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Clinical Manifestations, Risk Factors, and Disease Burden of Rickettsiosis, Cambodia, 2007–2020

Appendix

Appendix Table 1. Summary of AUI patients and study participants tested for rickettsioses by enrolment year and season (wet and dry) as part of a cross-sectional prevalence study in Cambodia from 2007 – 2020*

Year	TOTAL AUI PATIENTS			PARTICIPANTS TESTED FOR RICKETTSIA		
	Overall	Dry ¹	Wet ¹	Overall ¹	Dry ²	Wet ³
2007	790 (100.0%)	282 (35.7%)	508 (64.3%)	429 (54.4%)	225 (79.8%)	204 (40.2%)
2008	3,337 (100.0%)	1,053 (31.6%)	2,284 (68.4%)	1,520 (45.6%)	356 (33.8%)	1,164 (51.0%)
2009	5,853 (100.0%)	1,795 (30.7%)	4,058 (69.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
2010	4,849 (100.0%)	1,679 (34.6%)	3,170 (65.4%)	354 (7.3%)	159 (9.5%)	195 (6.2%)
2011	3,564 (100.0%)	1,142 (32.0%)	2,422 (68.0%)	317 (8.9%)	278 (24.3%)	39 (1.6%)
2012	4,379 (100.0%)	1,596 (36.4%)	2,783 (63.6%)	236 (5.4%)	126 (7.9%)	110 (4.0%)
2013	2,987 (100.0%)	1,071 (35.9%)	1,916 (64.1%)	210 (7.0%)	88 (8.2%)	122 (6.4%)
2014	2,211 (100.0%)	1,057 (47.8%)	1,154 (52.2%)	574 (26.0%)	350 (33.1%)	224 (19.4%)
2015	2,528 (100.0%)	1,044 (41.3%)	1,484 (58.7%)	512 (20.3%)	327 (31.3%)	185 (12.5%)
2016	2,207 (100.0%)	941 (42.6%)	1,266 (57.4%)	1,166 (52.8%)	666 (70.8%)	500 (39.5%)
2017	3,059 (100.0%)	1,227 (40.1%)	1,832 (59.9%)	1,974 (64.6%)	969 (79.0%)	1,005 (54.9%)
2018	3,119 (100.0%)	1,075 (34.5%)	2,044 (65.5%)	1,711 (54.9%)	829 (77.1%)	882 (43.2%)
2019	2,101 (100.0%)	803 (38.2%)	1,298 (61.8%)	1,016 (48.4%)	438 (54.5%)	578 (44.5%)
2020	1,237 (100.0%)	677 (54.7%)	560 (45.3%)	224 (18.1%)	152 (22.5%)	72 (12.9%)
TOTAL	42,221 (100%)	15,442 (36.6%)	26,779 (63.4%)	10,243 (24.3%)	4,963 (32.1%)	5,280 (19.7%)

*AUI, acute undifferentiated febrile illness.

Appendix Table 2. Summary of participant Rickettsioses co-infection status among patients with acute undifferentiated febrile illness presenting to study site health facilities and testing positive for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 - 2020

Infection type	Status	Participants (n = 42,221)
Rickettsial Infection	No	41,419 (98.1%)
	Yes	802 (1.9%)
Rickettsial Co-infection	No	42,177 (99.9%)
	Yes	44 (0.1%)
Rickettsial Co-infection (number of groups)	1 Group	758 (1.8%)
	2 Groups	43 (0.1%)
	3 Groups	1 (0.0%)

Appendix Table 3. Association between Rickettsial infection by group among patients with acute undifferentiated febrile illness presenting to study site health facilities and testing positive for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 – 2020*

Type	Status	STG			TG			SFG		
		OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
TG	No	—	—					—	—	
	Yes	2.36	1.34, 3.88	0.001				2.50	1.49, 3.97	<0.001
SFG	No	—	—		—	—				
	Yes	6.13	3.06, 11.1	<0.001	2.50	1.49, 3.97	<0.001			
STG	No	—	—		—	—		—	—	
	Yes				2.36	1.34, 3.88	0.001	6.13	3.06, 11.1	<0.001

*Determined by generalized linear model for binomial regression. — represents the reference group of each section. OR, odds ratio; SFG, spotted fever group; STG, scrub typhus group; TG, typhus group.

Appendix Table 4. Seroprevalence of Rickettsial infection by type and participant characteristic among patients with acute undifferentiated febrile illness presenting to study site health facilities and testing positive for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 - 2020

Category	Characteristic	STG			TG			SFG			Rickettsia All
		STG Sero	STG 4fold	All	TG Sero	TG 4fold	All	SFG Sero	SFG 4fold	All	
Age Breakdown	≤15 y (n = 4111)	30 (1.7%)	6 (0.3%)	36 (2.0%)	94 (5.2%)	13 (0.7%)	107 (5.9%)	36 (2.0%)	2 (0.1%)	38 (2.1%)	118 (2.9%)
	16–25 y (n = 1805)	13 (0.7%)	4 (0.2%)	17 (1.0%)	85 (4.8%)	12 (0.7%)	97 (5.5%)	32 (1.8%)	4 (0.2%)	36 (2.0%)	168 (9.3%)
	26–35 y (n = 1763)	10 (0.9%)	3 (0.3%)	13 (1.2%)	108 (9.9%)	12 (1.1%)	120 (11%)	28 (2.6%)	4 (0.4%)	32 (2.9%)	144 (8.2%)
	36–45 y (n = 1096)	30 (2.0%)	12 (0.8%)	42 (2.9%)	138 (9.4%)	21 (1.4%)	159 (11%)	16 (1.1%)	10 (0.7%)	26 (1.8%)	156 (14.2%)
	≥46 y (n = 1468)	53 (1.1%)	15 (0.3%)	68 (1.4%)	195 (4.0%)	26 (0.5%)	221 (4.5%)	54 (1.1%)	8 (0.2%)	62 (1.3%)	216 (14.7%)
Gender	Female (n = 4936)	51 (1.0%)	17 (0.3%)	68 (1.3%)	295 (5.6%)	41 (0.8%)	336 (6.3%)	78 (1.5%)	14 (0.3%)	92 (1.7%)	335 (6.8%)
	Male (n = 5307)	70 (1.1%)	21 (0.3%)	91 (1.5%)	342 (5.5%)	34 (0.5%)	376 (6.1%)	71 (1.1%)	15 (0.2%)	86 (1.4%)	467 (8.8%)
Education	Lower primary school (n = 6182)	20 (0.9%)	9 (0.4%)	29 (1.3%)	92 (4.1%)	18 (0.8%)	110 (4.9%)	35 (1.6%)	3 (0.1%)	38 (1.7%)	529 (8.6%)
	Primary school (n = 2224)	6 (0.6%)	0 (0%)	6 (0.6%)	33 (3.5%)	7 (0.7%)	40 (4.3%)	17 (1.8%)	2 (0.2%)	19 (2.0%)	162 (7.3%)
	Lower secondary school (n = 938)	8 (1.0%)	2 (0.2%)	10 (1.2%)	22 (2.7%)	7 (0.9%)	29 (3.6%)	9 (1.1%)	2 (0.2%)	11 (1.3%)	63 (6.7%)
	High school (n = 816)	0 (0%)	0 (0%)	0 (0%)	1 (1.2%)	1 (1.2%)	2 (2.4%)	0 (0%)	0 (0%)	0 (0%)	46 (5.6%)
	Diploma or university (n = 83)	87 (1.0%)	28 (0.3%)	115 (1.3%)	461 (5.2%)	60 (0.7%)	521 (5.8%)	131 (1.5%)	18 (0.2%)	149 (1.7%)	2 (2.4%)
Employment status	Unemployed (n = 8907)	17 (1.3%)	4 (0.3%)	21 (1.6%)	29 (2.2%)	7 (0.5%)	36 (2.7%)	1 (<0.1%)	4 (0.3%)	5 (0.4%)	743 (8.3%)
	Employed (n = 1336)	43 (0.8%)	10 (0.2%)	53 (1.0%)	140 (2.5%)	21 (0.4%)	161 (2.9%)	43 (0.8%)	5 (<0.1%)	48 (0.9%)	59 (4.4%)
Marriage status	Single (n = 5492)	53 (1.2%)	17 (0.4%)	70 (1.6%)	328 (7.3%)	41 (0.9%)	369 (8.2%)	87 (1.9%)	17 (0.4%)	104 (2.3%)	248 (4.5%)
	Married (n = 4483)	8 (3.3%)	5 (2.1%)	13 (5.4%)	21 (8.8%)	5 (2.1%)	26 (11%)	2 (0.8%)	0 (0%)	2 (0.8%)	513 (11.4%)
	Widowed (n = 240)	0 (0%)	0 (0%)	0 (0%)	1 (3.6%)	0 (0%)	1 (3.6%)	0 (0%)	0 (0%)	0 (0%)	40 (16.7%)
	Divorced (n = 28)	37 (0.8%)	12 (0.3%)	49 (1.1%)	220 (4.9%)	31 (0.7%)	251 (5.6%)	68 (1.5%)	10 (0.2%)	78 (1.7%)	1 (3.6%)
Season	Dry (Nov–Apr) (n = 4497)	67 (1.2%)	20 (0.3%)	87 (1.5%)	270 (4.7%)	36 (0.6%)	306 (5.3%)	64 (1.1%)	12 (0.2%)	76 (1.3%)	358 (8.0%)
	Wet (May–Oct) (n = 5746)	60 (1.1%)	7 (0.1%)	67 (1.3%)	308 (5.8%)	22 (0.4%)	330 (6.2%)	57 (1.1%)	13 (0.2%)	70 (1.3%)	444 (7.7%)
Area	Rural (n = 5331)	44 (0.9%)	25 (0.5%)	69 (1.4%)	182 (3.7%)	45 (0.9%)	227 (4.6%)	75 (1.5%)	9 (0.2%)	84 (1.7%)	456 (8.6%)
	Urban (n = 4912)	88 (1.0%)	27 (0.3%)	115 (1.3%)	380 (4.3%)	53 (0.6%)	433 (4.9%)	100 (1.1%)	21 (0.2%)	121 (1.4%)	346 (7.0%)
Had traveled	No (n = 8872)	16 (1.2%)	5 (0.4%)	21 (1.5%)	110 (8.0%)	14 (1.0%)	124 (9.0%)	32 (2.3%)	1 (<0.1%)	33 (2.4%)	637 (7.2%)
	Yes (n = 1371)	72 (1.0%)	25 (0.3%)	97 (1.3%)	191 (2.5%)	40 (0.5%)	231 (3.1%)	82 (1.1%)	18 (0.2%)	100 (1.3%)	165 (12.0%)
Traveled to forest	No (n = 7508)	32 (1.2%)	7 (0.3%)	39 (1.4%)	299 (11%)	27 (1.0%)	326 (12%)	50 (1.8%)	4 (0.1%)	54 (2.0%)	396 (5.3%)
	Yes (n = 2735)	4 (0.5%)	3 (0.3%)	7 (0.8%)	9 (1.0%)	1 (0.1%)	10 (1.2%)	9 (1.0%)	4 (0.5%)	13 (1.5%)	406 (14.8%)
Terrestrial Ecosystem	Southeastern Indochina dry evergreen forests (n = 869)	0 (0%)	2 (0.7%)	2 (0.7%)	2 (0.7%)	1 (0.3%)	3 (1.0%)	0 (0%)	1 (0.3%)	1 (0.3%)	29 (3.3%)

Category	Characteristic	STG Sero	STG 4fold	STG All	TG Sero	TG 4fold	TG All	SFG Sero	SFG 4fold	SFG All	Rickettsia All
	Cardamom Mountains rain forests (n = 292)	71 (1.0%)	24 (0.4%)	95 (1.4%)	422 (6.2%)	60 (0.9%)	482 (7.1%)	97 (1.4%)	17 (0.3%)	114 (1.7%)	5 (1.7%)
	Central Indochina dry forests (n = 6791)	29 (1.3%)	3 (0.1%)	32 (1.4%)	57 (2.5%)	5 (0.2%)	62 (2.7%)	26 (1.1%)	0 (0%)	26 (1.1%)	656 (9.7%)
	Tonle Sap-Mekong peat swamp forests (n = 2291)	100 (1.0%)	29 (0.3%)	129 (1.3%)	459 (4.7%)	64 (0.7%)	523 (5.4%)	128 (1.3%)	21 (0.2%)	149 (1.5%)	112 (4.9%)
Antibiotic use in last 30 d	No (n = 9736)	4 (0.8%)	3 (0.6%)	7 (1.4%)	31 (6.1%)	3 (0.6%)	34 (6.7%)	4 (0.8%)	1 (0.2%)	5 (1.0%)	757 (7.8%)
	Yes (n = 507)	21 (0.5%)	7 (0.2%)	28 (0.7%)	65 (1.6%)	9 (0.2%)	74 (1.8%)	20 (0.5%)	2 (<0.1%)	22 (0.5%)	45 (8.9%)

*SFG, spotted fever group; STG, scrub typhus group; TG, typhus group.

Appendix Table 5. Participant age-related distribution and association with Rickettsial infection among patients with acute undifferentiated febrile illness presenting to study site health facilities and tested for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 – 2020*

Age (yrs)	Total Tests	Negative Result	Positive Case	Unadjusted OR	p-value
<5	1,362	1,334	28	ref	p < 0.001
6 – 10	1,701	1,657	44	1.27	
11 – 15	1,048	1,002	46	2.19	
16 – 20	804	728	76	4.97	
21 – 25	1,001	909	92	4.82	
26 – 30	1,048	969	79	3.88	
31 – 35	715	650	65	4.76	
36 – 40	673	574	99	8.22	
41 – 45	423	366	57	7.42	
46 – 50	528	450	78	8.26	
51 – 55	341	299	42	6.69	
56 – 60	259	220	39	8.45	
61 – 65	167	133	34	12.18	
>66	173	150	23	7.31	
Total	10,243	9,441	802		

*OR, odds ratio.

Appendix Table 6. Association between key participant characteristics and Rickettsial infection by type among patients with acute undifferentiated febrile illness presenting to study site health facilities and testing positive for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 – 2020*

Category	STG			TG			SFG		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Year	0.95	0.90, 1.00	0.045	1.09	1.05, 1.12	<0.001	1.00	0.95, 1.05	>0.9
Age breakdown									
<15 y	—	—		—	—		—	—	
16–25 y	4.31	2.43, 7.70	<0.001	3.13	2.21, 4.43	<0.001	4.96	2.79, 9.00	<0.001
26–35 y	2.00	1.00, 3.86	0.043	2.66	1.87, 3.78	<0.001	5.47	3.07, 9.91	<0.001
36–45 y	2.26	1.08, 4.53	0.025	3.87	2.75, 5.48	<0.001	6.83	3.76, 12.6	<0.001
>46 yrs	4.97	2.89, 8.62	<0.001	3.55	2.55, 4.96	<0.001	4.05	2.18, 7.57	<0.001

Category	STG			TG			SFG		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Gender									
Female	—	—		—	—		—	—	
Male	1.00	0.70, 1.43	>0.9	1.19	0.98, 1.45	0.075	1.17	0.83, 1.66	0.4
Education									
Lower primary school	—	—		—	—		—	—	
Primary school	0.71	0.44, 1.12	0.2	0.78	0.61, 0.99	0.043	1.04	0.68, 1.56	0.9
Lower secondary school	0.28	0.11, 0.62	0.004	0.70	0.48, 1.00	0.059	1.16	0.66, 1.95	0.6
High school	0.44	0.20, 0.88	0.028	0.59	0.38, 0.88	0.013	0.77	0.37, 1.45	0.4
Diploma or university	0.00	0.00, 0.00	>0.9	0.42	0.07, 1.41	0.2	0.00	0.00, 0.00	>0.9
Employment status									
Unemployed	—	—		—	—		—	—	
Employed	1.46	0.82, 2.50	0.2	0.71	0.47, 1.04	0.086	0.21	0.07, 0.48	0.001
Season									
Dry (Nov–Apr)	—	—		—	—		—	—	
Wet (May–Oct)	1.41	1.0, 2.03	0.057	0.95	0.80, 1.14	0.6	0.76	0.55, 1.04	0.089
Area									
Rural	—	—		—	—		—	—	
Urban	1.88	0.88, 4.17	0.11	1.54	0.90, 2.69	0.12	6.67	2.24, 28.7	0.003
Had traveled									
No	—	—		—	—		—	—	
Yes	1.22	0.69, 2.07	0.5	0.65	0.49, 0.87	0.004	1.29	0.77, 2.10	0.3
Traveled to forest									
No	—	—		—	—		—	—	
Yes	0.79	0.48, 1.30	0.3	2.64	2.07, 3.40	<0.001	0.66	0.42, 1.03	0.066
Terrestrial Ecosystem									
Southeastern Indochina dry evergreen forests	—	—		—	—		—	—	
Cardamom Mountains rain forests	0.97	0.14, 4.33	>0.9	1.87	0.41, 6.33	0.4	0.23	0.01, 1.23	0.2
Central Indochina dry forests	1.39	0.65, 3.45	0.4	8.52	4.69, 17.4	<0.001	0.83	0.45, 1.65	0.6
Tonle Sap-Mekong peat swamp forests	0.88	0.33, 2.57	0.8	4.49	2.10, 10.3	<0.001	0.17	0.04, 0.56	0.008
Area*Terrestrial Ecosystem									
Urban * Cardamom Mountains rain forests	N/A			N/A			N/A		
Urban * Central Indochina dry forests	0.45	0.18, 1.15	0.10	0.77	0.41, 1.41	0.4	0.16	0.04, 0.55	0.008
Urban * Tonle Sap-Mekong peat swamp forests	N/A			N/A			N/A		
Antibiotic use in last 30 d									
No	—	—		—	—		—	—	
Yes	0.87	0.36, 1.77	0.7	1.14	0.76, 1.65	0.5	0.66	0.23, 1.49	0.4

*Determined by generalized linear model for binomial regression. — represents the reference group of each section. N/A, not applicable due to limited/insufficient data and results were omitted from the modeling; OR, odds ratio; SFG, spotted fever group; STG, scrub typhus group; TG, typhus group.

Acute Visit Questionnaire	Page 1 of 3	Hospital Center Code	Patient Number -
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Symptoms, Including First Symptom (PLEASE CIRCLE) Duration (days)																														
Fever	Y	N	Don't Know	1	2	3	4	>4 _____																						
Malaise	Y	N	Don't Know	1	2	3	4	>4 _____																						
Chills	Y	N	Don't Know	1	2	3	4	>4 _____																						
Muscle Aches	Y	N	Don't Know	1	2	3	4	>4 _____																						
Rash	Y	N	Don't Know	1	2	3	4	>4 _____																						
				Face	Trunk	Extremities	Other																							
Lesion	Y	N	Don't Know	1	2	3	4	>4 _____																						
# of lesions (PLEASE CIRCLE): 1-5 6-20 >20				Ulcer	Pustule	Nodule	Other																							
Joint Pain	Y	N	Don't Know	1	2	3	4	>4 _____																						
Headache	Y	N	Don't Know	1	2	3	4	>4 _____																						
Seizures	Y	N	Don't Know	1	2	3	4	>4 _____																						
Sore Throat	Y	N	Don't Know	1	2	3	4	>4 _____																						
Cough	Y	N	Don't Know	1	2	3	4	>4 _____																						
Shortness of Breath	Y	N	Don't Know	1	2	3	4	>4 _____																						
Nausea	Y	N	Don't Know	1	2	3	4	>4 _____																						
Vomiting	Y	N	Don't Know	1	2	3	4	>4 _____																						
				Number of episodes last 24h → _____																										
Abdominal Cramp*	Y	N	Don't Know	1	2	3	4	>4 _____																						
Diarrhea *	Y	N	Don't Know	1	2	3	4	>4 _____																						
				Number of episodes last 24h → _____																										
Bloody Stools *	Y	N	Don't Know	1	2	3	4	>4 _____																						
				Number of episodes last 24h → _____																										
Bloody Urine	Y	N	Don't Know	1	2	3	4	>4 _____																						
Bleeding (e.g. gums)	Y	N	Don't Know	1	2	3	4	>4 _____																						
Jaundice	Y	N	Don't Know	1	2	3	4	>4 _____																						
Other (specify):	Y	N	Don't Know	1	2	3	4	>4 _____																						

➤ Please collect stool sample for symptoms with * next to them

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MEDICATIONS			
Please mark all medications taken in the last 30 days ☐ Yes ☐ No ☐ Don't Know			
☐	Antibiotics	<i>Specify all</i>	_____
☐	Anti-malaria	<i>Specify all</i>	_____
☐	Steroids	<i>Specify all</i>	_____
☐	Chemotherapy	<i>Specify all</i>	_____
☐	Anti-HIV	<i>Specify all</i>	_____
☐	Other	<i>Specify all</i>	_____
TRAVEL HISTORY			
Please mark ALL places the patient has traveled in the last two months ☐ Yes ☐ No ☐ Don't Know			
☐	Regional	☐	Specify: _____
☐	Other province	☐	Specify: _____
☐	Other country	☐	Specify: _____
Was the patient in the Jungle in the last two weeks? ☐ Yes ☐ No ☐ Don't Know			
EXPOSURE HISTORY			
<i>What is the patient's occupation(s)? (Please check the primary occupation only)</i>			
☐	Grain Farmer	☐	Poultry Farmer
☐	Fisherman	☐	Office Worker
☐		☐	Cattle/Sheep Farmer
☐		☐	Factory Worker
☐		☐	Driver
☐		☐	Other _____

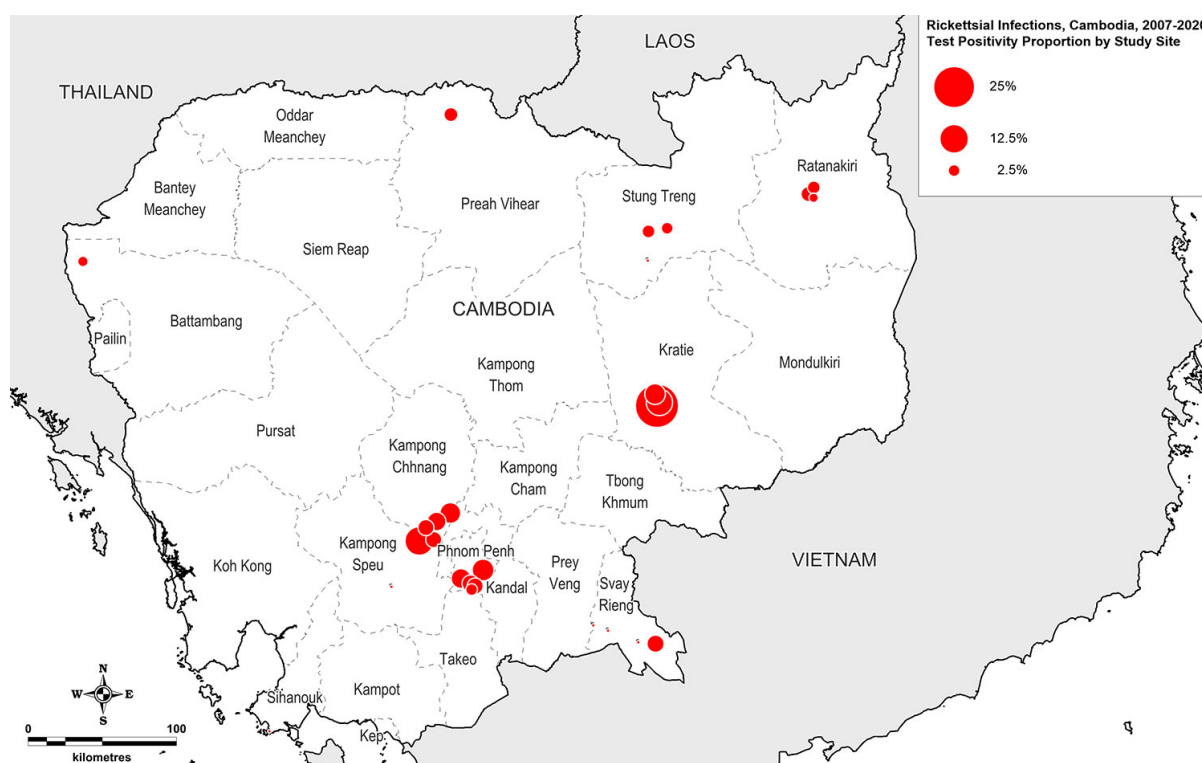
Does the patient report contact with a person who complained of or showed similar symptoms?		
☐ No	☐ Yes	☐ Don't Know
☐ Household member	☐ Neighbor	☐ Other
Does report a history of any the following activities? ☐ Yes ☐ No ☐ Don't Know		
IV Drug Use N Y DK	Alcohol (>2 drinks/d)	N Y DK
Smoking N Y DK	Unprotected Sex (with some one other than spouse)	
	N Y DK	
PHYSICAL EXAMINATION		
Temp _____°C RR _____ (Breaths/minute)		
Pulse Rate _____ (Beats/minute)		
Blood Pressure (mmHg) ____/____		
CLINICAL ASSESSMENT: Diagnosis most consistent with patient's clinical syndrome (pls check ONLY ONE)		
☐ Tuberculosis	☐ Pneumonia	☐ Diarrhea
☐ Upper Respiratory Infection	☐ Genital infection	☐ Dysentery
☐ Pharyngitis	☐ Malaria	☐ Other Infection (<i>specify</i>)

Acute Visit Questionnaire	Page 3 of 3	Hospital Center Code	Patient Number
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☐ Bronchitis	☐ Typhoid	
☐ Influenza	☐ Dengue	
PATIENT DISPOSITION		
☐ Outpatient Follow Up ☐ Admitted to Hospital ☐ Send to Referral Hospital ☐ Patient Dead		
FOLLOW UP VISIT INFORMATION		
		☐ Patient Refuses or is Not Available
Date	dd mm yy	Time 24-hour clock

Interviewed by : _____ / _____ / _____
Name / Signature / Date (dd/ mm/ yy)

Appendix Figure 1. Acute clinical assessment questionnaire template used as part of a cross-sectional prevalence study of patients with acute undifferentiated febrile illness presenting to study site health facilities and tested for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 - 2020.



Appendix Figure 2. Test positivity proportions by study site of patients with acute undifferentiated febrile illness presenting to study site health facilities and tested for rickettsioses as part of a cross-sectional prevalence study in Cambodia from 2007 - 2020.