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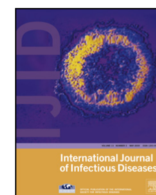

Investigation of an outbreak of rickettsial febrile illness in Guatemala, 2007☆

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SUMMARY

Objectives: We describe an outbreak of spotted fever group (SFG) rickettsiosis that occurred in 2007 in a farming community in southeastern Guatemala. We identified 17 cases of an acute febrile illness, among which 10, including two fatalities, were confirmed or probable cases of rickettsial disease (case–fatality proportion 12%).
Methods: PCR, a microimmunofluorescence assay (IFA), and Western blotting were performed on patient samples, and PCR was performed on ticks.
Results: Using an indirect IFA, seven of 16 (44%) ill persons tested had both IgM and IgG antibodies reacting with one or more *Rickettsia spp* antigens; the other nine (56%) had only IgM titers or were seronegative. Antibodies to SFG protein and lipopolysaccharide were detected by Western blotting with antigens of *Rickettsia typhi*, *Rickettsia rickettsii*, and *Rickettsia akari*. Only one sample, from an ill person who died, tested positive by PCR for a SFG *Rickettsia*. PCR analysis of *Amblyomma cajennense* ticks from domestic animals in the area detected the presence of SFG *Rickettsia* DNA in one of 12 ticks collected.
Conclusions: Further studies in Guatemala are warranted to establish the prevalence of rickettsioses and to fully characterize the identity of the etiologic agents and vectors.
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1. Introduction

In the Americas, several rickettsiae of the spotted fever group (SFG) are endemic and pathogenic. The best known of these are *Rickettsia rickettsii*, the cause of Rocky Mountain spotted fever (RMSF), *Rickettsia akari*, the cause of rickettsialpox, and *Rickettsia felis*, the agent of flea-borne spotted fever.[1–5] Recently, another species of SFG, *Rickettsia parkeri*, has been found to cause an eschar-associated rickettsiosis in the USA and in South America.[6–8] The application of more versatile and accurate molecular tools for the identification of rickettsiae from human specimens and ectoparasite samples has permitted the identification of new rickettsial agents and new tick vectors implicated in the transmission of various rickettsiae, thus expanding our knowledge of both the types of disease caused by rickettsiae and the geographic range of rickettsioses.

Although *R. rickettsii* causing RMSF has been found in Mexico, Costa Rica, and Panama, where the disease is known as 'fiebre

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manchada',[9–12] the presence and characteristics of rickettsiae in Guatemala have not been well studied.In 1898, an outbreak of epidemic louse-borne typhus was reported from Quetzaltenango, which continued to experience small outbreaks of this disease between 1943 and 1956.[13] Vaccination, sanitation, and louse-control measures apparently eliminated epidemic typhus by 1959.[13,14] Subsequent surveillance identified epidemic typhus in several municipalities of Quetzaltenango between 1970 and 1972;[13] however, some of these later cases may have represented recrudescent Brill–Zinsser disease. To date, neither SFG rickettsiae nor clinical cases resembling RMSF have been reported from Guatemala.

We describe an outbreak of febrile illness in the peri-urban farming community of Moyuta in the Department of Jutiapa, Guatemala. The outbreak occurred from March to May 2007 and resulted in illness in 17 persons, with two deaths. Here, we present evidence that 10 of the 17 patients apparently suffered from a rickettsial disease.

2. Materials and methods

2.1. Epidemiological investigation

Moyuta is a peri-urban community approximately 80 km from the capital, Guatemala City, in the department of Jutiapa on the border with El Salvador, situated at 1283 m above sea level (Figure 1). The maximum daily temperature averages 34 °C and minimum temperature 21 °C, with minimal seasonal variations. Total annual rainfall averages 1400 mm per year, with one rainy season between May and October. The small town of 17 499 inhabitants is surrounded by pastures and farmland and is served by one health center. On May 16, 2007, the Health Area of Jutiapa was informed of the second of two deaths in the previous month

Figure 1. Guatemala and neighboring countries. The location of Moyuta is shown on the map.

from a febrile illness of unknown origin among employees of a dairy farm. The Guatemala National Health Laboratory performed preliminary testing, and the presence of significantly high levels of IgG and IgM antibodies reacting to *Rickettsia typhi* and *R. rickettsii* antigens was detected using an indirect microimmunofluorescence assay (IFA) in the blood of four ill persons, including the first fatality (Castillo, unpublished data). To identify the etiologic agent and prevent further cases, the Guatemalan Ministry of Public Health and Social Welfare (Ministerio de Salud Pública y Asistencia Social, MSPAS), along with the Health Area of Jutiapa and the Centers for Disease Control and Prevention (CDC) Regional Office for Central America and Panama, initiated an investigation to identify additional cases. This effort involved identifying additional suspect cases, collecting appropriate biological samples from ill persons and domestic and wild animals, collecting ectoparasites, and conducting laboratory diagnostics.

For the purposes of this outbreak investigation, a case was defined as fever and headache in a resident of the neighborhood surrounding the dairy farm (population 763) between March 1 and May 16, 2007. Household interviews were conducted in all 197 houses in the neighborhood using a questionnaire addressing type and duration of symptoms, demographic characteristics of the ill persons and their occupations, and their domestic, peri-domestic, or occupational exposure to pets, domestic animals, rodents, and ectoparasites.

2.2. Collection of specimens from ill persons

When possible, acute and convalescent ethylenediaminetetraacetic acid (EDTA) whole blood, serum and/or plasma were obtained from each resident meeting the case definition. For each serum sample, an aliquot was shipped to the Guatemala National Health Laboratory (Guatemala City, Guatemala) and the remainder was frozen at -70 °C and shipped to the CDC, Atlanta, Georgia, USA, for further testing.

2.3. Collection of ectoparasites and animal samples

Domestic animals (cows, horses, dogs, and cats) were examined for ticks and fleas; ectoparasites were collected and refrigerated in cryovials until they could be frozen at -70 °C and shipped to CDC in Atlanta. Flagging for ticks was conducted in the fields surrounding the dairy farm where the two deceased individuals had worked, as well as in the cow barns where they milked cows. One hundred live traps were placed around the farm to capture small mammals.

2.4. Serological procedures

Seroreactivity to rickettsial antigens was evaluated with an IFA assay conducted according to standard procedures, and further confirmed by Western blotting (WB). *R. rickettsii* strain Sheila Smith and *R. typhi* Wilmington grown in embryonated chicken eggs and *R. akari* MK cultivated in African green monkey kidney (VERO) cells were used as antigens, as previously described.[15] For the IFA, fluorescein isothiocyanate (FITC)-labeled, goat anti-human IgG (gamma-chain specific) and anti-human IgM (mu-chain specific) antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, MD) were used at dilutions of 1:200 and 1:100, respectively. For the detection of IgM antibodies, IgG was absorbed with a Mini Rapi-Sep-M kit in accordance with the manufacturer's instructions (Panbio Diagnostics, Columbia, MD). Samples were tested at consecutive twofold serum dilutions starting at 1:32, and titers were represented as the reciprocal of the last dilution exhibiting reactivity with a specific antigen. IgG and IgM titers $\geq$64 were considered positive for this investigation.

For WB, rickettsial cells purified from host cell debris were solubilized at room temperature and separated through 10% sodium dodecyl sulfate (SDS) polyacrylamide gels, as described previously,[15] and were transferred to 45 μm nitrocellulose membranes (Schleicher and Schuell Bioscience Inc., Keene, NH). Non-specific protein binding sites were blocked with 5% non-fat dry milk, followed by incubation with serum diluted at 1:100 and then with horseradish peroxidase (HRP)-labeled goat anti-human IgG (gamma-chain specific) and IgM Fc5 μ antibody (Chemicon-Millipore) at 1:2500 dilution. Bound enzyme was detected by reaction with substrate solution containing 0.015% 4-chloro-1-naphthol (Bio-Rad, Hercules, CA), 0.015% hydrogen peroxide, and 16.7% methanol in 0.02 M Tris-buffered solution (pH 7.5). The developed membranes were washed in water, dried between filter papers, and digitized. All serological tests were repeated twice and identical results were obtained on different dates by two different technicians.

2.5. PCR analysis of human specimens

DNA was extracted from 200 μl of EDTA-whole blood using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA). When blood specimens were not available, serum or plasma was centrifuged for 10 min at 14 000 $\times$ g and the resulting pellet, suspended in 200 μl of homologous supernatant, was used for DNA extraction. PCR assays for the rickettsial 17 kDa antigen gene were used for the detection of spotted fever and typhus group *Rickettsia* DNA using Amersham Ready-to-Go-Beads (Amersham Biosciences, Piscataway, NJ) or Qiagen Master Mix (Qiagen), as described elsewhere.[6,16] Semi-nested amplification of the 70–602 nt fragment of the rickettsial outer membrane protein (OmpA) gene was performed for species identification of the SFG rickettsiae.[17] Genomic DNA of *Rickettsia prowazekii, Rickettsia slovaca*, or *R. rickettsii* was used as a positive control for the PCR assays. Amplification of *omp*B, *sca*4, and *glt*A was performed as described elsewhere; amplified fragments were sequenced and analyzed as described previously.[17] Sequences generated in this study were submitted to the NCBI GenBank under the following accession numbers: 17 kDa protein gene: **JF437645**; OmpA gene: **JF437646**.

2.6. Laboratory confirmed case definitions

A clinical case of acute febrile illness was considered a probable case of rickettsiosis if there was a single positive titer from either acute or convalescent serum. A confirmed case of rickettsiosis was a positive PCR result or a fourfold increase in titer between acute and convalescent sera.

2.7. Testing of tick specimens

Ticks were identified to the genus using standard taxonomic keys.[18] Morphological identification of *Amblyomma cajennense* ticks was confirmed by direct sequencing of the mitochondrial 12S ribosomal DNA gene (GenBank accession number **JF437644**), as described previously.[19]

Amblyomma tick DNA was extracted using the QiaMiniAmp Kit (Qiagen) following the manufacturer's instructions, eluted with 200 μl AE elution buffer and stored at 4 °C before testing. *Rhipicephalus microplus* DNA was extracted using the Wizard SV 96 Genomic DNA Purification System (Promega, Madison, WI) and a Biomek 2000 Robotic Workstation (Beckman Coulter, Fullerton, CA), and was eluted with 120 μl sterile nuclease-free water.[20] PCR testing for typhus and SFG rickettsiae was performed as described above.

Table 1
Clinical and epidemiological characteristics of ill persons from Moyuta, Department of Jutiapa, Guatemala

Patient ID	Sex	Age	Occupation	Date of onset	Symptoms (days of duration)[a]							Sought medical attention/ hospitalization (days)	Treatment (days)	Exposure factors		Outcome[b]	Category[c]
					Fever	Headache	Chills	Myalgia	Nausea/ vomiting	Rash	Abdominal pain			Animal contact	Arthropod contact		
1	F	24	Teacher	3/15/2007	Yes (4)	Yes (5)	Yes	Yes	None	None	Yes (5)	Yes/no	Amoxicillin, acetaminophen	Dogs, cats, rats	NR	R	P
2	F	68	Housewife	3/25/2007	Yes	Yes	Yes	No	None	None	Yes	NR	NR	Rats	NR	R	P
3	M	34	Farm worker	4/10/2007	Yes (3)	Yes (3)	Yes (2)	No	No/yes (4)	None	None	Yes/no	Amoxicillin (2)	Rats, dogs, chickens, cows	Flea bites	R	P
4	F	39	Housewife	4/10/2007	Yes (5)	Yes (3)	Yes (6)	Yes	None	Petechial (5)	None	Yes/no	None	Dogs, cats, chickens, sheep	Flea bites	R	P
5	M	36	Farm worker	4/13/2007	Yes (4)	Yes (6)	None	NR	No/yes (1)[d]	Petechial (6)	None	Yes/no	Amoxicillin (1 dose)	Rats, dogs, chicken	Flea bites	D	P
6	F	2	Child	4/19/2007	Yes (7)	Yes (7)	Yes (7)	No	Yes (1)/no	Maculopapular (9)	None	Yes/no	Acetaminophen	Rats, dogs, chickens	None	R	P
7	M	23	Farm worker	4/20/2007	Yes (4)	Yes (5)	Yes (5)	No	Yes (4)/no	None	Yes (4)	No/no	Aspirin (8)	Dogs, rats	NR	R	N
8	M	8	Student	4/20/2007	Yes (18)	Yes (12)	Yes (9)	No	Yes (4)/yes (4)	Macular (20)	Yes (6)	Yes/yes (9)	Penicillin, erythromycin, doxycycline (9)	Dogs, chickens, rats	NR	R	P
9	M	78	Farm worker	4/22/2007	Yes (18)	Yes (6)	Yes (6)	Yes	None	None	Yes (6)	No/no	Acetaminophen	Rats, dogs, chickens	NR	R	P
10	M	7	Student	4/28/2007	Yes (16)	Yes (4)	None	Yes	None	None	None	Yes/no	Trimethoprim (7)	Rats	None	R	N
11	M	23	Farm worker	5/2/2007	Yes (15)	Yes (4)	None	NR	Yes (4)/no	None	None	No/no	None	Rats, dogs	NR	R	N
12	F	18	Housewife	5/5/2007	Yes (2)	Yes (2)	Yes (2)	Yes (2)	None	None	None	Yes/no	Acetaminophen	Dogs	None	R	N
13	M	17	Farm worker	5/5/2007	Yes (9)	Yes (9)	Yes (5)	No	Yes (6)/yes (6)	Petechial (2)	None	Yes/yes (1)	Doxycycline (1 dose)	Dogs, cows	Flea bites	D	C
14	F	47	Housewife	5/9/2007	Yes	Yes	Yes	Yes	None	None	None	NR	NR	Dogs, pigs	NR	R	N
15	F	18	Librarian	5/12/2007	Yes (5)	Yes (2)	Yes (3)	No	Yes (2)/no	None	None	Yes/no	Diclofenac, ibuprofen	Rats	Flea bites	R	N
16	F	28	Housewife	5/12/2007	Yes (2)	Yes (3)	Yes (1)	NR	NR	NR	NR	Yes/no	Doxycycline (3)	Rats, dogs, chickens	NR	R	P
17	F	11	Student	5/15/2007	Yes (2)	Yes (3)	None	Yes (3)	Yes (1)/no	None	None	Yes/no	Doxycycline (2), acetaminophen	Rats, dogs, chickens	Flea bites	R	N

F, female; M, male; NR, no records available.
[a] Duration of symptoms is provided if records were available.
[b] R, recovered; D, died.
[c] P (probable): single positive serology test or convalescent positive IgG and IgM serology with at least one antigen; C (fatal confirmed): PCR-positive; N (recovered suspect): IFA-negative or only IgM-positive serology.
[d] Patient 5 also experienced diarrhea lasting for 46 h.

2.8. Cell culture isolation of rickettsiae

Frozen tick samples were thawed and their triturates were inoculated into a 25-cm^2 flask containing a confluent monolayer of Vero E6 cells (CRL 1587/Vero 76, American Type Culture Collection, Manassas, VA). Cells were maintained in RPMI-1640 cell culture medium (Gibco, Invitrogen Corp., Grand Island, NY) supplemented with 2% fetal bovine serum (HyClone Laboratories Inc., Logan, UT), 5% tryptose phosphate broth, and 1 mM L-glutamine at 32 °C and 34 °C for several passages.[17] The presence of rickettsiae was monitored by PCR and microscopic observation of acridine orange-stained smears of the cultures.[17]

3. Results

3.1. Epidemiological and clinical case findings

Seventeen cases including two fatalities (case–fatality proportion 12%) were identified between April 10 and May 15, 2007, with illness onset dates from March 15 through May 15 (Table 1). In addition to headache and fever, ill persons also reported chills (76%), nausea (44%), abdominal pain (31%), vomiting (25%), and rash (31%). Symptoms persisted 1–20 days (median 4 days) following the onset of illness. A petechial rash with unspecified location was present during the illnesses of the two persons who died (residents 5 and 13) for 6 and 2 days, respectively. Similarly, petechiae were observed for 5 days in another adult resident (resident 4) who recovered; two ill children (residents 6 and 8) developed a maculopapular rash lasting for 9 and 20 days, respectively, that persisted for several days following resolution of fever. Eschar or eschar-like lesions were not reported for the two persons who died, nor were they noticed by the other patients in their retrospective self-reporting.

Of the 17 ill persons, eight (47%) were male, including the two fatalities. Their median age was 23 years (range 2–78 years). Six (35%) worked on the same dairy farm and 12 (71%) lived in the residential area of the farm. The two fatalities occurred in men who had been employed to milk the dairy cows; the second man had been hired to replace the first man after his death. Fourteen (82%) ill persons had known exposure to animals living on the farm, including cows, pigs, dogs, and chickens. Fifteen (88%) ill persons reported seeing rats during the day and/or evening hours or being bitten by fleas, or both. The presence of body lice or head lice was not reported by any ill person; however, respondents acknowledged being able to recognize ticks and seeing them routinely on animals. Twelve (80%) ill persons sought medical care at the local health center. Of these, eight received antibiotics, including amoxicillin (three patients), penicillin (one patient), erythromycin (one patient), trimethoprim (one patient), and doxycycline (four patients). One pediatric patient (resident 8) was hospitalized and treated with three different antibiotics. One of the ill persons who died (resident 5) was treated with amoxicillin, and the second (resident 13) received a single dose of doxycycline 1 h before he died. Five ill persons were given, or self-medicated with acetaminophen, diclofenac, ibuprofen, and aspirin; two ill persons recovered without medication.

3.2. Testing of human specimens

At the CDC, serum samples of seven (44%) ill persons tested positive by IFA for both IgM and IgG antibodies, reacting with antigens of *R. typhi*, *R. rickettsii*, and/or *R. akari*, with titers ranging from 64 to 4096 (Table 2). No IFA antibody was detected in two samples from days 5 and 7 post-onset for the second fatality (resident 13). The sample from the other fatality (resident 5) tested IFA-positive for IgG and IgM antibody to *R. rickettsii* antigen at the Guatemala National Laboratory (Castillo, unpublished data), but the sample was not available for confirmatory testing at CDC. Sera of seven tested ill persons (44%) had only low IgM titers to one or more antigens, and elevated IgM antibody reacting with *R. akari* antigen was only present in the serum of one of these persons (resident 16). IgG-reactive sera were collected at 22 to 46 days after the onset of the febrile illness and were classified as convalescent based on the time course of the illnesses. When all

Table 2
Laboratory assessment of serum and blood specimens for antibodies reacting against rickettsiae and for rickettsial DNA by PCR performed at the CDC

Patient ID	Days after onset	PCR[a]	IgM-IFA[b]			IgG-IFA[b]		
			RT	RR	RAK	RT	RR	RAK
1	56	Neg (P)	256	512	32	<32	32	64
2	46	Neg (P)	512	32	256	1024	<32	1024
3	NR	Neg (S)	128	256	64	<32	512	<32
4	31	Neg (P)	512	1024	128	4096	2048	1024
	49	NT	1024	512	256	32	2048	256
5[c]	NR	NT	NT	NT	NT	NT	NT	NT
6	22	NT	128	256	<32	512	256	128
	40	NT	64	64	64	<32	128	<32
7	25	Neg (P)	32	64	64	<32	<32	<32
8	25	Neg (P)	256	128	128	32	<32	512
	38	NT	512	64	128	256	32	64
9	24	Neg (P)	32	64	64	<32	<32	256
10	18	NT	<32	64	32	<32	<32	<32
11	16	Neg (P)	64	<32	64	<32	<32	<32
12	18	Neg (P)	32	<32	32	<32	<32	<32
13	5	Pos (P)	32	32	<32	<32	<32	32
	7	Neg (S)	32	<32	32	<32	<32	32
14	5	Neg (P)	64	32	64	<32	<32	<32
15	2	Neg (P)	<32	64	32	<32	32	<32
	13	NT	64	64	32	<32	<32	<32
16	2	Neg (P)	32	<32	256	<32	<32	<32
17	3	Neg (P)	32	<32	<32	<32	32	32
	16	NT	64	64	32	<32	<32	32

CDC, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; NR, not recorded.
[a] P, plasma pellet; S, serum pellet; NT, not tested because of insufficient sample, no sample, or sample not suitable for PCR.
[b] Reciprocal IgM and IgG titers against antigen of *R. typhi* (RT), *R. rickettsii* (RR), and *R. akari* (RAK).
[c] Samples from patient 5 were not available for testing at the CDC.

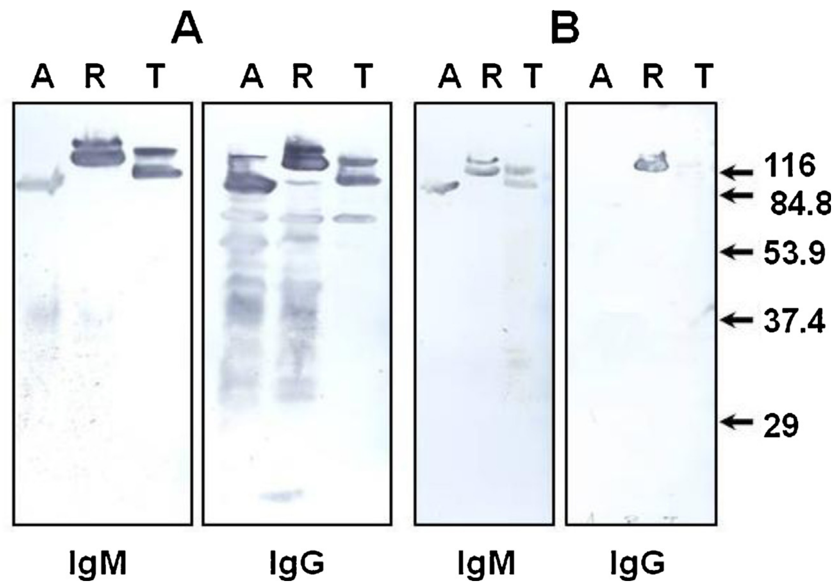

Figure 2. Western blotting reactivity of representative serum specimens. Panels A and B show the two different patterns of Western blotting reactivity observed in IFA-positive persons: resident 4 (panel A) and resident 8 (panel B). Arrows indicate the positions of the protein molecular weight markers with the following sizes, from the top to the bottom: 116 kDa, 84.8 kDa, 53.9 kDa, 37.4 kDa, and 29.0 kDa (Bio-Rad). The following antigen abbreviations are used: A, *R. akari*; R, *R. rickettsii*; T, *R. typhi*.

IFA-positive sera were tested by WB, the antibodies were primarily directed against high molecular weight surface protein antigens ($\geq$110 kDa) of each of three rickettsiae (Figure 2, A and B). Some sera exhibited weak IgM antibody reactivity to *R. akari* and *R. rickettsii* lipopolysaccharide (LPS) antigens at approximately 37.4 kDa and strong IgG antibody reactivity against LPS as washboard at 84–32 kDa (Figure 2A). Negative and positive serum controls were processed for each serologic assay and they performed as expected.

PCR was performed on 15 DNA samples from 14 ill persons whose blood samples were collected 2 to 56 days after the onset of illness (Table 2). Only the DNA from a precipitate of the serum of one of the fatalities (resident 13; day 5 after onset of the illness) tested positive for SFG rickettsiae using 17 kDa protein gene nested PCR (GenBank accession number **JF437645**). The nucleotide fragment of the fragment amplified had the greatest similarity (99%) with the highly conserved homologous gene fragments of the core SFG rickettsiae and differed from that of *R. akari*, *R. typhi*, and *R. prowazekii*. Other fragments typically used for molecular identification of *Rickettsia*, including *glt*A, *omp*A, *omp*B, and *sca*4, could not be amplified.

3.3. Environmental assessment and testing of tick samples

Five rats were trapped during the environmental investigation; they tested negative by PCR for *Leptospira* spp. at the Guatemalan National Laboratory (Castillo, unpublished data).

A. cajennense and *R. microplus* ticks were collected from a horse and a cow, respectively, cared for by the two farm workers who died. No ticks were collected by flagging. Twelve *A. cajennense* and 85 *R. microplus* tested negative for DNA of typhus group rickettsiae. DNA from one *A. cajennense* tested PCR-positive for the *omp*A fragment of SFG rickettsiae. The nucleotide sequences of the *omp*A PCR amplicon had the greatest genetic similarity with *omp*A sequences of *Rickettsia africae* and *Rickettsia sibirica* cluster (GenBank accession number **JF437646**). Other fragments of *Rickettsia*, including 17 kDa protein gene, *glt*A, *omp*B, and *sca*4, could not be amplified from this tick DNA. Inoculation of the triturate of the PCR-positive tick into Vero cell cultures did not yield a rickettsial isolate following three consecutive blind passages at 32 °C and 34 °C. No fleas were collected during this investigation.

4. Discussion

To our knowledge, we report the first confirmed outbreak of spotted fever rickettsiosis in Guatemala. The diagnosis was made by identification of SFG *Rickettsia* DNA in a specimen from one of two persons who worked on the same dairy farm and who died of an acute febrile illness, and in a tick found on a farm animal. In total, 17 ill residents with similar clinical manifestations were identified from a small community in the Department of Jutiapa from March through May 2007. Evidence for significant rickettsial exposure (IgG IFA antibodies, PCR, WB) was obtained for 10 of them, including the two residents who died.

Autopsies were not performed on the two deceased residents. Serum specimens were only obtained from one of the two deceased, and no specimens were available for testing at CDC from the first fatal case. The rickettsial etiology of this illness was confirmed by detection of rickettsial DNA in a specimen of serum collected on day 5 after onset of illness from the second fatality. This patient had not seroconverted to rickettsiae when he died on day 7 of illness. In contrast, IFA tests performed at the Guatemala National Health Laboratory on serum collected 5 days after onset of illness from the first fatality, detected high titers of antibodies reacting with both *R. typhi* and *R. rickettsii*. Negative testing for leptospirosis and ehrlichiosis in this patient and in other patients ruled out other etiologies responsible for this illness (not shown).

Limitations of the samples available for analysis restricted confirmation of the specific rickettsial etiologic agent(s) of these illness(es). Laboratory results were obtained at CDC for 16 ill persons; however, standard paired acute and early convalescent serum specimens were available for only two ill persons (residents 15 and 17), who both exhibited only a weak IgM titer of 64 to *R. typhi* and *R. rickettsii* antigens. For other ill persons, only either acute or convalescent serum specimens were available. One DNA sample (from one of the fatalities) of the five DNA samples made from acute IFA-negative serum specimens tested positive by PCR for rickettsiae. Microimmunofluorescence (also called IFA) is presently the gold standard for the serological diagnosis of rickettsial diseases;[21] however, its sensitivity depends on the timing of sample collection relative to illness onset. Serum samples obtained early in the infection are often negative or so close to the

cutoff limit that results are difficult to interpret; this was the case for some ill persons in this investigation. The sensitivity of the IFA is estimated at 94–100% after 14 days of illness, and testing paired acute and convalescent samples substantially increases confidence in the results. In the case of less severe rickettsial infections, such as rickettsialpox and *R. parkeri* or *R. africae* infection, convalescent sera often do not demonstrate significant titers until 25–28 days after the onset of illness.[6,22] The duration of antibody persistence after recovery from infection is also variable. In RMSF, IgG and IgM antibody titers increase concurrently by the second week of illness, and IgM antibodies disappear after 3–4 months, whereas IgG titers may persist from 7–8 months to several years.[23,24] Unfortunately, while we used *R. typhi*, *R. rickettsii*, and *R. akari* as surrogate antigens for the unknown etiologic agent(s) in this study and confirmed the presence of antibodies reacting with expected immunogenic rickettsial antigens in serum specimens by WB, it is not possible to define the rickettsial agent responsible for this seroconversion by serology, nor to exclude the possibility that titers were pre-existent in ill persons.[25,26] In this outbreak, extensive cross-reactivity was observed with antigens of typhus and SFG rickettsiae by both IFA and WB. This could be due to a novel rickettsial species or to multiple exposures to a variety of rickettsial agents on different occasions during the residents' lifetimes. Appropriate case–control studies and serological surveys are needed to characterize exposure to rickettsial agents found in different regions of Guatemala. We were not able to complete the molecular identification of the rickettsial agent causing this outbreak due to the lack of appropriate specimens such as whole blood, skin biopsy collected from the rash area, or autopsy specimens.

Testing of arthropods collected from residents, pets, or the immediate environment of the suspected cases may allow the detection of rickettsial agents. Of the two tick species collected during this investigation, *A. cajennense* and *R. microplus*, only one *Amblyomma* tick tested PCR-positive for a SFG *Rickettsia* and it was genetically closely related to *R. africae* and *R. sibirica* rather than other more common species like *Rickettsia amblyommii*.[20,27] Until relatively recently, only *A. cajennense*, *Haemaphysalis leporispalustris*, and *Rhipicephalus sanguineus* were implicated as vectors for the transmission of *R. rickettsii* in Mexico and Central America.[28–30] However, in Brazil and the USA, a more extensive list of tick species has been implicated in the natural cycles of many rickettsial agents pathogenic for humans.[1,31–33] Beside *A. cajennense*, *Amblyomma aureolatum* and *Amblyomma oblongogutatum* are involved in the natural cycles of *R. rickettsii*, while *R. parkeri* has been detected in *Amblyomma triste*.[27] Furthermore, recently reported SFG rickettsial agents causing *R. parkeri*-like illness in Brazilian patients from the Atlantic rain forest were found in *Amblyomma ovale* and *R. sanguineus*.[34] Small to medium size mammals are most frequently considered as reservoirs for many SFG rickettsiae. On the other hand, capybaras (*Hydrochoerus hydrochaeris*), the largest known rodent, are demonstrated to be a primary reservoir for *R. rickettsii* in Brazil.[27] Also, a recent study in Mississippi, USA, suggested that cattle can serve as a transportation mode for the dissemination of *R. parkeri*-infected *Amblyomma maculatum* ticks.[35]

Preliminary studies indicate that *R. felis*[36] and *Rickettsia hoogstraalii* are also present in arthropods from Guatemala (Estevez and others, unpublished data). Based on experience in the Yucatan Peninsula,[37,38] it is reasonable to expect that cases of cat flea rickettsiosis may also occur in Guatemala. Whether differences in the genetic or nutritional status of local human populations may also influence the clinical manifestations or morbidity of rickettsial diseases in Central America remains to be evaluated.

In conclusion, we report the first confirmed outbreak of SFG rickettsiosis in Guatemala. Our data indicate a need for further rickettsial disease surveillance and education of the medical community about the signs and symptoms of rickettsial diseases and proper patient management, particularly when fever of unknown origin is present.

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