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Molecular detection of *Bartonella henselae/quintana* DNA

Bartonellae are fastidious, Gram negative intraerythrocytic organisms in the genus *Bartonella,* which can often be difficult to grow and identify in the laboratory.

Since reclassification in 1993, the number of recognised species has increased from one to forty-five, some with designated subspecies; and additional vectors and animal reservoirs are expanding the repertoire ever further[1].

At least eleven species have currently been associated with disease in humans (Table 1).

Table 1: Species of *Bartonella* associated with human disease

Species	Known reservoir	Human pathophysiology
B. bacilliformis	Humans	Carrion's disease, (Oroya fever, Verruga peruana)
B. quintana	Humans	Trend fever, bacillary angiomatosis, and endocarditis
B. henselae	Domestic cat	Cat scratch disease, bacillary angiomatosis, peliosis hepatis, endocarditis, bacteremia with fever, neuroretinitis, meningitis, encephalitis
B. clarridgeiae	Domestic cat	Cat scratch fever
B. *elizabethae*	Rat	Endocarditis
B. grahamii	Mouse	Endocarditis and neuroretinitis
B. koehlerae	Domestic cat	
B. naantaliensis	Daubenton's bat	
B. vinsonii	Mouse, dog, cat	Endocarditis and bacteriaemia
B. washoensis	Squirrel	Myocardidits
B. rochalimae	Unknown	Carrion's disease-like symptoms

Bartonella henselae

The most clinically significant of these in the UK is *Bartonella henselae* which is the pathogen responsible for Cat Scratch Disease (also known as 'catch scratch fever', 'Teenys Disease', 'Inoculation lymphoreticulosis' and 'subacute lymphadenitis'). It is also the most common cause of lymphadenopathy in children and young adolescents. Although found as a commensal in many wild animals, *B. henselae* is usually transferred to humans from cats (particularly kittens) and is thought to infect humans by flea faecal contamination of cat scratches, licks or bites. Infected individuals may present with low-grade fever, enlarged tender lymph nodes that develop 1-3 weeks after exposure and/or a papule or pustule at the inoculation site. In most cases of cat scratch disease, infection will be resolved without treatment; however, some patients may develop complications from disseminated disease. It is therefore of clinical interest to be able to recognise infection so that antibiotics can be prescribed where appropriate. Azithromycin in particular has been shown to decrease lymph node volume more rapidly compared to no treatment[2].

Bartonella quintana

Also medically significant is *Bartonella quintana*, the pathogen responsible for Trench fever during WWI (also known as 'Meuse Fever', 'Wohlhynia Fever' and 'Quintan Fever'). *B. quintana* is passed to humans via a louse vector, and is thus most often found in those living under poor conditions; for example homelessness. Symptoms of infection with *B. quintana* include: fever, headache, rash and bone pain, mainly in the shins, neck and back[3].

In some cases, bacillary angiomatosis (caused by *B. henselae* or *B. quintana*) and bacillary peliosis (caused by *B. henselae*) occur in immunocompromised people, such as those with advanced HIV infection. Bacillary angiomatosis may present as lesions in the skin, subcutaneous tissue, bone, or other organs. Bacillary peliosis causes vascular lesions in the liver and spleen.

Other *Bartonella* spp.

Globally, the third species of significance is *Bartonella bacilliformis*, the cause of Bartonellosis, a biphasic disease transmitted by *Phlebotomus* sandflies and endemic in Andean regions such as Peru, Columbia and Ecuador. Additionally, many of the *Bartonella* species listed can cause subacute endocarditis (infection of the heart valves), which is often culture negative[4].

In all these cases, qualitative PCR based detection can prove extremely useful in the identification of a Bartonella causative agent.

Our assay

At Micropathology Ltd we use a nested PCR utilising standard block PCR on a thermocycler, with visualisation by agarose gel electrophoresis followed by ethidium bromide staining for qualitative detection of *Bartonella henselae/quintana*. Subsequent amplicons generated are sequenced for speciation of detected organisms. Although the assay was optimised for *Bartonella henselae/quintana* it will also detect many strains of each of the clinically relevant *Bartonella* listed in Table 1 where sequences are available (e.g. *B. bacilliformis*). Species can be differentiated using sequencing and are reported to species level.

The assay is validated to ISO15189:2022 standards to include sample types EDTA whole blood, tissue specimens, vitreous and aqueous fluids and pus.

References

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2. CDC (2015) 'Bartonella infection (Cat Scratch Disease, Trench Fever, and Carrion's Disease) *For Health Care Providers*' Available at: https://www.cdc.gov/bartonella/clinicians/index.html [Accessed: 22/12/2017].
3. Greub G. and Raoult D. (2002) '*Bartonella*: new explanations for old diseases' *Journal of Medical Microbiology* **51** pp.915-923.
4. CDC (2015) 'Bartonella infection (Cat Scratch Disease, Trench Fever, and Carrion's Disease) *Symptoms*' Available at: https://www.cdc.gov/bartonella/symptoms/index.html [Accessed: 22/12/2017].