



## A review of emerging flea-borne bacterial pathogens in New Zealand

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### Abstract

Recent studies have shown that *Rickettsia typhi*, *R. felis*, *Bartonella henselae*, and *B. clarridgeiae* occur in New Zealand. To raise awareness of these emerging and re-emerging flea-borne bacterial pathogens among New Zealand health workers, we review the clinical features, diagnosis, and treatment of infections, and the biology of the major flea vectors.

Fleas are the vectors of emerging and re-emerging bacterial pathogens. Four such pathogens are known to occur in New Zealand; *Rickettsia typhi*, *R. felis*, *Bartonella henselae*, and *B. clarridgeiae*. To raise awareness of these emerging pathogens among New Zealand health workers, in this review we describe the clinical features, diagnosis and treatment of infections and the biology of the major flea vectors.

### Flea-borne bacterial pathogens

#### *Rickettsia typhi*

This is an obligate intracellular Gram-negative bacterium that is the agent of murine or endemic typhus. The disease occurs world-wide and is increasingly being recognised in travellers, and in people in Australia, parts of the United States and more recently in New Zealand.<sup>1-3</sup> The major vector of *R. typhi* is the oriental rat flea, *Xenopsylla cheopis*, in which the organism multiplies in the midgut and is excreted in the faeces where it remains viable for years.<sup>4</sup> Although *X. cheopis* can transmit *R. typhi* by biting,<sup>5</sup> infections usually result from inhalation or ingestion of infected flea faeces or inoculation of faeces into pruritic flea bite lesions. *Rattus* rats are the main reservoir hosts and animals of all ages are highly susceptible to infections, which persist for 2 to 3 weeks but cause no ill-effects.

**Clinical manifestations**—*Rickettsiae* multiply in the vascular endothelium and induce vascular injury, which accounts for most of the clinicopathologic findings. Patients with murine typhus have non-specific signs including fever, headache, myalgia, and a non-specific maculopapular rash.<sup>6</sup> The disease is seen more commonly in adults but also occurs in children. Laboratory abnormalities include elevated liver enzymes, thrombocytopenia, and mild leucopenia.

**Diagnosis**—The majority of cases of murine typhus go undiagnosed (as clinical signs are non-specific).<sup>6</sup> Definitive diagnosis of infections depends on a high level of suspicion of the disease by physicians and confirmatory laboratory tests. Serological testing for rickettsial infection using an immunofluorescence assay is performed at LabPlus, Auckland District Health Board. Cross-reactivity can occur with other bacteria and this may lead to difficulties with interpreting results.<sup>7,8</sup> Nucleic acid

amplification testing on whole blood by PCR using primers for the 17 kDa protein and citrate synthase gene is also available. Culture is not routinely performed.

**Treatment**—Patients respond rapidly to treatment with tetracycline, doxycycline or a fluoroquinolone. Untreated patients show signs for two to three weeks and a significant number are hospitalised, with up to 10% requiring intensive care.

### ***Rickettsia felis***

Previously known as the ELB agent, this is a recently described Gram-negative obligate intracellular bacteria which is a member of the spotted fever group (SFG) rickettsiae.<sup>9</sup> Although only recognised for a short time, *R. felis* has already been found in the USA,<sup>1</sup> Brazil, Germany, Spain, France, Ethiopia, United Kingdom, Thailand and, most recently, New Zealand.<sup>10</sup>

While ticks are the principal reservoirs and vectors of the SFG rickettsiae, *R. felis* is maintained in nature by the cat flea, *Ctenocephalides felis*. Up to 93% of commercial cat fleas are infected,<sup>1</sup> but the prevalence is lower in wild-caught fleas and is 10% in New Zealand.<sup>10</sup> *R. felis* is transmitted transovarially by *C. felis* which can maintain infections for at least 12 generations without feeding on an infected host.<sup>11</sup> *R. felis* seems to be transmitted by flea bites<sup>12</sup> but flea faeces contain viable organisms and transmission might occur with faecal inoculation into pruritic flea bites.

**Clinical manifestations**—While infections in cats appear to be subclinical, people infected with *R. felis* may develop severe signs, presumably as organisms multiply in the vascular endothelium. The first case of “flea-borne spotted fever” occurred in Texas in 1994 but patients have now been described in Mexico (3), Brazil (2), Germany (2), France (2), and Thailand (1).<sup>13</sup> Mostly, there is no history of recent contact with fleas and clinical signs are non-specific, most commonly including fever, headache and rash. Other signs include marked fatigue, myalgia, photophobia, conjunctivitis, abdominal pain, vomiting, and diarrhoea—as well as solitary, black crusted skin lesions surrounded by a livid halo. Laboratory abnormalities are similar to those seen with murine typhus.

**Diagnosis**—Diagnoses of infections have been made by PCR and sequencing of DNA extracted from blood or skin biopsy samples using primers for the 17 kDa protein<sup>14,15</sup>, citrate synthase<sup>15,16</sup> and PS 120 protein<sup>17</sup> genes found in rickettsiae. Recently, *R. felis* has been established in tissue culture (XTC-2 and Vero cells) and this has enabled serological testing which appears to be reliable and has been used to diagnose infections.<sup>16</sup> Serology and PCR for *R. felis* is available at LabPlus, Auckland District Health Board.

**Treatment**—Patients have been successfully treated with doxycycline.<sup>17</sup> *In vitro* studies have shown *R. felis* is sensitive to doxycycline, rifampin, thiamphenicol, and fluoroquinolones—but not to gentamicin, erythromycin, amoxicillin, or cotrimoxazole.

### ***Bartonella henselae***

This Gram-negative bacillus was first described in 1993. The domestic cat is the major reservoir of infection and around 10% to 40% of cats have chronic bacteremia,<sup>18</sup> which may persist for years. Most infected cats appear healthy but can infect people through scratches, bites, and licks of open wounds. The cat flea, *C. felis*,

also transmits infections, by feeding or when infected flea faeces are inoculated into skin wounds caused by pruritic fleabites. There is some evidence that *B. henselae* is transmitted by ticks but we have not found organisms in ticks in New Zealand (Kelly P, unpublished data, 2004).

Although *B. henselae* was first recognised as an agent of bacillary angiomatosis in AIDS patients, the organism is now known to cause a wide variety of other disease syndromes. The nature and severity of the conditions correlate with the immune status of the patient—those with intact or only immature immune systems develop more localised infections, while immunocompromised patients (e.g. AIDS patients) often develop systemic infections which may be fatal.

**Clinical manifestations**—Bartonellosis are suspected to be the most common bacterial zoonoses acquired from companion animals.

- Cat scratch disease (CSD)

*B. henselae* is the major etiological agent of CSD which is probably the most common cause of chronic, benign, lymphadenopathy in children and young adults in developed countries.<sup>19</sup> The initial lesion consists of a papule, pustule or vesicle that develops 2 to 3 weeks after a cat bite or scratch, usually on the arms.<sup>20</sup> Although the initial lesion heals uneventfully, regional lymphadenopathy (the hallmark of the disease) develops 1 week later and persists for 2 weeks to 3 months before resolving spontaneously. In 75% of patients the adenopathy occurs with mild systemic symptoms including fever, malaise, fatigue, headache, anorexia, weight loss, and emesis that usually resolve within 2 weeks. Enlarged lymph nodes can be tender, and up to 20% of these nodes suppurate. Most cases are self-limiting with the adenopathy resolving spontaneously in 2 to 4 months.

Atypical manifestations of CSD occur in about 15% of patients. The most common is Parinaud's oculoglandular syndrome where there is unilateral conjunctivitis with pre-auricular lymphadenopathy that probably results from inoculation into the conjunctiva rather than the skin. In some patients, dissemination of the organism can occur to cause granulomas or abscesses in the liver, spleen, bone, or mesenteric lymph nodes. Systemic manifestations of CSD include hepatosplenomegaly, glomerulonephritis, and pleural effusion.<sup>19</sup> Various neurological syndromes have been reported with CSD including generalised seizures, transverse myelitis, encephalopathy, neuroretinitis, facial nerve paresis, and peripheral neuritis.<sup>21,22</sup>

- Bacillary angiomatosis-peliosis

Bacillary angiomatosis is a potentially fatal pseudoneoplastic vascular proliferative disease that occurs principally in immunocompromised patients, particularly those in the later stages of HIV infection.<sup>23</sup> There is considerable evidence that infections result from contact with cats.

Although the skin is most commonly affected, there may be osseous, gastrointestinal, respiratory, lymph node, central nervous system, and bone marrow bacillary angiomatosis (with or without accompanying skin lesions). In the skin, bacillary angiomatosis usually presents clinically as erythematous papules and nodules that may be localised or diffuse and bleed profusely when punctured. They may mimic Kaposi's sarcoma in appearance. In the disseminated

forms of bacillary angiomatosis there may be fever, weight loss, and enlargement of the affected organs.

Bacillary peliosis is a very rare condition caused by *B. henselae* infection of parenchymal vasculature which results in development of cystic, blood-filled spaces in the liver, spleen, or lymph nodes.<sup>24</sup> The disease is associated with immunosuppression and traumatic exposure to cats.

- Bacteremia

Recent studies have shown *B. henselae* is a cause of fever in HIV-infected people that might not be associated with lymphadenopathy or hepatosplenic involvement.<sup>25</sup> In a recent report from South Africa, 10% of people attending an HIV clinic were found to be PCR positive for *B. henselae*.<sup>26</sup> In HIV-uninfected patients (regardless of whether they are immunocompetent or pharmacologically immunosuppressed), *B. henselae* may cause an acute onset of febrile illness with arthralgia, myalgia, and headaches which may persist or become relapsing. In some patients, *B. henselae* infections result in long-term asymptomatic persistent bacteraemia. Contact with cats is a major risk factor for *B. henselae* bacteraemia.<sup>25</sup>

- Endocarditis

Endocarditis due to *B. henselae* occurs most often in patients with pre-existing valvulopathies who have contact with cats and their fleas.<sup>27</sup> The mortality rate is high (25%) and most patients require valve replacement surgery.

**Diagnosis**—There are no pathognomonic clinical features of *B. henselae* infections, and definitive diagnoses can only be made using laboratory testing. Histology of biopsy samples may provide a presumptive diagnosis, particularly if organisms are demonstrated within characteristic lesions with Warthin-Starry silver impregnation staining. Immunocytochemical techniques are used to detect *B. henselae* in tissue sections and cytological specimens in specialised laboratories.<sup>28</sup>

*B. henselae* can be cultured on most blood-enriched media, but primary isolates are usually only observed after 12 to 14 days of culture, sometimes as long as 45 days.<sup>29</sup> Lysis-centrifugation or freeze-thawing of blood samples greatly increases the recovery of organisms. Cultures from immunocompetent people and those previously treated with antibiotics are often negative. Identification of isolates is difficult using standard bacteriological methods and is best accomplished using molecular methods which are also sensitive and specific tests for detecting organisms in tissues and blood.<sup>29</sup> A variety of primers have been used in PCR analyses, including those against regions of the 16S rRNA, 17-kDa antigen, and citrate synthase (*gltA*) genes and the 16S/23S intragenic spacer region.<sup>30,31</sup>

Serology is a cheap, sensitive, and safe means of supporting clinical diagnoses of *Bartonella* infections, in particular endocarditis<sup>27</sup> and CSD.<sup>28</sup> Serological cross-reactivity occurs between *Bartonella* spp. and tests should be performed against all species known to occur in an area before an etiological diagnosis is made. Generally, highest titres are against the species causing the infection. Immunocompromised patients might not mount significant antibody responses. Culture, PCR, and serology for *B. henselae* are available in New Zealand.

**Treatment**—*In vitro*, *B. henselae* is susceptible to most antibiotics although only aminoglycosides are bactericidal.<sup>32</sup> Most typical cases of CSD, however, respond very poorly to antimicrobial therapy and the disease generally resolves spontaneously within 4 months.<sup>33</sup> Although treatment with azithromycin has been suggested for patients with pronounced lymphadenopathy, no therapeutic advantage has been demonstrated.<sup>34</sup> There is debate as to whether patients with complicated CSD benefit from antibiotics.<sup>35</sup> In immunocompromised patients with CSD or immunocompetent patients with central nervous system involvement, a combination of doxycycline and rifampin has been suggested but there is little data to support the recommendation.<sup>35</sup> In patients with suppurative lymph nodes, needle aspiration is an appropriate treatment.

The currently recommended treatment for bacillary angiomatosis and peliosis hepatis is oral erythromycin. Most HIV positive patients with bacillary angiomatosis respond well to antibiotics with complete remissions in 65% of patients within 30 days of treatment.<sup>23</sup> Relapses may occur when short (<15 days) courses of treatment are given.

Successful therapy for bartonella endocarditis usually entails replacement of the extensively damaged valve. The optimum antibiotic treatment is not known, but the use of gentamicin with a beta-lactam has been recommended.<sup>36</sup>

### ***Bartonella clarridgeiae***

This organism was first described in 1996<sup>37</sup> after it was isolated from the cat of an HIV-positive patient with CSD due to *B. henselae*. The cat is the reservoir of the organism and up to 12% of cats might have concurrent *B. henselae* infections. The vectors of *B. clarridgeiae* are unknown, but cat fleas infected with the organism have been found in France, the Netherlands, the Philippines, Indonesia, and now New Zealand.<sup>10</sup> Fleas containing DNA of both *R. felis* and *B. clarridgeiae* have been found in France.

**Clinical manifestations**—Serological testing has indicated *B. clarridgeiae* might be an agent of CSD in people.<sup>38</sup> In dogs, the organism has been implicated as an agent of hepatitis and endocarditis.

**Diagnosis**—The organism can be cultured on solid media and detected by PCR and sequencing.<sup>39</sup>

**Treatment**—There is no reliable data on appropriate treatment.

### **Fleas**

Fleas are insects in the order Siphonaptera that have a number of developmental stages in their life cycle. The adults live permanently on the host and feed on its blood. The immature stages develop in the environment and feed on organic debris. The cat flea, *C. felis*, is the most important ectoparasite of domestic cats and dogs in New Zealand and world-wide. It has a wide host range—readily feeding on people, pets, domestic animals, wild mammals, and rodents.<sup>40</sup> Adults can reproduce for up to 3 months and females lay up to 50 eggs per day, which hatch in the environment and develop to pupae in as little as 10 days. Adult fleas may remain in the cocoon for many months before emerging in response to vibration and/or heat. The adults are non-selective feeders and are attracted to their hosts by visual and thermal cues.

People are mainly bitten by newly emerging adults; direct transfer of fleas between hosts is uncommon.

The main host of the oriental rat flea, *X. cheopis*, are *Rattus* rats but sometimes they occur on the house mouse, *Mus musculus*. The number of eggs laid per day varies with the species of the host but is less than with *C. felis*. As with the cat flea, pupae can survive many months of starvation while awaiting a host.

## **Current information on flea-borne bacterial pathogens in New Zealand**

To date, only infections with *R. typhi* and *B. henselae* have been diagnosed in people in New Zealand. Locally acquired murine typhus due to *R. typhi* was first reported in 1991.<sup>3</sup> Subsequently, 20 patients from the greater Auckland region have been diagnosed with the disease (unpublished data, S Roberts). The majority of patients lived in northwest Auckland around the Kaipara Harbour, but three cases have occurred in the Coromandel Peninsula region. It is highly likely that it is present in other regions throughout New Zealand, especially those close to current or historic ports. *R. typhi* DNA has been detected by PCR in rats captured on the properties of two patients but not in fleas obtained from rats or domestic animals.<sup>2,10</sup>

Two patients infected with *B. henselae* have been reported in New Zealand; both had neuroretinitis<sup>22</sup> and one also had encephalopathy.<sup>21</sup> DNA of *B. henselae* has been identified in the lymph nodes of 3 patients with suspected CSD (unpublished data, S Roberts). Also, the organism has been isolated from 17% of cats in Auckland, and has been identified in 3% of cat fleas collected from dogs and cats presenting to the Veterinary Teaching Hospital of Massey University in Palmerston North.<sup>10</sup>

In nearby Australia, there have also been relatively few reports of clinical cases of *B. henselae* infections, although 5% of blood donors are seropositive and 35% of cats from Sydney are bacteremic.<sup>41</sup> While infections may be under-reported, recent genotyping studies have shown there is a high level of diversity amongst strains of *B. henselae* and those isolated from people are more genotypically homogeneous than those associated with feline reservoirs.<sup>18</sup> There might, then, be specific genotypes that are more likely to cause clinical infections in people and further studies are indicated to determine the types present in New Zealand.

*R. felis* and *B. clarridgeiae* have only recently been reported to occur in their vectors in New Zealand,<sup>10</sup> and there have been no reports of human infections.

## **Prevention of infections**

Prevention of infections with flea-borne bacterial pathogens is best achieved by minimising contact with the vectors and reservoirs of the organisms. In the case of *R. typh*, steps should be taken to eliminate rodents in the household. Rodent fleas (particularly *X. cheopis*) must be controlled simultaneously, otherwise outbreaks of murine typhus will occur after the rats die and their fleas seek alternative hosts.

Prevention of infections with *B. henselae*, *B. clarridgeiae*, and *R. felis* is best achieved by control of their cat flea vector. Veterinary advice should be sought on the variety of highly effective products, which can eliminate cat fleas from the household and are readily available in New Zealand. Bacteremic cats may also be a source of infection with direct transmission resulting from bites and scratches. Although these cats can be

identified by blood cultures, no single antibiotic or combination of antibiotics can reliably eliminate infections from cats. While infected animals can be removed from a household, cats play an important role in improving the quality of life of their owners, particularly children and immunosuppressed people.

Therefore, rather than families being deprived of their cats, the risk of infection can be greatly decreased by instituting effective flea-control strategies and preventing situations where scratches or bites are likely to occur, such as with rough play or during teasing.

A vaccine against *B. henselae* for cats has been reported but subsequent studies have shown lack of protection following infection and challenge with the different strains of the organism.<sup>42</sup>

## Conclusions

The development of new culture and molecular biology techniques has facilitated the investigation of flea-borne bacterial diseases of people. Application of these techniques in New Zealand has shown that *R. typhi* and pathogenic *Bartonella* species are present in the country and that infections occur in people. Also, the pathogenic flea-borne SFG rickettsia, *R. felis*, has been shown to occur in relatively high proportions of cat fleas. Health workers in New Zealand should, then, be aware of the possibility of infections with these organisms in their patients and diagnostic laboratories in the region should provide appropriate diagnostic tests.

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