DETECTION AND CHARACTERIZATION OF
BARTONELLA SPECIES IN WESTERN AUSTRALIA

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University,
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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

............................................................

(Gunn Kaewmongkol)
Abstract

In this study, the prevalence and genetic diversity of *Bartonella* species in various arthropod vectors from both wild and domestic animals in Australia were investigated using nested-polymerase chain reaction (PCR) assays and multilocus sequence analysis (MLSA). Previous studies on *Bartonella* species in Australia have been confined to mammalian hosts, including humans, cats, native rodents and eastern grey kangaroos. However, little is known about the status of bartonellae in arthropod vectors, which is essential in understanding the transmission dynamics of the organisms.

To facilitate the investigation, ectoparasites (ticks and fleas) were collected from both wild and domestic animals from various locations in Australia. All ectoparasites were screened for *Bartonella* species using newly designed nested-PCRs targeting the *gltA* gene (citrate synthase) and the ribosomal internal transcribed spacer (ITS) region, developed as part of the present study. Multilocus sequence analysis of the 16S ribosomal RNA (rRNA), citrate synthase (*gltA*), cell division protein (*ftsZ*) and RNA polymerase beta-subunit (*rpoB*) genes and the ribosomal ITS region was applied to identify and confirm the status of all *Bartonella* species identified in this study. Multilocus sequence analysis of the cytochrome oxidase subunit I (*COI*) and 18S ribosomal RNA (rRNA) genes of flea vectors harbouring a diversity of *Bartonella* species were analysed to characterize the extent of genetic diversity in the flea vectors and to elucidate vector-parasite associations.

A phylogenetic analysis of the 5 concatenated loci identified 3 novel *Bartonella* species in flea vectors from marsupials in Western Australia. *Candidatus* Bartonella antechini was detected in fleas (*Acanthopsylla jordani*) from mardos (*Antechinus flavipes* - also
called the yellow-footed antechinus). *Candidatus* Bartonella woyliei was detected in fleas (*Pygiopsylla hilli*), from brush-tailed bettongs (*Bettongia penicillata*– also called woylies), and *Candidatus* Bartonella bandicootii was detected in *Pygiopsylla tunneyi* fleas from western barred bandicoots (*Perameles bougainville*). Furthermore, a potential novel species, *Bartonella* sp. strain WC2 was detected in ticks (*Ixodes australiensis*) from woylies based on the criterion of a genetic similarity of less than 96% of the *gltA* locus compared with other validated *Bartonella* species. In the present study, the grouping of marsupial-derived *Bartonella* species confirmed the existence of a marsupial cluster of *Bartonella* species in Australia, which appears to have evolved separately to *Bartonella* species in other mammals.

The detection of the known zoonotic *Bartonella* species, *B. henselae* and *B. clarridgeiae* in red foxes and their fleas (*Ctenocephalides felis*), indicated that red foxes could be an important reservoir of *Bartonella* infections for other animals and humans in the same geographical locality. *Bartonella henselae* and *B. clarridgeiae* DNA were also detected from fleas collected from pet cats in the same area. The genetic association of these zoonotic *Bartonella* species detected in wildlife and pet animals has demonstrated and confirmed the distribution of zoonotic *Bartonella* species in fleas from both wild and domestic animals in this region and a possible ecological association between the animal species.

The genetic clustering of *Bartonella* species and flea vectors with their Australian fauna hosts suggests co-evolution of hosts, fleas and *Bartonella* species in Australia. In conclusion, the close association between Australian fauna, Australian fleas and *Bartonella* species suggests adaptation by *Bartonella* species to a specific ecological
niche, comprised of specific mammalian hosts and specific flea vectors in particular environments.
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Publications

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