Hainan province. Dis Surveill 2011;26(1):18 – 22. http://dx.doi.org/10.3784/j.issn.1003-9961.2011.01.007. (In Chinese).

13. Zhao W, Lao SJ, Wang ZG, Jia PB, Zeng LH, Li SG, et al. Investigation of rodents and their ectoparasites in Hainan province. Chin J Vector Biol Control 2007;18(6):519 – 21. http://dx.doi.org/10.3969/j.issn.1003-4692.2007.06.031. (In Chinese).
14. Chen Z, Yang XJ, Bu FJ, Yang XH, Yang XL, Liu JZ. Ticks (acari: ixodoidea: argasidae, ixodidae) of China. Exp Appl Acarol 2010;51(4):393 – 404. http://dx.doi.org/10.1007/s10493-010-9335-2.
15. Zhou S, Lin Y, Zu HY, Wang JH. Identification of tick species and investigation of *Anaplasma* spp. in some areas of Hainan. J Trop Biol 2023;14(2):159 – 65. http://dx.doi.org/10.15886/j.cnki.rdswxb.2023.02.004. (In Chinese).
16. Sun DW, Wang SQ, Zhao W, Wang GR, Wang YY, Zeng LH, et al. Investigation of seasonal fluctuation of ticks on bulls and dogs in Chengmai County in Hainan Province. China Trop Med 2011;11(9):1081 – 2. http://dx.doi.org/10.13604/j.cnki.46-1064/r.2011.09.005. (In Chinese).
17. An LP, Pei YX, Suo PH, Han Q, Zhao JG. Biological characteristics and artificial laboratory rearing of *Rhipicephalus sanguineus* in Hainan. China Trop Med 2022;22(6):522 – 8. http://dx.doi.org/10.13604/j.cnki.46-1064/r.2022.06.07. (In Chinese).
18. Zhang L, Wen S, Hou XX, Li S, Yang XN, Chen T, et al. Detection and genotyping of *Borrelia burgdorferi* in parasitic ticks in Qiongzhong area of Hainan province, China. Chin J Vector Biol Control 2020;31(4):426 – 9,464. http://dx.doi.org/10.11853/j.issn.1003.8280.2020.04.009. (In Chinese).
19. Chen SD, Wu BW, Chen JR, Lian JH, Lin DX. Isolation of Q *Rickettsia* from *Boophilus microplus* in Hainan province. J Guangdong Pharm Univ 1989;5(2):42 – 5. http://dx.doi.org/10.16809/j.cnki.1006-8783.1989.02.011. (In Chinese).
20. Sun DW, Wang SQ, Zhao W, Wang GR, Wang YR, Zeng LH, et al. Seasonal succession of ticks in small wild animals in Chengmai county of Hainan province. Chin J Public Health 2012;28(12):1620 – 2. http://dx.doi.org/10.11847/zgggws-2012-28-12-25. (In Chinese).
21. Huang LY, Zhou S, Liao HY, Wang JH. Molecular epidemiological investigation and genetic diversity analysis of Anaplasmas cattle in Hainan. Trop Agric Eng 2021;45(6):9-15. https://d.wanfangdata.com.cn/periodical/rdnygc202106003. (In Chinese).
22. Zhang LJ, Liu H, Xu BL, Lu QY, Li L, Chang LT, et al. *Anaplasma phagocytophilum* infection in domestic animals in ten provinces/cities of China. Am J Trop Med Hyg 2012;87(1):185 – 9. http://dx.doi.org/10.4269/ajtmh.2012.12-0005.
23. Wang SW, He J, Zhang LJ. Serological investigation of vector-borne disease in dogs from rural areas of China. Asian Pac J Trop Biomed 2012;2(2):102 – 3. http://dx.doi.org/10.1016/S2221-1691(11)60201-3.
24. Bi TL. Molecular epidemiological investigation study on cattle infected with *Anaplasma*, *Babesia* and *Theileria* in Qiongbei area [dissertation]. Haikou: Hainan University; 2021. http://dx.doi.org/10.27073/d.cnki.ghadu.2021.000933. (In Chinese).
25. Zhang L, Wen S, Hou XX, Li S, Yang XN, Chen T, et al. Detection and genotyping of *Borrelia burgdorferi* in parasitic ticks in Qiongzhong area of Hainan province, China. Chin J Vector Biol Control 2020;31(4):426 – 9,464. http://dx.doi.org/10.11853/j.issn.1003.8280.2020.04.009. (In Chinese).
26. Zhao HQ, Liu PP, Xue F, Lu M, Qin XC, Li K. Molecular detection and identification of *Candidatus* Ehrlichia hainanensis, a novel *Ehrlichia* species in rodents from Hainan province, China. Biomed Environ Sci 2021;34(12):1020 – 3. http://dx.doi.org/10.3967/bes2021.138.
27. Li JJ, Liu XX, Mu JQ, Yu XB, Fei YD, Chang J, et al. Emergence of a novel *Ehrlichia minasensis* strain, harboring the major immunogenic glycoprotein trp36 with unique tandem repeat and C-terminal region sequences, in haemaphysalis hystricis ticks removed from free-ranging sheep in Hainan Province, China. Microorganisms 2019;7(9):369. http://dx.doi.org/10.3390/microorganisms7090369..
28. Wei FR, Lan QX, Zhu D, Ye JH, Liu Q, Zhang Y. Investigation on *Babesia* in ticks infested on police dogs in selected areas of China. Chin J Parasitol Parasit Dis 2012;30(5):390-2. http://qikan.cqvip.com/Qikan/Article/Detail?id=43679584. (In Chinese).
29. Wang JM, Yang JF, Gao SD, Wang XX, Sun H, Lv ZY, et al. Genetic diversity of *Babesia* bovis MSA-1, MSA-2b and MSA-2c in China. Pathogens 2020;9(6):473. http://dx.doi.org/10.3390/pathogens9060473.
30. Wang JM, Yang JF, Liu JL, Wang XX, Xu JL, Liu AH, et al. Molecular detection and genetic diversity of *Theileria orientalis* in cattle in China. Parasitol Res 2018;117(12):3689 – 94. http://dx.doi.org/10.1007/s00436-018-6023-3.
31. Yang L, Wang JH, Upadhyay A, Zhao JG, Huang LY, Liao CH, et al. Identification of *Theileria* spp. and investigation of hematological profiles of their infections in goats in Hainan Island, China. Parasite 2022;29:13. http://dx.doi.org/10.1051/parasite/2022013.
32. Chen WZ, Qiu FX, Calisher CH, Liu JC, Chen HX, Li XD, et al. Twenty-eight alphavirus strains isolated from mosquitoes and ticks captured from Hainan Island, China. Chin J Exp Cin Virol 1997;11(2):144 – 6. http://dx.doi.org/10.3760/cma.j.issn.1003-9279.1997.02.117. (In Chinese).
33. Fang LQ, Liu K, Li XL, Liang S, Yang Y, Yao HW, et al. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis 2015;15(12):1467 – 79. http://dx.doi.org/10.1016/S1473-3099(15)00177-2.
34. Wu J, Wang SL, Jin YM, Ma Y, Zeng XJ, Jia PB, et al. Detection of groEL gene in spotted fever group Rickettsia of patients with fever in Hainan province. Mod Prev Med 2014;41(13):2417-9. https://d.wanfangdata.com.cn/periodical/xdyfyx201413037. (In Chinese).
35. Lin BH, Sun XJ, Zhan ZN, Lin YZ, Li WG. Serological and etiological studies on undefined rickettsiosis on Hainan island. J Hainan Med Univ 1997;3(3):97-102. https://kns.cnki.net/kns8/Detail?sfield=fn&QueryID=0&CurRec=1&recid=&FileName=HNYY703.000&DbName=CJFD9697&DbCode=CJFD&yx=&pr=&URLID=. (In Chinese).
36. Zhang L, Zhu X, Hou XX, Li H, Yang XN, Chen T, et al. Prevalence and prediction of Lyme disease in Hainan province. PLoS Negl Trop Dis 2021;15(3):e0009158. http://dx.doi.org/10.1371/journal.pntd.0009158.
37. Zhu X, Zhang L, Hou XX, Geng Z, Chen H, Chen T, et al. Investigation on Lyme Disease of patients with arthritis or neurological disorders in Hainan Province, China. Chin J Zoonoses 2015;31(4):353 – 6. http://dx.doi.org/10.3969/cjz.j.issn.1002-2694.2015.04.013. (In Chinese).
38. Zhang LJ, Liu H, Xu BL, Zhang ZL, Jin YM, Li WM, et al. Rural residents in China are at increased risk of exposure to tick-borne pathogens *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. Biomed Res Int 2014;2014:313867. http://dx.doi.org/10.1155/2014/313867.
39. Wu XB, Na RH, Wei SS, Zhu JS, Peng HJ. Distribution of tick-borne diseases in China. Parasit Vectors 2013;6:119. http://dx.doi.org/10.1186/1756-3305-6-119.

Perspectives

Advancements in Defining and Estimating the Reproduction Number in Infectious Disease Epidemiology

Kangguo Li[1,2]; Jiayi Wang[1,2]; Jiayuan Xie[1,2]; Jia Rui[1,2]; Buasiyamu Abudunaibi[1,2]; Hongjie Wei[1,2]; Hong Liu[1,2]; Shuo Zhang[1,2]; Qun Li[3]; Yan Niu[3,#]; Tianmu Chen[1,2,#]

The reproduction number (R) serves as a fundamental metric in the examination of infectious disease outbreaks, epidemics, and pandemics. Despite an array of available methods for estimating R, both newcomers and established public health professionals often encounter difficulties in comprehending the circumstances for their use and their constrictions. Consequently, this review intends to offer elementary guidance on R's selection and estimation approaches. To facilitate our review, we executed an extensive search on PubMed and Web of Science applying the following search approach: ["Basic Reproduction Number/classification"(Mesh)] AND ["Basic Reproduction Number/prevention and control" (Mesh)] OR ["Basic Reproduction Number/statistics and numerical data"(Mesh)]. Our search parameters were restricted to articles published from January 2013 to January 2023. This search rendered a total of 7,094 articles, of which we selected 60 that met our inclusion standards for further analysis.

CONCEPTUAL UNDERSTANDING OF *R*: AN ANALYSIS

R is a fundamental measure that indicates the average number of infections or cases resulting from contact with an infected individual, thus serving as an important gauge of the transmissibility of infectious diseases. There are three types of R: basic reproduction number (R_0), effective reproduction number (R_{eff}), and real-time or time-varying reproduction number (R_t). R_0 is utilized for evaluating the transmissibility of new pathogens or variants when they emerge (*1*). However, R_{eff} and R_t are employed to assess the effectiveness of public health and social measures (PHSMs), providing valuable insights for policymakers and public health officials (Figure 1) (*2–4*).

R_0, also known as the basic reproduction number, signifies the mean number of secondary infections attributed solely to a single infected individual within a susceptible population (*5–7*). It proves instrumental in predicting the probability and magnitude of disease outbreaks, plus the vaccination threshold required to establish herd immunity (*1*,*8*). Various factors like the frequency of contact among the population, sanitary practices, and seasonal changes may alter R_0 further (*9*). Altering the transmission rate (β), the recovery rate (γ or inverse of the mean infection period), or the contact rate substantially influences the estimated value of R_0 (*10*). It is essential to account for any pre-existing immunity within the given population while calculating R_0. Presently, there exists no standardized method for determining and reporting R_0, addressing the issue of its variability (*11*).

The concept of R_{eff} is similar to R_0 and often confused by researchers. The major distinction lies in the fact that R_{eff} is suitable for establishing a baseline for PHSMs or exposed populations, representing the actual immunity of the population (*12*). As a result, R_{eff} is usually smaller than R_0, because it primarily relies on not only the transmissibility of pathogens but also the levels of immunization within the population (*13*).

Anne Cori et al. (*14*) provided a more detailed breakdown of R_t, dividing it into the case reproduction number (R_c) and the instantaneous reproduction number (R_i). R_c represents the average number within R_t and reflects the transmissibility at a given time point. On the other hand, R_i represents the average number within R_t calculated under the assumption of no change after a specific time point, making it easier to estimate in real-time (*15*). R_t estimates the spread of pathogens by monitoring and tracking data that evolves over time during the course of an outbreak (*16*). R_t is also an important parameter for describing the epidemiological characteristics of a disease and evaluating the effectiveness of PHSMs (Figure 1) (*17–18*). The values of R_t vary due to factors such as changes in immunity and interventions across different populations, including interventions that impact personal contact networks (*19–20*). In practice, researchers must choose whether the main R_t index to

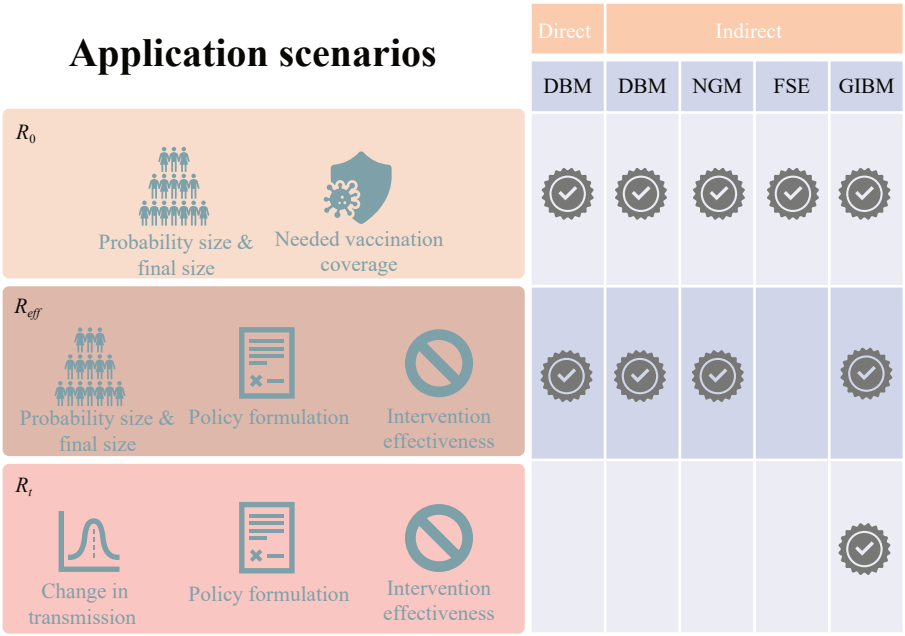


FIGURE 1. Comparison of application scenarios for various reproduction number methods.
Abbreviation: DBM=definition-based method; NGM=next-generation method; FSE=final-size equation; GIBM=generation interval-based method.

be obtained is R_c or R_i, and then select the appropriate modeling methods accordingly. Overall, both R_c and R_i represent the average number of individuals who are at risk of infection at a specific time (t), with R_c focusing on the attributes of infected individuals at the time t and being more widely used, while R_i emphasizes the temporal attribute at time t if the situation remains unchanged. Consequently, if the disease transmissibility declines at a particular point, R_i will transition from high to low, while R_c will smoothly decrease (*21*).

METHODOLOGY FOR CALCULATING *R*

Implementation of the Direct Method:

The direct method is used to estimate R by analyzing a clear transmission chain multiplying the β with the transmission probability per contact (p), contact rate (c), and infectious period (D) (*11*,*22*):

$$\beta = pc$$

$$R_0 = \beta D = pcD$$

The direct method is applicable to distinct scenarios that involve a minimal number of case generations within a brief time frame, or small sample sizes during the early phase of an epidemic or outbreak. This allows researchers the potential to separately calculate R for each possible transmission chain, analyze the distribution of R, and evaluate the contributions of different transmission chains to the spread of the disease. However, the direct method might be prone to bias resulting from small sample sizes and is subject to limitations related to the lack of time variation. Moreover, challenges regarding underreporting and fragmented data in real-time evaluations present potential issues (*23*).

Implementation of the Indirect Method

Methodology Based on Definitions: The definition-based method (DBM) is an indirect approach used to estimate the R value. This method is applied to various transmission dynamics models, including the Susceptible-Infectious-Recovered (SIR) model, the Susceptible-Exposed-Infectious-Recovered (SEIR) model, the Susceptible-Infectious-Recovered-Cross immune (SIRC) model, and the Susceptible-Infectious-Recovered-Susceptible (SEIS) model (*24*–*28*). Taking the SIR model as an example:

$$\frac{dS}{dt} = b_r N - \frac{\beta SI}{N} - d_r S$$

$$\frac{dI}{dt} = \frac{\beta SI}{N} - \gamma I - d_r I$$

$$\frac{dR}{dt} = \gamma I - d_r R$$

The secondary infections generated by an infected individual per unit of time are represented as $\beta S/N$,

which corresponds to the inflow process. On the other hand, the recovery or natural death of an infected individual per unit of time is denoted as $\gamma + d_r$, which corresponds to the outflow process. Thus, we can calculate R_{eff} as follows:

$$R_{eff} = \frac{Inflow\ process}{Outflow\ process} = \frac{\beta S}{N} \times \frac{1}{\gamma + d_r} = \frac{\beta S}{(\gamma + d_r)N}$$

R_0 refers to the R when nearly the entire population is susceptible, which means S is approximately equal to N:

$$R_0 = \frac{\beta}{d_r + \gamma}$$

The DBM calculates R by expressing it as a function of model parameters. This approach proves valuable in the advanced stages of an epidemic as it yields results with significant explanatory power. However, its applicability is limited to single-host and single-kinetic models, thus restricting its use in multi-host or co-kinetic models. The DBM incorporates both the disease's natural history and demographic parameters, rendering it meaningful for predicting and preventing outbreaks. Moreover, it is renowned for its simplicity, ease of comprehension, and minimal hardware or software requirements.

Methodology Based on Next-Generation: The next-generation method (NGM) serves as a prevalent approach for the estimation of R. This method utilizes the maximum eigenvalue of the next-generation matrix within a dynamic model following the method proposed by Van den Driesschie and Watmough (*29–33*). NGM is frequently applied across a range of dynamic models including, but not limited to, the SIR and SEIS models (*25*). Furthermore, it delivers quantitative accounts of secondary infections and can estimate the percentage of undetected cases across diverse outbreak scenarios (*29,34*). Compartments within these dynamic models are differentiated based on their infectivity. The 'x-group' signifies compartments possessing infectivity, whereas the 'y-group' denotes compartments devoid of infectivity. The equations corresponding to these groups are presented below:

$$\frac{dx_i}{dt} = F_i(x,y) - V_i(x,y)\ i = 1, \ldots, n$$

$$\frac{dy_j}{dt} = G_j(x,y)\ j = 1, \ldots, m$$

F_i represents the newly infected individuals in compartment i, V_i represents individuals who transit to other compartments. To illustrate NGM, we will continue using the SIR model as an example. In the SIR model, where n and m are 1 and 2, respectively, and with x = I and y = (S, R), the corresponding equations are as follows:

$$F_1 = \frac{\beta SI}{N}$$

$$V_1 = \gamma I + d_r I$$

$$G_1 = b_r N - \frac{\beta SI}{N} - d_r S$$

$$G_2 = \gamma I - d_r R$$

Taking derivatives of F and V to I, one obtains the Jacobi matrix: $F = \beta S/N$, and $V = \gamma + d_r$. And R_{eff} is the real part of the leading eigenvalue of the next-generation matrix (FV^{-1}) 25:

$$R_{eff} = \rho\left(FV^{-1}\right) = \frac{F}{V} = \frac{\frac{\beta S}{N}}{\gamma + d_r} = \frac{\beta S}{(\gamma + d_r)N}$$

$$R_0 = \frac{\beta}{\gamma + d_r}$$

Nevertheless, the application of the NGM method to multi-group or multi-host compartmental models exhibits certain limitations. This method exclusively ascertains the stability threshold of a disease-free equilibrium, displaying a deficiency in explicit explanatory power. Employing smaller data sets during the initial phases of an epidemic may result in the omission of pivotal information. Over time, there has been a noted enhancement in the quality and dependability of the NGM results. Hence, researchers must modify their methodologies based on specific scenarios. For instance, when studying diseases such as hand, foot, and mouth disease, it might be plausible to exclude certain factors like the short disease duration, mobility of patients, and spatial structure.

Equation for Determining Final Size: The final-size equation (FSE) is a valuable tool for comprehending the relationship between the outcome of an epidemic and R_0, while taking into account the proportions of susceptible and recovered individuals. In the SIR model, the calculation formula is as follows:

$$R_0 = \frac{ln\frac{S_0}{S_\infty}}{1 - S_\infty}$$

Where S_0 and S_∞ represent the initial and final proportions of susceptible individuals.

FSE is often employed in the SIR model to ascertain the ultimate scale of an epidemic (*35*). With its precise data output and straightforward equation form, it is well suited to facilitating initial estimates following the conclusion of an epidemic. Nonetheless, its use is

model-specific and necessitates fresh derivation for application for other models, which can prove challenging for complex dynamic models.

It has been definitively established that the FSE possesses a unique solution in three mean field models, namely homogeneous, pairwise, and heterogeneous. Moreover, linearizing the FSE facilitates the transformation of optimal vaccination issues into simpler knapsack problems, yielding practical insights for decision-makers and the general public when considering vaccination strategies (*36*–*37*). However, a gap exists with respect to the availability of an R package incorporating displacement or interaction for the calculation of R_t using the FSE approach (*38*).

Methodology Based on Generation Intervals: The method based on generation interval is frequently utilized to estimate R_t in the field of epidemiology. This approach leverages the concept of the generation gap, defined as the duration between the infection of a primary case and the consequent infection of secondary cases. This method streamlines the natural history of the illness by concentrating on the distribution of time intervals among generations. Within this framework, two key indicators are emphasized: the generation interval (GT) and the serial interval (SI). GT signifies the duration between infection incidents in an infector-infected pair, whereas SI symbolizes the time from symptom onset in these pairs (*39*). Accurate estimation of GT becomes demanding as it is dependent on an exhaustive investigation of contact history (*40*). In comparison, SI's determination is less challenging as symptoms can be readily detected during field epidemiological surveys (*41*). By quantifying the relationship between generations using SI, researchers can estimate R_t, R_{eff}, and R_0 (*42*–*44*).

Several R (version 4.3.0, R Core Team, Vienna Austria) packages, namely EpiEstim, EpiNow2, and R0, currently facilitate the computation of regeneration numbers based on GT or SI (*15*,*45*–*46*), thereby significantly lowering the barrier to their utilization. We have developed an interactive application for users unfamiliar with the R language, particularly grassroots disease control staff. This application, called Reproduction Number Calculator, enables access to these R packages without necessitating knowledge of programming (available at https://toolbox.ctmodelling.cn/). However, it is crucial to acknowledge the method's inherent limitations. Inaccuracies may arise if the assumed distribution of intergenerational times does not accurately reflect the dynamics of the disease (*42*). This uncertainty in distribution can potentially result in an underestimation of R's uncertainty (*15*). Oversights related to group immunity and infection staging can create bias when estimating R_{eff} (*42*). Further, the generation interval-based method comes with specific demands and limitations, such as a need for clear transmission chains, comprehensive and timely data, and an accurate intergenerational time distribution assumption. These factors may limit its utility in certain scenarios.

In conclusion, the generation interval-based method provides valuable insights into disease transmission dynamics and facilitates the estimation of R_t, R_{eff} and R_0. However, researchers should exercise caution in interpreting the results and consider the assumptions and data requirements associated with the method.

CONCLUSION

Choosing the correct approach to R estimation is critical in epidemiological research. Each model introduces its own unique strengths and weaknesses. The desired R, dictated by disease characteristics and accessible data, must be thoughtfully considered by researchers to identify the most fitting calculation method. This systematic strategy guarantees that the estimation procedure corresponds with the existing conditions and provides trustworthy outcomes.

Conflicts of interest: No conflicts of interest.

Funding: Supported by the National Key R&D Program of China (2021ZD0113903, 2021YFC2301604), Fundamental Research Funds for the Central Universities (20720230001).

doi: 10.46234/ccdcw2023.158

[#] Corresponding authors: Tianmu Chen, chentianmu@xmu.edu.cn; Yan Niu, niuyan@chinacdc.cn.

[1] State Key Laboratory of Vaccines for Infectious Diseases, Xiang An Biomedicine Laboratory, School of Public Health, Xiamen University, Xiamen City, Fujian Province, China; [2] State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, National Innovation Platform for Industry-Education Integration in Vaccine Research, Xiamen University, Xiamen City, Fujian Province, China; [3] Chinese Center for Disease Control and Prevention, Beijing, China.

Submitted: July 18, 2023; Accepted: September 11, 2023

REFERENCES

1. Dhungel B, Rahman S, Rahman M, Bhandari AKC, Le PM, Biva NA, et al. Reliability of early estimates of the basic reproduction number of COVID-19: a systematic review and meta-analysis. Int J Environ Res Public Health 2022;19(18):11613. http://dx.doi.org/10.3390/IJERPH191811613.

2. Gostic KM, McGough L, Baskerville EB, Abbott S, Joshi K, Tedijanto C, et al. Practical considerations for measuring the effective reproductive number, R_t. PLoS Comput Biol 2020;16(12):e1008409. http://dx.doi.org/10.1371/journal.pcbi.1008409.
3. Ferguson NM, Donnelly CA, Anderson RM. Transmission intensity and impact of control policies on the foot and mouth epidemic in Great Britain. Nature 2001;413(6855):542 – 8. http://dx.doi.org/10.1038/35097116.
4. Linka K, Peirlinck M, Kuhl E. The reproduction number of COVID-19 and its correlation with public health interventions. Comput Mech 2020;66(4):1035 – 50. http://dx.doi.org/10.1007/s00466-020-01880-8.
5. Pijpers FP. A non-parametric method for determining epidemiological reproduction numbers. J Math Biol 2021;82(5):37. http://dx.doi.org/10.1007/s00285-021-01590-6.
6. Navarro Valencia VA, Díaz Y, Pascale JM, Boni MF, Sanchez-Galan JE. Using compartmental models and Particle Swarm Optimization to assess Dengue basic reproduction number R_0 for the Republic of Panama in the 1999-2022 period. Heliyon 2023;9(4):e15424. http://dx.doi.org/10.1016/j.heliyon.2023.e15424.
7. Takahashi S, Liao QH, Van Boeckel TP, Xing WJ, Sun JL, Hsiao VY, et al. Hand, foot, and mouth disease in China: modeling epidemic dynamics of enterovirus serotypes and implications for vaccination. PLoS Med 2016;13(2):e1001958. http://dx.doi.org/10.1371/journal.pmed.1001958.
8. Anderson RM, May RM. Infectious diseases of humans: dynamics and control. Oxford: Oxford University Press. 1991. https://www.cambridge.org/core/services/aop-cambridge-core/content/view/S0950268800059896.
9. Zhang XB, Yan DY, Chen C, Jiang DX, Ding C, Lan L, et al. Research progress on the basic and effective reproductive number in the epidemiology of infectious diseases. Chin J Dis Control Prev 2021;25(7):753-7, 790. http://dx.doi.org/10.16462/j.cnki.zhjbkz.2021.07.003. (In Chinese).
10. Ridenhour B, Kowalik JM, Shay DK. Unraveling R_0: considerations for public health applications. Am J Public Health 2014;104(2):e32 – 41. http://dx.doi.org/10.2105/AJPH.2013.301704.
11. Guerra FM, Bolotin S, Lim G, Heffernan J, Deeks SL, Li Y, et al. The basic reproduction number (R_0) of measles: a systematic review. Lancet Infect Dis 2017;17(12):e420 – 8. http://dx.doi.org/10.1016/S1473-3099(17)30307-9.
12. Liu Y, Rocklöv J. The effective reproductive number of the Omicron variant of SARS-CoV-2 is several times relative to Delta. J Travel Med 2022;29(3):taac037. http://dx.doi.org/10.1093/jtm/taac037.
13. Green WD, Ferguson NM, Cori A. Inferring the reproduction number using the renewal equation in heterogeneous epidemics. J Roy Soc Interface 2022;19(188):20210429. http://dx.doi.org/10.1098/rsif.2021.0429.
14. Cori A, Ferguson NM, Fraser C, Cauchemez S. A new framework and software to estimate time-varying reproduction numbers during epidemics. Am J Epidemiol 2013;178(9):1505 – 12. http://dx.doi.org/10.1093/aje/kwt133.
15. Thompson RN, Stockwin JE, van Gaalen RD, Polonsky JA, Kamvar ZN, Demarsh PA, et al. Improved inference of time-varying reproduction numbers during infectious disease outbreaks. Epidemics 2019;29:100356. http://dx.doi.org/10.1016/j.epidem.2019.100356.
16. Nishiura H, Chowell G. The effective reproduction number as a prelude to statistical estimation of time-dependent epidemic trends. In: Chowell G, Hyman JM, Bettencourt LMA, Castillo-Chavez C, editors. Mathematical and statistical estimation approaches in epidemiology. Dordrecht: Springer. 2009. http://dx.doi.org/10.1007/978-90-481-2313-1_5.
17. Gressani O, Wallinga J, Althaus CL, Hens N, Faes C. EpiLPS: a fast and flexible Bayesian tool for estimation of the time-varying reproduction number. PLoS Comput Biol 2022;18(10):e1010618. http://dx.doi.org/10.1371/journal.pcbi.1010618.
18. Lal R, Huang WD, Li ZQ, Prasad S. An assessment of transmission dynamics via time-varying reproduction number of the second wave of the COVID-19 epidemic in Fiji. Roy Soc Open Sci 2022;9(8):220004. http://dx.doi.org/10.1098/rsos.220004.
19. Creswell R, Augustin D, Bouros I, Farm HJ, Miao S, Ahern A, et al. Heterogeneity in the onwards transmission risk between local and imported cases affects practical estimates of the time-dependent reproduction number. Philos Trans Roy Soc A Math Phys Eng Sci 2022;380(2233):20210308. http://dx.doi.org/10.1098/RSTA.2021.0308.
20. Eales O, Ainslie KEC, Walters CE, Wang HW, Atchison C, Ashby D, et al. Appropriately smoothing prevalence data to inform estimates of growth rate and reproduction number. Epidemics 2022;40:100604. http://dx.doi.org/10.1016/j.epidem.2022.100604.
21. Fraser C. Estimating individual and household reproduction numbers in an emerging epidemic. PLoS One 2007;2(8):e758. http://dx.doi.org/10.1371/journal.pone.0000758.
22. Dietz K. The estimation of the basic reproduction number for infectious diseases. Stat Methods Med Res 1993;2(1):23 – 41. http://dx.doi.org/10.1177/096228029300200103.
23. Gao DZ, Lou YJ, He DH, Porco TC, Kuang Y, Chowell G, et al. Prevention and control of zika as a mosquito-borne and sexually transmitted disease: a mathematical modeling analysis. Sci Rep 2016;6:28070. http://dx.doi.org/10.1038/srep28070.
24. Martcheva M. An introduction to mathematical epidemiology. New York: Springer. 2015. http://dx.doi.org/10.1007/978-1-4899-7612-3.
25. Guo XH, Guo YC, Zhao ZY, Yang ST, Su YH, Zhao BH, et al. Computing R_0 of dynamic models by a definition-based method. Infect Dis Model 2022;7(2):196 – 210. http://dx.doi.org/10.1016/j.idm.2022.05.004.
26. He SB, Peng YX, Sun KH. SEIR modeling of the COVID-19 and its dynamics. Nonlinear Dyn 2020;101(3):1667 – 80. http://dx.doi.org/10.1007/s11071-020-05743-y.
27. Goel S, Bhatia SK, Tripathi JP, Bugalia S, Rana M, Bajiya VP. SIRC epidemic model with cross-immunity and multiple time delays. J Math Biol 2023;87(3):42. http://dx.doi.org/10.1007/S00285-023-01974-W.
28. Jain S, Kumar S. Dynamic analysis of the role of innate immunity in SEIS epidemic model. Eur Phys J Plus 2021;136(4):439. http://dx.doi.org/10.1140/epjp/s13360-021-01390-3.
29. Tang B, Wang X, Li Q, Bragazzi NL, Tang SY, Xiao YN, et al. Estimation of the transmission risk of the 2019-nCoV and its implication for public health interventions. J Clin Med 2020;9(2):462. http://dx.doi.org/10.3390/jcm9020462.
30. Zhou WK, Wang AL, Xia F, Xiao YN, Tang SY. Effects of media reporting on mitigating spread of COVID-19 in the early phase of the outbreak. Math Biosci Eng 2020;17(3):2693 – 707. http://dx.doi.org/10.3934/mbe.2020147.
31. Yakob L, Clements ACA. A mathematical model of chikungunya dynamics and control: the major epidemic on Réunion Island. PLoS One 2013;8(3):e57448. http://dx.doi.org/10.1371/journal.pone.0057448.
32. Rudge JW, Webster JP, Lu DB, Wang TP, Fang GR, Basáñez MG. Identifying host species driving transmission of schistosomiasis japonica, a multihost parasite system, in China. Proc Natl Acad Sci USA 2013;110(28):11457 – 62. http://dx.doi.org/10.1073/pnas.1221509110.
33. Li Y, Zhang JH, Zhang XN. Modeling and preventive measures of hand, foot and mouth disease (HFMD) in China. Int J Environ Res Public Health 2014;11(3):3108 – 17. http://dx.doi.org/10.3390/ijerph110303108.
34. Unwin HJT, Cori A, Imai N, Gaythorpe KAM, Bhatia S, Cattarino L, et al. Using next generation matrices to estimate the proportion of infections that are not detected in an outbreak. Epidemics 2022;41:100637. http://dx.doi.org/10.1016/j.epidem.2022.100637.
35. Bidari S, Chen XY, Peters D, Pittman D, Simon PL. Solvability of implicit final size equations for SIR epidemic models. Math Biosci 2016;282:181 – 90. http://dx.doi.org/10.1016/j.mbs.2016.10.012.
36. Penn MJ, Donnelly CA. Asymptotic analysis of optimal vaccination policies. Bull Math Biol 2023;85(3):15. http://dx.doi.org/10.1007/s11538-022-01114-3.

37. Shaw CL, Kennedy DA. What the reproductive number R_0 can and cannot tell us about COVID-19 dynamics. Theor Popul Biol 2021;137:2 – 9. http://dx.doi.org/10.1016/j.tpb.2020.12.003.
38. Nash RK, Nouvellet P, Cori A. Real-time estimation of the epidemic reproduction number: scoping review of the applications and challenges. PLoS Digit Health 2022;1(6):e0000052. http://dx.doi.org/10.1371/journal.pdig.0000052.
39. Griffin J, Casey M, Collins Á, Hunt K, McEvoy D, Byrne A, et al. Rapid review of available evidence on the serial interval and generation time of COVID-19. BMJ Open 2020;10(11):e040263. http://dx.doi.org/10.1136/bmjopen-2020-040263.
40. Boëlle PY, Ansart S, Cori A, Valleron AJ. Transmission parameters of the A/H1N1 (2009) influenza virus pandemic: a review. Influenza Other Respir Viruses 2011;5(5):306 – 16. http://dx.doi.org/10.1111/j.1750-2659.2011.00234.x.
41. Svensson Å. A note on generation times in epidemic models. Math Biosci 2007;208(1):300 – 11. http://dx.doi.org/10.1016/j.mbs.2006.10.010.
42. Koh WM, Bogich T, Siegel K, Jin J, Chong EY, Tan CY, et al. The epidemiology of hand, foot and mouth disease in Asia: a systematic review and analysis. Pediatr Infect Dis J 2016;35(10):e285 – 300. http://dx.doi.org/10.1097/INF.0000000000001242.
43. Nouvellet P, Cori A, Garske T, Blake IM, Dorigatti I, Hinsley W, et al. A simple approach to measure transmissibility and forecast incidence. Epidemics 2018;22:29 – 35. http://dx.doi.org/10.1016/j.epidem.2017.02.012.
44. Townsend SE, Sumantra IP, Pudjiatmoko, Bagus GN, Brum E, Cleaveland S, et al. Designing programs for eliminating canine rabies from islands: Bali, Indonesia as a case study. PLoS Negl Trop Dis 2013;7(8):e2372. http://dx.doi.org/10.1371/journal.pntd.0002372.
45. Abbott S, Hickson J, Funk S, Badr HS, Monticone P, Ellis P, et al. epiforecasts/EpiNow2: 1.3.4 release. Zenodo; 2023. http://dx.doi.org/10.5281/zenodo.7611804.
46. Obadia T, Haneef R, Boëlle PY. The R0 package: a toolbox to estimate reproduction numbers for epidemic outbreaks. BMC Med Inform Decis Mak 2012;12:147. http://dx.doi.org/10.1186/1472-6947-12-147.

Notifiable Infectious Diseases Reports

Reported Cases and Deaths of National Notifiable Infectious Diseases — China, May 2023*

Diseases	Cases	Deaths
Plague	0	0
Cholera	3	0
SARS-CoV	0	0
Acquired immune deficiency syndrome†	5,455	1,933
Hepatitis	141,492	170
Hepatitis A	1,076	0
Hepatitis B	115,934	17
Hepatitis C	20,963	151
Hepatitis D	20	0
Hepatitis E	2,822	2
Other hepatitis	677	0
Poliomyelitis	0	0
Human infection with H5N1 virus	0	0
Measles	109	0
Epidemic hemorrhagic fever	399	0
Rabies	10	7
Japanese encephalitis	0	0
Dengue	21	0
Anthrax	25	0
Dysentery	3,753	0
Tuberculosis	69,068	343
Typhoid fever and paratyphoid fever	547	0
Meningococcal meningitis	2	0
Pertussis	1,334	0
Diphtheria	0	0
Neonatal tetanus	0	0
Scarlet fever	1,898	0
Brucellosis	9,067	0
Gonorrhea	9,077	0
Syphilis	53,258	10
Leptospirosis	8	0
Schistosomiasis	3	0
Malaria	212	0
Human infection with H7N9 virus	0	0
Influenza	212,889	2
Mumps	8,930	0
Rubella	73	0

Continued

Diseases	Cases	Deaths
Acute hemorrhagic conjunctivitis	2,311	0
Leprosy	27	0
Typhus	171	0
Kala azar	32	0
Echinococcosis	314	0
Filariasis	0	0
Infectious diarrhea[§]	115,898	0
Hand, foot and mouth disease	91,259	0
Total	727,645	2,465

* According to the National Bureau of Disease Control and Prevention, coronavirus disease 2019 (COVID-19) is not included.
† The number of deaths of acquired immune deficiency syndrome (AIDS) is the number of all-cause deaths reported in the month by cumulative reported AIDS patients.
§ Infectious diarrhea excludes cholera, dysentery, typhoid fever and paratyphoid fever.
The numbers of cases and cause-specific deaths refer to data recorded in National Notifiable Disease Reporting System in China, which includes both clinically-diagnosed cases and laboratory-confirmed cases. Only reported cases of the 31 provincial-level administrative divisions in Chinese mainland are included in the table, whereas data of Hong Kong Special Administrative Region, Macau Special Administrative Region, and Taiwan, China are not included. Monthly statistics are calculated without annual verification which is usually conducted in February of the next year for de-duplication and verification of reported cases in annual statistics. Therefore, 12-month cases could not be added together directly to calculate the cumulative cases because the individual information might be verified via National Notifiable Disease Reporting System according to information verification or field investigations by local CDCs.

doi: 10.46234/ccdcw2023.127

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

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

CCDC weekly

Vol. 5 No. 37 Sept. 15, 2023

Published since November, 2019

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

Sponsor
Chinese Center for Disease Control and Prevention

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

Printing
Beijing Kexin Printing Co., Ltd

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