



- Most commercial labs are unable to differentiate one spotted fever infection from another using these serologic methods.

#### *Persistent Antibodies*

- Antibodies to *R. rickettsia* might remain elevated for many months after the disease has resolved.
- In certain people, high titers of antibodies against *R. rickettsia* have been observed up to four years after the acute illness.
- Ten percent or more of healthy people in some areas might have elevated antibody titers due to past exposure to *R. rickettsii* or other SFR.
- Comparison of paired, and appropriately timed, serologic assays provides the best evidence of recent infection.
- Single or inappropriately timed serologic tests, in relation to clinical illness, can lead to misinterpretation of results.

#### *PCR*

- Polymerase chain reaction (PCR) amplification is performed on DNA extracted from whole blood and might also be used to amplify DNA from a skin biopsy of a rash lesion, or in post-mortem tissue specimens.
- *R. rickettsii* infect the endothelial cells that line blood vessels and may not circulate in large numbers in the blood until the disease has progressed to a severe phase of infection.
- Although a positive PCR result is helpful, a negative result does not rule out the diagnosis, and treatment should not be withheld on the basis of a negative result.

#### *IHC and Culture*

- Culture and immunohistochemistry (IHC) assays can also be performed on skin biopsies of a rash lesion, or post-mortem tissue specimens.
- Culture isolation and IHC assays of *R. rickettsii* are only available at specialized laboratories; routine hospital blood cultures cannot detect the organism.

***Treatment requires tetracycline-class of antibiotics (i.e. doxycycline). Doxycycline treatment is recommended for persons of all ages, including children. Beta lactam antibiotics and fluoroquinolones are contraindicated. Immediate empiric therapy is recommended and should not be delayed while awaiting diagnostic results.***

## **Tularemia**

Tularemia is caused by *Francisella tularensis*. This agent is found in nature in rabbits, muskrats, prairie dogs and other rodents. Human infection occurs through several routes, including tick or deer fly bites, skin contact with infected animals, bites from infected cats, ingestion of contaminated water, or inhalation of contaminated dusts or aerosols. Nebraska has reported a median of 7 cases annually (range: 5–16) over the last 5 years (2016–2020).

Disease signs and symptoms vary depending on how the bacteria enter the body. Illness ranges from mild to life-threatening. All forms are accompanied by fever, which can be as high as 104°F. Forms of this disease include:

- **Ulceroglandular** This is the most common form of tularemia and usually occurs following a tick or deer fly bite or after handling of an infected animal. A skin ulcer appears at the site where the bacteria entered the body. The ulcer is accompanied by swelling of regional lymph glands, usually in the armpit or groin.

- **Glandular** Similar to ulceroglandular tularemia but without an ulcer. Also generally acquired through the bite of an infected tick or deer fly or from handling sick or dead infected animals.
- **Oculoglandular** This form occurs when the bacteria enter through the eye. This can occur when a person is butchering an infected animal and touches his or her eyes. Symptoms include irritation and inflammation of the eye and swelling of lymph glands in front of the ear.
- **Oropharyngeal** This form results from eating or drinking contaminated food or water. Patients with oropharyngeal tularemia may have sore throat, mouth ulcers, tonsillitis, and swelling of lymph glands in the neck.
- **Pneumonic** This is the most serious form of tularemia. Symptoms include cough, chest pain, and difficulty breathing. This form results from breathing dusts or aerosols containing the organism. It can also occur when other forms of tularemia (e.g. ulceroglandular) are left untreated and the bacteria spread through the bloodstream to the lungs.
- **Typhoidal** This form is characterized by any combination of the general symptoms (without the localizing symptoms of other syndromes).

#### Laboratory diagnosis:

- Growth of *F. tularensis* in culture is the definitive means of confirming the diagnosis of tularemia. Depending on the form of illness, appropriate specimens include swabs or scrapings of skin lesions, lymph node aspirates or biopsies, pharyngeal swabs, sputum specimens, or gastric aspirates. Paradoxically, blood cultures are often negative.
- A presumptive diagnosis of tularemia may be made through testing of specimens using IFA, IHC staining, or PCR.
- The diagnosis of tularemia can also be established serologically by demonstrating a 4-fold change in specific antibody titers between acute and convalescent sera. Convalescent sera are best drawn at least 4 weeks after illness onset; hence this method may be useful for confirming the diagnosis but not for clinical management.

In patients that present with symptoms and/or history highly suggestive of tularemia, clinicians should consider culture which will facilitate typing if an isolate is recovered. For surveillance purposes, typing of isolates is highly advantageous. If tularemia is suspected, laboratory staff should be alerted to ensure safety precautions are in place to prevent exposure and infection in the lab workers. *Although tularemia can be life-threatening, most infections are successfully treated with antibiotics. While streptomycin is the drug of choice, gentamicin is an acceptable alternative. Tetracyclines may be a suitable alternative to aminoglycosides for patients who are less severely ill.*

#### Ehrlichiosis

Ehrlichiosis is caused most commonly by *Ehrlichia chaffeensis* (less commonly *Ehrlichia ewingii*), an intracellular bacterium that grows within cytoplasmic phagosomes of white blood cells, and can cause leukopenia. This bacteria is transmitted via the tick bite of *Amblyomma americanum* (“Lone star tick”). Symptoms may include severe malaise, fever, and headache. Although rare, severe outcomes including death are possible. **Nebraska reported its first ehrlichiosis-associated death in 2019.** Nebraska has reported a median of 7 cases annually (range: 2–9). However with the expansion of the Lone star tick (*Amblyomma americanum*) in Nebraska, this disease is likely underdiagnosed. **Providers suspecting a SFR or RMSF diagnosis should also consider ehrlichiosis as a potential diagnosis.**

## Laboratory diagnosis:

### *Serology*

- The reference standard serologic test for diagnosis of ehrlichiosis is the IFA assay for IgG.
- IgG IFA assays should be performed on paired acute and convalescent serum samples collected 2–4 weeks apart to demonstrate evidence of a 4-fold seroconversion.
- Antibody titers are frequently negative in the first week of illness. Ehrlichiosis cannot be confirmed using single acute antibody results.
- IgM IFA assays offered by reference laboratories are not necessarily indicators of acute infection and might be less specific than IgG antibodies.
- Antibodies, particularly IgM antibodies, might remain elevated in patients for whom no other supportive evidence of a recent rickettsiosis infection exists. For these reasons, IgM antibody titers alone should not be used for laboratory diagnosis.

### *Persistent Antibodies*

- Antibodies against *Ehrlichia* species might remain elevated for many months after disease has resolved.
- Comparison of paired, and appropriately timed, serologic assays provides the best evidence of recent infection.
- Single or inappropriately timed serologic tests, in relation to clinical illness, can lead to misinterpretation of results.

### *Cross Reactivity*

- Closely related organisms, including those in the *Ehrlichia* and *Anaplasma* genera, share similar antigens such that antibodies directed to one of these antigens can cross-react.
- Most commercial labs are unable to differentiate between *Ehrlichia* species.
- In areas endemic for Ehrlichiosis and Anaplasmosis, IFA using antigen from both *Ehrlichia* and *Anaplasma* species should be run side-by-side.

### *PCR*

- PCR amplification is typically performed on whole blood specimens, but might also be used to amplify DNA in solid tissue and bone marrow specimens.
- This method is most sensitive in the first week of illness and decreases in sensitivity following the administration of appropriate antibiotics (within 48 hours).
- Although a positive PCR result is helpful, a negative result does not rule out the diagnosis, and treatment should not be withheld on the basis of a negative result.

### *IHC and Culture*

- Culture isolation and IHC assays of *Ehrlichia* species are only available at specialized laboratories; routine hospital blood cultures cannot detect the organism.
- PCR, culture, and IHC assays can also be applied to post-mortem specimens.
- If a bone marrow biopsy is performed as part of the investigation of cytopenias, immunostaining of the bone marrow biopsy specimen can diagnose ehrlichiosis.

### *Blood-smear Microscopy*

- During the first week of illness, a microscopic examination of a peripheral blood smear might reveal morulae (microcolonies of *Ehrlichiae*) in the cytoplasm of white blood cells and is highly suggestive of a diagnosis.
  - *E. chaffeensis* most commonly infects monocytes.
  - *E. ewingii* more commonly infects granulocytes.
- Blood smear examination is relatively insensitive and should not be relied upon solely to diagnose ehrlichiosis.
- The observance of morulae in a particular cell type cannot conclusively differentiate among *Ehrlichia* species or between *Ehrlichia* and *Anaplasma*

***Recommended therapy is with a tetracycline-class antibiotic (i.e. doxycycline). Immediate empiric therapy is recommended and should not be delayed while awaiting diagnostic results.***

### **Anaplasmosis**

Anaplasmosis is caused by *Anaplasma phagocytophilum*, an intracellular bacterium that targets neutrophils, altering their function, and forms morulae within vacuoles. Symptoms are similar to ehrlichiosis and include malaise, fever, and headache. If left untreated, anaplasmosis can be fatal, even in previously healthy people. Severe clinical presentations may include difficulty breathing, hemorrhage, renal failure or neurological deficits. Like Lyme disease, anaplasmosis is transmitted by the *Ixodes scapularis* tick. For the first time in Nebraska, established populations (meeting CDC criteria) of this tick were identified during 2019 in Douglas, Sarpy, and Saunders counties. **This fact increases suspicion that anaplasmosis, caused by *Anaplasma phagocytophilum*, might be acquired in eastern Nebraska. However, even with these established populations, Nebraska is presently considered a low prevalence state for anaplasmosis. At this time, local human health risk is unknown, but clearly of increased concern.** Nebraska has reported from zero to two cases annually, and most recently zero in 2020.

### **Laboratory diagnosis:**

#### *Serology*

- The reference standard serologic test for diagnosis of ehrlichiosis is the IFA assay for IgG using *A. phagocytophilum* antigen.
- IgG IFA assays should be performed on paired acute and convalescent serum samples collected 2–4 weeks apart to demonstrate evidence of a 4-fold seroconversion.
- Antibody titers are frequently negative in the first week of illness. Anaplasmosis cannot be confirmed using single acute antibody results.
- IgM IFA assays may also be offered by reference laboratories, however, are not necessarily indicators of acute infection and might be less specific than IgG antibodies.
- Antibodies, particularly IgM antibodies, might remain elevated in patients for whom no other supportive evidence of a recent rickettsiosis infection exists. For these reasons, IgM antibody titers alone should not be used for laboratory diagnosis.

#### *PCR*

- PCR is typically performed on DNA extracted from whole blood specimens, but might also be used to amplify DNA in solid tissue, bone marrow, and autopsy tissue specimens.
- This method is most sensitive in the first week of illness, and decreases in sensitivity following the administration of appropriate antibiotics (within 24–48 hours).
- Although a positive PCR result is helpful, a negative result does not rule out the diagnosis, and treatment should not be withheld on the basis of a negative result.

### *Persistent Antibodies*

- Antibodies to *A. phagocytophilum* might remain elevated for many months after the disease has resolved.
- In certain people, high titers of antibodies against *A. phagocytophilum* have been observed up to four years after the acute illness.
- Between 5–10% of healthy people in some areas might have elevated antibody titers due to past exposure to *A. phagocytophilum* or similar organisms.
- Comparison of paired, and appropriately timed, serologic assays provides the best evidence of recent infection.
- Single or inappropriately timed serologic tests, in relation to clinical illness, can lead to misinterpretation of results.

### *Blood-smear Microscopy*

- During the first week of illness, a microscopic examination of a peripheral blood smear might reveal morulae (microcolonies of anaplasmae) in the cytoplasm of granulocytes and is highly suggestive of a diagnosis.
- However, blood smear examination is relatively insensitive and should not be relied upon solely to diagnose anaplasmosis.
- The observance of morulae in a particular cell type cannot conclusively differentiate between *Anaplasma* and *Ehrlichia* species.

### *IHC and Culture*

- IHC assays of *A. phagocytophilum* are only available at specialized laboratories; routine hospital blood cultures cannot detect the organism.
- Culture and IHC assays can also be applied to autopsy tissue specimens.

***Recommended therapy is with a tetracycline-class antibiotic (i.e. doxycycline). Immediate empiric therapy is recommended and should not be delayed while awaiting diagnostic results.***

## **Lyme Disease**

This has been addressed in a separate HAN and can be found here:  
<https://dhhs.ne.gov/han%20Documents/ADVISORY03312021.pdf>.

## **Southern Tick-Associated Rash Illness (STARI)**

A red, expanding “bull’s-eye” rash similar to those seen in patients with Lyme disease has also been observed in people bitten by *Amblyomma americanum*, often referred to as the lone-star tick. The condition has been named Southern Tick-Associated Rash illness. Occasionally patients may also experience fever, malaise and headache. Whether the lesions and illness described in patients following an *Amblyomma americanum* tick bite are infectious or allergic/toxin mediated remains speculative. Studies have shown that **the rash in such patients is not caused by *Borrelia burgdorferi***, the causative agent of Lyme disease. Though once thought to be caused by another species of *Borrelia*, research has not supported this hypothesis. While experts including CDC are uncertain as to the necessity for antibiotic treatment for this condition, since its etiology is unknown, a 21-day course of a tetracycline-class antibiotic is often prescribed (<https://www.cdc.gov/stari/symptoms/index.html>).

## **Heartland and Bourbon Virus**

Heartland and Bourbon viruses are both RNA viruses. Heartland virus was first discovered in 2009 in Missouri and is believed to be transmitted by *Amblyomma americanum* (“Lone star

tick”). Initial symptoms are similar to ehrlichiosis which include fever, fatigue, anorexia, nausea, and diarrhea. Bourbon virus was discovered in Bourbon County, Kansas in 2014. Like Heartland virus, Bourbon virus is also believed to be transmitted by *Amblyomma americanum*. Symptoms reported from patients include fever, fatigue, anorexia, nausea, vomiting, and maculopapular rash. Leukopenia, thrombocytopenia, and mild to moderate elevation of liver transaminases have also been seen in patients diagnosed with Heartland or Bourbon viruses. To date, no cases of Heartland or Bourbon virus have been identified in Nebraska residents. Infection with Heartland or Bourbon virus should be considered in patients being treated for suspected tick-borne disease who do not respond to treatment (e.g. doxycycline). Presently, no routine testing is available commercially for Heartland or Bourbon viruses. However, protocols are in place to allow people to be tested for evidence of infection through the CDC. **Providers must contact their local health department to determine if suspected patients meet CDC criteria for testing.**

**CDC specimen submission criteria for Heartland and Bourbon virus testing:**

Testing for Heartland or Bourbon virus should be considered for patient with an acute febrile illness within the past 3 months AND at least one epidemiologic criterion AND at least one clinical criterion:

- Epidemiologic criteria
  - Known tick bite, finding tick on body, or potential exposure to ticks through outdoor activities in the 3 weeks prior to illness onset during spring through fall (April–October); OR
  - Resides in or recently traveled to an area with previous evidence of Heartland or Bourbon virus. These areas can be found here: <https://www.cdc.gov/heartland-virus/statistics/index.html>
- Clinical criteria
  - Leukopenia (white blood cells <4,500 cells/μL) or thrombocytopenia (platelets <150,000 cells/mL) not explained by another known condition; OR
  - Suspected tickborne disease (e.g. RMSF, ehrlichiosis) with no clinical response to appropriate treatment (e.g. doxycycline).

Samples collected >3 months after symptom onset will not be tested at this time based on limitations of current understanding of antibody kinetics.

As of July, 2018 the following tests for Heartland and Bourbon virus are available at CDC:

Test	Heartland virus	Bourbon virus
RT-PCR	Yes	Yes
IgM MIA	Yes	Not available
IgG MIA	Yes	Not available
PRNT	Yes	Yes

**Alpha-gal Syndrome (Red meat allergy)**

Alpha-gal syndrome is an allergy first described in 2009 to the alpha-gal molecule. Alpha-gal (galactose-α-1,3-galactose) is a sugar molecule found in most mammals (except in people, apes, and monkeys) and can be found in products made from mammals (including some medications, cosmetics, vaccines, gelatin, and milk products). Allergic reactions typically occur after people eat meat from mammals that have alpha-gal or are exposed to products made from mammals. Symptoms can appear 3–6 hours after eating meat or exposure to products containing alpha-gal,

may not occur after every exposure, and may vary from person to person. Common symptoms include: rash, hives, difficulty breathing, drop in blood pressure, dizziness or faintness, nausea or vomiting, and severe stomach pain. **Alpha-gal allergies can be severe, and even life threatening.** Both children and adults can develop alpha-gal allergy; however, most cases of alpha-gal allergy appear to be in people >50 years of age. In the US, growing evidence suggests that Alpha-gal syndrome may be triggered by the bite from a lone star (*Amblyomma americanum*) or blacklegged (*Ixodes scapularis*) tick. It is thought that these ticks carry the alpha-gal molecule in their saliva after feeding on mammalian blood. People that are bitten by these ticks, especially those that are bitten repeatedly, are at risk of becoming sensitized and producing the antibody (IgE) necessary to then cause allergic reactions.

Tick	Distribution	Associated Illness	Infectious Agent
<i>Dermacentor variabilis</i> (American dog tick or wood tick)	Statewide	Rocky Mountain spotted fever	<i>Rickettsia rickettsii</i>
		Tularemia	<i>Francisella tularensis</i>
<i>Dermacentor andersoni</i> (Rocky Mountain wood tick)	NW Nebraska	Rocky Mountain spotted fever	<i>Rickettsia rickettsii</i>
		Tularemia	<i>Francisella tularensis</i>
<i>Amblyomma americanum</i> (Lone star tick)	Southern and central Nebraska	Ehrlichiosis (formerly human monocytic ehrlichiosis)	<i>Ehrlichia chaffeensis</i>
		Southern Tick Associated Rash Illness (STARI)	Unknown etiology
		Tularemia	<i>Francisella tularensis</i>
		Heartland virus disease	<i>Heartland Virus (Phlebovirus)</i>
		Bourbon virus disease	<i>Bourbon Virus (Thogotovirus)</i>
		Alpha-gal Allergy	<i>Alpha-gal sugar</i>
<i>Ixodes scapularis</i> (deer tick or blacklegged tick)	Three counties with established populations in Eastern Nebraska	Lyme disease	<i>Borrelia burgdorferi</i>
		Anaplasmosis (formerly human granulocytic ehrlichiosis)	<i>Anaplasma phagocytophilum</i>



**For more information please visit relevant CDC pages as follows:**

- RMSF: <https://www.cdc.gov/rmsf/>
- Tularemia: <https://www.cdc.gov/tularemia/>
- Ehrlichiosis: <https://www.cdc.gov/ehrlichiosis/>
- Anaplasmosis: <https://www.cdc.gov/anaplasmosis/index.html>
- Heartland Virus: <https://www.cdc.gov/heartland-virus/index.html>
- Bourbon Virus: <https://www.cdc.gov/ncezid/dvbd/bourbon/index.html>
- STARI: <https://www.cdc.gov/stari/>
- Alpha-gal: <https://www.cdc.gov/ticks/alpha-gal/index.html>
- Tickborne Diseases of the US: A Reference Manual for Health Care Providers, Fourth Edition (2017): <https://www.cdc.gov/lyme/resources/TickborneDiseases.pdf>

**Other Resource:**

- World Health Organization Coronavirus Disease (COVID-19) Advice for the Public, Myth Busters: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/myth-busters>