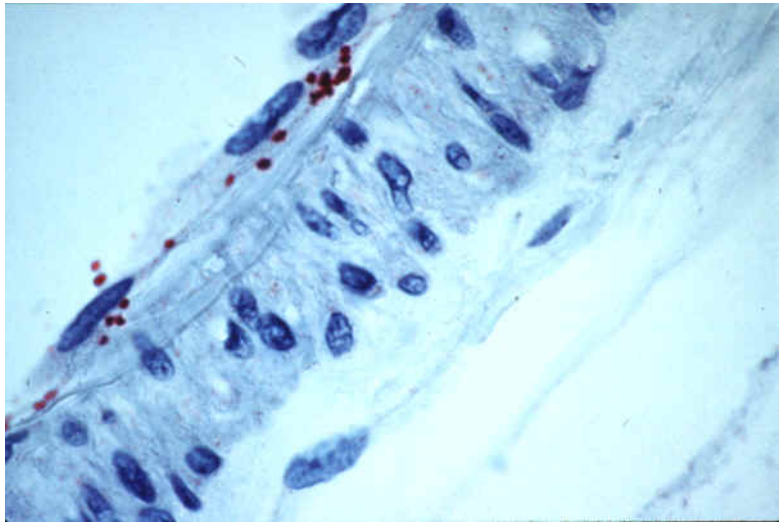
Rocky Mountain Spotted Fever (RMSF): Laboratory Testing



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Objectives

- Discuss laboratory testing options for RMSF
- Review the interpretation of acute and convalescent tests
- Review sample collection for RMSF testing, including autopsy specimens
- Discuss some common pitfalls in RMSF laboratory testing

Background

- Rickettsial infections pose difficult diagnostic challenges to clinicians and laboratorians.
- Confirmatory assays validate the accuracy of the clinical diagnosis but are not timely treatment decisions
- Laboratory confirmation of infection is vital to understanding the epidemiology and public health impact of RMSF.
- Several laboratory methods are available to diagnose RMSF
- Test results should be interpreted in the context of the patient's illness and the epidemiologic setting.

Polymerase Chain Reaction (PCR)

- PCR of skin biopsy of rash collected before 48 hours of treatment
- Whole blood collected from an acutely ill or deceased patient
- Pathology specimens collected during autopsy

PCR Limitations

- Although highly specific, sensitivity is low
- Usually only positive in extremely ill or deceased patients
- Treatment should always be started immediately, and sometimes the rash appears late
- Skin biopsy is more invasive than blood draws
- If patients have severe thrombocytopenia, collecting a skin biopsy may be impossible

Immunohistochemical (IHC) Staining

- For patients with a rash, IHC staining of a skin biopsy for RMSF is 100% specific and 70% sensitive in diagnosing RMSF
- Can be used to diagnose fatal and nonfatal cases of RMSF
- Like PCR, the IHC specimens should be collected within the first 48 hours after antibiotic therapy has been initiated

IHC Staining Limitations

- Rickettsiae might be focally distributed in tissue, this test might not always detect the agent
- Some patients develop rash late or not at all, limiting ability to collect specimens
- If patients have severe thrombocytopenia, collecting a skin biopsy may be impossible

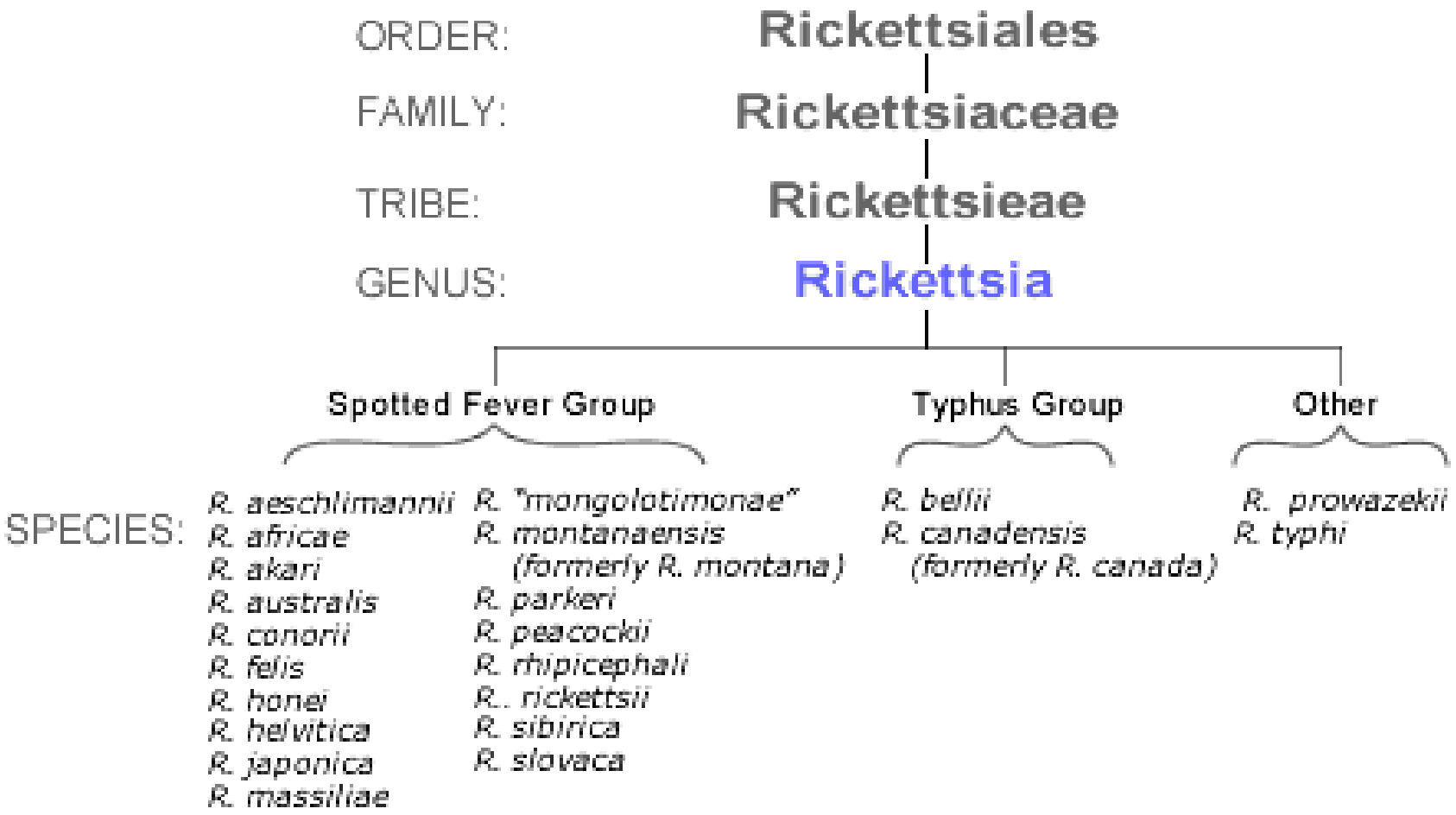
Serology

- Gold standard is IFA for IgG antibodies in paired serum samples
- First sample should be obtained in the first week and will be low or negative
- Second sample should be obtained 2-4 weeks later and should show a four fold increase in IgG if positive
- Both IgG and IgM begin to rise at the end of the first week of symptoms

Limitations

- Both IgG and IgM tend to rise together at the end of the first week and may remain elevated for years
- An elevated IgG or IgM titer in the first week is poor evidence for current disease
- About 3-20% of healthy people will have an elevated titer at any given time due to past illness or related or unrelated cross-reactivity

Rickettsia Taxonomy



Limitations Continued

- Serology is sensitive, but lacking in specificity
- Serology should only be used when patient has symptoms of Rickettsial disease
- If both an acute AND a convalescent sample are not obtained properly and compared, this test has very little value

Limitations of Serology

- A four fold increase in properly obtained IgG titers is 97% specific for Rickettsial disease
- IgM is not a good indicator of acute illness, and is highly nonspecific
- Cross reactivity between RMSF, other spotted fever group Rickettsiae and Typhus is possible

Limitations of Serology

- Rise in antibodies may be slower in people who were treated early, a third sample may be helpful in some cases
- This test will never be useful to make a treatment decision, but very useful in confirming and reporting cases
- This test is the only test available that can be used for non-acutely ill patients without rash

Summary of RMSF Laboratory Testing

- **Serology (RMSF titer)**
  - Indirect immunofluorescence assay (IFA)
  - Requires paired sera (acute and convalescent)
  - Look for a change (4-fold) in IgG antibody titers for confirmed infections
  - Positive single titers or titers that do not rise are considered probable cases
- **IHC**
  - Available at CDC. Can give a rapid result (48 hours)
  - Skin biopsy (2-4mm)
  - Patient must have rash
- **PCR**
  - Available at CDC. Can give a rapid result (48 hours)
  - Skin biopsy (2-4mm)
  - Whole blood of severely ill/fatal cases
  - *Negative PCR does not rule-out RMSF*

Specimen Requirements for PCR Testing

- 10 – 15mL of whole blood should be collected aseptically in a purple top EDTA vacutainer tube
- Biopsy specimen is a 3 mm punch, deep shave, or excisional biopsy specimen from the eschar or a representative rash lesion in a paraffin block
- Fresh tissue: unprocessed in 10% neutral buffered formalin
  - Fresh-frozen tissue should be sent separately on ice

Specimen Requirements for IHC Testing

- Biopsy specimen is a 3 mm punch, deep shave, or excisional biopsy specimen from the eschar or a representative rash lesion in paraffin block
- Fresh tissue: unprocessed in 10% neutral buffered formalin
  - Fresh-frozen tissue should be sent separately on ice
- Unstained slides: If paraffin block are unavailable unstained sections cut at 3–5 microns (10 slides per block) for IHC
  - Can not be used for molecular diagnostic assays (e.g. PCR)

Specimen Requirements for Serology Testing

- Serologic testing requires 3-5 mL in a red or tiger top tube
  - Tubes with additives (e.g. heparin, citrate sodium fluoride, etc.) can not be used
- If serum separation is not available, 10 – 15mL of whole **blood** should be collected aseptically in a red top vacutainer
- Best read of results is when acute and paired sera are run together

Post Mortem Specimens

- Whole blood can be used for PCR
- Post-mortem serum can not be used
- If possible, post-mortem tissue specimens should be collected from suspect RMSF patients
- Minimally invasive tissue collection techniques are available if full autopsy not performed

Post Mortem Specimens

- Mini-incision method (for lung and liver only)
  - Lung: Make a 3-4 cm-long skin cut parallel to the costal margin and immediately followed by a deeper incision through the intercostal muscle into the pleural cavity
  - Liver: a 3-4 cm-long skin cut which runs the seventh or eight intercostal space (corresponds to the second or third space above the right costal margin) and then a deeper incision into the abdominal cavity.
- For all tissues and for a better visual field, a 10 cm-long skin cut is recommended if accepted by the patient's family.
- Place the tissue directly into 10% neutral buffered formalin (NBF), at least 10 times the amount of the specimens, and gently shake the bottle to allow the tissue to mix well with the fixing solution, leave at room temperature.

Post Mortem Specimens

- Transcutaneous needle or puncture using soft tissue biopsy needle (for all tissues)
  - Lung: insert a 14-or 16 gauge soft tissue biopsy needle percutaneously into the thorax.
  - Liver: insert a 14- or 16-gauge soft tissue biopsy needle percutaneously into the abdomen between the seventh or eighth intercostal space and for kidney, from the posterior at T12 to L3 region, with the needle tip pointing toward the head of the body.
- The entire collection procedure should be repeated 4-5 times
- Each time the needle should be inserted at different angles (i.e. between 45-90°) against the body in order to collect a sufficient amount of tissue.
- Place the tissue directly into 10% neutral buffered formalin (NBF), at least 10 times the amount of the specimens, and gently shake the bottle to allow the tissue to mix well with the fixing solution, leave at room temperature.

Best Strategy

- At the first visit obtain an acute biopsy (if rash is present) for IHC and an acute serum sample for IFA
- *If the patient is acutely ill collect whole blood for PCR*
- **ALWAYS** Begin treatment at first visit
- Follow-up visit: obtain serum for second IFA sample

Contact information

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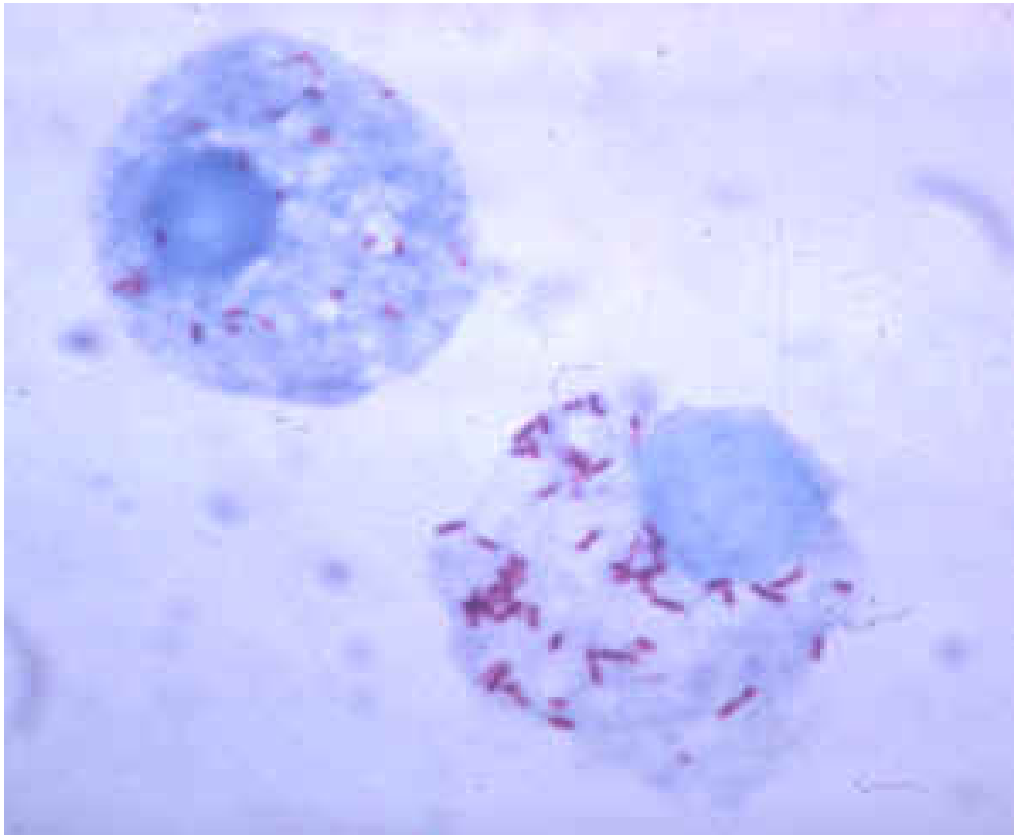
ADHS RMSF Website:
http://www.azdhs.gov/phs/oids/vector/rmsf/index.htm

Questions?



Rickettsia rickettsii – in tick hemolymph cells