Vital Surveillances

The Prevalence of *Rickettsial* and *Rickettsial*-Like Diseases in Patients with Undifferentiated Febrile Illness — Hainan Province, China, 2018–2021

Xiaofei Xie[1,2,3]; Ying Zhang[4]; Zhongqiu Teng[2]; Biao Duan[5]; Yan Hai[6]; Mingliu Wang[7]; Zhujun Shao[2]; Weili Liang[2]; Biao Kan[2]; Feifei Yin[1,#]; Aiping Qin[2,#]

ABSTRACT

Introduction: *Rickettsial* and *Rickettsial*-like diseases, resulting from obligate intracellular Gram-negative bacteria, pose a growing public health threat in China. To assess the current prevalence of these diseases on Hainan Island, a study was conducted on 9 bacterial pathogens found in patients with undifferentiated febrile illness (UFI) treated in Haikou between 2018 and 2021 using a TaqMan Polymerase Chain Reaction (TaqMan PCR) array.

Methods: Blood samples (*n*=503) were collected from patients with UFI between 2018 and 2021. The samples were screened for *Rickettsia* spp., *Orientia tsutsugamushi* (*O. tsutsugamushi*), *Anaplasma. phagocytophilum* (*A. phagocytophilum*), *Ehrlichia chaffeensis*, *Coxiella burnetii*, *Chlamydia psittaci*, *Brucella* spp., *Burkholderia pseudomallei*, and *Borrelia burgdorferi* using a TaqMan PCR array. Positive samples (Ct<35) underwent confirmation through nested PCR, sequencing, and phylogenetic analysis.

Results: *O. tsutsugamushi* and *A. phagocytophilum* were detected in the patients at positive rates of 14.51% (73/503) and 5.57% (28/503), respectively. Co-infection of *O. tsutsugamushi* and *A. phagocytophilum* was identified in scrub typhus (ST) positive populations from Hainan (10.96%, 8/73), Guangxi (61.54%, 8/13), and Yunnan (5.36%, 3/56) provincial-level administrative divisions (PLADs) of China.

Conclusion: An increased prevalence rate of ST and a decreased prevalence of rickettsioses were observed in patients with UFI in Hainan compared to a decade ago. The co-infection of *O. tsutsugamushi* and *A. phagocytophilum* poses a current public health threat in China.

Hainan Island, a tropical province in China, is situated in the "Tsutsugamushi Triangle Area" and is known for being an endemic region for ST, rickettsioses, and related diseases. From 2009 to 2011, the prevalence rates of ST and rickettsioses [spotted fever group (SFG) and typhus group (TG)] among undifferentiated febrile illness (UFI) patients in Hainan were reported as 5.5% and 5.9%, respectively (*1*). Initially considered endemic mainly to southern China, ST has increasingly spread northward, with a notable rise in national case numbers from 1,375 in 2006 to 23,474 in 2017 (*2*). Clinical features of ST and rickettsioses show similarities to other rickettsia-like diseases, including anaplasmosis, ehrlichiosis, Q fever, and chlamydiosis, as well as various bacterial, viral, and parasitic infections like influenza, malaria, and Lyme disease (*3*). Despite this, the involvement of these rickettsia-like pathogens in UFI remains to be thoroughly investigated.

We previously developed a TaqMan PCR array to identify bacterial pathogens in patients with UFI from Inner Mongolia Autonomous Region, China (*4*). In this study, we expanded our investigation to include the detection of 9 significant bacterial pathogens — *Orientia tsutsugamushi*, *Rickettsia* spp., *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Coxiella burnetii*, *Chlamydia psittaci*, *Brucella* spp., *Burkholderia pseudomallei*, and *Borrelia burgdorferi* — in UFI patients treated in Haikou City, Hainan Province between 2018 and 2021. Our findings highlight the current patterns of ST and rickettsioses in Hainan, as well as the prevalence of co-infections involving *O. tsutsugamushi* and *A. phagocytophilum* in China.

METHODS

Sample Collection, DNA Extraction, and TaqMan PCR Screening

Blood samples and demographic data were obtained

from 503 UFI outpatients at the Affiliated Hospital of Hainan Medical School between June 2018 and August 2021. Blood from putative ST patients (*n*=13), along with their eschar swabs (*n*=6) samples were provided by the Center for Disease Control and Prevention of Guangxi in September 2020. In June 2021, 624 blood samples from healthy volunteers in Yunnan Province were collected by the Institute of Endemic Diseases Control and Prevention of Yunnan. DNA extraction was performed according to standard protocols (*4*).

The primer and probe sequences were detailed in Supplementary Table S1 (available at https://weekly.chinacdc.cn/). TaqMan PCR arrays were carried out as previously described (*4*). Primary screening utilized pooled samples (*n*=5) based on experimental validation. Pools with Ct values <40 in the initial screening underwent individual testing to identify positive cases. A Ct value of ≤35 was considered positive, Ct values between 35 and 40 were considered putative positive, and Ct values ≥40 were considered negative (*4*).

Nested PCR and Amplicon Sequencing

TaqMan PCR positive samples (Ct≤35) were confirmed through verification using nested PCR and subsequent amplicon sequencing. The nested PCR reaction included 10 μL of 2× TSINGKE Master Mix, 2.5 μL of each forward and reverse primer (10 μmol/L), 2 μL of the template, made up to 25 μL with water. PCR cycling was carried out on a Thermo-Cycler from SensoQuest GmbH., Germany, involving an initial denaturation step at 98 ℃ for 5 min, 30 cycles of 94 ℃ for 20 s, 50 ℃ for 20 s, and 72 ℃ for 20 s, and a final extension at 72℃ for 10 min. The products from the first PCR round (2 μL) were utilized as templates for the subsequent PCR round. Amplicons from the nested PCR were assessed on a 1% agarose gel before sequencing (Tsingke Biotechnology Co. Ltd.). Phylogenetic trees were generated using MEGA 10.1.8.

Statistical Analysis

We utilized SPSS software (version 25.0, IBM, NewYork, USA) to conduct statistical analysis (4). The Pearson's chi-squared test was employed to assess the differences in incidences based on age, gender, and occupation. Statistical significance was determined at $P<0.05$.

RESULTS

Detection of Pathogens in Clinical Samples

Among 503 patients with UFI, they were from 17 out of 18 counties in Hainan Island. A total of 73 (14.51%) and 28 (5.57%) were positive for *O. tsutsugamushi* and *A. phagocytophilum*, respectively (Table 1). *O. tsutsugamushi* infections were detected year-round, peaking from September to October (Figure 1). Farmers had the highest *O. tsutsugamushi* infection rate, while adolescents showed a higher *A. phagocytophilum* infection rate. Co-infection with *O. tsutsugamushi* and *A. phagocytophilum* was observed in 1.59% (8/503) of the UFI patients, mainly from August to October (Figure 1). Notably, other pathogens including *Rickettsia* spp., *E. chaffeensis*, *C. burnetii*, *C. psittaci*, *Brucella* spp., *B. pseudomallei*, and *B. burgdorferi* were not detected in these UFI patients.

To further investigate co-infection, we collected blood samples from 13 clinically suspected ST patients and 6 eschar samples in the Guangxi Zhuang Autonomous Region. All 13 blood samples tested positive for *O. tsutsugamushi* (Supplementary Table S2, available at https://weekly.chinacdc.cn/). Surprisingly, 61.5% of these ST patients also tested positive for *A. phagocytophilum*, indicating a high co-infection rate. While eschars are typically associated with *O. tsutsugamushi* infection in ST-endemic regions, 3 out of the 6 eschar samples tested positive for *A. phagocytophilum*, suggesting a potential shared vector. Additionally, we found a co-infection rate of 0.48% (3/624) in blood samples from apparently healthy volunteers in Yunnan. The positivity rates for *O. tsutsugamushi*, *A. phagocytophilum*, and *Rickettsia* spp. were 8.97% (56/624), 3.69% (23/624), and 2.88% (18/624), respectively.

Sequence Comparison and Phylogenetic Analysis

TaqMan PCR positive samples (Ct≤35) for *O. tsutsugamushi* or *A. phagocytophilum* underwent further validation using nested PCR and sequencing. The sequences obtained were assigned GenBank accession numbers: OP698261–OP698267 for the partial 56-kDa gene of *O. tsutsugamushi* and OP690506–OP690511 along with OP709787 for the partial *msp2* gene of *A. phagocytophilum*. These sequences were aligned via the Basic Local Alignment Search Tool (BLAST) and subjected to analysis using

TABLE 1. Prevalence of *Anaplasma phagocytophilum* and *Orientia tsutsugamushi* in patients with UFI from Hainan Province, China, between 2018 and 2021.

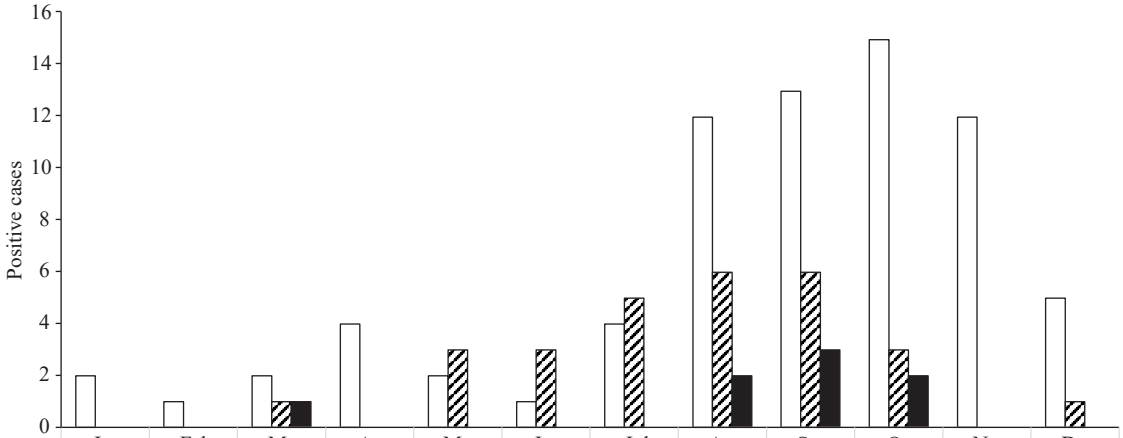
Variables	Patient No.	*Anaplasma phagocytophilum*		*Orientia tsutsugamushi*		Co-infection*	
		Positive† No.	Positive rate (%)	Positive No.	Positive rate (%)	Positive No.	Positive rate (%)
Gender§							
Male	320	20	6.25	48	15	1	0.31
Female	183	8	4.37	25	13.67	7	3.83
Age, years							
<20	25	4	16	3	12	0	—
20–29	44	0	—	2	4.54	0	—
30–39	78	4	5.13	10	12.82	0	—
40–49	86	8	9.3	14	16.28	4	4.65
50–59	86	4	4.65	15	17.44	1	1.17
60–69	98	5	5.1	15	15.3	2	2.04
70–79	61	1	1.64	11	18.03	1	1.64
Unknown	25	2	8	3	12	0	—
Occupation§							
Student	21	3	14.29	3	14.29	0	—
Farmer	185	13	7.03	36	19.46	6	3.24
Retirement	35	1	2.86	6	17.14	1	2.85
Worker	53	2	3.77	7	3.21	0	—
Self-employed	9	0	—	0	—	0	—
Unknown	200	9	4.5	21	10.5	1	0.05

Note: "−" means positive rate cannot be calculated.
Abbreviation: UFI=Undifferentiated Febrile Illness.
* Co-infection with *Orientia tsutsugamushi* and *Anaplasma phagocytophilum*.
† TaqMan PCR Ct<40.
§ Gender and occupational distribution of co-infection were statistically significant (P<0.05).

MEGA 10.1.8 software. A phylogenetic tree constructed based on the partial *msp2* sequences demonstrated genetic diversity among *A. phagocytophilum* strains in Hainan, Yunnan, Guangxi, and Inner Mongolia provincial-level administrative divisions (PLADs) (Figure 2). Notably, a distinct genetic branch comprising isolates OP690506 and OP690510 from Inner Mongolia, OP690507 and OP690508 from Guangxi, OP690509 from Yunnan, and OP709787 from Hainan appears to be predominant in *A. phagocytophilum* infections in China. Furthermore, isolate OP690511 from Hainan clustered separately, suggesting the co-circulation of different *A. phagocytophilum* strains on Hainan Island.

CONCLUSIONS

In this study, nine bacterial pathogens were investigated using a previously established TaqMan PCR array in patients with UFI in Hainan Province, where ST and rickettsioses have historically been prevalent. Among the tested samples, *Orientia tsutsugamushi* was detected in 14.51% (73/503), representing a more than 2-fold increase compared to the prevalence in 2009–2011 (5.5%, 13/236) (*1*). ST remains the top and ever-increasing threat to the population of Hainan. The increased incidence of ST could be due to the different sensitivities of the TaqMan PCR used in this study and the nested PCR used in the previous study (*1*), but is more likely attributable to changes in environmental factors such as temperature and rainfall (*5*). Previous studies have reported seroprevalences of TG *Rickettsia* (11.4%, 27/236) in Hainan (*6*). Surprisingly, *Rickettsia* spp. were not detected in the present study, although the conserved 17-kDa protein gene of *Rickettsia sibirica* was successfully used as a target for the detection of *Rickettsia* spp. (SFG/TG) in clinical samples from Yunnan (in this study) and Inner Mongolia PLADs



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Orienta	2	1	2	4	2	1	4	12	13	15	12	5
Anaplasma	0	0	1	0	3	3	5	6	6	3	0	1
Co-infection	0	0	1	0	0	0	0	2	3	2	0	0
Monthly cases	19	8	5	15	26	22	59	83	77	65	62	52

FIGURE 1. Month-wise distribution of positive cases of *Orientia tsutsugamushi*, *Anaplasma phagocytophilum*, and co-infection in Hainan, 2018–2021.

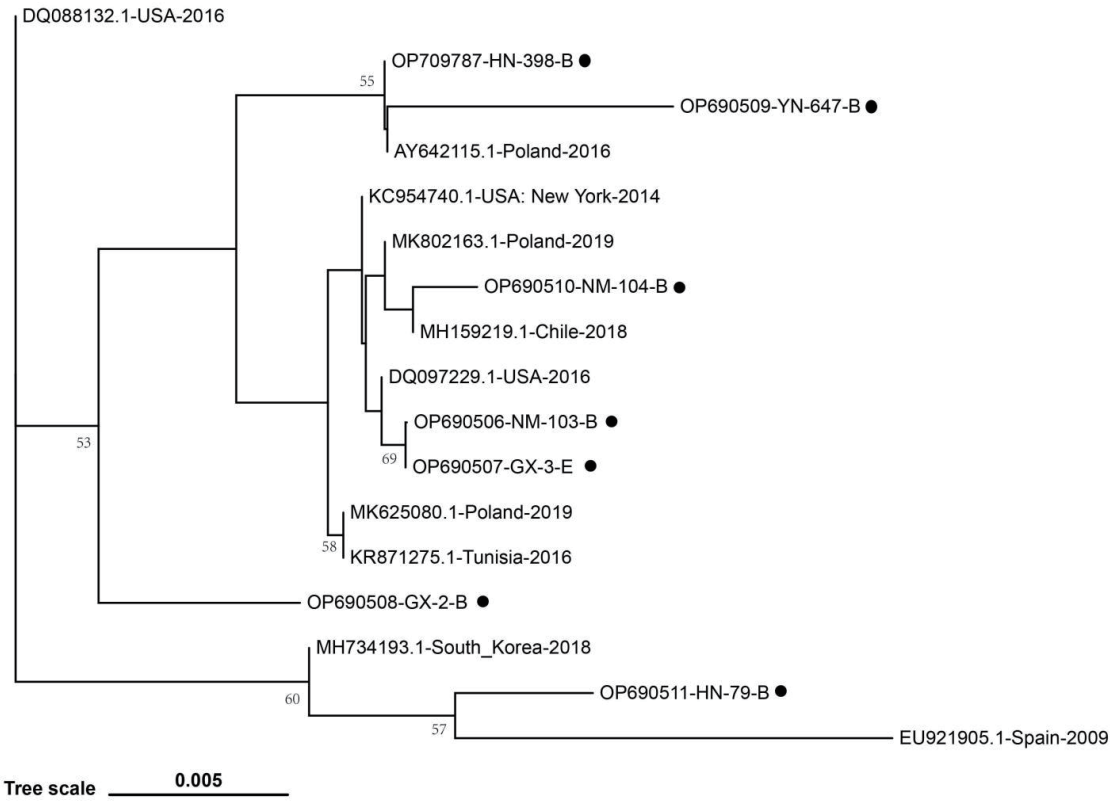


FIGURE 2. Phylogenetic analysis of the partial msp 2 sequences of Anaplasma phagocytophilum amplified from patients with undifferentiated febrile illness in Hainan (HN), Inner Mongolia (NM), Guangxi (GX), and apparent healthy population in Yunnan (YN).

(*4*). In contrast, the incidence of TG infection in UFI in 2009–2011 was 5.9% (14/236) (*1*). This decline in rickettsioses could be attributable to rapid urbanization in Hainan over the last decade and the associated

reduction in exposure to fleas and rats, which are the vectors and hosts of TG, respectively. Although further investigation and continuous monitoring are needed, the current results illustrate a shift in the epidemic trends of ST and rickettsioses in Hainan. It is worth noting that only 2 out of the 9 tested pathogens were detected in the UFI patients from Hainan. However, positive infections of *Ehrlichia chaffeensis*, *Borrelia burgdorferi*, *Coxiella burnetii*, *Chlamydia psittaci*, *Brucella* spp., and *Burkholderia pseudomallei* have previously been documented in the Hainan region (*6*–*10*). Therefore, caution should be exercised when interpreting the current results. Although our data imply that the undetected pathogens may play minor roles in UFI patients, the potential risk of these pathogens cannot be ruled out due to the biased sample size collected each year and the limitations of the TaqMan PCR array employed in this investigation. It is recommended that alternative primer-probe sets of targeting genes for the TaqMan array be tested to validate the results. In addition, cutting-edge technologies such as next-generation sequencing-seminested recombinase polymerase amplification assay (snRPA-nfo) will be of great help in identifying pathogens from clinical samples (*11*).

Anaplasmosis is a tick-borne disease present throughout China. A study in Hainan reported a high *A. phagocytophilum* seropositivity rate of 39.2% (337/852) among individuals tested (*6*). This could be attributed to the prolonged presence of IgG antibodies against *Orientia*, *Rickettsia*, or *Anaplasma*, commonly found in both recovering patients and asymptomatic carriers (*12*). In the current investigation, 5.57% (28/503) of UFI patients tested positive for *A. phagocytophilum*. Hence, local health authorities should consider *A. phagocytophilum* infection in UFI cases.

Simultaneous infections with *A. phagocytophilum* and *B. burgdorferi*, *O. tsutsugamushi*, and Thrombocytopenia Syndrome virus have been documented (*13*–*15*). Co-infections pose a new public health challenge that often goes overlooked. Apart from cases identified in Taiwan, China (*14*), instances of co-infection with *O. tsutsugamushi* and *A. phagocytophilum* have been confirmed in Hainan (10.96%, 8/73 patients), Guangxi (61.5%, 8/13 patients), and Yunnan (5.36%, 3/56 positive population) PLADs of China. Notably, serological evidence of co-infection was found in suspected individuals from The Republic of Korea (2.9%, 8/274) (*15*). While current and past research highlights the prevalence of co-infection in South Asia, larger studies are needed to verify its extent across the Tsutsugamushi Triangle Area.

In conclusion, our findings provide insight into the ongoing epidemic patterns of *rickettsial* and *rickettsia*-like diseases in Hainan. Moreover, they underscore the significant public health risk posed by co-infections involving *O. tsutsugamushi* and *A. phagocytophilum* in China.

Conflicts of interest: No conflicts of interest.

Acknowledgements: The authors wish to acknowledge all the donors, medical lab and administrative personnel of the Affiliated Hospital of Hainan Medical School for their help in sample collection. We would like to thank the colleagues in the departments or groups of ICDC for kindly providing reference DNA for this study.

Funding: his work was supported by grants from the Education 339 Department of Hainan Province [grant number Hys2020-360]; the National Natural Science Foundation of China [grant numbers 82060378, 81860367]; the Natural Science Foundation of Hainan Medical University [grant number JBGS202105]; and the National Institute for Communicable Disease Control and Prevention at the China CDC [grant number 131031102000015003].

doi: 10.46234/ccdcw2024.165

[#] Corresponding authors: Aiping Qin, qinaiping@icdc.cn; Feifei Yin, yinfeifeiff@163.com.

[1] Hainan Medical College, Haikou, Hainan Province, China; [2] State Key Laboratory of Infectious Diseases Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; [3] Cangzhou Central Hospital, Cangzhou City, Hebei Province, China; [4] Center for Disease Control and Prevention of Xilingol League, Xilinhaote, Inner Mongolia Autonomous Region, China; [5] Institute of Endemic Diseases Control and Prevention of Yunnan, Dali City, Yunnan Province, China; [6] General Center for Disease Control and Prevention of Inner Mongolia Autonomous Region, Hohhot City, Inner Mongolia Autonomous Region, China; [7] Center for Disease Control and Prevention of Guangxi, Nanning City, Guangxi Zhuang Autonomous Region, China.

Submitted: September 15, 2023; Accepted: July 02, 2024

REFERENCES

1. Wu J, Wang SL, Ma Y, Zeng XJ, Jia PB, Nie SF. Analysis of the detection results of rickettsial disease among patients with fever in Hainan province from 2009 to 2011. Chin J Health Lab Technol 2015;25(9):1311-13,1316. https://d.wanfangdata.com.cn/periodical/ChlQZXJpb2RpY2FsQ0hJTmV3UzIwMjQwNzA0EhF6Z3dzanl6ejIwMTUwOTAwNBoIbmt6bjc4ZWM%3D. (In Chinese).
2. Yue YJ, Ren DS, Liu XB, Wang YJ, Liu QY, Li GC. Spatio-temporal patterns of scrub typhus in mainland China, 2006–2017. PLoS Negl Trop Dis 2019;13(12):e0007916. https://doi.org/10.1371/journal.pntd.0007916.

3. Xie XF, Wang GY, Huang Y, Tang CN, Peng RY, Hu XY, et al. A review of the epidemic and clinical study on scrub typhus in China (2010–2020). J Hainan Med Univ 2023(19):57 – 61. https://doi.org/10.13210/j.cnki.jhmu.20220802.001.
4. Zhang Y, Hai Y, Teng ZQ, Xie XF, Xiong YW, Shao ZJ, et al. Investigation of 9 pathogenic intracellular bacteria in blood samples from outpatients with undefined febrile illness. Dis Surv 2022;37(4):457 – 63. https://doi.org/10.3784/jbjc.202201200010.
5. Van Peenen PFD, Lien JC, Santana FJ, See R. Correlation of chigger abundance with temperature at a hyperendemic focus of scrub typhus. J Parasitol 1976;62(4):653 – 4. https://doi.org/10.2307/3279442.
6. 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. https://doi.org/10.1155/2014/313867.
7. Li ZJ, Wang XM, Zhu X, Wang M, Cheng H, Li D, et al. Molecular characteristics of *brucella* isolates collected from humans in Hainan Province, China. Front Microbiol 2020;11:452. https://doi.org/10.3389/fmicb.2020.00452.
8. Zhu X, Chen H, Li S, Wang LC, Wu DR, Wang XM, et al. Molecular characteristics of *Burkholderia pseudomallei* collected from humans in Hainan, China. Front Microbiol 2020;11:778. https://doi.org/10.3389/fmicb.2020.00778.
9. 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. https://doi.org/10.1371/journal.pntd.0009158.
10. El-mahallawy HS, Kelly P, Zhang JL, Yang Y, Wei LJ, Tian LL, et al. Serological and molecular evidence of Coxiella burnetii in samples from humans and animals in China. Ann Agric Environ Med 2016;23(1):87 – 91. https://doi.org/10.5604/12321966.1196859.
11. Zhang Y, Hai Y, Duan B, Long H, Xie XF, Teng ZQ, et al. A seminested recombinase polymerase amplification assay to detect rickettsial pathogens in clinical samples. Diagn Microbiol Infect Dis 2023;107(4):116067. https://doi.org/10.1016/j.diagmicrobio.2023.116067.
12. Svendsen CB, Boye M, Struve C, Krogfelt KA. A novel fluorescent *in situ* hybridization technique for detection of *Rickettsia* spp. in archival samples. J Microbiol Methods 2009;76(3):301 – 4. https://doi.org/10.1016/j.mimet.2008.10.012.
13. Win AM, Nguyen YTH, Kim Y, Ha NY, Kang JG, Kim H, et al. Genotypic heterogeneity of *Orientia tsutsugamushi* in scrub typhus patients and thrombocytopenia syndrome co-infection, Myanmar. Emerg Infect Dis 2020;26(8):1878 – 81. https://doi.org/10.3201/eid2608.200135.
14. Tsai KH, Chung LH, Chien CH, Tung YJ, Wei HY, Yen TY, et al. Human granulocytic anaplasmosis in Kinmen, an offshore island of Taiwan. PLoS Negl Trop Dis 2019;13(9):e0007728. https://doi.org/10.1371/journal.pntd.0007728.
15. Heo DH, Hwang JH, Choi SH, Jeon M, Lee JH, Lee JH, et al. Recent increase of human granulocytic anaplasmosis and co-infection with scrub typhus or Korean hemorrhagic fever with renal syndrome in Korea. J Korean Med Sci 2019;34(11):e87. https://doi.org/10.3346/jkms.2019.34.e87.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Sequences of primers and probes for the TaqMan PCR array and nested PCR.

Pathogens	Targeting gene	Primers and probes	Sequence (5'–3')
Rickettsia sibirica (*1*)	17 kDa	17 kDa F	ACAGGATAGAAGACTTGCAGAGCTTACC
		17 kDa R	CAACTTGCCATTGTCCGTCAGG
		17 kDa P	FAM-ACAGCTCCTAGTGGTAGTAACGTAGAATGGC-BHQ1
		005 F†	TCTGTGAGGTAAGCTCTGCAAGTCTTCTATCC
		003 R†	GTAACGGTCCAGGCGGTATGAATAAACAAGG
Orientia tsutsugamushi (*1*)	56 kDa	56 kDa F	AGATGATAAGGATATTAAAGGGCATACAG
		56 kDa R	ATACACCCTCAGCAGCATTAATTG
		56 kDa P	FAM-CATGGTTGCATCAGGAGCACTTGGTG-BHQ1
		002 F†	GGCCAAGTTAAACTCTATGCTGAC
		002 R†	CAGCATTAATTGCTACACCAAGTGC
Ehrlichia chaffeensis (*1*)	*dsb*A	*dsb* F	GTGCAGCATGGTAGAACTCGATGTA
		dsb R	AGAGATTTTCCAATACTTGGAGAAG
		*dsb*P	FAM-TGCTAGTGCTGCTTGAACAGCTTTCAGTGAT-BHQ1
Anaplasma phagocytophilum (*1*)	*msp 2*	*msp* 2 F	ATGGAAGGTAGTGTTGGTTATGGTATT
		msp 2 R†	TTGGTCTTGAAGCGCTCGTA
		msp 2 P	FAM-TGGTGCCAGGGTTGAGCTTGAGATTG-BHQ1
		014 F†	GGGAGAGTAACGGAGAGACTAAGGC
		015 F†	ACTGGAACACTCCTGATCCTCGGAT
Coxiella burnetti (*1*)	23 sRNA	23 s F	CGGCTGAATTTAAGCGATTTATTTT
		23 s R	CGTAACCACACACGCATCTCA
		23 s P	FAM-CCGAACCCATTGCAA-BHQ1
*Chlamydia psittaci**	*ch 23 s*	*ch* 23 s F	CTGAAACCAGTAGCTTATAAGCGGT
		ch 23 s R	ACCTCGCCGTTTAACTTAACTCC
		ch 23 s P	FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA
Burkholderia pseudomallei (*2*)	*orf* 2	orf 2 F	GCTATGGTAATAAATGGACAATGAAATAA
		orf 2R	CGTGCACACCGGTCAGTATC
		Orf 2 P	FAM-CCGGAATCTGGATCACCACCACTTTCC-BHQ1
Brucella spp.(*1*)	*bcsp*31	*Bcsp* 31 F	GCTCGGTTGCCAATATCAATGC
		Bcsp 31 R	GGGTAAAGCGTCGCCAGAAG
		Bcsp 31 P	FAM-AAATCTTCCACCTTGCCCTTGCCATCA-BHQ1
*Borrelia burgdoferi**	*rec*A	*Lemrec*A F	GTTCTGCAACATTAACACCTAAAGCTT
		*Lemrec*A R	AGGTGGGATAGCTGCTTTTATTGAT
		*Lemrec*A P	FAM-ACAGGATCAAGAGCATG-BHQ1

Abbreviation: PCR=polymerase chain reaction.
* This study.
† For nested PCR.

SUPPLEMENTARY TABLE S2. Co-infection with *Anaplasma phagocytophilum* and *Orientia tsutsugamushi* in populations from Hainan, Yunnan, and Guangxi PLADs of China.

Sample			Positive rate (%)		
PLAD	**Type**	**No.**	**Scrub typhus**	**Anaplasmosis**	**Co-infection***
Guangxi	B	13	100.00	61.54	61.54
	E	6	83.33	50.00	60.00
Hainan	B	503	14.51	5.57	10.96
Yunnan	B	624	3.69	8.97	5.36

Abbreviation: PLAD=provincial-level administrative division; B=blood; E=eschar.
* Out of ST positive population.