Rickettsial Agents of Animal Disease; the Rickettsieae

Rickettsiales are minute obligate intracellular gram-negative bacteria. Their cell walls and energy-generating capability distinguish the genera *Rickettsia* and *Coxiella* from mycoplasmas and chlamydiae, respectively. Other members of the *Rickettsiales* grow on lifeless media, and still others lack cell walls. All multiply by binary fission and are associated with invertebrate vectors.

Three families are of veterinary interest. The family *Rickettsiaceae* includes parasites of the vascular endothelium (tribe *Rickettsieae*) and phagocytic cells (tribe *Ehrlichiaea*). *Bartonellaceae* are epicellular parasites and *Anaplasmataceae* are parasites of erythrocytes.

- Small obligate intracellular parasites
- Once considered to be viruses
- Separate unrelated genera
- Gram-negative bacteria
  - Stain poorly with Gram stain (Giemsa)
- “Energy parasites” but not obligate, have capacity to make ATP
  - Transport system for ATP is very efficient
- Reservoirs - animals, insects and humans
- Arthropod vectors (except *Coxiella*)

Cell Morphology and Staining

Cells measure up to 0.5 μm by 1.0 μm. Although structurally gram negative, preferred stains are Gimenez's, Macchiavello's, or Giemsa stains. The former two stain rickettsiae red, the latter purple. Electron microscopically and chemically, rickettsiae resemble other gram-negative bacteria. Endotoxic activity is present. Cells are nonmotile. The life cycle of *Coxiella burnetii* includes an endospore-like phase.

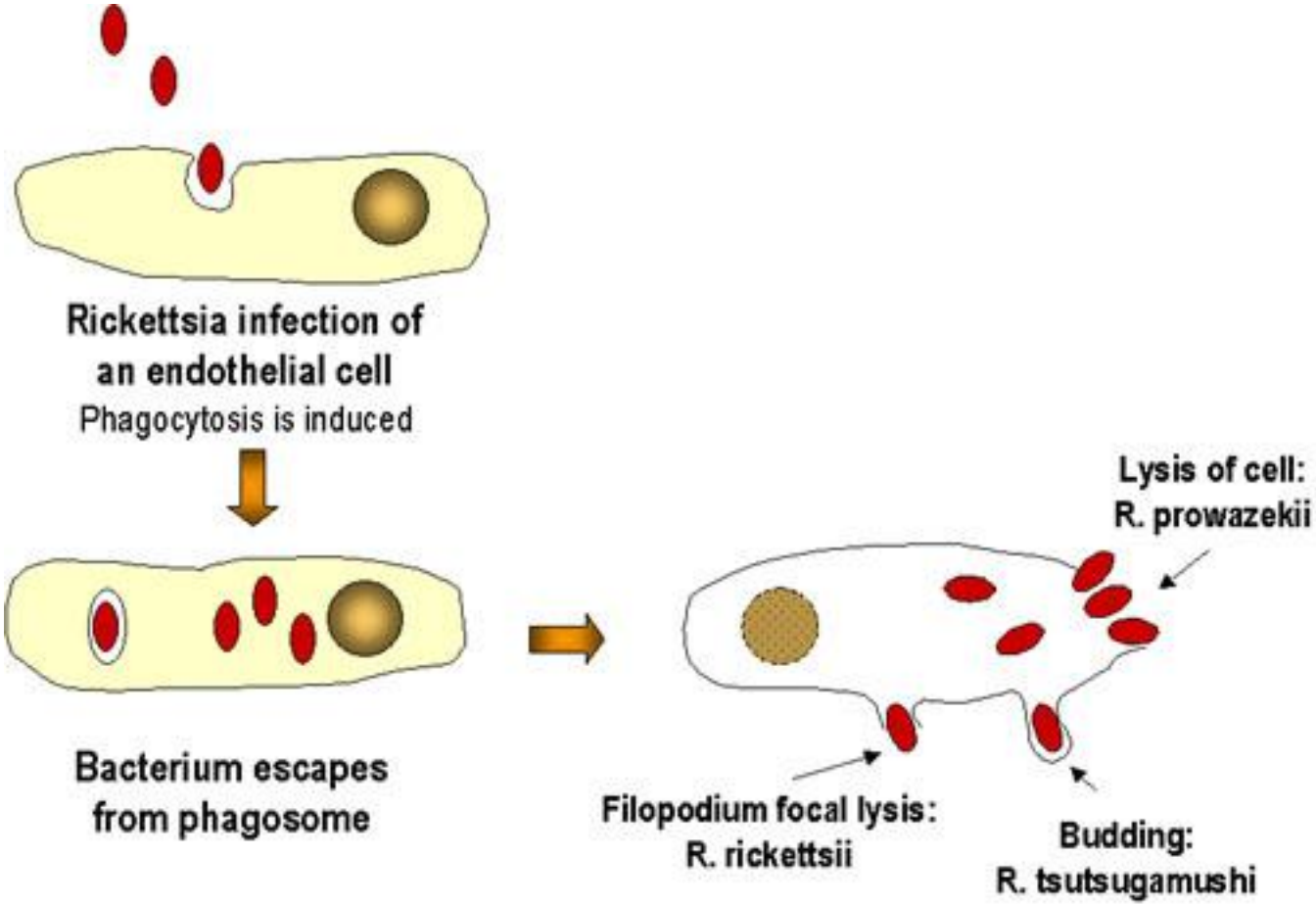
Members of the tribe *Rickettsieae*, except *C. burnetii*, cross-react with somatic antigens of certain *Proteus* (OX) strains, a phenomenon (Weil-Felix reaction) utilized in the diagnosis of rickettsial infections. This approach generally lacks species specificity. Rickettsial antigens used in complement fixation, immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and indirect hemagglutination tests can be group-specific or species-specific: extracts tend to give group reactions, while cell suspensions approximate species specificity.

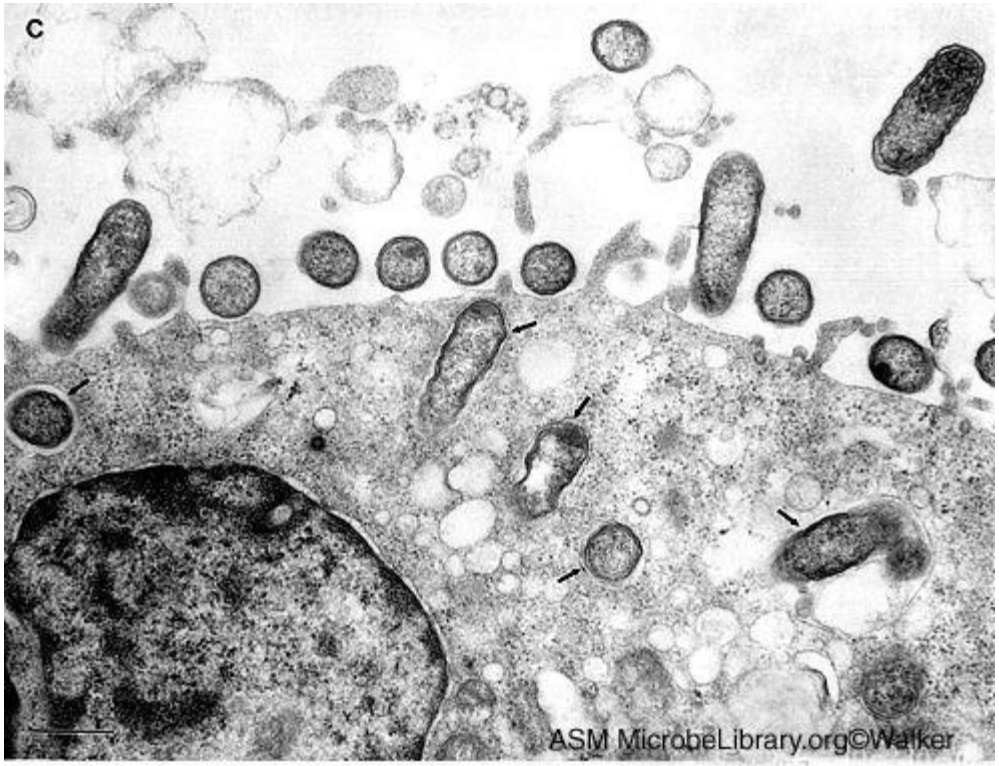
Growth Characteristics

Rickettsiae are propagated in yolk sacs of chick embryos, cell cultures, and laboratory animals, notably guinea pigs or mice. The optimal temperature is 33°C to 35°C, at which the generation time is about 9 hours. In cell culture, good growth may require incubation for several weeks, depending on the rickettsial species involved.

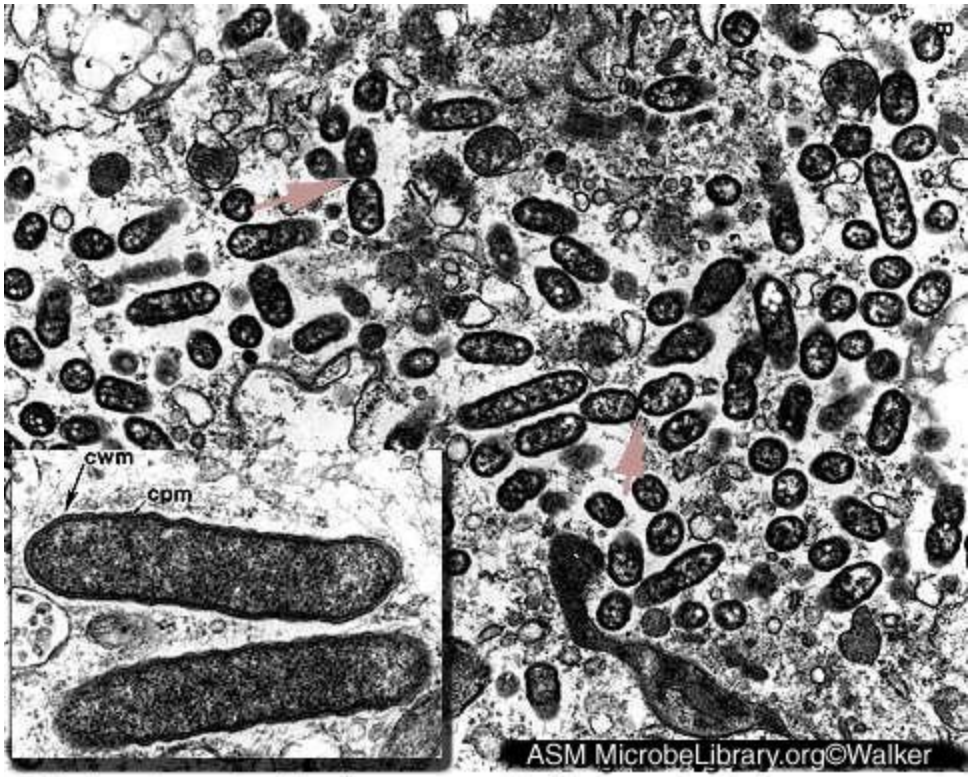
Pathogenesis

Mechanism and Pathology. Toxicity, neutralizable by antiserum, is characteristic of viable rickettsiae. They enter endothelial cells through endocytosis actively initiated by metabolizing rickettsial cells. They escape from the phagolysosome and multiply in the cytoplasm, and, in the case of spotted fever rickettsiae, in the nucleus. Replication results in endothelial cell necrosis and vasculitis, which leads to vascular disturbances, hemorrhages, edema, perfusion inadequacies, thrombosis, and necrosis. *Coxiella burnetii* multiplies within the phagolysosome, thanks to an enzyme system adapted to the low pH prevailing there (<5.0).

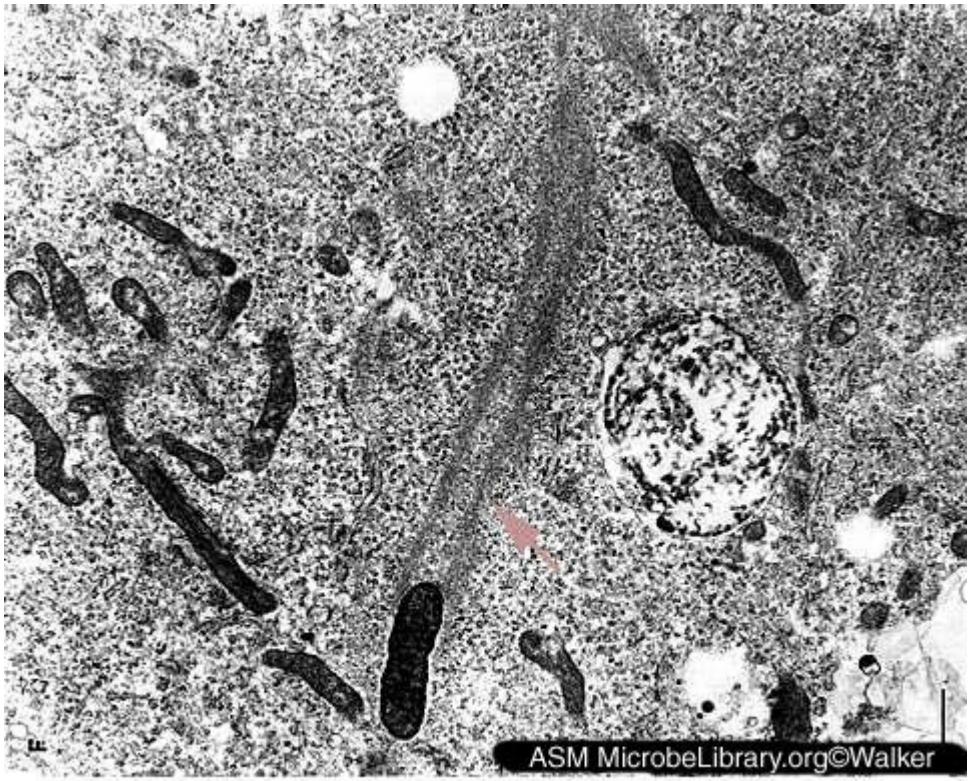

Rickettsia infection of
an endothelial cell
Phagocytosis is induced
Bacterium escapes
from phagosome
Lysis of cell:
R. prowazekii
Filopodium focal lysis:
R. rickettsii
Budding:
R. tsutsugamushi



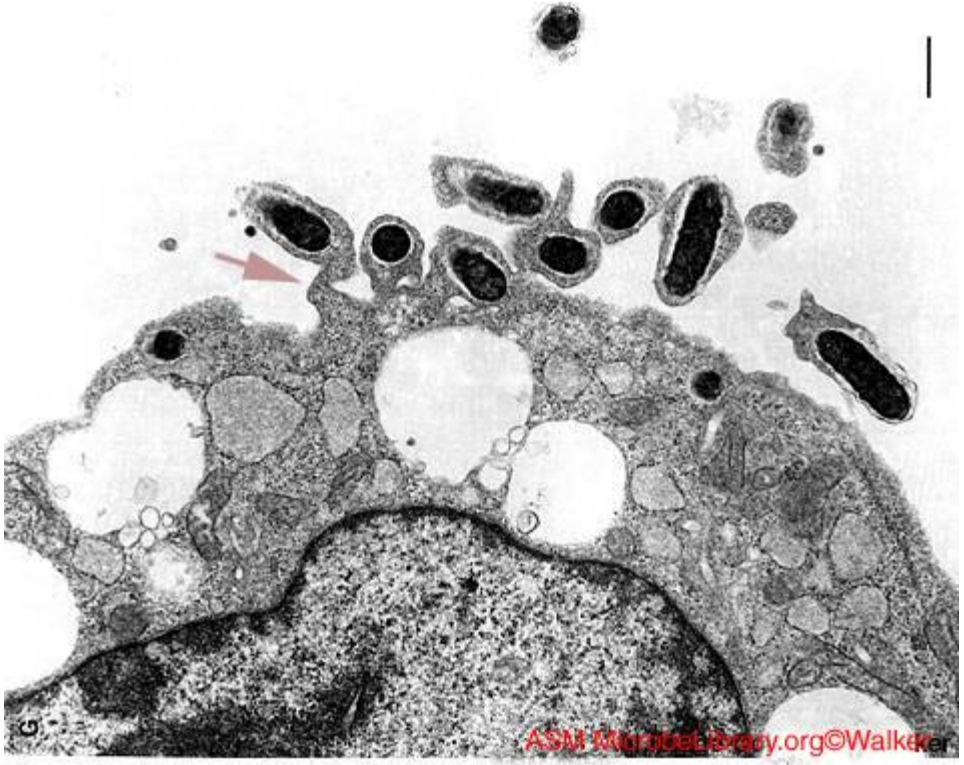
Attachment of rickettsiae to the surface of an endothelial cell is followed by their entry into the cell via rickettsia-induced phagocytosis. Following phagocytosis, the phagosome membrane (arrow) is lost and the rickettsiae escape into the host cell cytoplasm. Bar = 0.5 µm



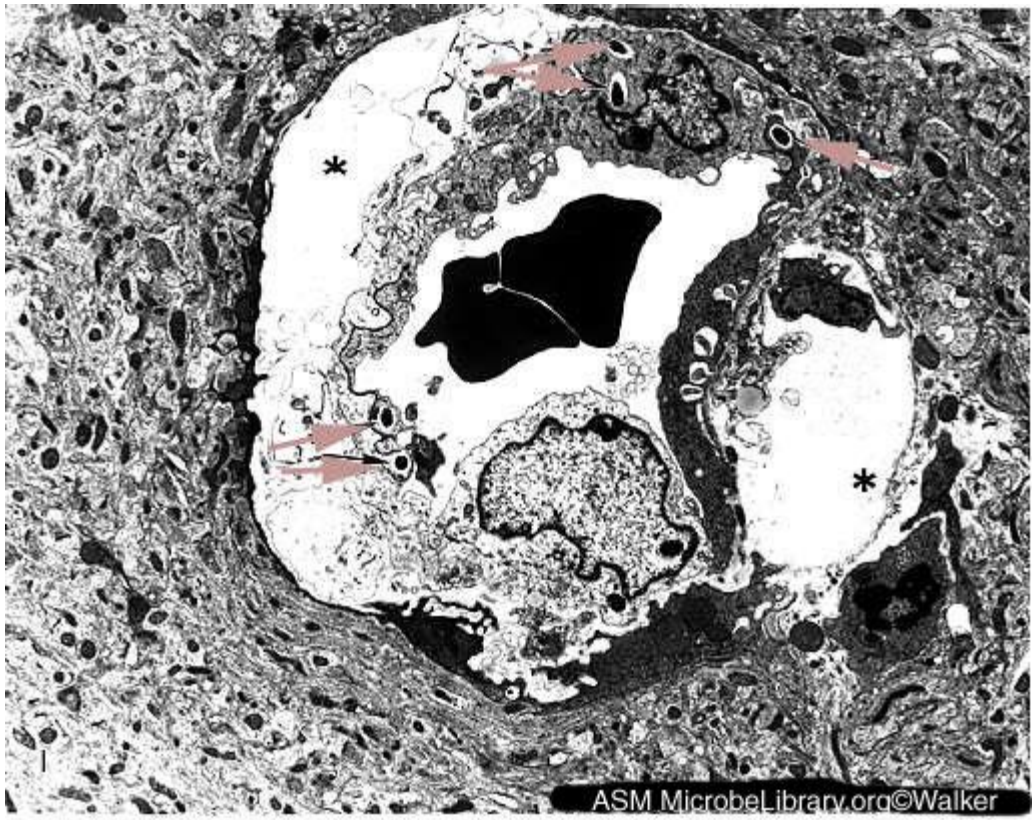
Following release from the phagosomes, rickettsiae grow free in the cytoplasm of cultured cells, dividing by binary fission (seen at arrows). Inset highlights the outer and inner membranes of rickettsia.



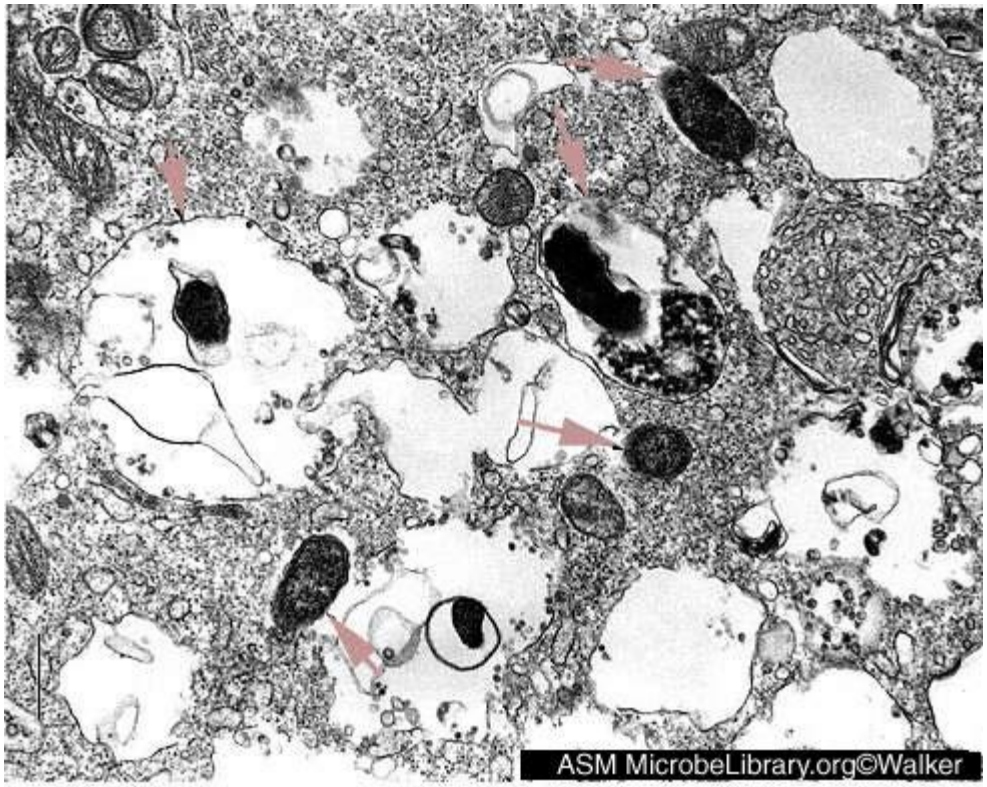
Rickettsiae are propelled through the host cell cytoplasm by stimulating the polymerization of host cell F actin, seen in the comet-like'tail'below (arrow).



Propulsion by F-actin into long host cell projections known as filopodia precedes the release of rickettsiae from the cell surface or their spread to adjacent endothelial cells.



Growth of rickettsiae (arrows) in the endothelium results in damage to vascular integrity and thus the leakage of fluid into a vital organ such as the brain. The accumulation of fluid (edema) in the perivascular space (asterisks) may result in clinical encephalitis.



Gamma interferon and tumor necrosis factor alpha, substances secreted by host immune cells, 'activate' the infected endothelial cell to kill intracellular rickettsiae via the creation of autophagosomes. Later, fusion of lysosomes with autophagosomes results in the digestion of dying rickettsia. (arrow)

Disease	Causative Agent	Vertebrate Reservoir	Vectors	Transovarian Passage	Clinically Affected Hosts
Epidemic typhus					
Classical	*Rickettsia prowazekii*	Humans (recovered)	Lice	–	Humans, lice
Sylvatic	*R. prowazekii*	Eastern flying squirrels	Fleas	–	Humans, lice
Endemic (murine) typhus	*R. typhi*	Rats, opossums, cats	Fleas	–	Humans
Endemic (murine) typhus-like	*R. felis* (ELB agent)	Opossums, cats	Fleas	–	Humans
Scrub typhus	*Orientia tsutsugamushi*	Mice, rats	Mites	+	Humans
Rocky Mountain spotted fever	*R. rickettsii*	Various feral mammals	Ticks	+	Humans (dogs, sheep)[a]
Q fever	*Coxiella burnetii*	Many mammals (birds?, fish?)	Arthropods,[b] ticks, mites, insects	?	Humans (ruminants)

[a] Occasionally affected.
[b] Airborne transmission commonly occurs in absence of vectors.

Immunologic Aspects

Immune complexes have been suspected in the pathogenesis of late vascular manifestations of RMSF. Humoral and cell-mediated responses occur. The latter especially are significant for removal of the agents by activated macrophages. No vaccines for animal rickettsioses are available. There is considerable interest in the development of a phase I Q fever vaccine, which has proven effective experimentally in ruminants and humans.

Laboratory Diagnosis

Direct demonstration of rickettsiae in cells is best done by direct immunofluorescence. In ruminant placentas, this establishes the presence of Q fever rickettsiae but not the cause of abortion. The use of Gimenez or other stains will not differentiate between *C. burnetii* and *Chlamydia psittaci*.

Isolation involves use of guinea pigs and mice, embryonated eggs, or cell culture. In surviving animals, a blood sample may contain antibody 2 to 3 weeks later. In eggs, tissue culture, and experimental animal tissues, direct immunofluorescent staining will identify an isolate.

Serology (complement fixation, microagglutination) is useful when paired samples can be obtained, and in recent infections a significant increase in antibody level is observed in the sample collected 10 to 14 days after the first. The Weil-Felix reaction is a helpful screening test for RMSF (not Q fever). Conclusions should be confirmed by specific rickettsial tests.