INVESTIGATION OF THE EMERGENCE OF TYPHUS GROUP RICKETTSIA IN CENTRAL TEXAS

A Dissertation

by

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ABSTRACT

Background: Typhus group rickettsia (TGR) can cause disease after exposure to *Rickettsia typhi*, or *Rickettsia prowazekii*, bacterium carried by fleas. Symptoms of TGR rickettsiosis are often non-specific, and there is evidence of the emergence of this pathogen in Texas. However, significant gaps in the knowledge of the epidemiology of disease exist.

Methods: Using data from Baylor Scott & White (BSW) hospitals and clinics, case-series and case-control analyses attempted to describe characteristics of diagnosed cases of acute TGR infections in central Texas and to model infection and make predictions about diagnosis. A laboratory investigation looked to identify potential animal reservoirs in Texas.

Results: The case-series analysis revealed discrepancies in diagnostic criteria between physicians, state health authorities, and other research groups. The most commonly reported symptom was fever (87.5%), and the majority of patients were found to have acute liver injury (68.8%).

Logistic regression models of TGR rickettsiosis patients compared to Rocky Mountain spotted fever (RMSF) patients revealed age (OR: 1.06; 95% CI: 1.01-1.10), acute liver injury (OR: 8.60; 95% CI: 2.06-35.96), and laboratory evidence of systemic infection (OR: 6.58; 95% CI: 1.21-35.89) to be associated with TGR diagnosis, while heart disease is more likely associated with RMSF diagnosis (OR: 0.13; 95% CI: 0.02-0.77). Comparing TGR rickettsiosis patients to patients testing negative for RMSF and TGR infections found living on a farm or ranch, or in a wooded area (OR: 31.05; 95% CI: 4.01-

240.17), acute liver injury (OR: 28.88; 95% CI: 3.46-241.14), and acute lung infection (OR: 17.78; 95% CI: 2.11-149.81) to be associated with TGR diagnosis.

DNA extraction product from 238 skunks, 196 canines, seven opossums, five mice, two raccoons, and one rat were tested with quantitative real-time polymerase chain reaction (PCR), but did not show evidence of infection with *R. typhi*.

Discussion: The challenge of confirming diagnosis of TGR infection makes characterizing, calculating prevalence, and modeling TGR infection difficult. Additionally, difficulty in identifying and describing infection in animal species contributes to gaps in epidemiological understanding of TGR rickettsiosis. Future research should involve active surveillance of patients and animal models of infection.

DEDICATION

I would like to dedicate this dissertation to my mother, Clare Sheffield, who has never once doubted that I was destined to do great things. She has encouraged me to never settle for less than that of which I was capable, and showed me that women can be strong and independent, even when society says otherwise. If given a million words, I would still not be able to adequately express my gratitude to you. I owe most of who I am today to your love, support, blood, sweat, and tears.

I would also like to dedicate this project to my grandfather, John "Doc" Sheffield. Granddaddy Doc, you set an example of the importance of education, and although we never had the opportunity to meet in this world, and it took me a while to get back to school and earn a terminal degree, I aspire to be as inspirational to the next generation as you have been to me.

I hope I have made you both proud.

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Contributors

This work was supervised by a dissertation committee consisting of Professor Jennifer Horney and Rebecca Fischer of the Department of Epidemiology and Biostatistics and Professor(s) Scott Lillibridge of the Department of Epidemiology and Biostatistics and Sarah Hamer of the Department of Veterinary Integrative Biosciences.

The data analyzed for Section 4 for the dissertation was completed by the student, in collaboration with Texas A&M University College of Veterinary Medicine and Baylor College of Medicine, and under the advisement of Rodion Gorchakov of the Department of Pediatric Tropical Medicine at Baylor College of Medicine.

All other work conducted for the dissertation was completed by the student.

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NOMENCLATURE

BIC	Bayesian Information Criterion
BSW	Baylor Scott & White
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
Ct	Cycle Threshold
DNA	Deoxyribonucleic Acid
DSHS	Department of State Health Services
EMR	Electronic Medical Records
ER	Emergency Room
HSR7	Health Service Region 7
IFA	Immunofluorescence Assay
LRT	Likelihood Ratio Test
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RMSF	Rocky Mountain Spotted Fever
SFGR	Spotted Fever Group Rickettsia
SHNF	Sam Houston National Forest
TGR	Typhus Group Rickettsia

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1. INTRODUCTION

1.1 Introduction

Typhus group rickettsia (TGR), comprised of the bacteria *Rickettsia typhi* and *Rickettsia prowazekii,* can cause disease after exposure to the bacteria. These are rod-shaped, gram-negative bacteria that multiply in the cytosol of endothelial cells.[1] The bacteria are carried by *Ctenocephalides felis* and *Xenopsylla cheopis* fleas, and their transmission cycle is maintained by animal hosts. Humans become infected when flea feces containing the bacteria enter the body through breaks in the skin, sometimes resulting clinical rickettsiosis. Throughout the world, rodents are the most common potential reservoirs; however, many researchers have detected previous exposure in cats, dogs, and opossums, implying they may act as effective intermediary hosts.[2-14] Areas of endemic TGR are often subtropical or tropical; however cases have been reported in other climates.[15, 16] In the United States, the majority of cases have been reported in California, Hawaii, and Texas, but there has also been detection of the bacteria in fleas in Oklahoma.[17]

Symptoms of TGR rickettsiosis are often non-specific and frequently include a "classic triad" of fever, headache, and rash.[18, 19] Other common symptoms are body aches, loss of appetite, nausea, vomiting, stomach pain, and cough. Symptoms typically appear between five and 14 days post infection. Rickettsiosis is likely to be mistaken for other infections, as the symptoms are common to other illnesses, including other rickettsial infections, such as RMSF.[20] Rickettsiosis can produce severe complications when not

treated properly. According to a study published by the Centers for Disease Control and Prevention (CDC) that described severe cases in south Texas, the most common complications were bronchiolitis, pneumonia, meningitis, septic shock, cholecystitis, pancreatitis, myositis, and rhabdomyolysis.[21]

In Texas, the number of reported cases of rickettsiosis has been on the rise, increasing from 27 per year in 2003 to 364 per year in 2016.[19, 22] The majority of reported cases occurred in counties located in the Rio Grande Valley near the Texas-Mexico border and near the Gulf of Mexico. Surveillance data from the Texas Department of State Health Services (DSHS) reflects a new geographic distribution of TGR, with cases occurring throughout central and south-central Texas.[22] Only a few studies have reported on the clinical manifestations associated with the increase of rickettsiosis, but they have focused on the metropolitan areas of Houston (Harris County), Galveston (Galveston County), Austin (Travis County), as well as the Rio Grande Valley along the Texas-Mexico border (Starr County, Hidalgo County, Willacy County, and Cameron County) (Figure 1).[3, 5, 18, 19, 21, 23-33]

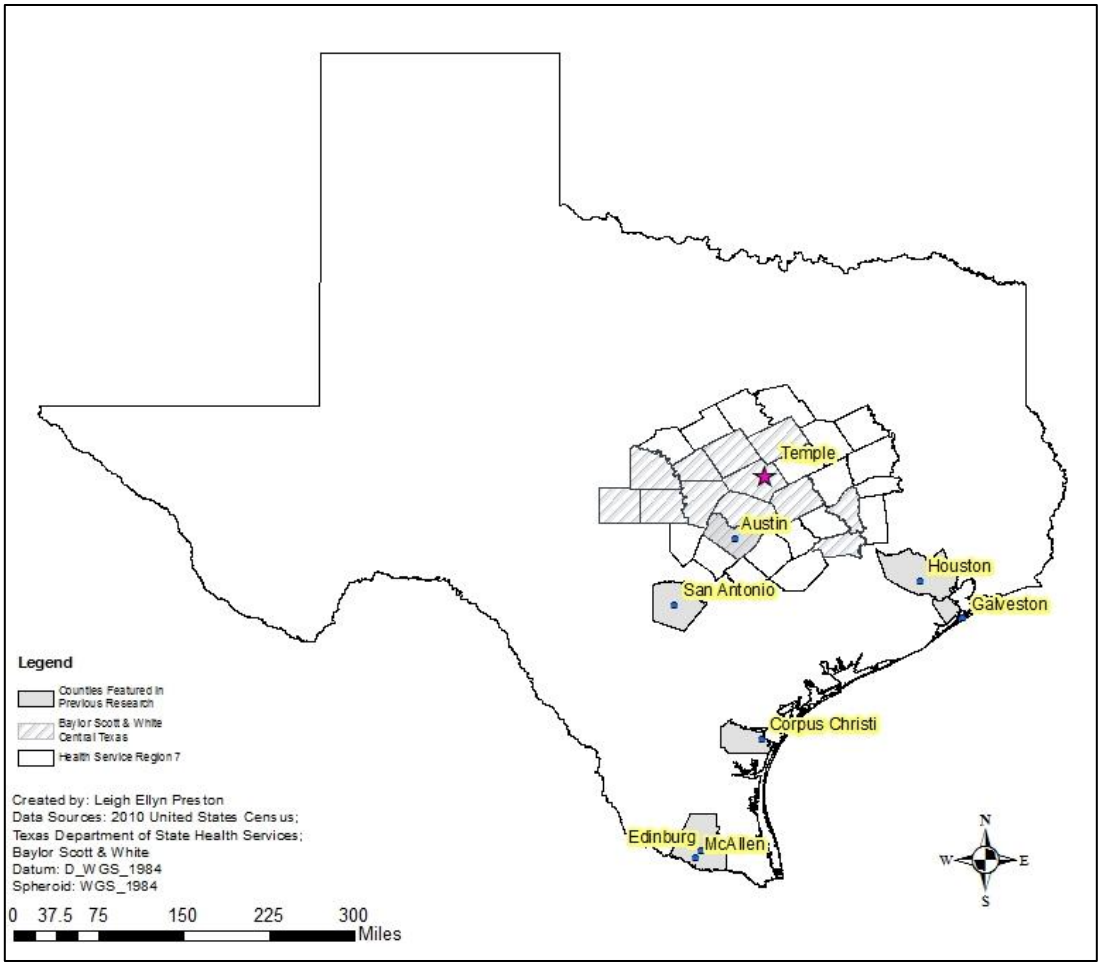


Figure 1. Map of Previous and Current Typhus Group Rickettsia Research Areas, 2002-2018.

Despite the recent emergence of TGR rickettsiosis in central Texas, a gap exists in the knowledge of clinical disease presentation, diagnostic criteria for clinical decision-making and surveillance purposes, and relevant potential animal reservoirs. Although rickettsiosis can be easily treated with doxycycline, delayed diagnosis and treatment can lead to severe consequences. The purpose of this study is to describe clinical manifestations in known cases of acute TGR rickettsiosis in central Texas, a novel geographic location of public health concern for disease, and to identify potential animal

reservoirs in the state. The prevalence of infection and characteristics of rickettsiosis among patients in central Texas is explored through a case-series analysis; predictions and modeling of rickettsiosis in central Texas are done with a case-control analysis; and possible animal reservoirs in Texas are explored through a laboratory analysis. The ultimate goal is to illuminate knowledge gaps specific to the region that can be used to improve disease diagnosis and surveillance and inform timely clinical management and disease prevention efforts.

1.2 Study Setting and Methods

This study investigates rickettsiosis in both humans and animals. Investigation of acute human cases of rickettsiosis was conducted in partnership with the Baylor Scott & White (BSW) healthcare system, and screening of TGR infection in animals was performed in collaboration with investigators at the Texas A&M University College of Veterinary Medicine and the Baylor College of Medicine Section of Tropical Medicine.

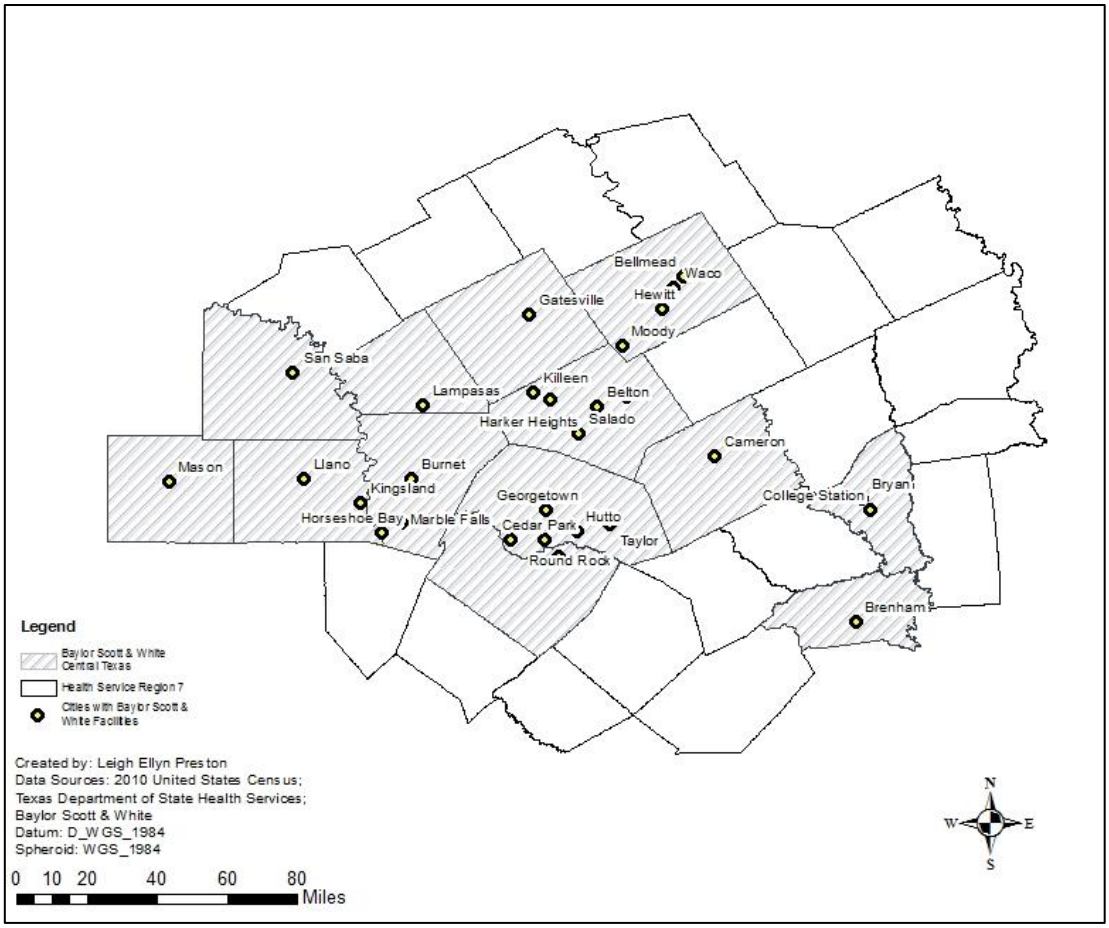


Figure 2. Baylor Scott & White Facilities in Central Texas and Health Service Region 7.

1.2.1 Study Region

The population served by BSW facilities in central Texas region mostly resides within the DSHS Health Service Region 7 (HSR7) (Figure 2). The one exception is Mason County, which is located in Health Service Region 9, to the west of HSR7. The distribution of BSW facilities throughout HSR7 makes it an ideal partner for the investigation of the emergence of TGR in central Texas. Many counties in HSR7 (58%) are considered rural, as defined by the nation's Human Resources & Services Administration's Federal Office of Rural Health Policy (FORHP), which uses a

combination of the United States Census Bureau and the Office of Management and Budget designations.[34]

According to 2017 census data, almost two-thirds (63%) of HSR7 residents report their ethnicity as non-Hispanic white, which is higher than in Texas overall (42%), and 23% report their ethnicity as Hispanic, which is lower than Texas overall (39.4%). The average percentage of the population in poverty is 15.5% (median, 15.3%; range, 5.8% to 25.6%), and the average percentage of the population under 65 years of age without health insurance is 20% (median, 20%; range, 11% to 27%). The overall prevalence of poverty for the state of Texas is 14.7%, and the uninsured under 65 percentage for the state is 19.4%.[35] Thus, demographics for the study area differ from state averages in that the majority of counties in the study area have smaller population densities, more residents report their ethnicity to be non-Hispanic white and fewer identify as Hispanic, a higher proportion of the population live in poverty, and slightly higher percentage of residents under age 65 are uninsured.

Individual counties included in the study region also differ from counties where TGR rickettsiosis cases have previously been described. Table 1 shows demographics for individual counties of interest to this investigation, and Figure 2 shows the region that is the subject of the current investigation in the geographical context of the counties from which data has already contributed to the understanding of TGR in Texas. Namely, counties in central Texas have a higher percentage of non-Hispanic white residents and lower percentage of Hispanic residents, fewer residents in poverty, and lower population densities. Importantly, this is the first investigation of TGR in rural counties. Figure 2 shows the region that is the subject of the current investigation in the geographical context

of the counties from which data has already contributed to the understanding of TGR in Texas.

Table 1. County Demographics[†]

County	Rural	Population Density (per mile^2)	Non-Hispanic White (%)	Hispanic (%)	Poverty (%)	Uninsured (%)
Health Service Region 7 Counties						
Bastrop	No	83.5	52.6	37.8	13.2	21.1
Bell*	No	295.2	45.6	24.8	12.9	13.7
Blanco	Yes	14.8	76.7	19.7	10.7	22.4
Bosque	Yes	18.5	77.6	18.7	15.8	22.7
Brazos*	No	332.8	55.9	25.8	24.9	16.5
Burleson	No	26.1	65.0	20.6	14.4	19.1
Burnet*	Yes	43.0	73.4	22.5	11.5	19.8
Caldwell	No	69.8	39.7	52.3	16.6	20.9
Coryell*	No	71.7	58.5	18.3	14.2	13.4
Falls	No	23.3	51.2	23.3	25.6	19.7
Fayette	Yes	25.8	71.0	21.3	11.1	19.3

Table 1. Continued

County	Rural	Population Density (per mile^2)	Non-Hispanic White (%)	Hispanic (%)	Poverty (%)	Uninsured (%)
Freestone	Yes	22.6	67.0	15.4	19.1	20.3
Grimes	Yes	33.8	58.3	24.4	16.7	21.0
Hamilton	Yes	10.2	84.6	12.5	15.1	22.9
Hays	No	231.7	53.8	39.0	12.4	15.6
Hill	Yes	36.6	70.3	20.8	16.2	22.6
Lampasas*	No	27.6	72.0	20.0	13.6	19.3
Lee	Yes	26.4	63.4	23.4	12.7	18.6
Leon	Yes	15.7	76.1	14.6	18.0	22.5
Limestone	Yes	25.8	58.3	22.4	21.1	20.7
Llano*	Yes	20.7	86.1	10.7	13.4	19.4
Madison	Yes	29.3	54.9	23.0	17.3	22.0
Mason*	Yes	4.3	72.7	25.4	13.2	26.7
McLennan*	No	226.5	26.4	0.1	18.5	18.4
Milam*	Yes	24.3	62.0	26.6	16.3	19.5

Table 1. Continued

County	Rural	Population Density (per mile^2)	Non-Hispanic White (%)	Hispanic (%)	Poverty (%)	Uninsured (%)
Robertson	No	19.4	57.5	20.9	18.7	19.6
San Saba*	Yes	5.4	64.4	30.3	20.1	26.4
Travis*	No	1034.4	49.0	33.9	12.2	15.2
Washington*	Yes	55.8	63.7	16.3	14.1	17.9
Williamson*	No	378.0	59.6	24.5	5.8	10.9
Previous Research Counties[3, 5, 18, 19, 21, 23-33]	**Rural**	**Population Density (per mile^2)**	**Non-Hispanic White (%)**	**Hispanic (%)**	**Poverty (%)**	**Uninsured (%)**
Bexar	No	1383.1	60.3	27.7	16.3	16.6
Galveston	No	769.9	24.6	57.3	12.9	15.7
Harris	No	2402.2	43.0	29.7	16.6	20.7
Hidalgo	No	493.2	92.2	6.2	31.2	29.7
Nueces	No	405.8	63.9	29.4	15.1	18.3

* Denotes Baylor Scott & White central Texas counties † Data taken from 2017 census data

1.2.2 Diagnostic Criteria for Human TGR Rickettsiosis

TGR rickettsiosis is a notifiable condition in the state of Texas, but reporting can be challenging for physicians for a number of reasons. First, there is no standard case definition. The state diagnostic criteria defines a confirmed case of TGR rickettsiosis as an individual with a clinically compatible presentation that is also laboratory confirmed. Laboratory confirmation is defined as either a four-fold increase in titers of *R. typhi*-specific antibodies from acute infection to convalescent phase (three to four weeks later); amplification of TGR DNA by PCR; visualization of *R. typhi*-specific antibodies in skin lesion or tissue specimen by immunofluorescence (IFA), or isolation of the causative bacteria. A probable case is defined as a clinically compatible case with IFA titers of ≥1:128, or a compliment fixation test result >1:16, or other, unspecified serology.[36] In clinical practice, however, physicians at BSW consider any patient with an IFA titer of ≥1:64 a case, and treat the patient accordingly. Another barrier to accurate reporting is that diagnostic tests used in the DSHS case definition involve repeat testing on patients (e.g., testing blood from suspected case-patients during both the acute and convalescent phases). This can be challenging and time consuming for physicians, whose main concern is treating the patient, and to patients who would require an additional clinical visit despite convalescence. Lastly, there is some testing cross-reactivity with spotted fever group rickettsia (SFGR), another subgroup of *Rickettsia spp.* that differ clinically and epidemiologically from TGR, so testing requires trained laboratory personnel and validated protocols.[29, 37, 38] For these reasons, it is likely that the number of cases reported to DSHS underrepresents the actual prevalence of TGR rickettsiosis in central Texas and throughout the state of Texas.

The lack of standardized case definition for TGR rickettsiosis is also reflected in the literature, as investigators apply different criteria for defining TGR rickettsiosis. Eight researchers define a confirmed case as a patient having a four-fold increase in acute and convalescent antibody titers,[3, 18, 19, 21, 23, 24, 26, 28, 31] isolation of *R. typhi*,[19, 24, 28] positive PCR,[18, 19, 24, 28] positive IFA in tissue,[19, 28] and/or a single *R. typhi*-specific antibody titer ≥1:1024.[18, 19, 24] Erickson et al. suggest the following criteria: a confirmed case is a patient with an IFA titer of ≥1:1024 and a RMSF-specific titer ≤ 2 times the TGR-specific titer; amplification of TGR DNA by PCR; ≥ 4-fold increase in acute and convalescent titers. A probable case is a patient with an IFA titer ≥ 1:128 and a RMSF-specific titer ≤ 2 times the TGR-specific titer, and a suspected case is a patient with an IFA titer of ≥1:64 and a negative RMSF-specific titer.[18] For this investigation, the diagnostic criteria used by BSW physicians will be compared to those defined by Erickson et al.[18] Case definitions are compared in Table 2. The many different case definitions used in TGR surveillance and research complicate understanding of TGR, clinically or epidemiologically, throughout the country.

Table 2. Diagnostic Criteria

Texas Department of State Health Services Criteria[36]	Confirmed Case:
	• Clinically compatible presentation with one or more of the following laboratory confirmations:
	  ○ Four-fold increase in in titers of *R. typhi*-specific antibodies from acute infection to convalescent phase
	  ○ Amplification of typhus group rickettsia DNA by polymerase chain reaction
	  ○ Visualization of *R. typhi*-specific antibodies in skin lesion or tissue specimen by immunofluorescence
	  ○ Isolation of the *R. typhi* bacteria
	Probable Case:
	• Clinically compatible presentation with one or more of the following laboratory confirmations:
	  ○ Immunofluorescence titers of ≥1:128
	  ○ Compliment fixation test result >1:16
	  ○ Other unspecified serology

Erickson et al. Criteria[18]	Confirmed Case: • Immunofluorescence titer of ≥1:1024 and a Rocky Mountain spotted fever-specific titer ≤ 2 times the typhus group rickettsia-specific titer • Amplification of typhus group rickettsia DNA by polymerase chain reaction • Four-fold increase in in titers of *R. typhi*-specific antibodies from acute infection to convalescent phase Probable Case: • Immunofluorescence titer ≥ 1:128 and a Rocky Mountain spotted fever-specific titer ≤ 2 times the typhus group rickettsia-specific titer Suspected Case: • Immunofluorescence titer of ≥1:64 and a negative Rocky Mountain spotted fever-specific titer
Baylor Scott & White Criteria	Clinically compatible presentation with immunofluorescence titer of ≥1:64

1.2.3 Data Collection

BSW provided a list of all patients who had been tested for rickettsial diseases from January 1, 2012 to August 15, 2018, at BSW facilities located in the central Texas region. Patients appearing on this list were separated into three groups: patients testing positive for TGR, patients testing positive for RMSF, and patients testing negative for both. Patient groups were cross-referenced so that no patient was included in more than one patient group, and multiple tests on a single patient were removed so that each patient only appeared once in the dataset. For this investigation, any patient who tested positive for both TGR and RMSF was placed in the TGR-positive group. TGR-positive patients serve

as cases in a case-series and a case-control analyses, RMSF-positive patients serve as one group of controls in a case-control analysis, and negative patients serve as another control group in a case-control analysis. Specific analysis plans are described in the respective chapters. This study was reviewed and approved by the Baylor Scott & White and Texas A&M University Institutional Review Boards (BSW: 018-501; TAMU: IRB2018-0526).

Banked blood samples from various animal species that could be potential reservoirs in Texas were analyzed for the final stage of this investigation. All specimens were previously collected by students in the laboratory of Dr. Sarah Hamer at the Texas A&M University College of Veterinary Medicine, or by Bonnie Gulas-Wroblewski from the Texas A&M University College of Veterinary Medicine and Angelo State University in San Angelo, Texas. Blood specimens were examined for the presence of *R. typhi* using quantitative real-time PCR. Samples from 196 canine samples were collected at Texas animal shelters, and samples from 15 wild animals were collected near the Stubblefield campsite in the Sam Houston National Forest (SHNF). Canine and wildlife specimens from Dr. Sarah Hamer's laboratory were collected from May 2013 to December 2014. Ms. Gulas-Wroblewski and Angelo State University collected skunk specimens throughout the state of Texas from April 2, 2012 to May 11, 2018. All samples were analyzed using the Life Technologies Applied Biosystems ViiA Real Time PCR System Version 1.2.4 (Carlsbad, CA). Specific information regarding the PCR assay can be found in Chapter 4. This work was reviewed and approved by the Texas A&M University and Baylor College of Medicine Institutional Biosafety Committees (TAMU: IBC2015-144; BCM: HAHC669).

1.3 Literature Review

1.3.1 Literature Review of Research Conducted in the United States

In order to gain a better understanding of the epidemiology and become familiar with the existing literature on TGR rickettsiosis in the United States, a systematic review was performed using PubMed to identify relevant research. Papers published in English in the last 16 years (2002 - 2018) were identified using the search terms "murine typhus," "flea-borne typhus," and "flea borne typhus." This search yielded 454 relevant papers. The title and abstract of each paper were searched to exclude studies performed outside of the United States. Twenty-four relevent studies were reviewed for the location of the study, the type of study, the study population, and the conclusions of the researchers. Information on the studies can also be found in the Appendix. Seventeen of the studies were conducted in Texas, or written about cases that occurred in Texas. [3-5, 18, 19, 21, 23-33] Seventeen involved human cases, and nine involved animal or flea investigation.[2-5, 9, 17-19, 21, 23-33, 39] None of these studies involved control groups or attempted case-control analysis. The studies focused on animals and fleas and involved testing blood samples or fleas for active infection or evidence of past infection.

The human case reports, case-series, or cross-sectional studies described typical symptoms of TGR rickettsiosis, as well as any unusual findings.[18, 19, 21, 23-27, 30, 32, 33] In all of the case reports, patients were hospitalized with complaints of fever, and most reported contact with animals that could serve as TGR reservoirs. All patients described presented with high fevers that lasted for several days, many had headaches, rashes, and stomach pains or vomiting and diarrhea. In one case, the diagnosis was made 72 days after the onset of symptoms and after treatment for a possible viral illness. The patient developed

long-term sequelae, including headaches, short-term memory impairment, and diagnosis with a learning disability, which were attributed to the long course of infection.[26] One study reviewed only cases of fatal TGR rickettsiosis and found that in all patients, fever was reported, and the majority had thrombocytopenia, elevated liver enzymes, nausea or vomiting, rash, and respiratory manifestations. [28] Forty-five percent of fatal cases also documented neurological manifestations. All 24 articles in this literature review emphasized the need to preempt the potentially serious and long-term health consequences that may occur with delayed treatment or in the absence of any treatment at all.

Included in this review were nine studies focusing on testing animals, as potential reservoir hosts, and fleas, as known vectors, of TGR in the United States.[2-5, 9, 15, 17, 39, 40] Researchers used either whole blood or serum to investigate active infection and past exposure in the animals and the vectors. No research groups in the United States have been able to detect TGR DNA by PCR in animal samples, but several have found evidence of past exposure to TGR in animal serum. Five studies have detected past exposure to TGR in opossums, dogs, cats, and rats.[3-5, 9, 15] In opossums, Adejemian et al. found that 12 out of 17 (71%) had previously been exposed to TGR, Blanton et al. found 66.7% (n=8), Maina et al. found 40.84% (n=107), and Boostrom et al. detected previous exposure in 8% (n=10) of tested opossums.[3-5, 9] Adejemian et al. also found evidence of past exposure in dogs (n=4, 44%) and cats (n=3, 18%), and Easterbrook et al. found that 7% (n=14) of live-trapped rats in Baltimore, Maryland had previously been exposed to TGR.[3, 15]

Seven studies have attempted to detect TGR DNA by PCR in fleas.[2, 3, 5, 9, 17, 39, 40] The reported prevalence of *R. typhi* DNA in fleas to be between 0 to 4.2%.[2, 3, 5, 9, 17, 39, 40] A study conducted by Noden et al. investigated R. typhi infection of fleas in central

Oklahoma.[17] Researchers collected 222 fleas taken from 52 owned felines and canines and discovered that 4.2% of the fleas tested positive for *R. typhi* DNA in PCR testing. This is the first evidence that the pathogen could be circulating in owned animals and their fleas in Oklahoma. However, while fleas are important in the transmission of TGR, this may be geographically specific, and their positivity does not necessarily translate to a high burden of TGR among animals.

1.3.2 Literature Review of Research Conducted Abroad

A separate literature review was performed to learn more about the clinical and epidemiological aspects of TGR rickettsiosis outside of the United States. This review consisted of 98 original articles published from 2002 to 2018 and included cross-sectional surveys, case reports, case series, and cohort studies of animals, humans, and ectoparasites. These studies are outlined in the Appendix. Many researchers also newly describe TGR in animals and suggested an expansion of potential reservoir host species, including red foxes, or found changing geographic restrictions on host species previously thought to exist, such as canines.[8, 12]

TGR clinical presentation is similar worldwide: fever is the most common symptoms, along with headache, and muscle weakness. All hospitalized case-patients had elevated liver enzymes, and several had signs of systemic infection. More serious presentations included involvement of the lungs or the central nervous system. Most of the serious cases developed due to delayed or incorrect diagnosis as enteric fever, dengue fever, encephalitis of unknown origin, influenza, viral pharyngitis, and otitis media,

underscoring the importance of considering rickettsia as a differential diagnosis in cases of fever of unknown origin where TGR has been documented.[41-45]

1.4 Significance and Specific Aims

1.4.1 Public Health Significance

The specific aims of this project are to describe the clinical and epidemiologic characteristics of TGR rickettsiosis in central Texas and to highlight the complexities of existing diagnostic algorithms and challenges to clinical management and surveillance of this important vector-borne disease in Texas. To our knowledge, this investigation is the first of its kind in the central Texas region and one of the first to apply a One Health framework by investigating TGR rickettsiosis as a human disease and in animal hosts. By including Burnet, Llano, Mason, Milam, San Saba, and Washington counties, it is also the first investigation of TGR in a Texas county classified as rural by the FORHP. The findings of this project may also be used by public health officials and physicians to improve surveillance efforts and patient care.

1.4.2 Specific Aims

The objectives of this project are to better understand the prevalence of TGR rickettsiosis and characteristics of TGR rickettsiosis in patients in central Texas and highlight the difficulties of diagnosis; to make predictions and model infections in order to assist physicians in making accurate and rapid diagnosis; and to explore possible animal reservoirs in Texas.

These objectives will be met through the following specific aims:

1. Describe the characteristics of TGR rickettsiosis patients diagnosed at BSW facilities in central Texas through a case-series analysis. Compare clinical characteristics of cases defined under the existing BSW diagnostic criteria to those among cases defined by stricter criteria used more widely in research. *This aim is addressed in Section 2.*
2. Compare characteristics of patients with laboratory confirmed TGR rickettsiosis to features of patients diagnosed with RMSF infection and to patients negative for any rickettsial diseases, and establish features more indicative of TGR rickettsiosis than of differential diagnoses. *This aim is addressed in Section 3.*
3. Describe the prevalence of *R. typhi* in animal species thought to be potential reservoirs of TGR in Texas. *This aim is addressed in Section 4.*

2. HUMAN CASES OF TYPHUS GROUP RICKETTSIOSIS IN CENTRAL TEXAS, 2012-2018

2.1 Introduction

Symptoms of TGR rickettsiosis are typically non-specific, with many patients presenting with an established "classic triad" of fever, headache, and rash.[18, 19] However, disease outcomes can be severe and even include death.[18] DSHS has reported eight deaths attributed to infection with TGR since 2003 (approximate case fatality rate 2.4 deaths per 1000 cases).[46] Other researchers have reported long-term neurological defects in patients.[26] Although the burden of TGR is low, in terms of the total number of infections, new evidence of an increasing incidence suggests the burden will grow in the coming years. Because of the non-specific nature of TGR rickettsiosis symptoms, and the possibility of severe complications and outcomes, understanding the epidemiology and clinical impact of TGR in Texas and further document its spread across the state is imperative.

Data suggest that TGR rickettsiosis recently emerged as an infectious disease in Texas, and some data supports its emergence in new regions of Texas. According to data from the DSHS, nine of the 30 counties in the central Texas region, HSR7, reported an increasing reports of TGR rickettsiosis to the state.[22] Reporting of TGR rickettsiosis to DSHS is complicated by the lack of a standard case definition and challenges to meeting specific diagnostic criteria, leading to a likely underreporting of the true number of TGR rickettsiosis individuals in central Texas.

The BSW network was chosen because it has a presence in most of the counties of HSR7. BSW facilities in central Texas include 50 clinics and hospitals throughout 13 counties (Figure 2). The major diagnostic hospital in the BSW network is located in Temple, situated near the middle of Bell County. United States Census Bureau data from 2017 reports a population of 202,338 in central Texas counties containing BSW facilities.[35] Of the 13 central Texas counties containing BSW facilities, six (46%) are considered rural by the FORHP. The average percentage of residents who report their ethnicity as non-Hispanic white in counties containing BSW facilities is 61%, the average percentage of residents in poverty is 15%, and the average percentage of residents under the age of 65 who are uninsured is 18%.[35] Demographic characteristics for central Texas counties containing BSW facilities and HSR 7 can be found in Table 1.

This investigation of characteristics of TGR rickettsiosis in central Texas seeks to further the understanding of the recent emergence of TGR by describing the case-patients identified through a network of hospitals and clinics in 13 counties in central Texas. In addition, the barriers to diagnosis and surveillance efforts in Texas are discussed.

2.2 Methods

2.2.1 Case Definition

The case definition for TGR rickettsiosis used by the BSW network designates any patient with an IFA ≥ 1:64 as a case. Another case definition for TGR rickettsiosis, proposed by Erickson et al., uses stricter case definitions to distinguish between rickettsial infections and account for unavailable convalescent IFA titers.[18] This stricter criteria designates a confirmed case to be a patient with an IFA titer of ≥1:1024 and a RMSF-

specific titer ≤ 2 times the TGR-specific titer; amplification of TGR DNA by PCR; or ≥ four-fold increase in acute and convalescent titers. A probable case is a patient with an IFA titer ≥ 1:128 and a RMSF-specific titer ≤ 2 times the TGR-specific titer, and a suspected case is a patient with an IFA titer of ≥1:64 and a negative RMSF-specific titer.[18] Results are presented using both BSW diagnostic criterion and diagnostic criteria proposed by Erickson et al.[18]

2.2.2 Data Collection

BSW laboratory tests for rickettsial diseases fall into two categories: Typhus Fever, which tests for the TGR pathogens, and RMSF, which tests for *Rickettsia rickettsii*. A list of all tests for rickettsial diseases from January 1, 2012 to August 15, 2018 that were ordered by physicians in the counties of interest was systematically reviewed by applying the case definition used by BSW: an IFA titer ≥1:64 specific for TGR pathogens. All test results for RMSF, duplicate patient test entries, and patients with TGR test results < 1:64 were removed from the dataset, leaving only the patients with a TGR-specific IgG or IgM titer of ≥ 1:64. Using BSW's electronic medical records (EMR) system, Epic, medical charts of identified case-patients were reviewed to determine the age of the patient at diagnosis, gender, hospitalization, length of hospital stay, symptoms, comorbidities, complications, the county in which the diagnosis occurred, the county of residence of the patients, and potential risk factors for descriptive analyses.

2.2.3 Data Analysis

Variables describing patient symptoms, comorbidities, laboratory findings, and risk factors were grouped into categories based on their similarities, and then recorded as binary (characteristic present, 1, or absent, 0) for analysis. The variable "hospitalization" was also recorded as binary (hospitalized, 1, or not hospitalized, 0), and length of hospitalization was recorded as a continuous variable. Information on classification of medical chart findings is found in the Appendix. Descriptive statistics were calculated using STATA 14.2 (Statacorp, LLC, College Station, TX), and geospatial analyses were made with ArcGIS 10.6 (ESRI, Redlands, CA). This study was reviewed and approved by the Baylor Scott & White and Texas A&M University Institutional Review Boards (BSW: 018-501; TAMU: IRB2018-0526).

2.3 Results

2.3.1 Description of Rickettsia Tests Performed

From January 1, 2012 to August 15, 2018, a total of 1,417 samples were submitted for testing for rickettsial diseases. Of those 1,417 samples, 476 (34%) were submitted for TGR. After removing patients with negative test results and multiple entries, a total of 16 unique TGR rickettsiosis patients were identified.

2.3.2 Description of Cases Using BSW Criteria

TGR rickettsiosis cases ranged in age from 18 to 90 years (mean=56.4 years; median=51.5 years). Three-quarters of the cases were male (n=12, 75%), and 13 (81%) of the patients were hospitalized. Two patients (13%) died within three months of their

diagnosis. Of the 13 patients that were hospitalized, the average length of hospitalization was 4.56 days, with a median of 4 days (range of 1 to 15 days). Additional case characteristics can be found in Table 3.

Table 3. Characteristics of Typhus Group Rickettsia Cases Using Baylor Scott & White Criteria

Characteristic	Number of Cases (%)
Gender	
Male	12 (75.0)
Female	4 (25.0)
Age (years)	
Minimum	18
Maximum	90
Average	56.4
Median	51.5
Hospitalized	13 (81.3)
Length of Hospitalization (days)	
Minimum	0
Maximum	15
Average	4.6
Median	4.0
Death	2 (12.5)
Risk Factors	
Living on a farm, ranch, or in a wooded area	9 (56.3)
Domestic animals in the home	8 (50.0)
Exposure to Other Wild Animals	5 (31.3)
Exposure to Livestock	3 (18.8)
Insect Bite	2 (12.5)
International Travel	2 (12.5)
Recreational Exposure	1 (6.3)
Yard Work/Gardening	1 (6.3)
Presenting Symptoms	

Table 3. Continued

Characteristic	Number of Cases (%)
Fever and/or Chills	14 (87.5)
Headache	7 (43.8)
Gastrointestinal Symptoms	7 (43.8)
Rash	6 (37.5)
Malaise	6 (37.5)
Myalgia/Arthralgia	5 (31.3)
Neurological Symptoms	3 (18.8)
Respiratory Symptoms	1 (6.3)
Laboratory Findings	
Acute Liver Injury	11 (68.8)
Abnormal Blood Cell Counts	11 (68.8)
Acute Kidney Injury	8 (50.0)
Laboratory Evidence of Systemic Infection	6 (37.5)
Abnormal Lipids	5 (31.3)
Laboratory Evidence of Systemic Inflammation	5 (31.3)
Acute Lung Infection	4 (25.0)
Comorbidities	
Heart Disease	4 (25.0)
Liver/Kidney Disease	3 (18.8)
Obesity	3 (18.8)
Disease of the Lungs	2 (12.5)
Tobacco/Drug/Alcohol Use	1 (6.3)
Diabetes Mellitus	1 (6.3)

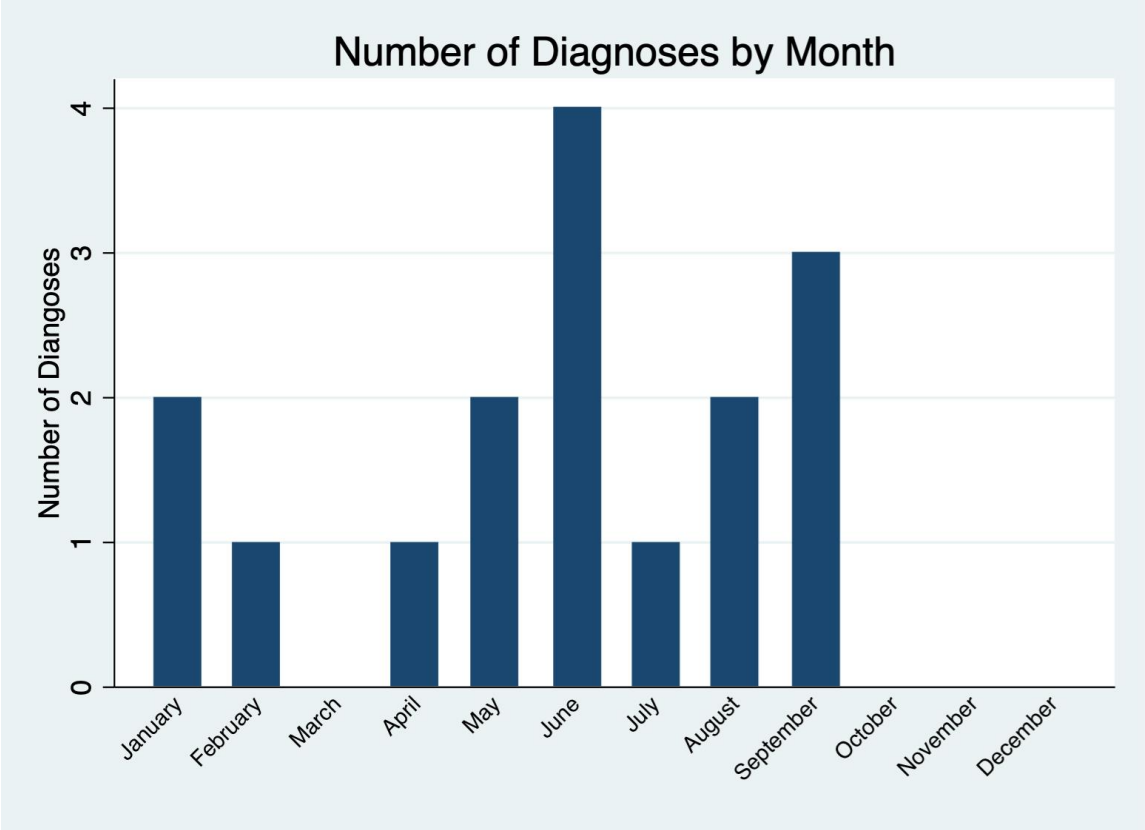
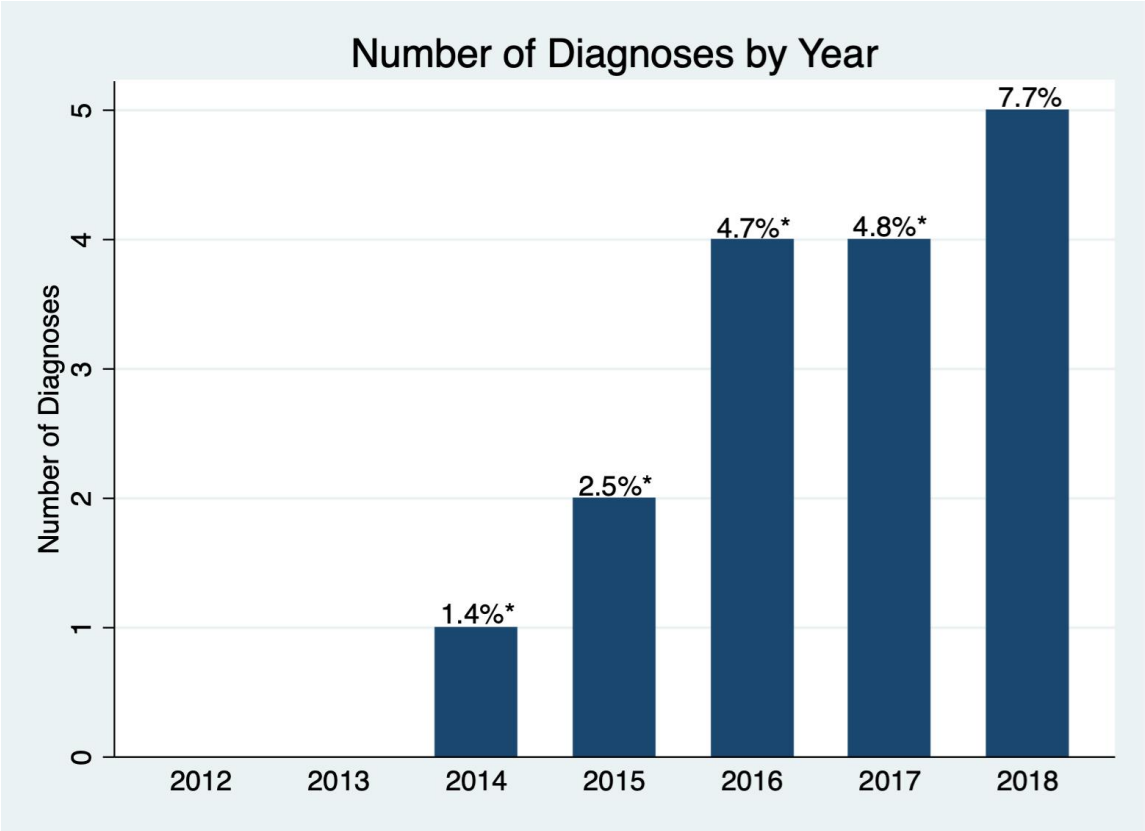


Figure 3. Typhus Group Rickettsia Cases Diagnosed at Baylor Scott & White Facilities in Central Texas by Month Using Baylor Scott & White Diagnostic Criteria, 2012-2018 (n=16).

The cases were diagnosed in eight months out of the year. No cases were diagnosed in March, October, November, or December. The majority of the cases (n=9; 56%) were diagnosed in the summer months (May, June, July, and August), with a peak in June (Figure 3). The year with the most diagnoses was 2018 (n=5; 31%), despite only having approximately six months of data for the year (Figure 4). Most cases (n=10; 63%) were diagnosed in Bell County, likely due to the large BSW hospital located in Temple, however, the origin of the cases was much more diverse. Most patients lived in Burnet County (n=4; 25%), followed by Bell County (n=3; 19%), and Brazos County (n=2; 13%).

One patient lived in Bastrop County, one in Comanche County, one in Lampasas County, one in Llano County, one in McLennan County, one in San Saba County, and one in Travis County (Figure 5 and 6).



*Statistically significant versus 2018.

Figure 4. Typhus Group Rickettsia Cases Diagnosed at Baylor Scott & White Facilities in Central Texas by Year Using Baylor Scott & White Diagnostic Criteria, 2012-2018 (n=16).

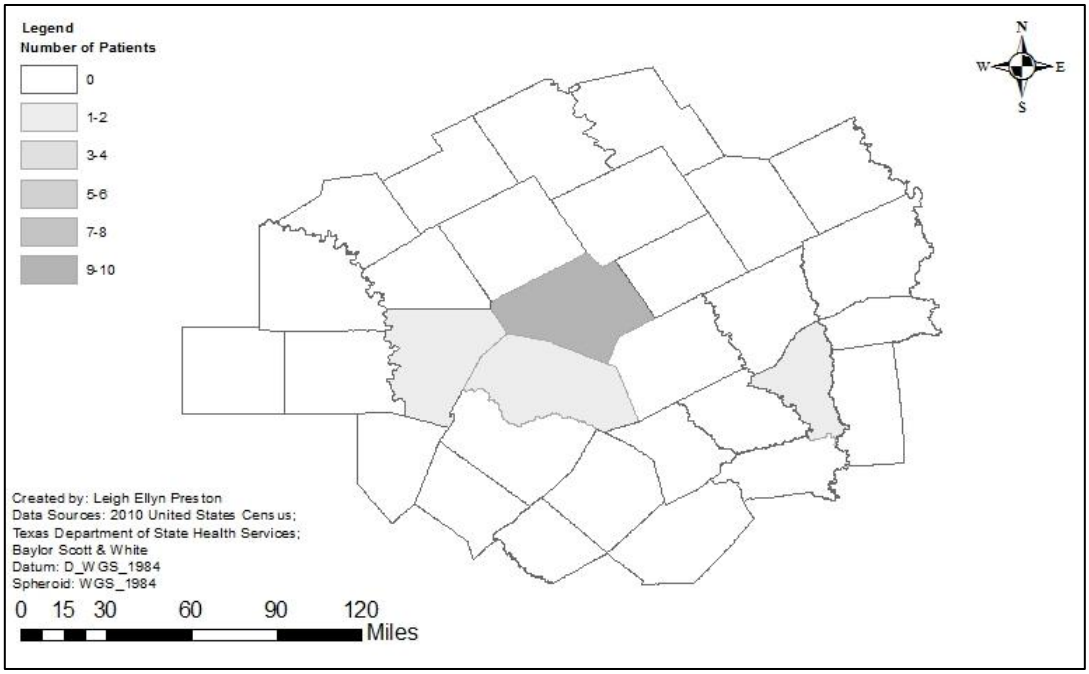


Figure 5. Frequency and Distribution of Typhus Group Rickettsia Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by County Where Diagnosis was Made (n=16).

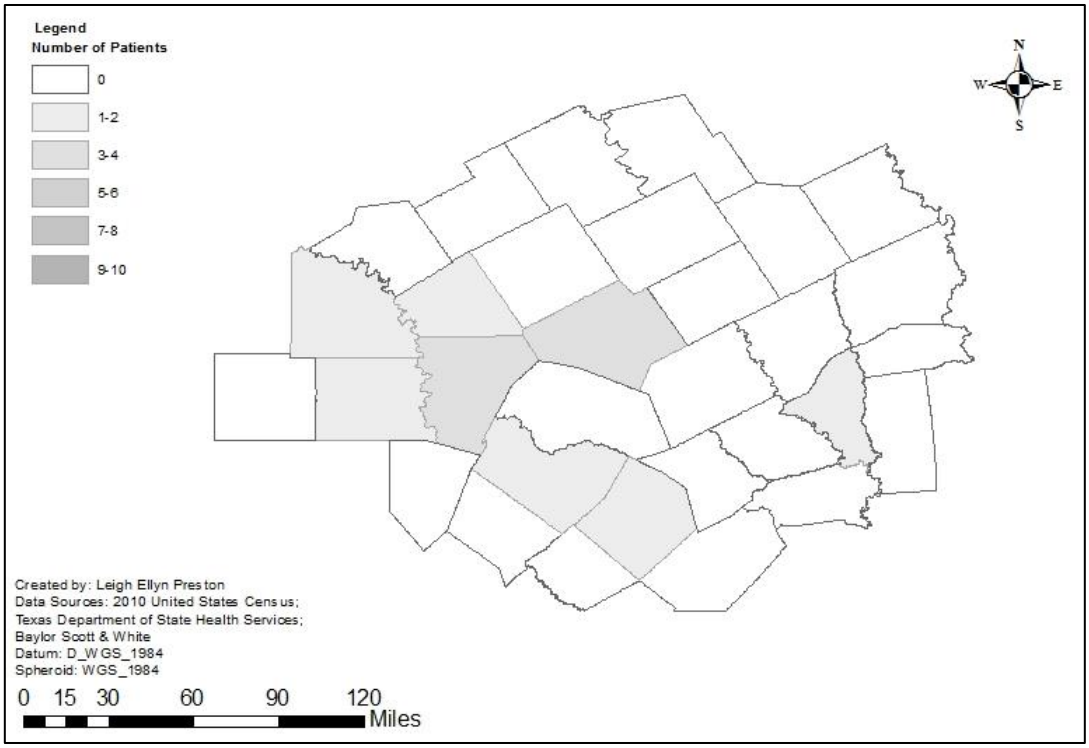


Figure 6. Frequency and Distribution of Typhus Group Rickettsia Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by County Where Patient Lived (n=16).

Clinical manifestations of TGR rickettsiosis in the 16 patients were fever (n=14; 88%), rash (n=6; 38%), and headache (n=7; 44%), as is commonly observed in other TGR rickettsiosis patients. Additional manifestations were gastrointestinal symptoms including diarrhea, vomiting, nausea, and abdominal pain (n=7; 44%), malaise or weakness (n=6; 38%), and myalgias and/or arthralgias (n=5; 31%). Typical laboratory findings in TGR rickettsiosis are acute liver injury with elevated liver enzyme levels, and abnormal blood cell counts with thrombocytopenia, leukopenia, and pancytopenia. BSW case-patients exhibited acute liver injury (n=11; 69%) showing elevated alanine transaminase, aspartate aminotransferase, or bilirubin levels, or acute hepatitis, and abnormal blood cell counts (n=11; 69%). Other laboratory findings are reported in Table 3.

Five patients (31%) had comorbidities including skin or prostate cancer, hypothyroidism, macular degeneration, prostatic hyperplasia, or depression. Four patients (25%) had histories of cardiovascular problems such as hypertension, aortic valve disease, heart murmurs, coronary artery disease, or congestive heart failure, and three patients (19%) had liver or kidney issues such as chronic kidney disease, non-fatty liver disease, hepatitis C virus, or stenosis of the liver. Two patients (13%) had histories of asthma or chronic obstructive pulmonary disorder. Due to the low number of cases, these proportions may not reflect the proportions of disease in the general population.

Cases in this study had many similar risk factors to cases reported in related studies. More than half of the patients lived on a farm, ranch, or in a heavily wooded area (n=9; 56%). Half of the patients reported exposure to domestic animals including dogs, cats, rabbits, or doves that reside in the home with the patient (n=8; 50%), and many reported exposure to wild animals such as rats, raccoons, and wild deer (n=5; 31%). Others reported exposure to livestock (n=3; 19%), having remembered an insect bite (n=2; 13%), or international travel (n=2; 13%).

2.3.3 Description of Cases Using Erickson Criteria

Using the diagnostic criteria suggested by Erickson et al., the dataset includes three confirmed cases, four probable cases, and one suspected case.[18] Case characteristics can be seen in Table 4.

Table 4. Characteristics of Typhus Group Rickettsiosis Cases Using Erickson Criteria

Characteristic	All Cases, n=8 (%)	Confirmed Cases, n=3 (%)	Probable Cases, n=4 (%)	Suspected Cases, n=1 (%)
Gender				
Male	5 (62.5)	2 (67.0)	2 (50.0)	1 (100.0)
Female	3 (37.5)	1 (33.0)	2 (50.0)	0 (0.0)
Age (years)				
Minimum	18	49	18	86
Maximum	90	90	54	
Average	56.9	65.3	43.3	
Median	53.0	57.0	50.5	
Hospitalized	8 (100.0)	3 (100.0)	4 (100.0)	1 (100.0)
Length of Hospitalization (days)				
Minimum	3	5	3	15
Maximum	15	12	5	
Average	4.7	9.0	3.5	
Median	5.0	10.0	3.0	
Death	2 (25.0)	1 (33.0)	0 (0.0)	1 (100.0)
Risk Factors				
Living on a Farm, Ranch, or in a Wooded Area	4 (50.0)	2 (67.0)	1 (25.0)	1 (100.0)
Domestic Animals	4 (50.0)	2 (67.0)	1 (25.0)	1 (100.0)
Exposure to Other Wild Animals	3 (37.5)	2 (67.0)	1 (25.0)	0 (0.0)
Exposure to Livestock	1 (13.0)	0 (0.0)	1 (25.0)	0 (0.0)
Insect Bite	1 (13.0)	1 (33.0)	0 (0.0)	0 (0.0)
International Travel	2 (25.0)	0 (0.0)	2 (50.0)	0 (0.0)
Recreational Exposure	1 (13.0)			1 (100.0)
Yard Work/Gardening	1 (13.0)	0 (0.0)	0 (0.0)	1 (100.0)
Presenting Symptoms				
Fever and/or Chills	7 (88.0)	3 (100.0)	3 (75.0)	1 (100.0)
Headache	4 (50.0)	2 (67.0)	2 (50.0)	0 (0.0)
Gastrointestinal Symptoms	4 (50.0)	1 (33.0)	3 (75.0)	0 (0.0)
Rash	3 (37.5)	0 (0.0)	3 (75.0)	0 (0.0)
Malaise	2 (25.0)	0 (0.0)	1 (25.0)	1 (100.0)
Myalgia/Arthralgia	2 (25.0)	1 (33.0)	1 (25.0)	0 (0.0)
Neurological Symptoms	1 (13.0)	1 (33.0)	0 (0.0)	0 (0.0)
Respiratory Symptoms	1 (13.0)	1 (33.0)	0 (0.0)	0 (0.0)
Laboratory Findings				
Acute Liver Injury	6 (75.0)	2 (67.0)	3 (75.0)	1 (100.0)
Abnormal Blood Cell Counts	5 (62.5)	1 (33.0)	3 (75.0)	1 (100.0)
Acute Kidney Injury	4 (50.0)	1 (33.0)	2 (50.0)	1 (100.0)
Laboratory Evidence of Systemic Infection	4 (50.0)	1 (33.0)	3 (75.0)	0 (0.0)
Abnormal Lipids	2 (25.0)	1 (33.0)	0 (0.0)	1 (100.0)

Table 4. Continued

Characteristic	All Cases, n=8 (%)	Confirmed Cases, n=3 (%)	Probable Cases, n=4 (%)	Suspected Cases, n=1 (%)
Laboratory Evidence of Systemic Inflammation	1 (13.0)	0 (0.0)	1 (25.0)	0 (0.0)
Acute Lung Infection	4 (50.0)	1 (33.0)	3 (75.0)	0 (0.0)
Comorbidities				
Heart Disease	1 (13.0)	1 (33.0)	0 (0.0)	0 (0.0)
Liver/Kidney Disease	1 (13.0)	0 (0.0)	1 (25.0)	0 (0.0)
Obesity	1 (13.0)	1 (33.0)	0 (0.0)	0 (0.0)
Disease of the Lungs	2 (25.0)	1 (33.0)	1 (25.0)	0 (0.0)
Tobacco/Drug/Alcohol Use	1 (13.0)	0 (0.0)	1 (25.0)	0 (0.0)
Diabetes Mellitus	1 (13.0)	1 (33.0)	0 (0.0)	0 (0.0)

In comparison to the BSW criteria, using the criteria proposed by Erickson et al., eight cases were diagnosed between April and December. One case was diagnosed in 2014, no cases were diagnosed in 2015, one in 2016, four in 2017, and two in the first half of 2018. The median age was 53, with a range from 19 to 90 years. The median length of hospital stay was five days with a range of 3 to 15 days. The cases were diagnosed in three counties (Figures 7 and 8): Bell (6), Brazos (1), and Burnet (1), and originated in five counties: Bell (2), Brazos (1), Burnet (3), Lampasas (1), and Llano (1).

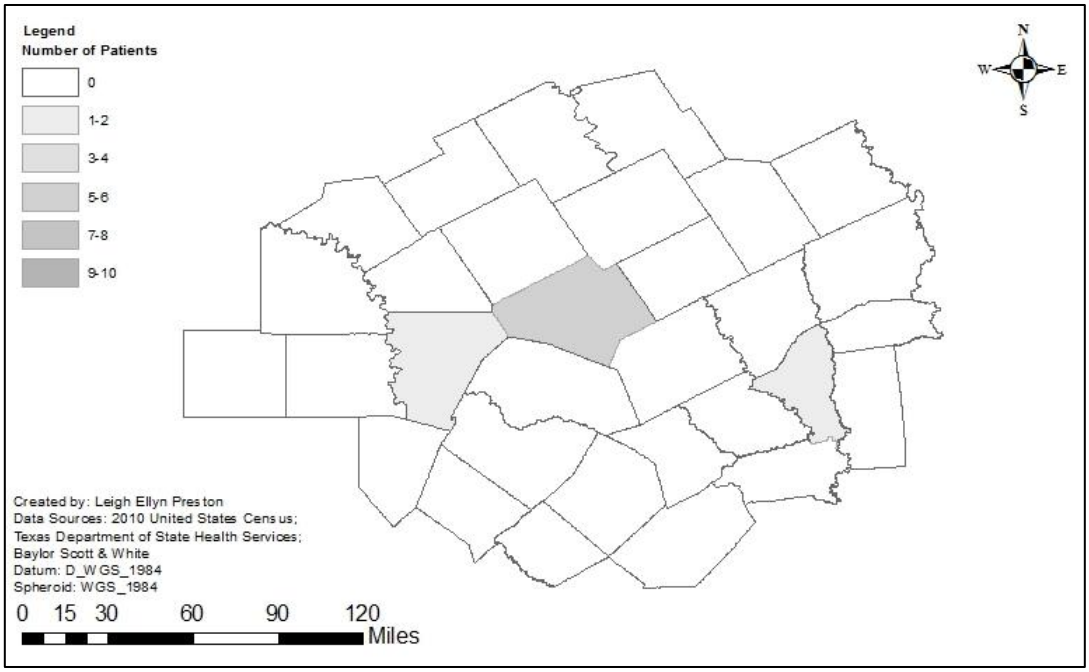


Figure 7. Frequency and Distribution of Typhus Group Rickettsia Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by County Where Diagnosis was Made Using Erickson Criteria (n=8).

Of the patients that are considered cases (confirmed, probable, or suspected) under the stricter diagnostic criteria, five (62.5%) were male, and three (37.5%) were female. All eight patients were hospitalized, and two patients (25%) died within three months of diagnosis. Seven (88%) of the cases presented with fever, four (50%) with headache, and three (37.5%) with rash.

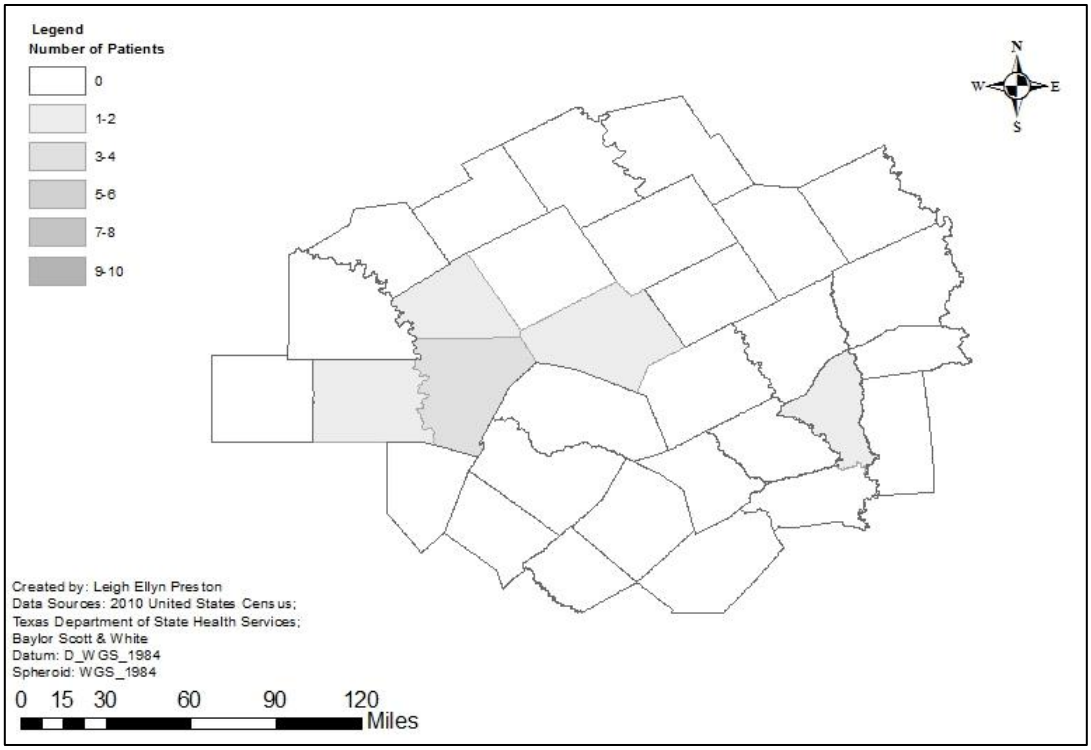


Figure 8. Frequency and Distribution of Typhus Group Rickettsia Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by County Where Patient Lived Using Erickson Criteria (n=8).

Half of the patients (n=4) reported gastrointestinal symptoms, two patients (25%) reported malaise and myalgia or arthralgias. Six (75%) patients had acute liver injury, and five (62.5%) had abnormal blood cell counts. Symptoms and laboratory findings are presented in Table 4.

Half of the patients (n=4) lived on a farm, ranch, or in a wooded area, or domestic animals living in the home. Three (37.5%) patients reported exposure to other wild animals, two (25%) patients reported international travel, and one (13%) patient reported exposure to livestock, insect bites, or yard work.

2.4 Discussion

As with other case reports and case series analyses, fever was the most frequently reported presenting symptom, and the majority of patients in this study had abnormal liver function and abnormal blood cell counts. BSW diagnostic locations included in this analysis were located in four counties: Bell, Brazos, Burnet, and Williamson. Cases lived in eight counties: Bastrop, Bell, Brazos, Burnet, Lampasas, Llano, San Saba, and Travis. Many cases were originally seen at a facility in a different county than that in which they were diagnosed. Patients initially seen in smaller facilities often had to be referred or transferred to larger facilities for diagnostic testing and treatment. Initial encounters occurred in four counties: Bell, Brazos, Burnet, and Williamson (Figure 9). Additionally, several patients lived on farms, ranches, or in wooded areas, implying that exposures are happening frequently in rural areas, and highlighting the necessity for increased diagnostic capabilities in smaller medical facilities that are typical in rural areas.

The number of cases diagnosed in the first half of 2018 is higher than any other year, despite only reflecting data for about half of the year. A number of factors could explain this relative increase. The number of TGR-specific tests ordered by physicians increased slightly each year, which could explain the increasing number of cases. An increase in awareness of the diagnosis, exposures, or symptoms would also explain both the increase in tests ordered and in the number of cases. Another explanation could be an increase in the number of infected fleas in the area, or an increase in exposure to the vector.

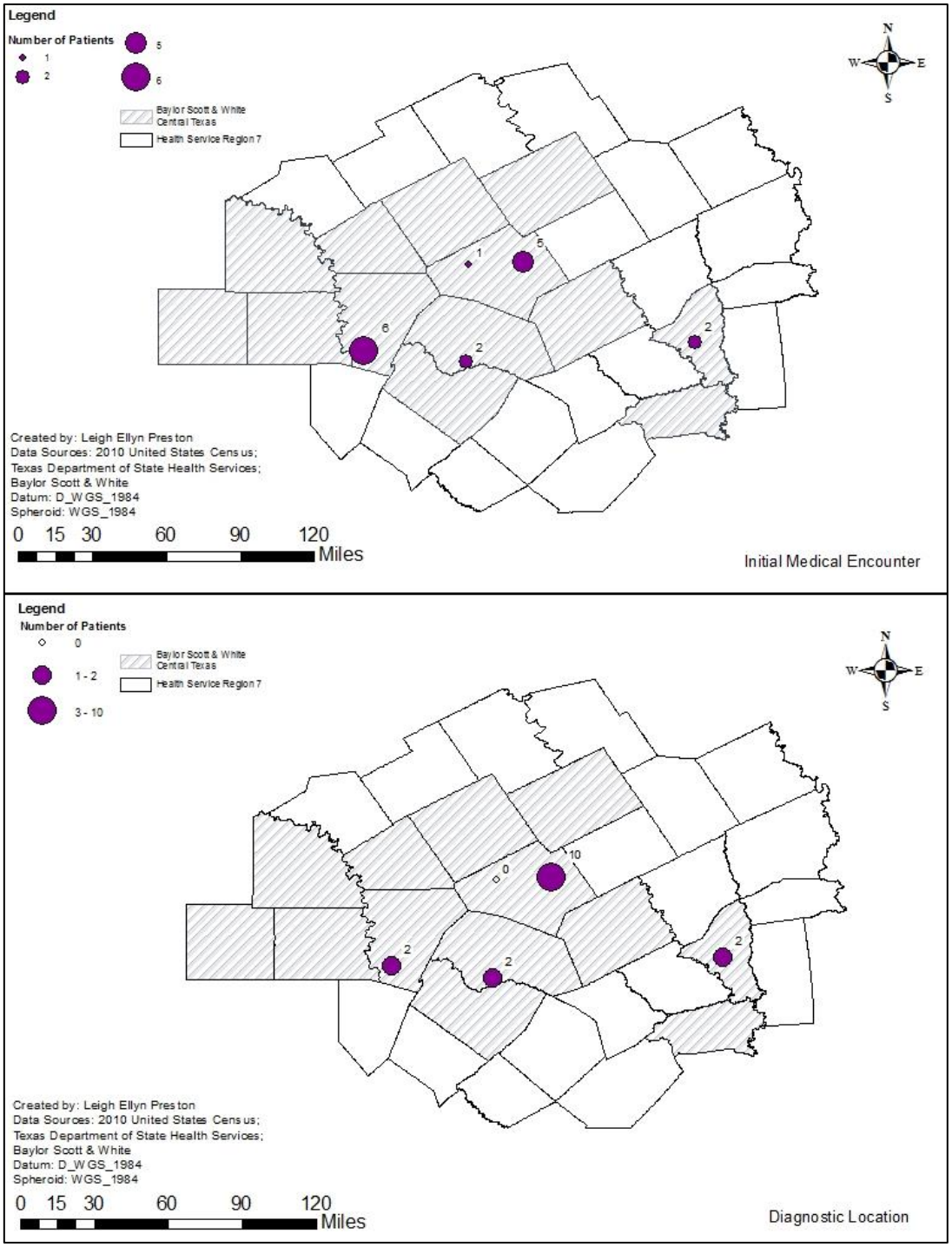


Figure 9. Frequency of Typhus Group Rickettsia Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by (top) Location of Initial Medical Encounter and (bottom) Location Where Diagnosis was Made (n=16).

Although RMSF and TGR infections are both treated with doxycycline, from an epidemiological standpoint, it is beneficial to know which pathogens are circulating in a population. There is a need for more specific diagnostic testing, as there is often cross-reaction between rickettsia antigens, and understanding the prevalence and incidence of each disease can illuminate patterns in the movement and involvement in human disease of the different vectors that carry the causative agent of each. [29, 37, 38] Increased specificity of diagnostic tests would also alleviate some of the issues caused by discrepancies in diagnostic criteria between physicians, state health officials, and TGR research groups.

This study has several important limitations. Because the data is based on EMRs, it does not include any data that was not recorded or evaluated by the physician. The data included in this study does not reflect any cases diagnosed outside of the central Texas region, or that were not seen by physicians or seen by physicians from other health care facilities in central Texas. Data from patients that were not tested for TGR rickettsiosis by a physician, and subclinical or asymptomatic cases are not included in this study, likely leading to an underrepresentation of disease prevalence in the area. The sample size is quite small, reducing the power of the analysis to detect any association, when present, between variables.

This is the first investigation of TGR rickettsiosis that we know of in many of the counties of central Texas. This investigation will provide some direction for future researchers as they identify populations in which to search for TGR rickettsiosis. The use of a stricter diagnostic criteria for comparison to the BSW diagnostic criterion helps to better understand the difference in data collected for surveillance and clinical diagnosis. Future research should expand the dataset by including more healthcare facilities in central

Texas, and possibly add an active surveillance components for residents of the area. Subsequent studies should also investigate the effect that the different diagnostic criterion used by clinicians and DSHS has on patients, treatment, and surveillance.

2.5 Conclusion

The data presented indicate a need for increased awareness of symptoms and exposures common in TGR rickettsiosis cases among primary care physicians in both rural and urban locations. The diagnostic capacity of smaller healthcare facilities and in rural counties should also be increased to adequately and promptly treat patients who test positive for TGR rickettsiosis. Prompt treatment could prevent systemic infection and severe complications. The relative increase in the number of cases in 2018 highlights the need to educate the general public about TGR transmission cycles to increase primary prevention efforts in the population. Discrepancies in diagnostic criteria hinder research efforts of the epidemiology of this emergent pathogen, and should be discussed by stakeholders. Overall, more research is needed to better understand *R. typhi* infection in Texas.

3. CASE-CONTROL STUDY OF TYPHUS GROUP RICKETTSIOSIS IN CENTRAL TEXAS

3.1 Introduction

Symptoms of TGR rickettsiosis can often be mistaken for the symptoms of other infections. For example, infections with TGR and RMSF present similarly and it is likely that one could be mistaken for the other.[20] While TGR and RMSF infections are both easily treated with doxycycline, misdiagnosis and delayed treatment still occur and can lead to complications that can be severe, including admission to the intensive care unit, long-term neurological disabilities, and even death.[18, 26, 41-46]

In Texas, the number of reported cases of TGR rickettsiosis have steadily risen from 27 per year in 2003 to 364 per year in 2016.[19] This is a 9.5% increase in disease incidence, while the population of Texas has increased 10.5%.[35] Recent publications on TGR emergence have focused on the metropolitan areas of Austin, Houston, Galveston, as well as the Texas-Mexico border area around the Rio Grande Valley. [3, 5, 18, 19, 21, 23-33] None of the publications included a control group, making the calculation of risk or odds of infection difficult and none of the publications focused on rural areas. The evidence of emergence, non-specificity of symptoms, and possibility for severe complications, make understanding the epidemiology and risk and odds of infection in the population in central Texas vital.

The aim of this case-control analysis is to improve understanding of TGR epidemiology by identifying risk factors and unique symptoms of TGR rickettsiosis, and differentiating signs, symptoms, and comorbidities that increase the odds of diagnosis with

TGR rickettsiosis. This is accomplished by analyzing cases identified through a network of hospitals and clinics in 13 counties throughout central Texas, and calculating odds ratios (OR) for exposures and symptoms of TGR rickettsiosis leading to improved patient care.

3.2 Methods

3.2.1 Data Collection

A list of all laboratory tests performed for rickettsial diseases at BSW facilities in central Texas from January 1, 2012 to August 15, 2018 was reviewed to determine the cases and controls for the study. TGR rickettsiosis and RMSF cases were defined by the BSW diagnostic criteria: TGR-specific IgG or IgM IFA antibody titer of ≥ 1:64, and RMSF-specific IgG or IgM IFA antibody titer of ≥ 1:64 (Table 5). Any patients with titers considered positive for both TGR and RMSF were defined as TGR rickettsiosis cases for this analysis. Patients with negative titers (≤ 1:64) for both infections were placed into a separate group. Duplicate patient test entries were deleted so that each patient received only one entry. Lists of TGR rickettsiosis patients, RMSF disease patients, and the negative patients were cross-referenced so no patients were in more than one group.

Table 5. Inclusion Criteria for Patient Groups

Patient Group	Inclusion Criteria
Typhus group rickettsiosis cases	• IgG or IgM immunofluorescence titer of ≥ 1:64 specific for typhus group rickettsia pathogens
Rocky Mountain spotted fever cases	• IgG or IgM immunofluorescence titer of ≥ 1:64 specific for Rocky Mountain spotted fever pathogens • IgG or IgM immunofluorescence titer of ≤ 1:64 specific for typhus group rickettsia pathogens
Negative patients	• IgG or IgM immunofluorescence titer of ≤ 1:64 specific for typhus group rickettsia pathogens • IgG or IgM immunofluorescence titer of ≤ 1:64 specific for Rocky Mountain spotted fever pathogens

There were approximately 4.3 RMSF disease case for every one TGR rickettsiosis case. Negative subjects were selected for inclusion using a simple random sample to satisfy a ratio of one TGR rickettsiosis patient to four negative patients. Medical charts were reviewed on all selected subjects to capture age of the patient at diagnosis, gender, hospitalization, symptoms, comorbidities, the county in which the diagnosis occurred, the county of residence of the patients, and relevant exposures for analysis. Information on classification of medical chart findings is found in the Appendix.

3.2.2 Data Analysis

ORs and confidence intervals (CI) were calculated for each variable against the binary outcome of TGR-positive test results and recorded in Table 6. Statistical models were built using forward, step-wise logistic regression. Regression models are useful because they allow predictions that control for multiple variables that may obscure the relationship between the variables and the outcome, TGR-positive test results. Variables were selected for the model building process based on a conservative p-value of 0.20 during bivariate analysis. All variables with a p-value ≤ 0.20 were checked in the model building process. The variable with the lowest p-value in bivariate analysis was added to the model in the first round of model building, and in subsequent rounds, the model with the lowest Bayesian Information Criterion (BIC) was selected for inclusion in the model. The BIC was used because it penalizes a fitted model for each variable included, thus preventing over fitting of models. A model that has been over fit fails to describe the relationship between variables, and instead describes random error in the data.

Each model was also checked using a Likelihood Ratio Test (LRT) before advancing to the next round. An LRT tests the null hypothesis that a reduced model that is nested within another model is a better model of the data. When the p-value of the LRT is statistically significant at the 0.05 level, it can be assumed that the additional variable in the full model is significant to the model. A reduced model was considered to be the final model when the p-value an LRT was no longer significant at the 0.05 significance level.

Statistical analyses were performed using STATA 14.2 (Statacorp, LLC, College Station, TX), and geospatial analyses were made with ArcGIS 10.6 (ESRI, Redlands, CA). This study was reviewed and approved by the Baylor Scott & White and Texas A&M University Institutional Review Boards (BSW: 018-501; TAMU: IRB2018-0526).

3.3 Results

3.3.1 Description of the Data

From January 1, 2012 to August 15, 2018, 1,417 serum samples were submitted to test for rickettsial diseases with 476 (34%) of those testing for TGR rickettsiosis, and 941 for RMSF. After inclusion criteria were applied, there were 16 cases of TGR rickettsiosis, and 69 cases of RMSF diagnosed in the designated period. A total of 180 patients tested during the study period were negative for both RMSF and TGR infections, and 60 were selected as a simple random sample for inclusion in the second control group.

The geographic range of TGR rickettsiosis, RMSF infection, and negative patients can be seen in Figures 10, 11, and 12. All characteristics, symptoms, and potential risk factors of patients in each of the study groups can be seen in Table 6.

3.3.2 Bivariate Analysis

In bivariate analyses comparing TGR rickettsiosis cases to RMSF cases, living on a farm, ranch, or in a wooded area (OR: 7.59; 95% CI: 2.30-25.03), laboratory evidence of systemic inflammation (OR: 4.03; 95% CI: 1.08-14.99), acute liver injury (OR: 10.45; 95% CI: 3.06-35.64), acute kidney injury (OR: 4.75; 95% CI: 1.49-15.17), acute lung infection (OR: 5.42; 95% CI: 1.19-24.68), and laboratory evidence of systemic infection (OR: 6.30; 95% CI: 1.69-23.43) were associated with TGR rickettsiosis.

In bivariate analyses using TGR rickettsiosis cases compared to patients testing negative, age (OR: 1.03; 95% CI: 1.00-1.06), hospitalization (OR: 4.61; 95% CI: 1.20-17.75), living on a farm, ranch, or in a wooded area (OR: 9.00; 95% CI: 2.62-30.93), acute liver injury (OR: 6.08; 95% CI: 1.84-20.07), and acute kidney injury (OR: 3.92; 95% CI: 1.24-12.44) were significant at the 0.05 significance level. Results of bivariate analyses can be seen in Table 7.

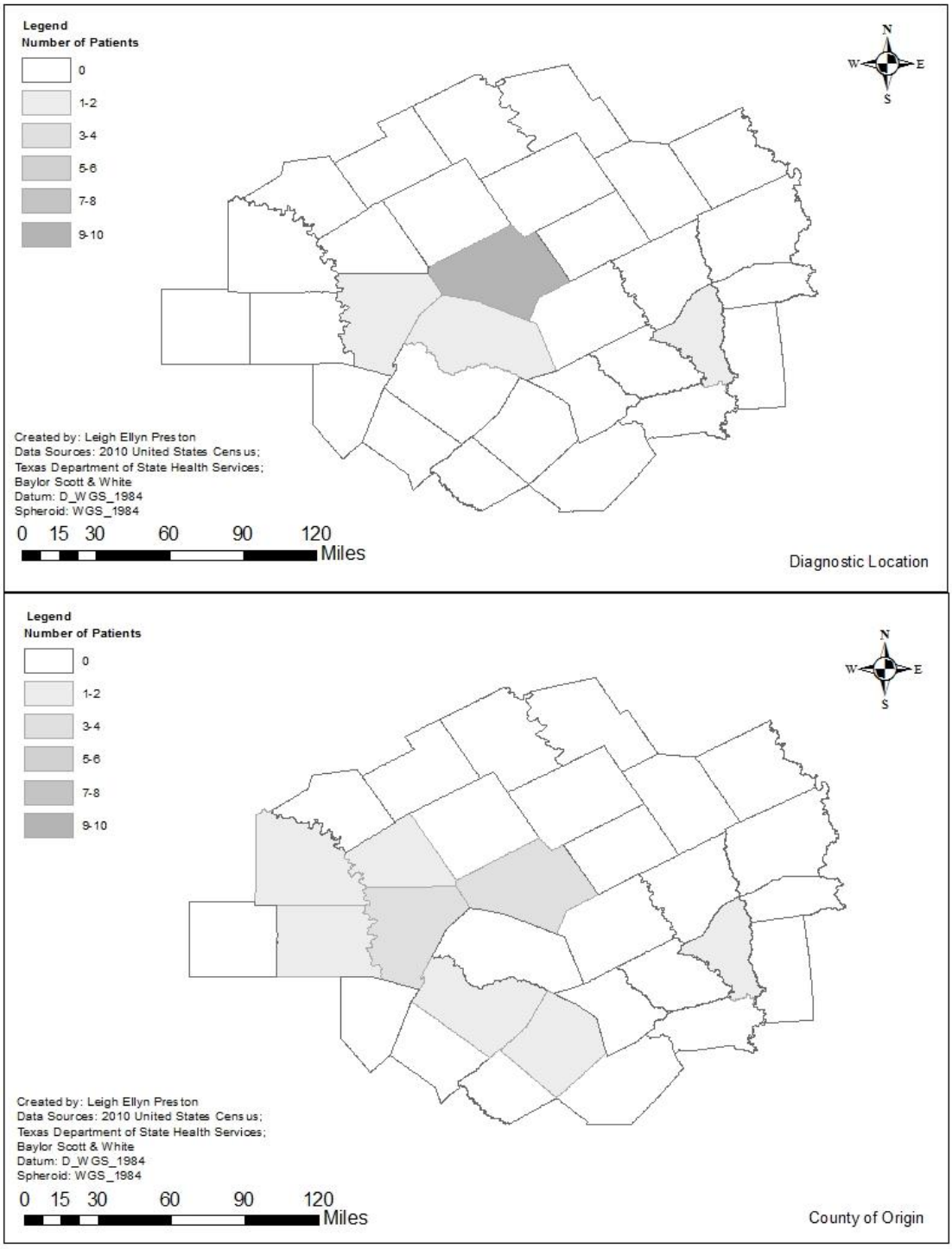


Figure 10. Frequency and Distribution of Typhus Group Rickettsia Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by (top) County Where Diagnosis was Made and (bottom) County Where Patient Lived (n=16).

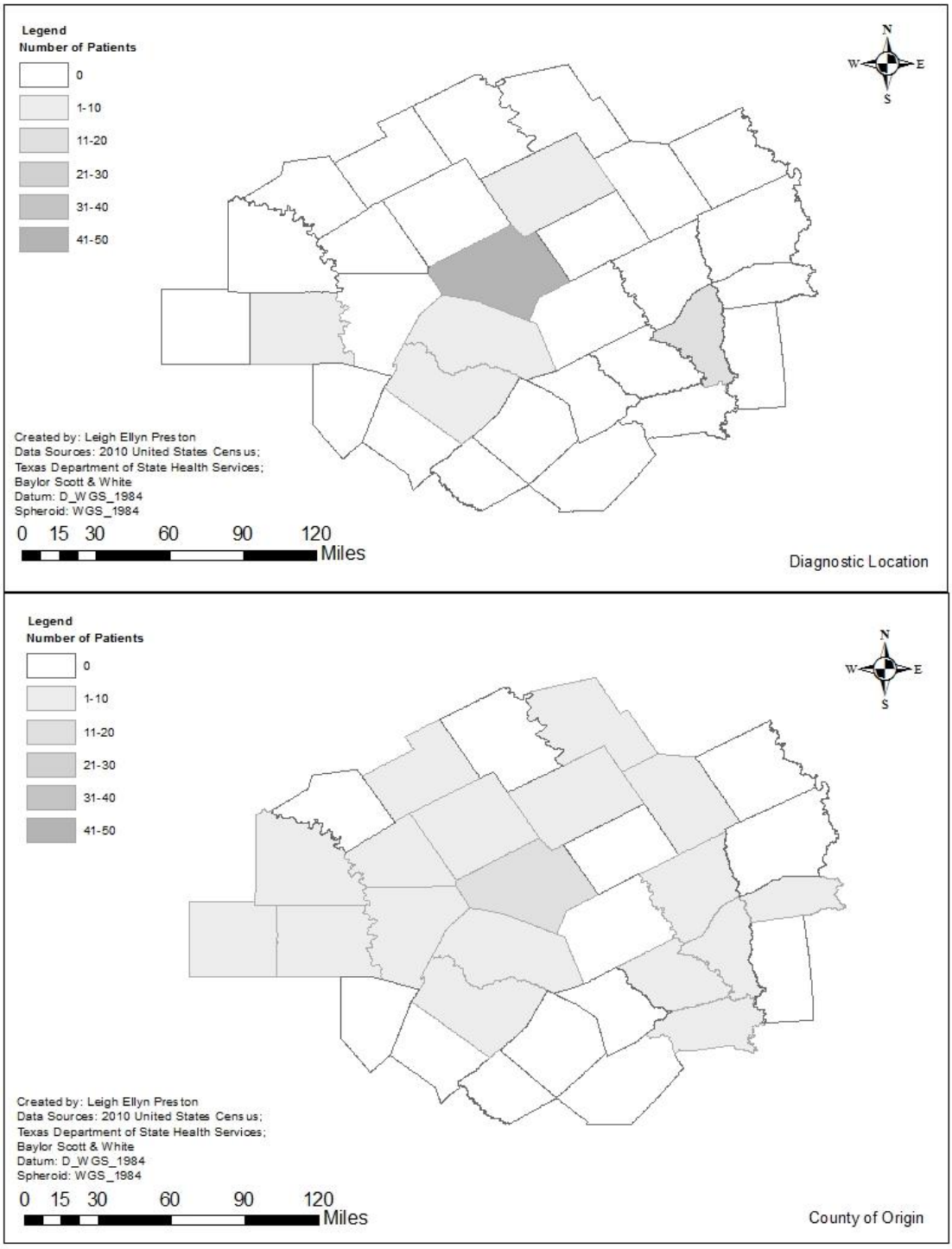


Figure 11. Frequency and Distribution of Rocky Mountain Spotted Fever Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by (top) County Where Diagnosis was Made and (bottom) County Where Patient Lived (n=69).

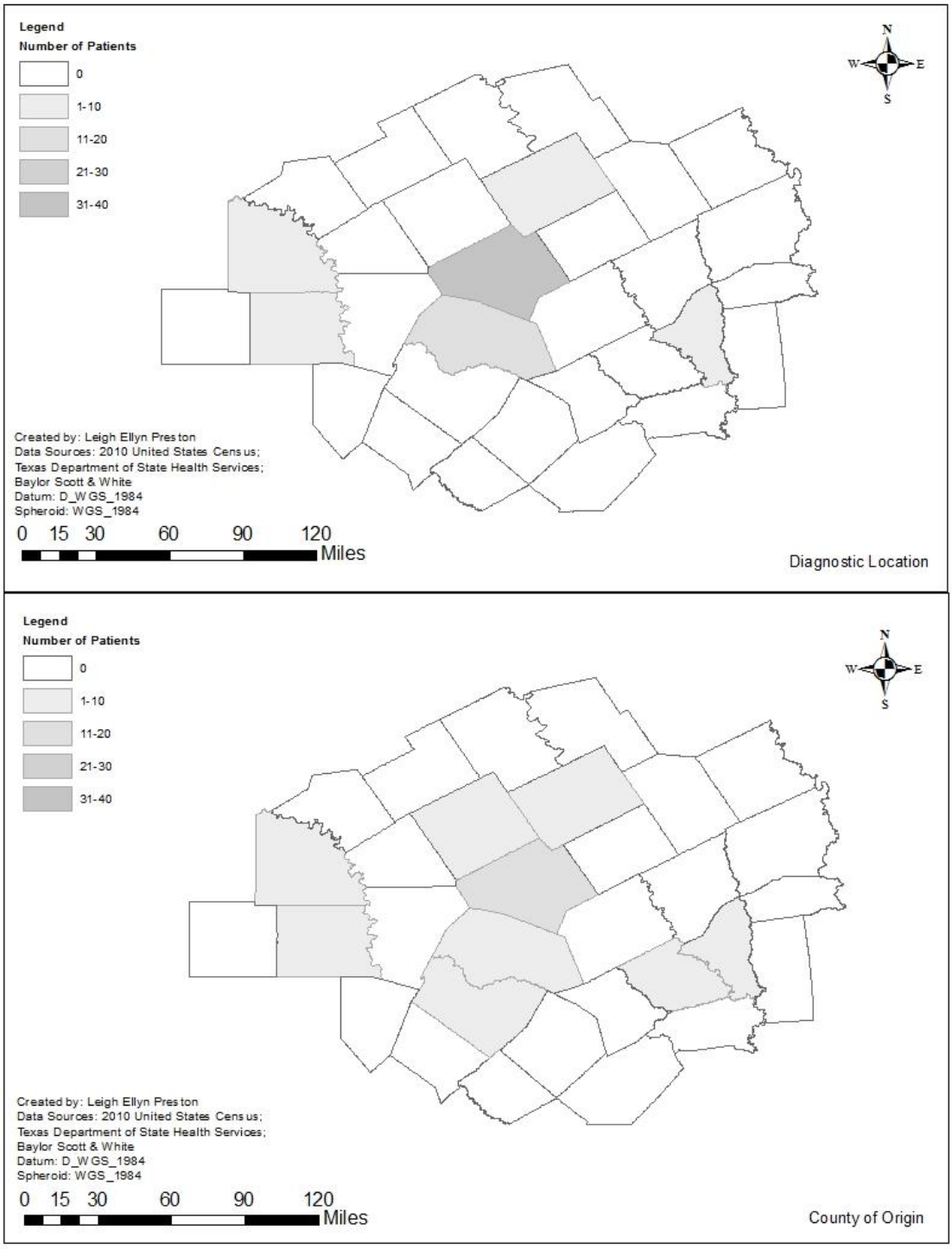


Figure 12. Frequency and Distribution of Negative Patient (Differential Diagnosis) Diagnoses at Baylor Scott & White Facilities in Central Texas, 2012-2018, by (top) County Where Diagnosis was Made and (bottom) County Where Patient Lived (n=64).

3.3.3 Multivariate Logistic Regression

The final logistic regression model comparing TGR rickettsiosis to RMSF infection included acute liver injury, laboratory evidence of systemic infection, age, and heart disease comorbidities. The odds of diagnosis with TGR rickettsiosis increased with each year increase in age (OR: 1.05, 95% CI: 1.01-1.10), as well as in patients with acute liver injury (OR: 8.60, 95% CI: 2.06-35.96) and those with laboratory evidence of systemic infection (OR: 6.58, 95% CI: 1.21-35.89). Patients with a comorbid heart condition were less likely to be diagnosed with TGR rickettsiosis (OR: 0.13, 95% CI: 0.02-0.77). When comparing TGR rickettsiosis patients to negative patients, the final model included living on a farm, ranch, or in a wooded area (OR: 41.8, 95% CI: 4.79-364.65), acute liver injury (OR: 30.47, 95% CI: 3.44-270.2), and acute lung infection (OR: 26.94, 95% CI: 3.08-235.27). In the model comparing TGR rickettsiosis patients to negative patients, age (OR: 1.04, 95% CI: 0.99-1.09) was included in the model because it is a common risk factor for negative disease outcomes. All ORs and CIs can be found in Table 8.

Table 6. Characteristics of Patients

Characteristic	Number of Typhus Group Rickettsiosis Patients, n=16 (%)	Number of Rocky Mountain Spotted Fever Patients, n=69 (%)	Number of Negative Patients, n=64 (%)
Gender			
Male	12 (75.0)	39 (57.0)	32 (50.0)
Female	4 (25.0)	30 (43.0)	32 (50.0)
Hospitalized	13 (81.3)	37 (54.0)	31 (48.0)
Death	2 (12.5)	2 (3.0)	2 (3.0)
Risk Factors			
Animals in the Home	8 (50.0)	24 (35.0)	20 (32.0)

Table 6. Continued

Characteristic	Number of Typhus Group Rickettsiosis Patients, n=16 (%)	Number of Rocky Mountain Spotted Fever Patients, n=69 (%)	Number of Negative Patients, n=64 (%)
Insect Bite	2 (12.5)	14 (20.0)	5 (8.0)
Living on a Farm, Ranch, or in a Wooded Area	9 (56.3)	10 (14.0)	8 (13.0)
International Travel	2 (12.5)	9 (13.0)	19 (30.0)
Recreational Exposure	1 (6.3)	20 (29.0)	11 (17.0)
Exposure to Livestock	3 (18.8)	9 (13.0)	5 (8.0)
Exposure to Other Wild Animals	5 (31.3)	0 (0.0)	12 (19.0)
Yard Work/Gardening	1 (6.3)	2 (3.0)	6 (9.0)
Occupational Exposure	0 (0.0)	13 (19.0)	11 (17.0)
Presenting Symptoms			
Fever and/or Chills	14 (87.5)	49 (71.0)	41 (64.0)
Rash	6 (37.5)	33 (48.0)	24 (38.0)
Myalgia/Arthralgia	5 (31.3)	23 (33.0)	28 (43.0)
Headache	7 (43.8)	24 (35.0)	17 (27.0)
Malaise	6 (37.5)	24 (35.0)	31 (48.0)
Gastrointestinal Symptoms	7 (43.8)	18 (26.0)	21 (33.0)
Respiratory Symptoms	1 (6.3)	7 (10.1)	16 (25.0)
Neurological Symptoms	3 (18.8)	6 (9.0)	16 (25.0)
Laboratory Findings			
Laboratory Evidence of Systemic Inflammation	5 (31.3)	7 (10.0)	7 (11.0)
Acute Liver Injury	11 (68.8)	12 (17.0)	17 (27.0)
Abnormal Blood Cell Counts	11 (68.8)	21 (30.0)	19 (27.0)
Acute Kidney Injury	8 (50.0)	12 (17.0)	13 (20.3)
Acute Lung Infection	4 (25.0)	4 (6.0)	5 (8.0)
Laboratory Evidence of Systemic Infection	6 (37.5)	6 (9.0)	10 (16.0)
Comorbidities			
Heart Disease	4 (25.0)	33 (48.0)	20 (31.0)
Disease of the Lungs	2 (12.5)	9 (13.0)	7 (11.0)
Liver/Kidney Disease	3 (18.8)	9 (13.0)	4 (6.0)
Obesity	3 (18.8)	9 (13.0)	9 (14.0)
Tobacco/Drug/Alcohol Use	1 (6.3)	16 (23.0)	11 (17.0)
Diabetes Mellitus	1 (6.3)	9 (13.0)	7 (11.0)

Table 6. Continued

Characteristic	Number of Typhus Group Rickettsiosis Patients, n=16 (%)	Number of Rocky Mountain Spotted Fever Patients, n=69 (%)	Number of Negative Patients, n=64 (%)
Age (years)			
Minimum	18	6	2
Maximum	90	88	83
Average	56.4	46.6	44.7
Median	51.5	49.0	44.0
Length of Hospital Stay (days)			
Minimum	0	0	0
Maximum	15	41	30
Average	4.6	4.5	3.6
Median	4.0	1.0	0.0

Table 7. Results of Bivariate Analysis

Characteristic	Typhus Group Rickettsiosis versus Rocky Mountain Spotted Fever Odds Ratio (p-value, 95% Confidence Interval)	Typhus Group Rickettsiosis versus Negative Patients (p-value, 95% Confidence Interval)
Gender	0.43 (0.18, 0.13-1.48) †	0.33 (0.08, 0.10-1.14) †
Age	1.03 (0.09, 0.99-1.06) †	1.03 (0.05, 1.00-1.06) * †
Hospitalized	3.75 (0.05, 0.98-14.34) †	4.61 (0.03, 1.20-17.75) * †
Length of Hospitalization	1.03 (0.53, 0.94-1.13)	1.00 (0.98, 0.93-1.08)
Death	4.79 (0.13, 0.62-36.91) †	4.43 (0.15, 0.57-34.19) †
Risk Factors		
Animals in the Home	1.88 (0.26, 0.63-5.62)	2.2 (0.17, 0.72-6.70) †
Insect Bite	0.56 (0.48, 0.11-2.76)	1.69 (0.56, 0.30-9.61)
Living on a Farm, Ranch, or in a Wooded Area	7.59 (0.001, 2.30-25.03) * †	9.0 (<0.001, 2.62-30.93) * †
International Travel	0.95 (0.95, 0.19-4.90)	0.34 (0.18, 0.07-1.64) †

Table 7. Continued

Characteristic	Typhus Group Rickettsiosis versus Rocky Mountain Spotted Fever Odds Ratio (p-value, 95% Confidence Interval)	Typhus Group Rickettsiosis versus Negative Patients (p-value, 95% Confidence Interval)
Recreational Exposure	0.16 (0.09, 0.02-1.32) †	0.32 (0.3, 0.04-2.69)
Exposure to Livestock	1.54 (0.56, 0.37-6.48)	2.72 (0.21, 0.58-12.86)
Exposure to Other Wild Animals	Omitted	1.97 (0.28, 0.57-6.73)
Yard Work/Gardening	2.23 (0.52, 0.19-26.27)	0.64 (0.69, 0.07-5.77)
Presenting Symptoms		
Fever and/or Chills	2.86 (0.19, 0.59-13.74) †	3.93 (0.09, 0.81-18.82) †
Rash	0.65 (0.46, 0.21-2.00)	1.00 (1.00, 0.32-3.10)
Myalgia/Arthralgia	0.91 (0.87, 0.28-2.93)	0.58 (0.37, 0.18-1.88)
Headache	1.46 (0.50, 0.48-4.40)	2.15 (0.19, 0.69-6.68) †
Malaise	1.13 (0.84, 0.36-3.47)	0.64 (0.44, 0.21-1.97)
Gastrointestinal Symptoms	2.2 (0.17, 0.71-6.78) †	1.59 (0.41, 0.52-4.87)
Respiratory Symptoms	0.59 (0.63, 0.07-5.17)	0.20 (0.13, 0.02-1.64) †
Neurological Symptoms	2.42 (0.25, 0.54-10.95)	0.69 (0.60, 0.17-2.74)
Laboratory Findings		
Laboratory Evidence of Systemic Inflammation	4.03 (0.04, 1.08-14.99) * †	3.70 (0.05, 0.99-13.81) †
Acute Liver Injury	10.50 (<0.01, 3.06-35.6) * †	6.08 (<0.01, 1.84-20.07) * †
Abnormal Blood Cell Counts	2.29 (0.14, 0.75-6.91) †	2.37 (0.13, 0.78-7.24) †
Acute Kidney Injury	4.75 (<0.01, 1.49-15.17) * †	3.92 (0.02, 1.24-12.44) * †
Acute Lung Infection	5.42 (0.03, 1.19-24.68) * †	3.93 (0.07, 0.92-16.83) †

Table 7. Continued

Characteristic	Typhus Group Rickettsiosis versus Rocky Mountain Spotted Fever Odds Ratio (p-value, 95% Confidence Interval)	Typhus Group Rickettsiosis versus Negative Patients (p-value, 95% Confidence Interval)
Laboratory Evidence of Systemic Infection	6.30 (<0.01, 1.69-23.43) *†	3.24 (0.06, 0.96-10.94) †
Comorbidities		
Heart Disease	0.36 (0.11, 0.11-1.24) †	0.73 (0.63, 0.21-2.56)
Disease of the Lungs	0.95 (0.95, 0.18-4.90)	1.16 (0.86, 0.21-6.22)
Liver/Kidney Disease	1.54 (0.56, 0.36-6.48)	3.46 (0.13, 0.69-17.36) †
Obesity	1.54 (0.56, 0.37-6.48)	1.41 (0.64, 0.33-5.95)
Tobacco/Drug/Alcohol Use	0.22 (0.16, 0.03-1.80) †	0.32 (0.3, 0.04-2.69)
Diabetes Mellitus	0.44 (0.46, 0.05-3.79)	0.54 (0.58, 0.06-4.76)

*Statistically significant at the 0.05 significance level †Statistically significant at the 0.20 significance level

Table 8. Multivariate Analysis

Typhus Group Rickettsiosis vs. Rocky Mountain Spotted Fever	Odds Ratio	p-value, 95% Confidence Interval
Acute Liver Injury	8.60	<0.01, 2.06-35.96
Laboratory Evidence of Systemic Infection	6.58	0.03, 1.21-35.89
Age	1.06	0.02, 1.01-1.10
Heart Disease	0.13	0.02, 0.02-0.77
Typhus Group Rickettsiosis vs. Negative		
Living on a Farm, Ranch, or in a Wooded Area	31.05	<0.01, 4.01-240.17
Acute Liver Injury	28.88	<0.01, 3.46-241.14
Acute Lung Infection	17.78	0.01, 2.11-149.81
Age	1.04	0.12, 0.99-1.09

3.4 Discussion

The results presented here can assist in differentiating between TGR rickettsiosis and other infections, decreasing time between clinical presentation and treatment, which can reduce the risk of severe complications of TGR rickettsiosis. Based on the conclusions of this study, physicians should consider rickettsia panel testing in patients with abnormal liver function who report living on a farm, ranch, or in a wooded area.

TGR rickettsiosis is a reportable condition in the state of Texas, however, the diagnostic criteria for reporting to DSHS is slightly different than that used by physicians for diagnosis and treatment. In clinically compatible cases, DSHS requires a four-fold increase in titers of *R. typhi*-specific antibodies from acute infection to convalescent phase (three to four weeks later); amplification of TGR DNA by PCR; visualization of *R. typhi*-specific antibodies in skin lesion or tissue specimen by immunofluorescence (IFA), or isolation of the causative bacteria. A probable case is defined as a clinically compatible case with IFA titers of ≥1:128, or a compliment fixation test result >1:16, or other, unspecified serology.[36] The differences in diagnostic criterion can complicate the reporting of cases to DSHS. Obtaining the convalescent IFA titers required for case confirmation might be complicated due to loss to follow up of emergency room (ER) patients, and the required travel time for patients to have the laboratory tests performed. Ten of the 16 TGR rickettsiosis patients were first seen in the ER, or were transferred to BSW diagnostic facilities from smaller clinics that lacked the diagnostic capability to determine TGR rickettsiosis diagnosis. Due to these factors, it is highly probable that the number of cases reported to DSHS is under representative of the true number of TGR rickettsiosis cases in central Texas.

Despite the differences in diagnostic criteria, there is some overlap in the geographic spread of BSW cases and DSHS cases, indicating that a number of BSW cases are included in DSHS surveillance data. Figure 13 highlights the geographic spread of TGR rickettsiosis cases according to BSW criteria (2012-2018) compared to the geographic spread of cases reported to DSHS (2008-2016). Because of the difference in diagnostic criteria, however, comparisons of these maps are difficult. Obtaining follow up IFA titers on BSW case-patients, or crosschecking the BSW data with DSHS data would allow for comparison.

Having a small sample size reduces the power of the analysis to detect an effect, when an effect is present, between the variables. The approximately 1:4 ratio of the TGR rickettsiosis cases with RMSF infected cases and negative controls was undertaken to compensate for this low power and to narrow the CIs. Power can be increased in future studies by increasing study areas, including more diagnostic facilities, or through active surveillance.

The difference in diagnostic criteria in TGR literature makes comparisons between findings in other studies difficult. Future research should explore the odds of various risk factors using other diagnostic criterion to corroborate the results of this and other TGR studies.

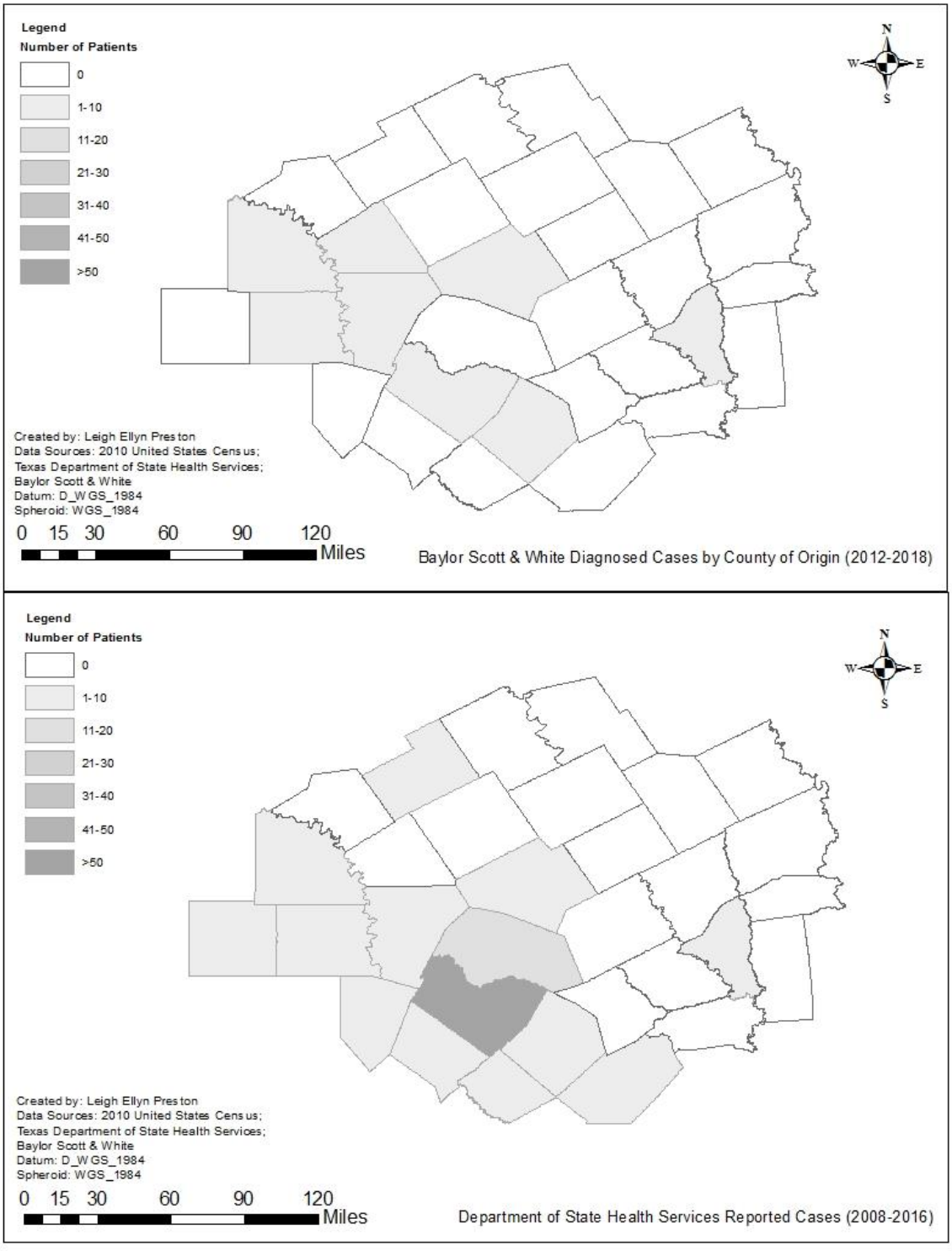


Figure 13. Frequency and Distribution of Typhus Group Rickettsia Diagnoses by (top) Baylor Scott & White Facilities in Central Texas, 2012-2018 (n=16) (bottom) Department of State Health Services, 2008-2016 (n=290).[22]

The use of EMRs to capture case information also limits data collection. EMRs will not capture any information not reported to or recorded by physicians, and also will not contain information on patients with subclinical or asymptomatic infections, patients who were not tested for TGR rickettsiosis by their physicians, or patients who did not seek treatment for infections. Active surveillance would be one way to reduce the impact of this limitation.

To our knowledge, this is the first case-control study of TGR rickettsiosis in central Texas. The use of BSW facilities in central Texas provides a representative sample of the population of central Texas, making the findings generalizable to the residents in the area. Despite the sample size, the variables included in the two logistic regression models were statistically significant, suggesting some confidence in the relationship between each included variable and TGR-specific positive IFA.

TGR rickettsiosis outcomes could be improved with increased understanding among physicians of risk factors and common symptoms. Enhancing diagnostic capacity in rural counties could also reduce the amount of time between disease onset and treatment, thereby further improving TGR rickettsiosis disease outcomes in central Texas. Within the communities of central Texas, educational campaigns to reduce contact with the vector, including emphasizing the importance of treating pets for fleas and encouraging residents to seek treatment when symptoms appear would likely improve surveillance and disease outcomes. Due to the status of TGR rickettsiosis as an emergent condition, it is probable that, without intervention, the number of reported cases will continue to increase in the coming years. Empowering the population to protect themselves against infection would prevent negative health outcomes moving forward.

Future research should involve more diagnostic facilities to increase the number of cases included. Working with physicians, state health authorities and other TGR research groups to improve diagnostic criteria would benefit surveillance efforts, and active sampling of patients would increase the understanding of the prevalence of the pathogen. Finally, testing of arthropod vectors for evidence of infection with *R. typhi* would provide a more complete picture of the transmission of TGR in central Texas.

3.5 Conclusion

There are many complications with diagnosing, treating, and reporting TGR rickettsiosis cases, including non-specific symptoms and differing diagnostic criteria. Considering rickettsia panel testing in patients with common symptoms and exposures, and streamlining the diagnostic criteria for TGR rickettsiosis would remove some of the barriers to understanding TGR epidemiology in Texas.

4. INVESTIGATION OF RICKETTSIA TYPHI INFECTION IN RESERVOIR ANIMALS

4.1 Introduction

R. typhi, a causative agent of TGR rickettsiosis, is spread through the feces of infected flea vectors after taking a blood meal. The transmission cycle of *R. typhi* includes many species of reservoir hosts, animals in which the pathogen survives and multiplies but does not necessarily cause symptomatic infection or result in disease. *R. typhi* infects many different mammalian species, but has only been documented to cause disease in humans. The animal species in which evidence of *R. typhi* infection exists, worldwide, are rats, dogs, and cats.[6, 7, 10-14, 47-42] Researchers have also found evidence of *R. typhi* infection in opossums and red foxes, but it is likely that any small mammal could act as a reservoir for the bacteria because of their frequent contact with the flea vector. [4, 8, 9]

In a literature review of studies conducted in the United States from 2002 to 2018, researchers focused on detecting *R. typhi* in opossums, rats, and cats.[2-5, 9, 15] Four of those studies also attempted to detect the bacteria in fleas, and one study tested fleas from owned cats and dogs in Oklahoma.[2, 3, 5, 9, 17, 39, 40] This group detected *R. typhi* in 4.2% of the fleas through PCR, and due to the manner in which the bacteria is spread, it is likely that the dogs and cats might be infected as well. In Texas, a study performed in 2003 found evidence of past exposure in opossums, and another performed in 2008 found evidence of past infection in dogs and cats in Texas through IFA.[3, 5] Since 2008, only one additional study has attempted to define animal reservoirs in Texas.[4] Blanton et al. detected *R. typhi* antibodies in eight (66.7%) of the opossums they tested in Galveston, Texas, near the Gulf

of Mexico.[4] These are the only studies to detect evidence of *R. typhi* infection in any potential reservoir species in Texas during the last ten years. While these studies provide evidence that these animals may be reservoirs, they only determine past exposure to the bacteria, and not current infection. Little is known about *R. typhi* infection in animals, and because of the evidence of the emergence of human rickettsial disease caused by *R. typhi* in novel regions of Texas, it is important to understand in which species, and to what extent, *R. typhi* infects potential animal reservoirs. Expanded knowledge of the epidemiology of TGR would inform strategies to disrupt the transmission of the bacteria and, ultimately, prevent human disease.

The focus of this analysis was to look for evidence of *R. typhi* in biologic samples collected in Texas from species that have been established as reservoirs in the literature in other geographic locations, as well as species that have not been established, but could plausibly act as reservoirs for *R. typhi* infection. Through this exploration, a greater understanding of animal reservoirs and the associated risks to human health can be determined.

4.2 Materials and Methods

Animal species selected for this investigation were chosen based on current knowledge of the transmission cycle of *R. typhi*. Because of their status as established reservoirs, canine and mouse samples were tested. Opossums have shown evidence of past infection, and thus were included in this investigation. Other small mammals, including skunks, raccoons, and rats were tested due to their potential to harbor the bacteria.

4.2.1 Sample Collection and Sample Size Analysis

Canine blood samples were collected from May 2013 to December 2014 from animals housed in animal shelters in Bryan/College Station, Dallas, Edinburg, El Paso, Fort Worth, Houston, and San Antonio, Texas, by Dr. Sarah Hamer at the Texas A&M University College of Veterinary Medicine.[53] Each shelter was visited three times for sample collection, and approximately 30 dogs were sampled from each shelter at each visit. To be included in the sampling, dogs had to have reached at least six months of age and had to be able to provide fresh feces to the researchers for use in another investigation. Members of Dr. Hamer's laboratory team drew approximately five milliliters of blood for research on *Trypanosoma cruzi, Dirofilaria immitis, Anaplasma spp., Ehrlichia spp.,* and *Borrelia burgdorferi* infection in the canines. Samples were taken within 14 days of the animals entering the shelter in an attempt to capture only cases acquired outside of the facility.

Blood samples from raccoons, opossums, mice, and rats were collected from animals trapped at the Stubblefield campgrounds of the SHNF on April 4, 2015 and April 18, 2015.

Additionally, blood samples from skunks were collected from several locations throughout Texas from April 2, 2012 to May 11, 2018 for an investigation into their role as possible reservoirs of *T. cruzi*. Bonnie Gulas-Wroblewski of Texas A&M University College of Veterinary Medicine and Dr. Robert Dowler at Angelo State University performed this study. Skunk blood samples from Angelo State University were collected as part of the natural history and teaching collections and samples from Ms. Gulas-Wroblewski were collected from road-kills, wildlife rehabilitation centers, or live-trapped

skunks. The possible prevalence detection level for each species (skunk, canine, and other wildlife) was calculated using the formula: $n=\ln(\alpha)/\ln(q)$, where n represents the sample size, q is 1- minimum detectable prevalence, and α is 0.05 for a confidence level of 95%.[54] Figures 14 and 15 show the sampling locations of the animal shelters, SHNF sample site (Figure 14), and either the specific location or the county of skunk sample collection location (Figure 15).

4.2.2 DNA Extraction and PCR Analysis

DNA was extracted from canine and SHNF animal samples by researchers in Dr. Sarah Hamer's laboratory using a commercial spin-column-based E. Z. N. A. Tissue DNA kit (Omega Bio-tek, Norcross, GA). DNA was extracted from skunk samples by Ms. Gulas-Wroblewski using DNeasy DNA extraction kits (Qiagen, Germantown, MD). Real-time quantitative PCR to detect *R. typhi* nucleic acid sequences was performed at the Baylor College of Medicine campus in Houston, Texas using the Life Technologies Applied Biosystems ViiA Real Time PCR System Version 1.2.4 (Carlsbad, CA).

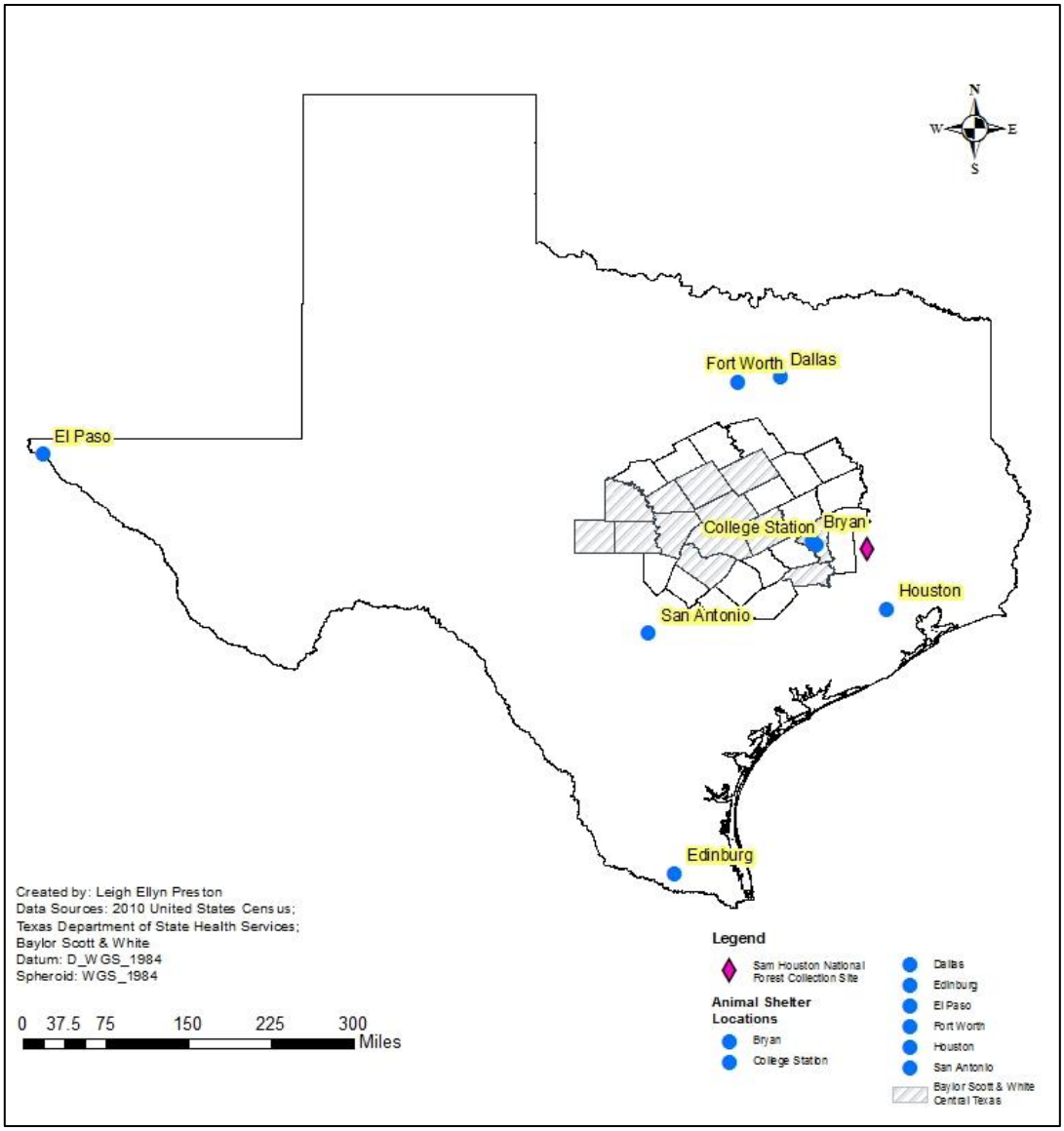


Figure 14. Distribution of Shelter Locations Where Canine Blood was Collected, 2013-2014 (n=196) and Sam Houston National Forest Sampling Site Where Other Wildlife Blood was Collected, 2015 (n=15).

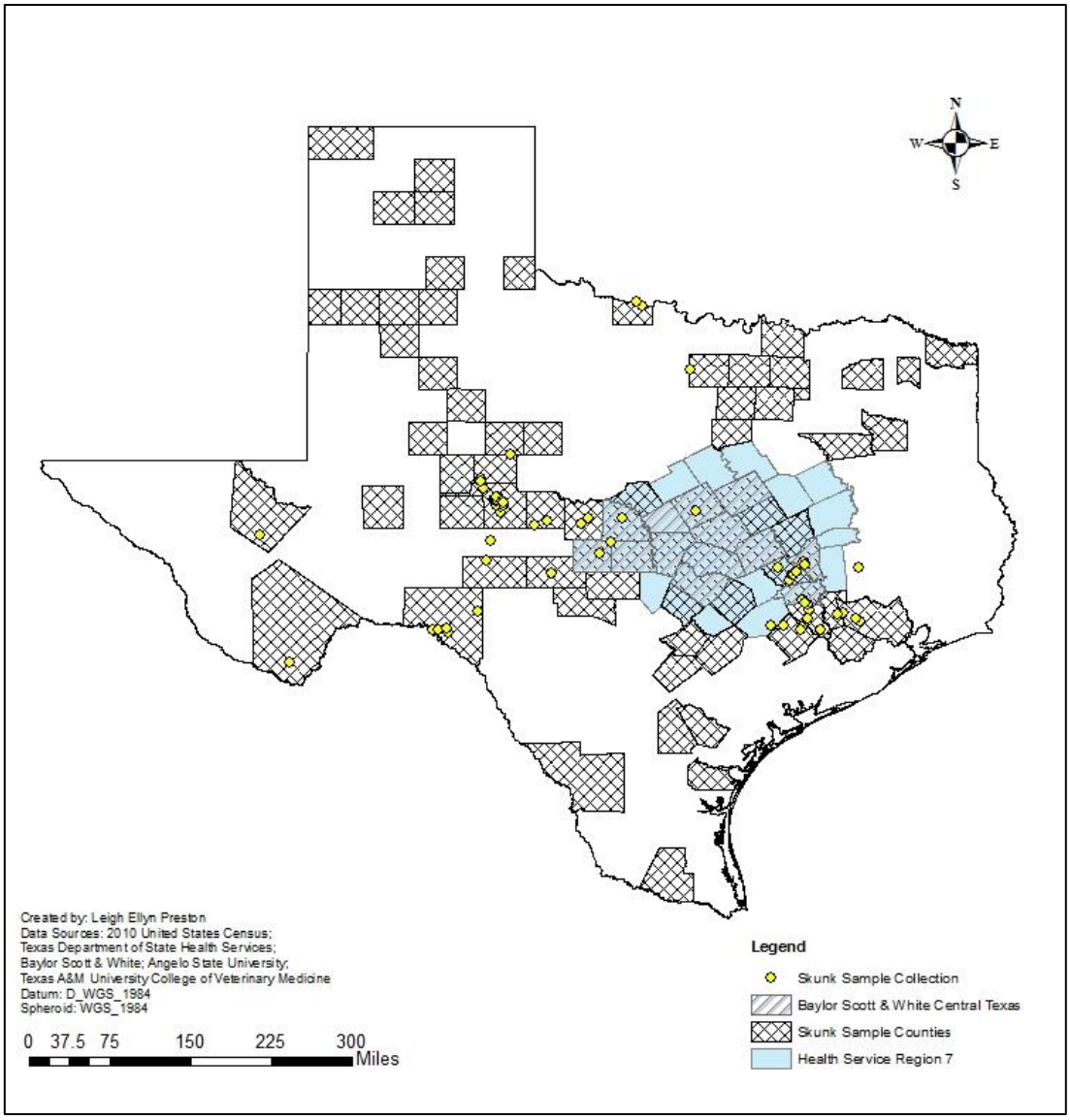


Figure 15. Distribution of Locations Where Skunk Blood Samples Were Taken, 2012-2018 (n=238).

Primers and probes for PCR were determined according to work done by Henry et al.[55] The protocol amplifies a 121 base pair region that differentiates *R. typhi* from other *Rickettsial* species. Each PCR assay was prepared using the optimized volume of 15 µl of master mix, including Fast Advanced master mix and *R. typhi* primers and TaqMan probes, and 5 µl of the DNA extraction product. Any sample was considered positive for *R. typhi*

if the cycle threshold (ct) value was less than 40 cycles. The University of Texas Medical Branch in Galveston, Texas provided a positive *R. typhi* DNA extraction product for use as a positive control, and each batch included a negative (sterile water) control. For quality control, each sample extraction product was also tested with a universal actin TaqMan assay as an internal positive control to confirm successful DNA extraction and absence of PCR inhibition. Actin assay protocol designates any sample with a ct value less than 38 positive. This work was reviewed and approved by the Texas A&M University and Baylor College of Medicine Institutional Biosafety Committees (TAMU: IBC2015-144; BCM: HAHC669).

4.3 Results

The DNA extraction products included 238 skunks, 196 canines, seven opossums, five mice, two raccoons, and one rat. A sample size of 238 skunks should detect a prevalence of *R. typhi* of 0.013, 196 canines should detect a prevalence of 0.015, and a sample size of 15 should detect a prevalence of 0.18 in other wildlife. None of the DNA extraction product amplified *R. typhi* DNA. All samples passed quality control checks, revealing that all samples contained DNA, that there was no PCR inhibition, and that laboratory practices are likely not the cause of negative results.

Table 9 evaluates the sample sizes necessary for different precision and confidence levels; a sample size calculator was used to calculate sample sizes necessary to detect a prevalence similar to the detectible ranges of previous literature.[56] Precision and statistical power are related to the probability of concluding that there is no effect when one actually exists. Because the prevalence of TGR in animals is not well characterized, these

calculations are difficult to determine, but based on the sample sizes in this study, we could detect approximately a 1% prevalence with a precision of ± 0.5% with 90% confidence in skunks, and a 3% prevalence with a precision of ± 3.0% with 95% confidence in canines.

Table 9. Sample Size and Precision Calculations

Prevalence (%)	Precision (%)	Confidence (%)	Sample Size
1	± 0.5	90	1072
1	± 0.5	95	1522
1	± 1.0	90	268
1	± 1.0	95	381
3	± 1.0	90	788
3	± 1.0	95	1118
3	± 3.0	90	88
3	± 3.0	95	125
5	± 1.0	90	1286
5	± 1.0	95	1825
5	± 2.0	90	322
5	± 2.0	95	457
5	± 5.0	90	52
5	± 5.0	95	73

4.4 Discussion

Because no samples were positive for *R. typhi* DNA, either the prevalence of the bacteria in animals throughout the state is very low or that *R. typhi* is not present in these animals at all. Researchers in the United States have been unable to detect *R. typhi* in potential reservoir species by PCR, but internationally, four researchers have found the bacteria in potential reservoir species (canines, cats, and rodents) at detectable prevalence

rates ranging from 0.01 to 0.26.[6, 10, 12, 52] The detectable prevalence rates of this study fall within the range of detectable prevalence rates found in other studies, indicating that it is unlikely that the species tested in this study are acting as potential reservoir species in Texas.

Although this analysis was unsuccessful at establishing current *R. typhi* infection in animal species in Texas, it highlights the difficulties of diagnosis of *R. typhi* infection through PCR assay of whole blood. Bacteria may be present in the reservoir species, but unless there are enough bacteria circulating in the blood, it is very difficult to detect the bacterial DNA in whole blood. This study included a wide range of potential reservoir species, and a large number of DNA extraction product collected from an extensive geographical range, suggesting a low prevalence of TGR in the animals investigated.

Another possible test that has been used by other researchers to investigate exposure to TGR in potential reservoir species is an IFA. An IFA would measure antibodies circulating in each species specific to the TGR antigen. With each animal species tested, a specific positive control must be used, as well as secondary antibodies specific to the species being tested. Because commercial kits for specific animal species are not yet available, IFA testing in potential animal reservoirs requires developing or acquiring the positive controls and secondary antibodies, which was beyond the scope of this study; however, IFA testing of potential animal reservoirs in Texas should be considered in future investigations.

Many researchers have successfully detected *R. typhi* in fleas through PCR, which would be a logical future investigation. Future research should also continue to search for evidence of bacterial infection in potential reservoir species, and expand the search to

include more mammalian DNA extraction product, especially from small wild mammals such as raccoons, opossums, mice, and rats. In general, more research is needed to understand the transmission cycle and infection of *R. typhi* in potential reservoir species to gain a better comprehension of the epidemiology of this emergent pathogen.

4.5 Conclusion

Infection with *R. typhi* in potential reservoir species remains elusive and difficult to uncover. With the rising number of human infections in new regions in Texas, understanding the circulation of the bacteria in both the vector and in potential reservoir species is an important component in disrupting the transmission cycle. Despite unsuccessful attempts to detect infection in reservoir species in this study, these findings can guide future investigations into the transmission cycle of *R. typhi*.

5. CONCLUSION

5.1 Summary

The purpose of this study was to describe clinical manifestations in known cases of acute TGR rickettsiosis in central Texas, and to identify potential animal reservoirs in Texas. These objectives were met through three specific aims. The first aim was to describe the characteristics of TGR rickettsiosis patients diagnosed at BSW facilities in central Texas through a case-series analysis and to compare clinical characteristics under existing BSW diagnostic criteria to those among cases as defined by criteria proposed by Erickson et al.[18] The second aim was to compare characteristics of patients with laboratory confirmed TGR rickettsiosis to features of patients diagnosed with RMSF and patients who tested negative for rickettsial diseases. The third aim was to describe the prevalence of *R. typhi* in potential reservoir species in Texas. The following is a summary of the findings of each specific aim.

5.1.1 Baylor Scott & White Typhus Group Rickettsia Case Characteristics

The case-series analysis revealed that clinical manifestations of patients treated for TGR rickettsiosis at BSW facilities were similar to the cases described in previous research. The most commonly reported symptom was fever (n=14, 87.5%), and 11 of the 16 patients (68.8%) were found to have acute liver injury, a common finding in TGR rickettsiosis. BSW TGR rickettsiosis cases also had common exposures of other TGR rickettsiosis cases, including exposure to domestic (n=8, 50%) or wild (n=5, 31%) animals.

Six of the 16 cases (38%) originated in counties designated as rural, and two cases (13%) were diagnosed in a county designated as rural.

When using the diagnostic criteria proposed by Erickson et al., the number of cases dropped to eight, but the cases according did not differ from the BSW cases. Using the stricter criteria, four of the cases (50%) originated in counties designated as rural, and one (13%) was diagnosed in a county designated as rural. The data in this study point to the need for increased awareness and diagnostic capability in clinics serving in rural counties.

Although we found no difference between the two groups of cases using BSW diagnostic criteria and the Erickson criteria, it does present interesting surveillance challenges. The gold standard for testing in a clinical setting is testing through IFA. In order for a patient to be considered a confirmed case by DSHS standards, patients would have to return approximately three weeks later to have another IFA done, or physicians would have to change their diagnostic testing to include PCR testing to detect TGR DNA or immunofluorescence of tissues. Detecting rickettsial infection by PCR is often difficult unless the infection is systemic, or the sample is taken from a skin punch where the infection is manifesting. Because of these difficulties, it is likely that the surveillance data for HSR7 is underestimating the actual burden of disease in the area.

5.1.2 Predicting Diagnosis of Typhus Group Rickettsiosis

In bivariate logistic regression analysis, TGR rickettsiosis patients were more likely to be hospitalized, more likely to live on a farm or ranch, or in a wooded area, and more likely to have abnormal liver function, and acute kidney or lung infection when compared with both RMSF and negative patients. When compared only to RMSF patients, TGR

rickettsiosis patients were more likely to live on a farm or ranch, or in a wooded area (OR: 7.59; 95% CI: 2.30-25.03), more likely to have laboratory evidence of systemic inflammation (OR: 4.03; 95% CI: 1.08-14.99), had higher odds of acute liver injury (OR: 10.50; 95% CI: 3.06-35.60), more often had acute lung infections (OR: 5.42; 95% CI: 1.19-24.68), and had higher odds of laboratory evidence of systemic infection (OR: 6.30; 95% CI: 1.69-23.43). When compared only to negative patients, TGR rickettsiosis patients were older (OR: 1.03; 95% CI: 1.00-1.06), they were more likely to be hospitalized (OR: 4.61; 95% CI: 1.20-17.75), more likely to live on a farm or ranch, or in a wooded area (OR: 9.00; 95% CI: 2.62-30.93), and had higher odds of acute liver injury (OR: 6.08; 95% CI: 1.84-20.07), and acute kidney injury (OR: 3.92; 95% CI: 1.24-10.94).

Multivariate logistic regression models of TGR rickettsiosis patients compared to RMSF infection patients revealed age (OR: 1.06; 95% CI: 1.01-1.10), acute liver injury (OR: 8.60; 95% CI: 2.06-35.96), and laboratory evidence of systemic infection (OR: 6.58; 95% CI: 1.21-35.89) to be associated with diagnosis of TGR rickettsiosis, while heart disease is more likely associated with RMSF diagnosis (OR: 0.13; 95% CI: 0.02-0.77). Comparing TGR rickettsiosis patients to negative patients found living on a farm or ranch, or in a wooded area (OR: 41.81; 95% CI: 4.79-364.65), acute liver injury (OR 30.47; 95% CI: 3.43-270.29), and acute lung infection (OR: 17.78; 95% CI: 2.11-149.81) to be associated with diagnosis of TGR rickettsiosis. This model also included age, although it was not statistically significant in the model (OR: 1.04; 95% CI: 0.99-1.09). Physicians should consider a rickettsia panel test for patients who report living on a farm, ranch, or wooded area, as well as those with abnormal liver function.

Comparisons of the results of this project to those of other research studies are difficult due to differences in diagnostic criteria. Although the variables associated with TGR rickettsiosis diagnosis identified in logistic regression models were statistically significant, the confidence intervals were wide as a consequence of the small sample size. Increasing the number of cases in the study would increase precision.

5.1.3 Investigating Infection in Potential Reservoir Species

In an attempt to detect active infection with *R. typhi* in potential animal reservoir species, DNA extraction product from 238 skunks, 196 canines, seven opossums, five mice, two raccoons, and one rat collected throughout Texas were analyzed by PCR. Bacterial infection was not detected in any of the samples, which is consistent with findings of previous research in the United States, and highlights the difficulties of using PCR to detect and diagnose TGR infection.

A sample size of 238 skunks should detect a prevalence of *R. typhi* of 1.3%, 196 canines should detect a prevalence of 1.5%, and a sample size of 15 should detect a prevalence of 18% in other wildlife. In precision and confidence calculations, the sample sizes in the analysis, can detect a 1% prevalence with a precision of ± 0.5% with 90% confidence in skunks, and a 3% prevalence with a precision of ± 3.0% with 95% confidence in canines. Due to the lack of understanding of the prevalence of TGR in animals, calculations are difficult to make with certainty.

Detection of infection with this bacterial pathogen in potential reservoir species remains difficult, while human infections continue to increase. Vector collection and

analysis for infection would likely prove to be a better approach to determining the source of human exposure to TGR.

5.2 Future Research

5.2.1 Characterizing and Predicting Human Infection with Typhus Group Rickettsia

Due to the challenges of confirming diagnosis of TGR rickettsiosis, characterizing the disease, calculating prevalence, and modeling infection is difficult. Future studies should involve active surveillance and prospective serum collection and analysis of participants suspected of rickettsial infection. Researchers should attempt to enroll larger numbers of participants through the inclusion of additional hospital networks and healthcare facilities. The expansion of studies into new geographic regions in which the bacteria may be circulating could also contribute more cases to future research. Characterizing and modeling infection with TGR is important to understanding the epidemiology of TGR rickettsiosis and preventing further spread of the pathogen.

5.2.2 Identifying Active Infection in Potential Reservoir Species

Determining active infection with *R. typhi* in potential reservoir species has been difficult for many researchers. Future research should focus on understanding infection and pathogenicity in those potential reservoir species. Future work should investigate infection in ectoparasites by collecting fleas off of potential reservoirs, before testing the reservoir. Future work should also include a wider range of species and increase the number of samples from those species. Building on the methods and procedures of past

investigations would increase the likelihood of detecting active infection in potential reservoir species.

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APPENDIX

Characteristic	Description in Medical Charts
Animals in the Home	Patient reported owning a dog, cat, rabbit, dove, or parrot.
International Travel	Patient reported travel to Mexico or to the Texas-Mexico border, the Philippines, Belize, Honduras, Caribbean, Korea, Costa Rica, Cambodia, Bahamas, South Africa, Peru, India, Haiti, Rwanda, Kuwait, Jamaica, Israel, or Grand Cayman Island.
Recreational Exposure	Patient reported visiting lakes, participating in outdoor activities, fishing, hunting, hiking, visiting National Parks, participates in outdoor sports, or attending street dances.
Occupational Exposure	Patient reported spending a significant amount of time outdoors as a contractor, surveyor, in a shop, as a National Parks employee, with the Army, or as a police officer, working as a farmer or rancher, an animal control officer, working on land, or working as a taxidermist.
Gastrointestinal Symptoms	Patient reported diarrhea, nausea, abdominal pain, upset stomach, or vomiting.
Respiratory Symptoms	Patient complained of shortness of breath, cough, sore throat, chest pain, congestion, dyspnea, or nasal congestion.
Neurological Symptoms	Patient reported parenthesis, altered mental status, seizures, vertigo, lightheadedness, photophobia, progressive numbness in the extremities, dizziness, loss of consciousness, facial tingling, numbness in face, reduced mental abilities, epilepsy, syncope, epistaxis, ataxia, peripheral neuropathy, intracranial mass, or numbness.
Laboratory Evidence of Systemic Inflammation	Physician reported encephalitis, systemic inflammatory response, leukocytosis, meningitis, vasculitis, elevated white blood cell count, or elevated inflammatory markers.
Acute Liver Injury	Physician reported elevated liver function, elevated liver enzymes, elevated bilirubin, hyperbilirubinemia, acute hepatitis, elevated transaminase, transaminitis, or hypoalbuminemia
Abnormal Blood Cell Counts	Physician reported thrombocytopenia, leukopenia, pancytopenia, or bicytopenia.
Acute Kidney Injury	Physician reported acute kidney injury, hypokalemia, hyponatremia, hydronephrosis, hyperpotassemia, kidney infection, acute kidney failure, acute renal failure, hyperphosphatemia, or nephrolithiasis.

Acute Lung Infection	Physician reported acute respiratory failure with hypoxemia, hilar adenopathy, hypoxemic respiratory failure, acute respiratory distress syndrome, pneumonia, bronchitis, health-care associated pneumonia, community-acquired pneumonia, acute pulmonary embolism, or hypoxia.
Laboratory Evidence of Systemic Infection	Physician reported lactic acidosis, metabolic acidosis, septic, sepsis, severe sepsis, sepsis with organ dysfunction, acute bacterial endocarditis, septic shock, or bacteremia.
Heart Disease	Patient had history of hypertension, heart murmur, coronary artery disease, valve replacement, bradycardia, congestive heart failure, atrial fibrillation, atypical chest pain, or valve prolapse.
Liver/Kidney Disease	Patient had history of nonalcoholic fatty liver disease, hepatitis C virus, stenosis of the liver, chronic kidney disease, end stage renal disease, malignant neoplasm of the liver, hepatitis, or alcoholic cirrhosis of the liver.
Disease of the Lungs	Patient had history of asthma, chronic obstructive pulmonary disorder, tuberculosis, emphysema, or obstructive lung disease.

Literature Review of Research Conducted in the United States

Author	Year, Location, Type	Subject(s), Study Population	Results
Abramowicz et al.[2]	2012, California, cross-sectional (animal)	177 opossums, 20 rats, 16 cats captured near reported human cases; 4,586 fleas	• No rickettsial DNA was detected in any animal by PCR; • *R. typhi* antibodies were not detected in any serum samples (IFA); • None of the animal tissues sampled tested positive for *R. typhi* DNA ○ In fleas: 1,950 pools tested, 29 (1.5%) were positive for *R. typhi* DNA only, and 33 (1.7%) were positive for both *R. typhi* and *R. felis*
Adjemian et al.[3]	2010, Austin, Texas, case series and environmental investigation	33 confirmed human cases out of 53 suspected human cases 56 animals: 17 cats, 9 dogs, 17 opossums, 9 raccoons, 4 rats (found near case-patient's homes)	• Suspected case: fever greater than or equal to 38 degrees C • Confirmed case: 4-fold rise in acute and convalescent antibody titers for *R. typhi* infection • 23 (70%) of cases hospitalized; complications included pneumonia, coagulopathy, and renal failure • Other commonly reported symptoms: malaise (76%), headache (73%), chills (61%), myalgia (61%), loss of appetite (58%), nausea (52%), rash (46%), vomiting (42%), diarrhea (36%) • 33 confirmed cases were clustered geographically in central Austin • 26 (79%) owned a dog or cat, recent opossum exposure reported by 11 (42.4%) • 19 (33.9%) of animals were seropositive: 3 cats, 4 dogs, 12 opossums; no raccoons or rats were seropositive ○ No evidence of *R. typhi* DNA in blood, tissue, or arthropod specimens

Afzal et al.[21]	2017, McAllen and Edinburg, TX, case series	90 adults and children diagnosed over 3-year period in 2 hospitals in South Texas	Diagnostic criteria: • Titer ≥ 1:128 • 4-fold increase of acute and convalescent IgG titers Initial symptoms: • 99% fever, • 82% malaise, • 77% headache, • 70% fatigue, • 68% myalgias, • 39% rash, • 28% complications; ○ Signs consistent with bacterial sepsis found in >70% of cases
Basra et al.[23]	2012, Galveston, case report	59 year old male	Diagnostic criteria: • 4-fold increase of acute and convalescent IgG titers • Presented to ER with headache and fever (headache for 2 weeks, fever 5 days) • Discharged with presumed viral illness, • Symptoms persisted and he was admitted; • Rash on chest and arms, evidence of systemic inflammation, • History revealed exposure to opossums in yard ○ MT diagnosis confirmed with IgG test one month after discharge

Billeter et al.[39]	2016, California, animal cross-sectional (fleas)	Flea collection from 130 cats (130 flea pools) from 3 counties in California – all shelters or rescues	Flea pools were analyzed using PCR; ○ *R. felis* was detected, but *R. typhi* was not
Blanton et al.[4]	2016, Galveston, cross-sectional (animal)	Opossums (13 – 1 was unable to provide blood)	Whole blood (PCR) & Serum (IFA); • No evidence of current infection, but 8 (66.7%) had reactive antibodies (past infection)

Blanton et al.[24]	2015, Galveston, case report	32 year old male in Galveston	• Patient had titer of 1:2048 • Lesions on palm, then fatigue and malaise followed by fever, chills and sweats; • Owned dog and cat regularly treated with flea prevention, noticed dead fleas in bedding, and • Rat and opossum activity in yard; • Black macule at base of right palm (epidermal necrosis); ○ Analyses found rickettsia in the cytoplasm of numerous infected cells

Blanton et al.[25]	2015, Galveston; case series and cross-sectional	12 active, 500 samples previously collected to determine local prevalence of *R. typhi* antibodies	• Diagnostic criteria:
			• Confirmed:
			• 4-fold increase of acute and convalescent serum levels
			• Positive PCR
			• Isolation of *R. typhi*
			• Probable:
			  ○ Serum titer levels ≥ 1:256
			• Active:
			  ○ 58% chills,
			  ○ 50% myalgias (muscle pain),
			  ○ 50% rashes,
			  ○ 57% elevated liver enzymes,
			  ○ 58% hospitalized,
			  ○ 17% ICU
			• Samples: Represent about 1% of population of Galveston
			  ○ IFA and Western blot showed 8 (1.6%) reactive

Boostrom et al.[5]	2002, Corpus Christi, Texas, animal cross-sectional	149 opossums live-trapped or collected from area residents and given to animal control facilities	Tested opossums by ELISA, titer >1:32 considered positive (previous study); sera also tested by IFA • ELISA: 38/149 positive for *R. typhi* • IFA: 10/125 (8%) positive for *R. typhi* Flea testing: 359 fleas tested by IFA: 20% positive • 529 fleas tested by PCR: 14 (2.6%) positive

Carr et al.[26]	2014, Corpus Christi/Tampa, FL, case report	17 year old female	Diagnostic criteria:
			• Confirmed:
			  ○ IgM titer > 1:150; IgG titer > 1:400
			  ○ 4-fold increase of acute and convalescent serum levels
			• Patient had IgG titer of 1:1024
			• Admitted to hospital in Tampa after 12 days of fever, headaches, and rashes;
			• Complained of vomiting, myalgia, and weakness –
			• Had returned 12 days earlier (day 1 of symptoms) from 2 week vacation in Corpus Christi,
			• Adopted stray kitten (said to be free of fleas),
			• Indication of systemic inflammation;
			• Released after 4 days sans fever, but daily headaches, readmitted 8 weeks later with vomiting, headache, double vision;
			• Released when symptoms improved,
			• Within a week, she had left-sided headache, confusion, slurred speech, and shaking of right hand, isolated ocular hemorrhages in periphery of retina and optic nerve (esp. left side)
			• Ordered *R. typhi* serology test and *R. typhi* was confirmed;
			• Released after 14 days (IV doxycycline);
			• Readmitted twice for headache and irritability;
			• Patient continues to have occasional headaches, short-term memory impairment, inability to focus (resulting in learning disability – home schooled)

Easterbrook et al.[15]	2007, Baltimore, MD, animal prevalence (cross-sectional)	201 live-trapped Norway rats	Rat IgG was measured by IFA for reactivity against *R. typhi* • 14/201 (7%) of rats had antibodies reactive to *R. typhi* • Authors indicate that there may be some cross-reactivity among *Rickettsia* antigens
Eremeeva et al.[40]	2008, Oahu, HI, flea prevalence (cross-sectional)	210 fleas combed from mice in summers of 2004, 2006, and 2007 and sent to CDC for PCR testing	4 fleas (1.9%) positive for *R. typhi* alone • 1 flea (0.05%) positive for *R. typhi* and *R. felis*

Eriksson et al.[18]	Jan. 1, 2008 – December 31, 2016; Houston/Harris county; case-series	TGR patients seen at Texas Children's Hospital – positive by IFA (36 TGR cases)	Diagnostic criteria: Confirmed: • ≥1:1024 and a titer for *R. typhi* ≥2-foldgreater than that for *R. rickettsii*; • Positive PCR • ≥4-fold increase of acute and convalescent serum titer Probable: • IFA titer ≥1:128 and a titer for *R. typhi* ≥2-fold greater than that for *R. rickettsii* Suspected: • IFA titer ≥1:64 and negative for *R. rickettsia* • 97% presented with fever • 31% with fever, rash, and headache • 86% elevated transaminases • 61% thrombocytopenia • Median 8 days between onset of symptoms and hospitalization • 83% hospitalized (about 5 days) • 19% developed severe illness • All but 2 reported contact with animals • 75% to dogs or cats • patients had contact with opossum • 8 had contact with fleas • Most cases happened in summer
Liddell et al.[27]	2012, Corpus Christi, TX; case report	54 year old male	Patient had titer > 1:64 • Flu-like symptoms 3 days prior to seeing physician, • Escorted to ER after 45 minutes observation and several episodes of loss of consciousness; • Patient reported traveling to McAllen area; • Over next 3 days, patient experienced headaches, chills, and fever, decline in white cells and platelets, also major increase in liver enzymes; • Infections disease physician diagnosed MT
Maina et al.[9]	2016, Orange County, California, cross-sectional (animal)	Opossums (262), fleas (597)	Quantitative real-time PCR assay and ELISA Fleas: 8 (1.3%) positive – PCR Opossum: all done using ELISA, no rickettsia DNA; • 107 (40.84%) IgG reactive

Murray et al.[19]	2017, Texas, case-series	1,762 patients with probable or confirmed MT	Diagnostic criteria: Confirmed: • 4-fold increase of acute and convalescent serum titer • Positive PCR • Isolation of *R. typhi* • Positive IFA in tissue • Single titer ≥ 1:1024 Probable: • IFA titer ≥ 1:128 • Complement fixation ≥ 1:16 In 2003, only 9 counties reported cases of MT, while by 2013, 41 counties had reported cases • Attack rate male: 6.7/100,000; female: 7.3/100,000 • 1,047 (59.6%) patients hospitalized • Commonly reported symptoms: • 99.7% fever • 77.2% headache • 70.1% chills • 64.1% malaise • 52.8% anorexia • 51.5% nausea/vomiting • 50.8% myalgia • Increasing geographic area of cases; highlights the importance of education on bite prevention and raising awareness of physicians
Noden et al.[17]	2017, Oklahoma, cross-sectional (fleas)	Fleas from 2 veterinary clinics in Enid and Oklahoma City (n=222) – collected from owned cats and dogs	Fleas from 52 animals found a prevalence of 4.2% • First recorded evidence of *R. typhi* in owned dogs and cats in Oklahoma area
Pieracci et al.[28]	2017, Texas, cross-sectional	11 cases	Diagnostic criteria: Confirmed: • 4-fold increase of acute and convalescent serum titer • Positive PCR • Isolation of *R. typhi* • Positive IFA in tissue • Single titer ≥ 1:1024 Probable: • Clinically compatible illness and IFA titer ≥ 1:64 11 fatal cases from 1985 – 2015, 8 male, 55% respiratory manifestations, 45% neurological manifestations, 100% fever, 55% nausea/vomiting, 55% rash, 82% thrombocytopenia, 63% elevated hepatic transaminases, 55% contact with flea or animal

Purcell et al.[29]	2007, Corpus Christi, Texas, cross-sectional (seroprevalence)	Serum from 513 children seen in Driscoll Children's Hospital; tested by IFA	Children 1-5 years of age: 13 (8.6%) positive for *R. typhi* IgG Children 6-11: 24 (13.3%) positive for *R. typhi* IgG Children 12-17: 25 (13.8%) positive for *R. typhi* IgG Total: 62 (12%) positive for *R. typhi* IgG • Author discusses cross-reactivity between *R. typhi* and *R. rickettsii* antibodies
Reynolds et al.[57]	2002, West Virginia and Georgia, case series	West Virginia: 44 year old man (February 2002) Georgia: 57 year old man (March 2002)	West Virginia: • Presented to ER with headache, fever, and chills • Findings after examination: hematuria, joint pain, abdominal pain and vomiting • Laboratory findings: elevated ALT and AST, elevated leukocytes and platelets, elevated sedimentation rate • Patient stated he was a hunter and had been in contact with flying squirrels (indirectly) one month prior to onset • Treated with doxycycline in hospital, symptoms did not improve • Serological testing for TGR at 27 days post onset: IgG=<1:64; IgM=1:512 – 53 days after onset: IgG=1:1024; IgM=1:128 for *R. prowazekii* Georgia • Visited doctor after 1 week of confusion associated with febrile illness; symptoms: rigors, malaise, myalgia, headache, vomiting, anorexia, cyclic fever • Patient treated with cefepime, ampicillin, and gentamicin until it was discovered that he had contact with a flying squirrel 2 weeks prior to onset – prescribed doxycycline • Serum tested at day 7, 13, and 23 after onset for *R. prowazekii* antigens; IgG=1:8192 at all time points
Seshadri et al.[30]	2014, Corpus Christi, case report	12 year old male	• Admitted after 9 day fever; • Seen by nurse practitioner and diagnosed with ear infection and pharyngitis; • Reported weakness in lower extremities and unable to walk on own; in hospital, diagnosed with myositis; • *R. typhi* confirmed in serum – elevated IgG and IgM

Silva et al.[32]	2016, Houston, cross-sectional	Texas Children's Hospital, Houston; 24 cases	• 58% female, • 50% white, • 41% Hispanic, • Age 4 – 19 (average = 11.3), • 75% had relevant animal/environmental exposure (most commonly dogs or cats), • 20% flea bites, • 91% fever, • 45% rash, • 37% lack of appetite, • 33% headache, • 29% vomiting

Stidham et al.[33]	2015, San Antonio, case report	Joint Base San Antonio (Army Air Force); 14 year old dependent	• Acute titer: 1:128; convalescent titer: 1:256 • Presented with fever (104) abdominal pain, emesis and diarrhea, • Extensive contact with companion felines as well as feral felines, • Tested negative for other rickettsial illness – positive for *R. typhi*

Abbreviations: B. henselae = Bartonella henselae; DNA = Deoxyribonucleic Acid; ELISA = Enzyme-linked Immunoassay; ER = Emergency Room; ICU = Intensive Care Unit; IFA = Immunofluorescence Assay; IV = Intravenous; MT = Murine Typhus; PCR = Polymerase Chain Reaction; *R. felis* = *Rickettsia felis*; *R. prowazekii* = *Rickettsia prowazekii; R. typhi* = *Rickettsia typhi*; TGR = Typhus Group Rickettsia

Literature Review of Research Conducted Abroad

Author	Year, Location, Type	Subject(s), Study Population	Results
Angelakis et al.[58]	2012, Sri Lanka, cross-sectional (fever cohort)	178 patients with fever of unknown origin – tested serum IFA	○ 3 (1.6%) were positive for *R. typhi* ○ Cases had an average of 4 days of fever, no rash, no recollection of tick bite
Anyfantakis et al.[59]	2013, Greece, case series	165 confirmed cases of MT – researchers looking specifically at liver enzyme values	○ 3 serum samples collected from the patients: first on admission, second around 2 weeks after admission, 3rd around 1 month after admission ○ First sample: 142 (86%) = elevated AST, 114 (69%) elevated ALT, 136 (82.4%) elevated LDH ○ Second sample: 126 (76.3%) elevated AST, 112 (67.8%) elevated ALT, 117 (70.9%) elevated LDH ○ Third sample: 14 (8.4%) elevated AST, 12 (7.2%) elevated ALT, 8 (4.8%) elevated LDH ○ Conclusions: elevated liver enzymes seen in many patients; without proper treatment, liver damage can become a problem in MT cases

Aouam et al.[60]	2015, Tunisia, characterization of cases (case series)	73 adult patients hospitalized for MT	○ 38 (52%) of patients lived in rural or suburban areas; ○ No reports of exposure to fleas or rats; ○ Most common symptoms were fever, headache, and myalgia; ○ 47 (64.4%) of patients reported rash; ○ 8 patients presented with interstitial pneumonia and 2 with lymphocytic meningitis; ○ Diagnosis was confirmed by IgM titer (indirect fluorescence assay) ○ Conclusions: MT is difficult to diagnose, and there can be major complications with delayed diagnosis/treatment
Azuma et al.[61]	2006, Japan, case report	54 year old male traveling in Vietnam	○ Patient reports fever began in Vietnam on May 3, rash appeared May 7, returned to Japan on May 9, admitted to hospital on May 10 ○ Blood collected on days 10, 11, 12, 14, 17, and 24 after diagnosis ○ Indirect immunofluorescence tests performed on day 10, 14, and 24 detected *R. typhi* IgM ○ PCR in convalescent phase serum (day 10) was negative ○ Subsequent tests to decipher between *R. typhi* and *R. prowazekii* were done including Western immunoblotting and cross-absorption tests – confirmed *R. typhi*

Badiaga et al.[62]	2012, Marseilles, France, cross-sectional (homeless population)	320 homeless persons enrolled, 299 provided serum (MIF)	○ 65 (22%) were positive for *R. typhi* antibodies (Western Blot); ○ MIF titer of greater than or equal to 1:25 were found in 70 participants ○ Conclusions: authors remark that the prevalence increased significantly from 2010 to 2011 (13% to 31%) indicating that this population (and population in general) is increasingly affected by this disease
Balleydier et al.[63]	2015, Reunion France, case series	8 typhus cases confirmed by IFA and Western Blot	All patients had fever (average 14.3 days); • 6 patients hospitalized; • 7 patients had arthromyalgia, • 6 had headaches; • 4 patients had rash; • 6 patients had elevated liver enzymes; • 5 had low lymphocyte counts • 4 had low platelets • None of the patients had traveled in the last 3 months; ○ Only one patient reported insect bite • Reported risk factors: 4 patients had domestic pets, 4 livestock in the surroundings, 4 presence of wild fauna in the surroundings, 3 rat exposures, 3 outdoor activities, 2 house cleaning • Conclusions: MT should be a consideration for fevers of unknown origin in Reunion

Bermúdez et al.[64]	2013, Panamá, cross-sectional	97 volunteers from 3 different areas of Panamá	○ 38 (39%) had antibodies for Rickettsia spp, (IFA) ○ No clear evidence of *R. typhi*
Bhengsri et al.[65]	2016, Thailand, retrospective cohort – fever of unknown origin	2,446 – greater than or equal to 7 years of age; patients had non focal infection and had not received immunization in the last 48 hours; no blood products in previous 6 months	○ 28 tested positive (IFA) for anti-*R. typhi* antibodies; ○ Besides fever, most patients reported headache and myalgia; ○ 39% reported cough and 14% rash; 32% reported arthralgia; 54% were hospitalized; 56% elevated ALT and 74% elevated AST
Chaliotis et al.[66]	2012, Greece, case series	90 adult patients with MT identified and described	• Most cases lived in rural (52%) or semi-urban (34%) areas; • Higher frequency in the summer • Triad (fever, headache, rash) in 64% of patients – within 2 days of hospital admission; • Low platelets (81%), pulmonary, neurological, and renal complications noted in 26% of cases • Overall cumulative incidence rate was 0.43 cases per 100 total patients, and 2.36 per 100 of acute-febrile illness patients • 91.1% of patients reported chills • IFA used to identify antibodies

Chang et al.[67]	2012, Taiwan, case series	81 cases of MT confirmed by serum (IFA)	• 78 (96.3%) cases reported fever, • 49 (61%) chills, • 42 (51.9%) headache, • 23 (28.4%) myalgia and rash, • 48 (67.6%) slow heartbeat • 74 patients were evaluated for liver function: acute hepatitis found in 52 (70.3%) • Most of the cases had elevated liver enzymes, but the enzyme diverged from other enzyme elevations seen in other cases
Chang et al.[68]	2017, Taiwan, cohort	(441 – 88 MT; 68 elderly and 373 non elderly) Cases of scrub or MT or Q fever; patients over 64 were the experimental group, control was between 18-64	• Elderly have significantly higher rate of severe complications, but not significantly higher mortality rate; • 5 cases of MT developed severe illness including meningitis (60%, 3), ARDS (40%, 2), ARF (100%, 5), and death (20%, 1); • Prothrombin time prolongation is a predictor for severe complications
Chansamouth et al.[69]	2016, Laos, cohort	250 pregnant women admitted with fever	• 10 (4%) of participants had confirmed diagnosis of MT; • 4 (2%) had confirmed diagnosis of Dengue and MT – confirmed by rapid diagnostic test and IFA, as well as PCR assay; • All but 2 of these mothers had healthy, full-term babies • 2 did not receive medication and had pre-term births

Chareonviriyaphap et al.[70]	2014, Thailand, animal cross-sectional	257 rodents (504 fleas) captured across Thailand	• 121 (47%) rodents positive (ELISA) for *R. typhi* (IgG or IgM or both); • 13 (2.58%) of fleas were positive for *R. typhi* by PCR
Chen et al.[71]	2014, China, cross-sectional of hemorrhagic fever cases (search for cause)	85 HF patients in an ID hospital in China	• 1 laboratory confirmed case (IFA) IgG
Choi et al.[72]	2005, South Korea, cross-sectional (seroprevalence)	200 previously collected serum samples that were positive for SFGR or TGR	• 7 (0.035) samples were positive for TGR DNA by PCR
Christou et al.[73]	2010, Cyprus, flea cross-sectional	457 fleas taken directly from rodents	• 19 (4%) were positive for *R. typhi* by PCR
Dieme et al.[74]	2015, Reunion Island, flea cohort	205 Fleas collected from 59 small terrestrial mammals: rats, shrews, mouse, tailless tenrec	• 3 (1.46%) were positive for *R. typhi* using PCR

Dittrich et al.[75]	2015, Laos, CNS cohort	1112 patients with CNS symptoms (or signs requiring lumbar puncture) – CSF collected from 1051 patients	• 28 (11%) tested positive for *R. typhi* or Rickettsia spp. by PCR or IFA/MAT
			• Patients with CNS infection caused by *R. typhi* presented later than patients with conventional bacteria;
			  ○ The mortality of these patients was nearly double the mortality rate of ST or leptospirosis, but similar to those with conventional bacterial infections (33%);
			  ○ Presented with significantly longer fever duration, more commonly had rash
			• Conclusions: *R. typhi/Rickettsia spp* are leading causes of bacterial CNS infections in Laos

Dzul-Rosado et al.[76]	2013, Yucatan, Mexico, case report	42 year old female and 12 year old son	• Patients presented with malaise, headache, fever, fibromyalgia, sore throat, fatigue and rash that spread after 6 days; • Dengue was the original diagnosis but couldn't be confirmed; • MT was diagnosed based on PCR and IFA

Dzul-Rosado et al.[56]	2017, Teabo, Mexico (rural), animal cross-sectional	10 dogs (free-roaming, but owned); adult male Rhipicephalus sanguineus ticks	• 100% of samples were positive for *R. typhi* using PCR; • Indicates that ticks and dogs can be involved in transmission
Forshey et al.[7]	2010, Peru, cross-sectional seroservy (humans); serological survey of fleas and animals	1195 human serum samples; 71 dogs and 13 cats 170 fleas and 43 lice	• Serum tested by ELISA • 123 (10.3%) positive for TGR IgG antibodies • 2 (2.8%) dogs were positive for *R. typhi*; 1 (7.7%) cat was positive for *R. typhi* • 1 dog positive by PCR for Rickettsial DNA, but species could not be identified • No fleas or lice were positive by PCR

Gasem et al.[77]	2009, Indonesia, fever cohort	137 outpatients from 2 primary health centers in Semarang with acute undifferentiated fever	• Inclusion criteria: fever at least 38 degrees Celsius for less than 14 days with no other apparent disease
			• Patient serum tested by IFA; blood sample taken at first visit, then again around 14 days later
			• IFA considered positive for Rickettsia if:
			  o Titers were ≥1:256 for IgG and ≥1:64 for IgM
			  o Observed seroconversion
			  o A ≥4-fold increase in titers between acute and convalescent serum collections
			• Cross-checked by PCR
			• All positive samples were tested by microscopic agglutination test and IgG ELISA
			• 9 (7%) patients had evidence of acute infection with *R. typhi* (none had rash)
			  o 6 of 67 hospitalized patients were diagnosed
			  o 3 of 70 outpatients were diagnosed
			• Other reported symptoms: headache (85%), myalgia (70%), nausea (64%), cough (44%), and abdominal pain (38%)

Gastellier et al.[78]	2014, France, case report	41 year old female returning from 5 week stay in Tunisia	• Hospitalized with fever; symptoms began 7 days • Prior to admission: severe headache, vomiting, no fever (at first); • Reported staying in rural area, contact with various domestic animals, but no recollection of flea bite; • Other symptoms: chills, sweating, nausea, myalgia, arthralgia, stiff neck, cough, abdominal pain for 2 weeks after onset • Clinical workup: no rash or flea bite mark – first suspected typhoid fever • Serum sampled 2 weeks after onset of symptoms and tested for Rickettsia species – revealed elevated IgG/IgM levels (PCR negative)
Gracia et al.[79]	2015, Spain, animal cross-sectional	89 cats; 458 fleas; cat samples collected from 24 veterinary practices throughout Spain	• None of the cats tested positive with PCR or IFA; • 34 tested positive for Rickettsia species
Hamaguchi et al.[80]	2015, Vietnam, cohort of suspected rickettsioses (descriptive study)	749 enrolled, 579 eligible for analysis (ELISA)	• 193 (33.3%) positive for MT; ○ 125 (64.8%) had fever > 7 days, 139 (72%) headache, 153 (79.3%) myalgia, 140 (72.5%) chills, 157 (96.3%) elevated liver enzymes

Hernandez-Cabrera et al.[81]	2004, Canary Islands, Spain, case series	22 patients 14 years of age and older at in- and out-patient of the Hospital Universitario Insular of Las Palmas	• Case definition:
			  ○ IgM titer ≥1:40
			  ○ 4-fold increase in IgG titers against *R. typhi* by IFA in 8 weeks
			  ○ Either of these or both
			• All patients reported contact with animals (13 dogs, 6 horses, 5 goats, 2 cats, 1 camel)
			• All patients had high fever; 90% had headache; 68% had rash; 45% arthromyalgia; 38% hepatomegaly; 28% cough; 18% diarrhea; 13% nausea/vomiting and abdominal pain; 13% insect bite; 13% confusion

Hidalgo et al.[82]	2013, Colombia, cross-sectional	682 volunteers in Caldas province	• 172 (25.2%) of participants were seropositive (IFA); 196 (33.3%) were reactive to both *R. typhi* and *R. felis* • Conclusions: high seroprevalence of flea-borne rickettsia diseases in north Caldas, Colombia and should be tested for to reduce morbidity and mortality
Himsworth et al.[16]	2015, Vancouver, Canada, animal cross-section	725 animals; 630 PCR samples; 553 serum samples; 404 blood clot samples	• All PCR testing for *R. typhi* was negative; • 2 of 553 (0.36%) of serum samples were reactive against TGR (originated from Norway rats)
Irwin et al.[83]	2013, New Zealand, fever cohort	57 patients with undifferentiated symptoms of febrile illness lasting greater than or equal to 72 hours; originally tested serum, then PCR of whole blood	• 5 tested positive for MT; • Rural residents, having seen rats in the 6 weeks prior to illness onset, low platelet count, rash, low creatinine associated with MT infection; • 4 of those 5 had PCR and 2 of the 4 were positive – the other 2 (neg) had begun antibiotic treatment prior to PCR
Jensenius et al.[84]	2013, Norway, cross-sectional	82,825 total Westerners who had traveled and sought care; 3,666 diagnoses included in the study (acute and potentially life-threatening illnesses)	• 16 (0.43%) were positive for MT – out of 820 potentially life-threatening bacterial diseases

Kho et al.[85]	2016, Malaysia, case-series	72 year old female living on a farm in Malaysia	• Presented with fever of unknown origin and interstitial pulmonary fibrosis; • Complained at admission of back ache, anorexia, diarrhea, abdominal pain, productive cough; • Developed atrial fibrillation and hypotension; low red cell count, increased plasma and liver enzymes; • CT scan suggested severe pneumonia; • PCR amplification revealed *R. typhi*
Khrouf et al.[86]	2016, Tunisia, cross-sectional	121 samples from 101 patients with acute fever and cutaneous rash; obtained from 5 different hospitals	• 5 (10.6%) patients tested positive for *R. typhi* by either qPCR or RLB • In test comparisons (between qPCR and RLB), 5 patients were positive by PCR and 1 by RLB • Authors conclude that qPCR and RLB can be used to effectively diagnose *R. typhi* infection
Kuan et al.[87]	2017, Japan, case-report	51 year old Japanese male	• 2-week history of rash, accompanied for 5 days by fever, headache, myalgia • Had been camping in Sri Lanka and bitten by insects • Leucocytosis, and thrombocytopenia also seen • IFA: greater than 1:1024
Kuchuloria et al.[88]	2016, Georgia (country), fever cohort	537 patients in 6 hospitals throughout Georgia – blood and serum samples collected as well as questionnaire for risk factors	• 39 (7%) were positive for anti-*R. typhi* IgM antibodies (ELISA); • Risk factors significant in multivariate regression: consumption of unpasteurized milk products, consumption of undercooked meat, sore throat, dyspnea

Kularatne et al.[89]	2013, Sri Lanka, cross-sectional study of all rickettsial infections	371 cases of rickettsial infections; 122 chosen for IFA to confirm diagnosis	• No subjects reacted only to *R. typhi* antibodies, but 1 patient was diagnosed with MT (not serologically confirmed); • 27 (25.7%) MT + other infection
Kuo et al.[48]	2012, Taiwan, animal cross-sectional	2.048 small mammals (shrews and rodents), 330 fleas	• 0.1% of small mammals tested positive by IFA for *R. typhi* alone, but • 9.4% tested positive for both *R. typhi* and R. conorii • No *R. typhi* detected in fleas (PCR)
Kuo et al.[49]	2015, Taiwan, animal cross-section	1064 rodents assayed for *R. typhi* exposure; 927 samples subjected to nested PCR	• 30 (2.8%) out of 1064 seropositive for *R. typhi*; • Most animals had more than one infection
Kuo et al.[90]	2017, Taiwan, cohort	Authors took national typhus data and mapped it to see if there was correlation with distance from the ports – 476 cases reported from 2000 – 2014	• Incidence rate = 0.14/100,000/year • Authors found the prevalence higher at international port locations
Lai et al.[91]	2014, Taiwan, cohort (suspected Q fever cases)	413 cases of suspected Q fever, Scrub Typhus, MT, leptospirosis, dengue	• 12 cases reported of MT, 9 confirmed cases by IFA; • Important to note that MT was not the outcome of interest, authors were investigating SFRG
Lai et al.[92]	2017, Taiwan, retrospective cohort	11109 participants – researchers looking for Q fever cases; 468 Q fever cases,	• Researchers were investigating Q fever, but also co-infections with MT (etc); • They found that there were 4 co-infections with Q fever & MT & scrub typhus; • 190 (2.4%) confirmed MT cases; Researchers note that these findings are similar to others

Lau et al.[93]	2016, American Samoa, cross-sectional	200 serum samples collected during a study on leptospirosis, analyzed retrospectively; participants also responded to a questionnaire to assess risk factors	• All sera were unreactive to *R. typhi* at a dilution of 1:100 (IFA recommended cut off) • Authors concluded that the prevalence of MT cannot be determined
Laudisoit et al.[50]	2014, Democratic Republic of the Congo, animal cross-sectional	126 rats, 193 fleas collected in 6 administrative districts	• 13% prevalence in rats; • 10.8% of flea pools positive for *R. typhi* (PCR) • Conclusion: highest prevalence in rodents known to date in the world
Leulmi et al.[94]	2014, Africa, flea cross-sectional	5,897 fleas collected from small mammals in central Africa	• Fleas were tested using PCR; • 4 fleas were positive for *R. typhi* Conclusions: • Researchers report that this is the first direct evidence of *R. typhi* in certain regions of Africa
Lim et al.[95]	2016, New Zealand, cohort	989 blood donors across New Zealand; also given questionnaire	• 106 positive by AARL slide test; • 7 (0.7%) positive by IFA; • 3 positive by Western blot; • 6 positive by cross-adsorption assay
Lledó et al.[8]	2016, Spain, animal cross-sectional	314 wild red foxes from Soria, Spain	• 6 (1.9%) showed antibodies for *R. typhi* through IFA – • Fleas were also collected from foxes, but researchers did not find the flea that is typically the vector for *R. typhi* • This study shows that not only can foxes act as a reservoir, but that there are potentially more species of fleas that can infect animals and humans

Luciani et al.[96]	2014, Italy, case report	75 year old woman	• Presented to ER with headache, tremors, difficulty speaking, rash, and fever for 5 days; • No reported travel or eating unpasteurized food – • Hospitalized; • Medical history=acid reflux, hypertension, depression; • Physical exam: low blood pressure, increased heart rate • Work up: increased white cells, liver enzymes • Patient's blood pressure dropped, and sepsis was suspected • Day 3, serum tested for Rickettsia species (negative) • Day 10 after admission, IgM assay was positive (IFA) • Conclusions: MT is emergent in non-endemic areas of low rat population in Italy

Malheiro et al.[97]	2017, Portugal (traveler from SE Asia), case report	38 year old female returning from Bali	• Risk factors: no pre-travel vaccinations or prophylaxis, contact with rats, mosquitos, sporadic dog and cat contact • Symptoms: presented with fever, chills, malaise, myalgia, and conjunctival congestion; normal chest x-ray • Work up: high white cell count, elevated liver aminotransferases, blood work originally negative • One day after admission, SOB, low blood pressure, headache, nausea and faint, rash; x-ray = bilateral interstitial infiltrate in lung parenchyma • Discharged on day 13; ○ MT diagnosis obtained post-discharge (confirmed using blood and serum collected on day 4 of disease and day 13 after discharge) positive PCR
Martínez-Ortiz et al.[10]	2016, Bolmay, Yucatán, Mexico (rural), cohort (animal)	128 dogs	• Whole blood samples from 128 dogs were taken for PCR amplification; • 7 (5.5%) were positive for *R. typhi*
Maude et al.[98]	2014, Bangladesh, cross-sectional	1,209 patients tested; participants were recruited from hospital admission reports and had to provide informed consent	• 805 (66.6%) were positive for R. tyhpi, • 77 (6.4%) positive for both R. tyhpi and O. tsutsugamushi (ELISA) • Conclusion: MT should be considered as diagnosis in febrile illness

Mayxay et al.[99]	2015, Laos, fever cohort	229 patients without malaria is Southern Laos	• 1 (0.4%) had positive serum (ELISA) – single infection; • 16 patients had multiple infections: 3 (19%) had MT + SFGR, 1 had Dengue + MT + leptospirosis • 5 (2.18%) overall positive for MT
McGready et al.[100]	2010, Thai-Burmese border, fever cohort	211 women (pregnant or had just given birth)	• Diagnostic criteria: o Positive PCR o Isolation of bacteria o 4-fold increase in IgM or IgG between acute and convalescent titers measured by IFA • 14/211 positive 7 by IFA, 7 by PCR
McGready et al.[101]	2014, Thailand, fever cohort (descriptive study based on previous literature)	26 pregnant women included in the descriptive study	• 14 (54%) MT confirmed by either serum or PCR; • 7 tested positive for both serology and PCR Pregnancy outcomes: • 1 still birth • Normal gestational age, • 1 premature • Live born 11 normal birth weight

Mokrani et al.[102]	2012, Algeria, fever cohort (cross-sectional)	108 patients with fever and rash were entered in the study	• Serum tested by MIF, whole blood with Western Blot • 4 (3.7%) were positive on both tests for MT • Conclusions: authors note the high incidence of typhus group rickettsia in the area

Mouffok et al.[103]	2008, Algeria, case series	42 year old male, and 25 year old male	• 42 year old:
			  ○ Reported contact with cats and dogs that had noticeable tick infestations
			  ○ Sought medical attention after 10 days of high fever, sweating, headache, arthralgia, myalgia, cough, and weight loss
			  ○ Only convalescent serum collected: IgG-1:2048; IgM 1:1024
			• 25 year old:
			  ○ Patient was a farmer; hospitalized for 5 day history of fever, headache, diarrhea, and did not respond to amoxicillin and acetaminophen
			  ○ Reported contact with cats and cattle
			  ○ Patient had rash and pharyngitis
			  ○ Laboratory results revealed elevated neutrophils
			  ○ Convalescent-phase serum (2-weeks after hospitalization): IgG 1:256, IgM 1:256
			  ○ Serum also tested positive for SFGR pathogens, but Western blot and cross-absorption studies confirmed *R. typhi*
			  ○ These confirmatory tests indicate cross-reactivity of antigens

Moy et al.[104]	2015, Singapore, case report	39 year old male from India, and 27 year old Filipino male	Patient 1: • Admitted at day 9 of fever; • Symptoms=night sweats, neck pain, double vision, vomiting (on day of hospital admission); • No recent insect bites or skin rashes; workup showed typical signs of MT (low cell count, elevated liver enzymes; • MRI showed increased cranial pressure (day 2 after admission); • On day of discharge, serum collected on day 2 after admission tested positive for anti-*R. typhi* antibodies, • Serum collected on day 11 after admission was even higher (consistent with MT infection) Patient 2: • 3 day history of fever and headache followed by 1 day history of vomiting and drowsiness; • Recent travel to Indonesia (5 day trip) symptoms appeared day after return; • No contact with animals or insect bites; hepatitis b carrier; increased pulse, slightly decreased BP; consciousness level steadily decreased; • Rickettsia titer performed day 4 after admission, negative; • Neural examination revealed bilateral lateral gaze palsy and dysmetria; • 2 month follow up, repeated *R. typhi* titer (IgG) elevated, confirming diagnosis

Musso et al.[105]	2014, Tahiti, cross-sectional	472 French Polynesian blood donors, 178 ticks, 36 cat fleas	• No significant detection of *R. typhi* (IFA) at established cutoff levels for diagnosis; • All tick and flea samples (PCR) negative for *R. typhi*
Nanayakkara et al.[11]	2013, Sri Lanka, cross-sectional (animal)	123 serum samples from dogs were collected (IFA) – all dogs were owned	• Only 3 (2%) of dogs had antibodies to *R. typhi*, however many 60 (49%) were positive for Rickettsia species • Conclusion: Any detection of *R. typhi* in dogs is significant as they are not a typical reservoir for the pathogen, and shows the spreading of the bacteria
Nogueras et al.[12]	2013, Spain, animal cross-sectional	221 cats, 80 fleas collected from cats; coastal areas of Spain	• 217 cat samples tested, 35 (16%) positive (IFA); • Whole blood was collected from 23 seropositive cats, 5 of these were PCR positive for *R. typhi* • 44 (55%) of fleas were positive by PCR for *R. typhi* • Conclusion: authors state that in many areas, high prevalence of *R. typhi* in cats correlates with high prevalence in human disease
Nogueras et al.[13]	2013, Spain, animal cross-sectional	201 dogs were sampled from veterinary offices and shelters across Spain	• 9 (4.48%) positive for *R. typhi* by IFA; • *R. typhi* DNA was obtained from 2 cultures
Nogueras et al.[106]	2014, Spain, cross-sectional	341 HIV+ patients evaluated to find seroprevalence of anti-*R. typhi* antibodies (IFA)	• 7.6% were positive for *R. typhi* • Findings were consistent with numbers found in similar locations from healthy subjects

Osterloh et al.[107]	2016, Germany, experimental study (immune-compromised mice)	T and B cell deficient mice (n~21)	• Researchers were investigating the animal model of *R. typhi* infection in immune-compromised mice; • They found that *R. typhi* can be cleared by innate immune responses (without T or B cell responses), but that persistent infections caused CNS disorders at 3 to 4 months post infection; • Most mice showed stroke-like symptoms in the initial stages of infection; • Mice died between 80 and 120 days after infection; • High levels of *R. typhi* were isolated from brain tissue, but not from other organs (new finding)
Parola et al.[108]	2003, Thai-Myanmar border, fever cohort	46 patients at the Kwai River Christian Hospital Clinical Center (20 years and older)	• Patients were included if they had: o Rash or eschar o Arthropod bites or recent exposure to the jungle o Negative malaria smear o Positive serum for rickettsia • Serum tested by IFA at day 0 and day 21 of fever • Serum was cross-checked by Western blot and cross-adsorption • 4 (9%) tested positive for *R. typhi*
Peniche-Lara et al.[51]	2015, Mexico, animal cohort	42 rodents were collected from homes of patients diagnosed with fever of unknown origin; 10 fleas	• 8 (19%) rats were positive (PCR) – active *R. typhi* infection; • 3 fleas were positive (PCR)
Persichetti et al.[14]	2016, Italy, cohort (animal & vector)	42 cats; 28 fleas or flea pools; 73 ticks or tick pools	• No cats were seropositive for *R. typhi*; • 5 ticks and 23 fleas were positive for Rickettsia spp. but specific *R. typhi* evidence was not found

Phatharodom et al.[109]	2015, Thailand, case report	69 year old Indian business man – transplant recipient, coronary bypass (immune-suppressed)	• 5 years after transplant, presented with 4-day history of fever; chills, fatigue, malaise; no travel history; high pulse; low red cell count • Admitted – broad-spectrum antibiotics (7 days), no change; • Day 8 of hospitalization, non-productive cough, dyspnea; chest x-ray=bilateral interstitial infiltration; • Blood drawn, serum tested, revealed high titers of IgG/IgM for *R. typhi*
Phommasone et al.[110]	2013, Laos, case report	20 year old female rice farmer – one of the participants in the non-malarial fever of unknown origin study	• Patient's home was surrounded by gardens; • Presented after 14 days of headache, 3 days of fever, myalgia, vomiting; • Suspected scrub typhus; ○ Serum collected at admission showed antibodies for Scrub typhus, but was slight pos for MT; • PCR showed mixed DNA for Scrub typhus and MT
Phongmany et al.[111]	2006, Laos, fever cohort	427 adults (older than 15 years of age) admitted to Mahosot Hospital with a fever	• Patients had blood drawn on admission, then taken about 1 week later tested by IFA • 41 (9.6%) were positive for *R. typhi* • Conducted multiple logistic regression comparing MT with scrub typhus • Symptoms of MT patients: headache (95%), abdominal pain (43%), nausea (45%), cough (35%), back pain (38%), myalgia (85%), palpable liver (73%)

Prabhu et al.[112]	2011, Tanzania, fever cohort	870 patients total, 450 tested for SFGR and TGR	• Pediatric patient inclusion criteria (age 2 months to 13 years of age): history of fever in the last 48 hours (>37.5 C and at least 38 C respectively); clinical officer collected demographic data, clinical history, exam findings, provisional diagnosis (collected within 24 hours of admission) • Serum tested for *R. typhi* reactivity by IFA; considered positive if ≥4-fold increase in IFA titer; if repeat titers were not available, or titers did not meet the case definition, titers ≥1:64 were considered "exposed" to TGR • 2/207 (1%) of adults had acute TGR; 9/395 (2.3%) had TGR exposure
Pradhan et al.[41]	2012, Nepal, fever cohort (children)	1084 children less than 15 years who presented to an outpatient clinic with fever greater than 39 degrees C enrolled (excluded if hospitalized within 24 hours of outpatient treatment; 164 (15%) pathogens identified by PCR	• 22 (2% of total tested, 13% of identified) were PCR positive for *R. typhi*; • Most commonly diagnosed with enteric fever (45%); • These patients were more likely to present with nausea or vomiting or diarrhea; none presented with rash

Premaratna et al.[113]	2014, Sri Lanka, fever cohort	• Group 1: ○ 57 male military members stationed in Sri Lanka, admitted to a teaching hospital for injuries during field activities • Group 2: 49 male military members stationed in Sri Lanka, who developed febrile illness during field activities	All participants tested for *R. typhi* by IFA, none were positive

Psaroulaki et al.[114]	2012, Cyprus, case series and environmental investigation	193 laboratory confirmed MT cases by PCR or IFA	• Annual Incidence –
			  ○ 2000: 7.1/100,000
			  ○ 2008: 1.4/100,000
			  ○ Average: 2.7/100,000/year
			• Most common symptoms –
			  ○ Fever (189 – 97.9%)
			  ○ Headache (146 – 75.6%)
			  ○ Malaise (192 – 99.5%)
			• 145 (75.1%) required hospitalization
			• Rural areas had more cases (153 – 79.3%)
			• Environmental Surveillance
			  ○ Fleas in 113 (58.5%) of homes
			• Flea bites reported by 58 (30.1%) of patients

Punda-Polic et al.[115]	2007, Croatia, case-series	57 patients diagnosed between 1982 and 2002	• Medical records searched (retrospectively) to find diagnosed cases • All patients had fever • 75% headache; 70% rash, 53.2% malaise, 43.6% arthralgia/myalgia • 26 patients (45.6%) had regular contact with domestic animals; 25 (43.9%) reported seeing rats and mice in living areas
Punjabi et al.[116]	2012, Indonesia, fever cohort (cross-sectional)	226 patients enrolled (non-malarial fever)	• 13 confirmed (ELISA) cases of MT; • 1 case of bacterial pneumonia due to MT; • 2 urinary tract infections positive for MT

Raby et al.[117]	2013, Australia, case series	53 year old male, 59 year old female returning from vacation in Bali	Case 1: • Became ill 1 week after returning home from 2 weeks in Bali; • Initially had fever, headache, myalgia, arthralgia (symptoms lasted 5 days); • After 5 days, developed nausea, vomiting, dry cough, then the next day, he became confused and developed rash – these symptoms prompted him to seek medical attention • Work up – bilateral pulmonary infiltrates on chest x-ray; • Blood results showed renal failure, slightly elevated liver enzymes, low platelets; • 10 days in ICU; • IFA titer revealed reaction to *R. typhi* (increasing number over 2 weeks) • Discharged after 18 days hospitalization • Risk factors – reported close contact with birds, orangutan, elephant, and lion; swimming in water slide park and ocean, received multiple mosquito bites Case 2: • Presented 2 weeks after returning from Bali, 1 week of illness • Symptoms – myalgia, headache, fever; developed rash – presumed dengue or Chikungunya infection • Fever worsened, patient developed dry cough, increasing dyspnea • Work up – hepatitis, increased liver enzymes – suspected legionnaires disease due to bilateral pulmonary infiltrates on chest x-ray • MT confirmed by IFA at 10 days after onset of illness

Rakotonanahary et al.[52]	2017, Madagascar, cross-sectional	31 participants from an urban area and 31 participants from a rural area in Madagascar; 52 small mammals; 133 fleas	• 39% of human samples were positive for *R. typhi* – no significant difference between rural and urban (ELISA) • 2% of rats (n=2) tested positive for *R. typhi* (ELISA) • 26% of rat fleas tested positive for *R. typhi* (PCR)
Reeves et al.[118]	2015, Japan, flea cross-sectional	>1,600 fleas collected from military facilities throughout Japan	• 1 was positive for *R. typhi* specifically, but many were positive for Rickettsia species
Sakaguchi et al.[119]	2004, Japan, case report	56 year old man	• Sought medical care for fever and rash on trunk and upper limbs; treated with lincomycin and cefditoren pivoxil (no improvement) • On day 3, patient reported he had been in bamboo grove on days 1-11 prior to onset • Admitted to hospital on day 6 of illness • Serum samples taken at days 5, 6, 9, 20, and 34 – IgM titer of 1:200; IgG positive for TGR • Day 6 (1:80), day 9 (1:160), day 20 (1:160), day 34 (1:80) • Diagnosis was confirmed with Western blot and absorption tests

Sakamoto et al.[42]	2012, Japan, case report	56 year old Japanese traveler returned from Thailand	• Patient developed symptoms (fever, headache, fatigue) 1 day after returning from visit; • Admitted to hospital 5 days later with suspected malaria; • Patient reported that he had a 5 day fever of unknown origin when in Thailand where he was a language instructor; • Exam: temp rose 3 degrees Celsius within 4 hours, low blood pressure, high pulse, conjunctivitis, rash • Work up: low platelets, elevated liver enzymes; chest x-ray= left pleural effusion • Suspected dengue fever and rickettsial infection (and septic shock); • Patient did not respond to treatment and went in to respiratory distress • Diagnosis confirmed first by PCR – serum not stored so could not be tested

Sansyzbayev et al.[120]	2017, Kazakhstan, vector analysis	2,963 fleas from gerbils were collected from 5 different regions, pooled and analyzed for different rickettsial diseases	No *R. typhi* was detected in the fleas
Schulze et al.[121]	2011, Germany, case report	33 year old female who returned from 5 weeks of field research in Nepal	• Patient admitted to hospital after 7 days of fever up to 39.7 C and flu-like symptoms • Low blood pressure and high pulse; light rash all over her body that developed 4 days after initial symptoms • Complained of myalgia and "feeling drunk" • Chest x-ray showed signs of pneumonia – treated with moxifloxacin but fever continued • Rickettsial tests ordered on day 12 of illness (day 5 of hospitalization) • IgG results: 1:640 (IFAT); IgM: 1:80 – retrospective testing on serum drawn on day of admission: IgG-1:40, IgM 1:20 – Repeat titers: IgG-1:1280 (2.5 weeks after admission) and 1:640 (9 weeks after admission) • DNA sequencing matched 100% with *R. typhi*

Spernovasilis et al.[122]	2017, Kasos Island, Greece, case report	54 year old male from a rural area	• Risk factors: frequent contact with pigs and chickens as well as bees
			• Symptoms: fever, low blood pressure, high heart rate, rash, mild lung tenderness
			• Work Up: low cell counts, mild anemia, mild elevation of liver enzymes, mild proteinuria, normal chest x-ray;
			  o Day 2 of hospitalization, abdominal ultrasound=thickened gallbladder wall;
			  o Day 3 after hospitalization, IFA revealed high titer for anti-*R. typhi* IgM – no IgG

Stockdale et al.[123]	2011, United Kingdom, case report	29 year old male returning after 11 days in Indonesia and Hong Kong	• 8-day history of fever, rigors, night sweats, myalgia, and headache; developed dry cough and shortness of breath; rash developed 2 days after hospital admission • Reported frequently seeing rats in living areas, reported multiple insect bites • TGR IFA ordered (negative upon admission) convalescent serology (1 month later) showed IgM 1:1024 and IgG 1:2048
Syhavong et al.[124]	2010, Laos, cross-sectional	392 patients admitted to the hospital	• Serology performed for rickettsia (IFA) • Evidence of MT in 14/382 patients (3.7%)

Teoh et al.[125]	2017, Australia, cross-sectional	131 veterinarians from across Australia (answered questionnaire and provided blood sample)	• Questionnaires given to assess risk factors for disease; • Blood samples collected for IFA testing for past MT infection; • 4.6% were positive for *R. typhi*; 35.1% were positive for both *R. typhi* and *R. felis* • Higher percentage were positive for *R. felis*

Theunissen et al.[126]	2017, Belgium, case report	2 patients returning from travel: Patient 1=37 year old woman returning from Indonesia and Malaysia Patient 2=34 year old female returning from Ethiopia	Patient 1- • Risk factors: none reported • Symptoms: fever (presented in outpatient clinic on day 8 of fever), hospitalized the following day with signs of sever hepatitis • Hospitalization: given broad-spectrum antibiotics but did not improve, day 4 after admission, developed tinnitus, and perceptive hearing loss on left side; tested for MT • On day 10 post-admission – positive serology confirmed by PCR from blood taken upon admission Patient 2- • Risk factors: hiking in Awash National Park • Symptoms: fever (presented in ER after 7 days of fever on day 8 post return), admitted to hospital • Hospitalization: condition worsened after treatment with ceftriaxone: blurred vision, hepatitis, splenomegaly, acute kidney disease, pleuro-pericarditis, signs of retinal bleeding; o 16 days after admission, antibody titer tested positive for *R. typhi*, confirmed by PCR

Thompson et al.[127]	2015, Kathmandu, Nepal, cohort within a RCT	125 patients randomly selected for the cohort; 627 enrolled in RCT	• 21 (17%) tested positive by ELISA or IFA; • 12 of these were also confirmed by PCR • Conclusions: rickettsial infections are an important cause of fevers of unknown origin in Nepal
Tsioutis et al.[128]	2014, Greece, cohort (looking at symptoms and outcomes)	49 patients aged 65 or older diagnosed with *R. typhi* infection	• 46 (93.3%) resided in rural or semi-urban areas; • 20 (41%) reported frequent contact with animals, • None reported flea bites or contact with ticks; • 26 (53.1%) had underlying chronic diseases; • All patients presented with fever • 41 (83.7%) had headache, • 36 (73.5%) had rash, • Muscle weakness in 42 (85.7%); • Severe infection with complications reported in 16 (32.7%) including CNS in 10 (20%) • Conclusions: Complications occur more frequently in elderly patients, but researchers did not observe unfavorable outcomes in any of the participants

Vallée et al.[129]	2010, Laos, cross-sectional/case-control/seroprevalence	2002 adults in Vientiane Capital City with residency in the area greater than 5 years	• Researchers administered a survey to participants and drew blood by finger prick • Anti-*R. typhi* ELISA performed • 20.6% positive for IgG MT • MT prevalence higher in more urbanized areas • Multivariate analysis comparing scrub typhus to MT "cases"

van der Vaart et al.[130]	2014, The Netherlands, case report	40 year old male returning from travel to Borneo	• Presented to hospital with fever, headache, seating, nausea (started 1 day previous); • Traveling in Borneo for 1 month; reported frequent insect bites and exposure to fresh water; • Took malarial prophylaxis and pre-travel vaccines • Physical exam: no fever, but had rash; • Low lymphocytes, low platelets • Patient admitted the next day, condition worsened, increased dyspnea, respiratory failure; • Day 2 chest x-ray=bilateral interstitial abnormalities • Serum collected day 1 after admission weakly positive IgG for *R. typhi*, after 7 days, IFA increased 4-fold (WHO definition of "case") • Patient made a full recovery

Walter et al.[131]	2012, Marseille, case series	32 confirmed cases (MIF); 42,276 samples tested	• 2008: 0.06% prevalence • 2009: 0.07% prevalence • 2010: 0.1% prevalence • Most infections 8/32 (25%) occurred in August • All cases acquired the infection while traveling to either Africa or Asia • 15 (47%) reported rash, 11 (34%) headache, 31 (of 31 100%) reported fever; ○ Classic triad in only 4 (12%) of cases • One patient presented with septic shock on admission and another with myocarditis • Elevated transaminases in 18 (56%) of cases • Western Blot was positive in all cases, PCR only performed for one case

Xu et al.[43]	2014, China, case report	74 year old male, farmer	• Admitted to hospital for impaired consciousness preceded by fevers and headaches that lasted for days; • Presented with headaches, fever, dizziness, and nausea; MRI showed lesions in the brain • Diagnosed with encephalitis of unknown origin; • Serum sample collected on day 2 of hospitalization; • Neurologic exam showed mild coma and neck stiffness; intracranial lesions increased, and MRI revealed hemorrhage; • Day 3 of hospitalization, daughter mentioned father had been in an undeveloped area 10 days prior to onset of illness and had complained of itch from bug bite; • 2 weeks after onset of illness, blood was collected and serum was used for IgG reaction (IFA); baseline was positive, then 2 weeks later IgG titer was higher; active *R. typhi* confirmed by PCR

Yang et al.[44]	2012, China, outbreak investigation	76 serum samples from persons attending a drug detoxification program (76 of 430 patients in one ward reported fever of unknown origin)	• 35 (40%) serum samples were positive for anti-R.typhi (IgM), 29 (38%) positive for IgG antibodies; • 12 (16%) of samples were positive in PCR • All patients were men who worked in clothing manufacturing facility and reported frequent contact with rats and cats in the cafeteria • Patients reported headache, dizziness, myalgia, fever, shivers, no rash, none remembered being bitten by an insect • Initial diagnosis was thought to be influenza (treated with antiviral meds)
Yoshimura et al.[132]	2015, Japan, case report	43 year old male returning from travel to Bali (Indonesia) – travel lasted 10 days	• Patient presented at hospital 10 days after returning; symptoms began 5 days after returning • Symptoms: high fever, fatigue, severe headache, rash discovered on examination; no history of flea bites; • Chest x-ray showed nodular lesion and pleural effusion in the lung; • Elevated liver enzymes; • 34th day of illness, elevated IgG/IgM (IFA)

Zavala-Castro et al.[45]	2009, Mexico, case report	3 year old female	• Patient seen in the ER for fever lasting 3 days (39.8 C); child had exposure to cat and dog, mice in home, flea bites noticed on legs 2 days before onset of symptoms • Also reported abdominal pain, headache, fatigue, and myalgia • Patient misdiagnosed with viral pharyngitis and otitis media; treated outpatient with nimesulide, amoxicillin, and trimethoprim (did not respond to treatment) • 10 days after initial ER visit, hospitalized and diagnosed with *Salmonella typhi* infection; treated with sulfonamide, cephalosporin, and acetaminophen • Laboratory findings: low platelets, neutrophilic leukocytosis, elevated liver enzymes • Diagnosed with *R. typhi* by PCR; also performed IFA 20 days after onset of illness (IgM 1:256; IgG 1:128)
Zavala-Castro et al.[133]	2014, Mexico, case report	23 year old male	• Patient presented with fever (suspected of rickettsial infection); • Reported having cats in the house, no fleas or ticks observed; had rash; • PCR was positive for *R. typhi*
Zhang et al.[134]	2008, China, cross sectional	365 health farm workers in close contact with domestic animals, vectors, or rodents (220 samples tested)	• Participants given a questionnaire and provided serum for testing • 4.1% (9/220) serum samples were positive for *R. typhi*

Zimmerman et al.[135]	2008, Nepal, fever cohort	756 adults (greater than 13) with febrile illness treated at Patan Hospital in Kathmandu, Nepal	• Patients with fever greater than 38 C were recruited to the study • Blood drawn from patients and tested for IgM antibodies against *R. typhi* and by PCR • Diagnosis of MT required positive PCR result • 85 (11%) had IgM antibodies, 50 (7%) had positive PCR • Logistic regression comparing MT with enteric fever patients: age (odds ratio-1.07), living in Kathmandu (odds ratio 14.37), and diagnosis in winter season (odds ratio-28.93) were significant
Znazen et al.[136]	2013, Tunisia, case series	43 serologically confirmed cases – 1024 patients were initially screened, 43 (4.2%) are described in this report	• 58.1% were from rural areas, no patients reported exposure to rats or rat fleas, more cases during the summer and the fall • Most frequent complaint was fever (67.5%), rash reported in 44.1% and headache in 60.5%; • 44.1% presented with thrombopenia and 47.2% had elevated liver enzymes; • Hospitalization occurred in 36 (83.7%) of cases; • Interstitial syndrome occurred in 10 (23.2%) cases, 33 (58.1%) had signs of systemic inflammation

Abbreviations: AARL = Amino Acid Reference Locus; ALT = Alanine Amiotransferase; ARDS = Acute Respiratory Distress Syndrome; AST = Aspartate Aminotransferase; CNS = Central Nervous System; CSF = Cerebrospinal Fluid; CT = Computed Tomography; DNA = Deoxyribonucleic Acid; ELISA = Enzyme-linked Immunoassay; ER = Emergency Room; IFA – Immunofluorescence Assay; LDH = Lactose Dehydrogenase; MAT = Monocyte Activation Test; MIF = Micro Immunofluorescence; MRI = Magnetic Resonance Imaging; MT = Murine Typhus; O. tsutsugamushi = Orientia tsutsugamushi; PCR = Polymerase Chain Reaction; qPCR = Quantitative Polymerase Chain Reaction; *R. conorii* = *Rickettsia conorii*; RCT = Randomized Controlled Trial; *R. felis* = *Rickettsia felis*; RLB = Reverse Line Blot; *R. typhi* = *Rickettsia typhi*; SFRG = Spotted Fever Rickettsia Group; TGR = Typhus Group Rickettsia