Rickettsial Disease Diagnostics and Epidemiology

Bonny Mayes, MA
Zoonosis Control Branch
Department of State Health Services
Austin, Texas
Rickettsial Infections (Spotted & Typhus Fevers)

- **Typhus Group**
  - *Rickettsia prowazekii* (louse-borne, epidemic, sylvatic typhus)
  - *R. typhi* (flea-borne, endemic, murine typhus)

- **Spotted Fever Group (tick-borne)**
  - *R. aeschlimannii* (Rickettsiosis)
  - *R. africae* (African tick-bite fever)
  - *R. australis* (Queensland tick typhus)
  - *R. conorii* (Mediterranean spotted fever, Boutonneuse fever)
  - *R. heilongjiangensis* (Far Eastern spotted fever)
  - *R. Helvetica* (Aneruptive fever)
  - *R. honei* (Flinders Island spotted fever, Thai tick typhus)
  - *R. japonica* (Japanese spotted fever)
  - *R. marmionii* subspecies (Australian spotted fever)
  - *R. massiliae* (Mediterranean spotted fever-like disease)
  - *R. parkeri* (Maculatum infection)
  - *R. rickettsii* (Rocky Mountain Spotted Fever)
  - *R. sibirica* (North Asian tick typhus, Siberian tick typhus)
  - *R. sibirica mongolotimonae* (Lymphangitis-associated rickettsiosis)
  - *R. slovaca* (Tickborne lymphadenopathy)
  - *Rickettsia* species 364D

http://www.cdc.gov/otherspottedfever/
Flea-borne Typhus

- **Etiologic Agent:**
  - bacterium *Rickettsia typhi* (and possibly *R. felis*)

- **Vectors:**
  - primarily rat fleas (*Xenopsylla cheopis*) and cat fleas (*Ctenocephalides felis*)

- **Reservoirs:**
  - rats, opossums, domestic cats, and other small mammals

- **Modes of transmission:**
  - *transmission to humans can occur when flea feces, containing the bacteria, are scratched into the bite site or other abrasion in the skin, or are rubbed into the conjunctiva*
  - another possible mode of transmission is inhalation of dried rat or cat flea feces

*Infection by *R. felis* has been attributed to flea saliva rather than feces* (http://www.ehtjournal.net/index.php/ehtj/article/view/7168/9204#CIT0013)
Flea-borne Typhus
History

• Also known as murine or endemic typhus
• Worldwide distribution
• First identified in the United States in 1913
• Over 5,000 cases were reported annually through the mid-1940’s mainly in SE states and California
• 1945 US Public Health Service initiated campaign to control rats and their fleas – by late 1980’s, case counts reduced to less than 100 cases/yr
• Most states discontinued reporting flea-borne typhus after the drastic reduction of cases
• Not nationally reportable, so reliable case counts are not known
• Still reportable in some states, including Texas, California and Hawaii, where the majority of cases are thought to occur
Flea-borne Typhus Disease in Humans

- Symptoms occur from 6 to 14 days after exposure
- Most common symptoms include:
  - Fever
  - Headache
  - Malaise
  - Anorexia
  - Myalgia
  - Nausea and/or vomiting
  - Rash – occurs in ~50% of those infected
    - Generally starts on trunk and spreads to the arms and legs but usually does not occur on the face, palms or soles
  - Thrombocytopenia*
  - Elevated liver enzymes**
- Typhus-associated deaths are rare but may occur in 5% of those infected

*reported in 16% of cases in Texas, 2003-2013
** reported in 27% of cases in Texas, 2003-2013
Flea-borne Typhus
Epidemiology in Texas

- Flea-borne typhus has been included in the Annual Summary of Notifiable Diseases in Texas since 1946.
- From 1946 to 2014, there were 6,729 cases reported (mean=97.5); highest number of cases reported in 1946 (n=1147) and lowest in 1994 (n=9).
- Typhus cases occur year round, but majority of cases occur from May to July.
- Most cases occur in the southern portion of the state, from Nueces County southward to the Rio Grande Valley.
Tick-borne Spotted Fever
Group Rickettsia (SFGR)

- **Etiologic Agents (in the U.S.):**
  - Rickettsia sp. Bacteria
    - *Rickettsia rickettsii* (Rocky Mountain Spotted Fever)
    - *Rickettsia parkeri* (Maculatum infection)
    - *Rickettsia* species 364D (Eschar-associated illness)
    - *Rickettsia amblyommii*?? (detected in many ticks; pathogenicity has not been determined)

- **Vectors:**
  - *Rickettsia rickettsii* - mainly:
    - The American dog tick (*Dermacentor variabilis*), Rocky Mountain dog tick (*Dermacentor andersoni*), brown dog tick (*Rhipicephalus sanguineus*), cayenne tick (*Amblyomma cajennense*)
  - *Rickettsia parkeri*:
    - Gulf Coast tick (*Amblyomma maculatum*)
  - *Rickettsia* species 364D
    - Pacific Coast tick (*Dermacentor occidentalis*)

- **Reservoirs:**
  - ticks; dogs and rodents

- **Modes of transmission:**
  - transmitted to a vertebrate host via the bite of an infected tick
  - generally, the tick must be attached and feeding for about 24 hours before the bacteria can be transmitted to the host
SFGR

History

- Rocky Mountain spotted fever (RMSF), the prototypic disease of the spotted fever group, has been a reportable disease in the US since the 1920’s
- This disease was first described in the Rocky Mountain region of the US, but has been reported throughout most of the contiguous US
- The majority of cases (>60%) are reported from only five states (North Carolina, Oklahoma, Arkansas, Tennessee and Missouri)

- As of 2010, RMSF has been included in a broader category called Spotted Fever Group Rickettsiosis (SFGR) - many of the cases being reported as RMSF were not actually identified as being specifically *Rickettsia rickettsii*

[Source: www.cdc.gov/rmsf/stats/](http://www.cdc.gov/rmsf/stats/)
Disease in Humans

- Disease onset averages one week following the bite of an infected tick (for RMSF, first symptoms typically begin 2-14 days after tick bite)
- Most common symptoms include:
  - Fever
  - Headache
  - Myalgia
  - Anemia
  - Myalgia
  - Nausea and/or vomiting
  - Rash – occurs in >80% of those infected with RMSF
    - generally starts on ankles and wrist and spreads to the torso; often present on the palms and soles
  - Thrombocytopenia
  - Elevated liver enzymes
- RMSF can be fatal in as many as 20% of untreated cases
Spotted Fever
Epidemiology in Texas

*Rocky Mountain Spotted Fever/Spotted Fever Group Rickettsiosis has been included in the Annual Summary of Notifiable Diseases in Texas since 1951

From 1951 to 2014, there were 1,353 cases reported (mean=21); highest number of cases reported in 1983 (n=108) and lowest in 1958, 1962, 1965 and 2001 (n=0)
Flea-borne Typhus and SFGR in Texas, 2008-2014

Spotted Fever Group Rickettsiosis Cases
2008-2014

Flea-borne Typhus
2008-2014
Flea-borne typhus is a rickettsial disease whose course resembles that of louse-borne typhus, but is generally milder. The onset is variable, often sudden and marked by headache, chills, fatigue, fever and general pains. A macular eruption may appear on the 5th or 6th day, initially on the upper trunk, followed by spread to the entire body, but usually not to the face, palms or soles. Absence of louse infestation, geographic and seasonal distribution and sporadic occurrence of the disease help to differentiate it from louse-borne typhus.
Clinical evidence: Any reported acute onset of fever and one or more of the following: headache, myalgia, anorexia, rash, nausea/vomiting, thrombocytopenia, or any hepatic transaminase elevation.

Confirmed: Clinically compatible case that is laboratory confirmed

Probable: Clinically compatible case with supportive laboratory results:
• IFA serologic titer of ≥1:128, OR
• A single CF of >16, OR
• Other supportive serology (single titer ≥1:128 by an LA, IHA, or MA test)

Note: Because serologies for rickettsial diseases can be cross-reactive, specimens should be tested against a *panel of Rickettsia antigens, including, at a minimum, R. rickettsia and R. typhi, to differentiate between SFGR and non-SFGR Rickettsia spp. In addition, according to CDC, Rickettsial IgM tests lack specificity (resulting in false positives); thus, IgG titers are considered to be much more reliable.

* Specimens can be forwarded to the DSHS Serology lab for Rickettsial panel testing.
Flea-borne Typhus Epi Case Criteria 2016  
Laboratory Confirmation Tests

Serological evidence of an elevation (four-fold change) in immunoglobulin G (IgG)-specific antibody titer reactive with *Rickettsia typhi* or *Rickettsia felis* by IFA, complement fixation (CF), latex agglutination (LA), microagglutination (MA), or indirect hemagglutination antibody (IHA) test in acute – and convalescent – phase specimens ideally taken at least 3 weeks apart,

OR
Positive PCR assay to *R. typhi* or *R. felis*,

OR
Demonstration of positive *R. typhi* or *R. felis* IF of skin lesion (biopsy) or organ tissue (autopsy),

OR
Isolation of *R. typhi* or *R. felis* from clinical specimen
Spotted fever group rickettsioses (SFGR) are a group of tick-borne infections caused by some members of the genus *Rickettsia*. The most well-known SFGR is Rocky Mountain spotted fever (RMSF), an illness caused by *Rickettsia rickettsii*. Disease onset for RMSF averages one week following a tick bite. Illness is characterized by acute onset of fever and can be accompanied by headache, malaise, myalgia, nausea/vomiting, or neurologic signs; a macular or maculopapular rash may appear 4-7 days following onset in many (~80%) patients, often present on the palms and soles. RMSF can be fatal in as many as 20% of untreated cases, and severe fulminant disease can occur. In addition to RMSF, human illness associated with other spotted fever group *Rickettsia* species, including infection with *Rickettsia parkeri*, has also been reported. In these patients, clinical presentation appears similar to, but can be milder than, RMSF; the presence of an eschar at the site of tick attachment has been reported for some other SFGR.
**Clinical evidence:** Any reported acute onset of fever and one or more of the following: rash, eschar, headache, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation.

**Confirmed:** Clinically compatible case (meets clinical evidence criteria) that is laboratory confirmed.

**Probable:** Clinically compatible case (meets clinical evidence criteria) with serological evidence of elevated IgG or IgM antibody reactive with *R. rickettsii* or other spotted fever group antigen* by IFA (serologic titer of ≥1:128),

**Note:** Because serologies for rickettsial diseases can be cross-reactive, specimens should be tested against a *panel of Rickettsia antigens, including, at a minimum, R. rickettsia and R. typhi, to differentiate between SFG and non-SFG Rickettsia spp. In addition, according to CDC, Rickettsial IgM tests lack specificity (resulting in false positives); thus, IgG titers are considered to be much more reliable.

*Specimens can be forwarded to the DSHS Serology lab for Rickettsial panel testing.*
Serological evidence of an elevation (four-fold change) in immunoglobulin G (IgG)-specific antibody titer reactive with *Rickettsia rickettsii* or other spotted fever group antigen* between paired serum specimens (one taken in the first week of illness and a second 2-4 weeks later), as measured by a standardized indirect immunofluorescence assay (IFA),

OR

Detection of *R. rickettsii* or other spotted fever group DNA* in a clinical specimen by polymerase chain reaction (PCR) assay,

OR

Demonstration of spotted fever group antigen* in a biopsy/autopsy specimen by IHC,

OR

Isolation of *R. rickettsii* or other spotted fever group rickettsia* from a clinical specimen in cell culture


Spotted fever group species **excluded** from this condition are: *R. felis and R. akari.*
# Typhus & SFGR - Clinical Evidence

<table>
<thead>
<tr>
<th>Flea-borne Typhus</th>
<th>Spotted Fever Group Rickettsiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Must have Fever</strong></td>
<td>Must have <strong>Fever</strong></td>
</tr>
<tr>
<td>Headache</td>
<td>Headache</td>
</tr>
<tr>
<td>Myalgia</td>
<td>Myalgia</td>
</tr>
<tr>
<td>Chills</td>
<td>Anemia</td>
</tr>
<tr>
<td>**Anorexia (<strong>reported in 52% of cases in Texas, 2003-2013</strong>)</td>
<td>Malaise</td>
</tr>
<tr>
<td>Rash, if present, typically starts on upper trunk and spreads, but generally not to palms/soles (<strong>reported in &lt;50% of cases in Texas, 2003-2013</strong>)</td>
<td>Rash occurs in ~80% of patients with RMSF; typically begins on ankles and wrists and spreads to trunk – may be on palms/soles</td>
</tr>
<tr>
<td>Nausea/vomiting (<strong>reported in 51% of cases in Texas, 2003-2013</strong>)</td>
<td>Nausea/vomiting</td>
</tr>
<tr>
<td>Thrombocytopenia (<strong>reported in 16% of cases in Texas, 2003-2013</strong>)</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Elevated liver enzymes (<strong>reported in 27% of cases in Texas, 2003-2013</strong>)</td>
<td>Elevated liver enzymes</td>
</tr>
</tbody>
</table>
Serologic Testing for Rickettsial Diseases

• IFA is considered the gold standard of serologic testing for rickettsial diseases (94-100% sensitive after 14 days – may lack titers in first 7 days of illness)

• HOWEVER, “serological testing is limited by antibody cross-reactivity with RMSF and typhus antigens, leading to false positives by both ELISA and IFA” which can contribute to misdiagnosis and misreporting of both diseases

• IgM titers are not reliable (CDC: “We do not recommend IgM testing for rickettsial diseases, especially in the absence of IgG testing, as false positives and false negatives are common.”)

• If cost restrictions limit testing, IgG is preferred over IgM!

• Physicians need to collect both acute and convalescent serum specimens and order IFA tests

• Should test for both (order rickettsial panel)
# 2014 Probable Typhus Cases - titers

<table>
<thead>
<tr>
<th>Onset/Blood Collected Date</th>
<th><em>R. typhi</em> IgM</th>
<th><em>R. typhi</em> IgG</th>
<th><em>R. rickettsia</em> IgM</th>
<th><em>R. rickettsia</em> IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/8, 12/15</td>
<td>1:512</td>
<td>1:256</td>
<td>1:512</td>
<td>&lt;1:64</td>
</tr>
<tr>
<td>3/25, 4/1</td>
<td>1:128</td>
<td>1:64</td>
<td>1:128</td>
<td>&lt;1:64</td>
</tr>
<tr>
<td>1/22, 2/3</td>
<td>1:512</td>
<td>1:512</td>
<td>1:64</td>
<td>1:128</td>
</tr>
<tr>
<td>4/29, 5/2</td>
<td>1:128</td>
<td>1:128</td>
<td>1:64</td>
<td>1:128</td>
</tr>
<tr>
<td>12/31, 1/7</td>
<td>1:512</td>
<td>1:256</td>
<td>1:256</td>
<td>1:64</td>
</tr>
</tbody>
</table>

Of the first 45 case investigation forms I reviewed, ~25% had + RMSF titers (and not all had RMSF testing done!)
# Laboratory Criteria

<table>
<thead>
<tr>
<th>Flea-borne typhus</th>
<th>Spotted Fever Group Rickettsiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Fourfold or greater increase in IgG Ab titer in acute and convalescent sera (C)</td>
<td>*Fourfold or greater increase in IgG Ab titer in acute and convalescent sera (C)</td>
</tr>
<tr>
<td>*IFA – IgM or IgG Titer of ≥1:128 (P)</td>
<td>*IFA – IgM or IgG Titer of ≥1:128 (P)</td>
</tr>
<tr>
<td>ELISA (EIA) only good for screening purposes—values do not reflect accurate quantification of Ab titers</td>
<td><strong>PCR on punch skin biopsy or swab of rash is best test for RMSF! (CDC)</strong></td>
</tr>
<tr>
<td>PCR on skin biopsy or swab (CDC)</td>
<td>**PCR on skin biopsy or swab (CDC)</td>
</tr>
<tr>
<td>Immunohistochemistry or culture (CDC)</td>
<td>Immunohistochemistry or culture (CDC)</td>
</tr>
</tbody>
</table>

*a single specimen cannot be used to differentiate between SFGR and flea-borne typhus with confidence

**must collect during acute phase of illness no more than two days after doxycycline treatment begins**
Rickettsial Disease Case Investigation Example

- Patient with fever/chills, headache, anorexia, photophobia, malaise, myalgia, thrombocytopenia, elevated liver function tests, rash (spread from arms/legs to trunk)
- Doctor orders RMSF IgG and IgM
  - IgG 1:128
  - IgM 1:256
- No convalescent testing
- No known tick or flea exposure, no exposure to wild animals, dogs are present at residence
- Classified as probable SFGR
• No typhus testing done
• ZCB requested that the serum be forwarded from commercial lab to DSHS for rickettsial panel testing
• Results:
  - *R. rickettsii* IgG 1:128
  - *R. typhi* IgG 1:1024
• Changed condition to probable flea-borne typhus!
How would you classify this case?

<table>
<thead>
<tr>
<th>Onset/Blood Collected Date</th>
<th><em>R. typhi</em> IgM</th>
<th><em>R. typhi</em> IgG</th>
<th><em>R. rickettsia</em> IgM</th>
<th><em>R. rickettsia</em> IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/8, 1/16</td>
<td>1:256</td>
<td>1:128</td>
<td>1:256</td>
<td>1:128</td>
</tr>
</tbody>
</table>

- Patient has fever, headache, nausea/vomiting, malaise, thrombocytopenia
- Patient does not report exposure to fleas or ticks
- Patient does not have rash
- Patient from area with both typhus & spotted fever occur
- Patient did not travel
- Physician unable to get convalescent sample for testing
Flea-borne typhus and spotted fever group rickettsioses (SFGR) are a group of vector-borne infections caused by some members of the genus *Rickettsia*. These infections can be difficult to differentiate clinically and serologically (due to antibody cross-reactivity). Illness is characterized by acute onset of fever that may be accompanied by: headache, malaise, myalgia, nausea and/or vomiting, anorexia, and rash.

**Clinical evidence:** Any reported acute onset of fever and one or more of the following: rash, headache, nausea/vomiting, anorexia, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation.
“Rickettsia, unspecified” Epi Case Criteria 2016
Case Definition/Case Classification (cont.)

**Probable:** Clinically compatible case (meets clinical evidence criteria) with serological evidence of elevated IgG or IgM antibody reactive with spotted fever AND typhus group antigens by IFA (serologic titers of ≥1:128) that cannot be classified as either flea-borne typhus or SFGR.

**Note:** For “Rickettsia, unspecified”, an undetermined case can only be classified as probable. This occurs when a case has compatible clinical criteria with laboratory evidence to support infection, but spotted fever and typhus fever group titers are equal and exposure history, diagnosis and clinical information are ambiguous.
**Spotted Fever Group Rickettsia (SFGR) Classification Algorithm**

Fever

- Yes
  - Fourfold or greater rise in IgG antibody titer to *Rickettsia rickettsii* or other *SFGR* antigen by IFA in acute AND convalescent sera specimens OR positive PCR assay to *R. rickettsii* or other SFGR spp. OR demonstration of SFGR antigen in a biopsy/autopsy specimen by IHC OR isolation of *R. rickettsii* or other SFGR from a clinical specimen in cell culture
    - Yes
      - Confirmed SFGR
    - No
      - NOT A CASE

- No
  - NOT A CASE

At least one of the following:
  - rash, eschar, headache,
  - myalgia, anemia,
  - thrombocytopenia, or any hepatic transaminase elevation

Serologic evidence of elevated (titer ≥1:128) IgM** or IgG antibody reactive with *R. rickettsii* or other SFGR antigen by IFA

- No
  - NOT A CASE

- Yes
  - If positive by ELISA/EIA, request Rickettsial panel testing (ELISA is only a screening test)

Are typhus titers higher than SFGR titers?

- No
  - Are typhus and SFGR titers equal and are exposure history, diagnosis and clinical presentation ambiguous?
    - Yes
      - Probable “Rickettsia, unspecified”
    - No
      - Probable typhus
      - NOT A CASE of SFGR
  - Yes
    - Probable typhus
    - NOT A CASE of SFGR

Was flea-borne typhus testing done?

- Yes
  - Are typhus titers negative or lower than SFGR titers?
    - No
      - Probable SFGR
    - Yes
      - Probable typhus
      - NOT A CASE of SFGR
  - No
    - Request ***typhus testing; if no sample available for testing, will technically meet case definition

*IgM titers are not reliable: false negatives and false positives are common; if only a low IgM titer, look for alternate explanation for illness

***Cannot rule out flea-borne typhus without testing because antibodies cross react

IFA=immunofluorescence antibody
PCR=polymerase chain reaction
IHC=immunohistochemistry
ELISA=enzyme-linked immunosorbent assay
EIA=enzyme immunoassay

Flea-borne Typhus Algorithm

1. Fever
   - Yes
     - One of the following: rash, headache, nausea/vomiting, myalgia, anorexia, anemia, thrombocytopenia, or any hepatic transaminase elevation
       - Yes
         - Fourfold or greater rise in IgG Ab titer to *Rickettsia typhi* or *R. felis* antigen by IFA, CF, LA, IHA in acute and convalescent sera specimens OR positive PCR assay to *R. typhi* or *R. felis* OR demonstration of positive *R. typhi* or *R. felis* IF of skin lesion (biopsy) or organ tissue (autopsy) OR isolation of *R. typhi* OR *R. felis* clinical specimen
           - Yes
             - Confirmed Flea-borne Typhus
           - No
             - NOT A CASE
       - No
         - NOT A CASE
   - No
     - NOT A CASE

Rev. 02-16
Rickettsial Disease Case Investigation

- Make sure ALL rickettsial lab reports are in NEDSS
- If only spotted fever testing done, see if sample can be forwarded to DSHS rickettsial panel testing
- Need to contact patient
  - Information about exposure to vectors
  - Travel history
- Treatment information
- Make sure all required fields are completed in NEDSS
  (refer to Data Entry Guidelines – Quick Reference section for patient demographics/lab report)
Disease Reporting/Communication

- Regional ZC should be the liaison between the LHDs and ZCB – if responsible jurisdiction is LHD, information should flow through regional ZC office
  - Completed case investigation forms
  - Questions
  - Issues with classification or missing documentation/information

- Regional ZC all have different preferences and are involved to varying degrees

- If ZCB communicates directly with LHD, regional ZC should at least be notified (cc’d if email)
References

• Centers for Disease Control and Prevention website: www.cdc.gov/rmsf/

• Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever, Ehrlichioses, and Anaplasmosis --- United States. A practical Guide for Physicians and Other Health-Care and Public Health Professionals: www.cdc.gov/mmwr/preview/mmwrhtml/rr5504a1.htm