

Short Report: Molecular Epidemiology of *Leptospira borgpetersenii* Serovar Arborea, Queensland, Australia, 1998–2005

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Abstract. *Leptospira borgpetersenii* serovar Arborea is an emerging cause of leptospirosis in Australia. It was not previously recognized as an endemic serovar before the 1990s, but at that point, human infections with the serovar increased significantly. Using fluorescent-amplified fragment-length polymorphism (FAFLP) molecular typing, human and rodent isolates were compared genetically. Typing revealed 11 unique profiles among the 23 isolates examined; however, there was no clonality revealed between the human and rodent isolates. There was clonality among rodent isolates from geographically related areas. This study highlights the utility of *Leptospira* culture combined with FAFLP for the examination of the epidemiology of this disease.

Leptospirosis is the disease caused by the gram-negative spirochete *Leptospira*. *Leptospira* are divided into 20 species that are determined by DNA–DNA hybridization and 16S rRNA analysis; serologically, there are approximately 200 serovars.^{1,2} The majority of leptospirosis infections in Australia occurs in the state of Queensland with an incidence rate of approximately 3.2 cases per 100,000 people, and the majority of infections occur in the far northeastern coastal areas.³ Of particular interest is the emergence of novel serovars of *Leptospira*, particularly those that affect areas where leptospirosis is not endemic; one such serovar is *L. borgpetersenii* serovar Arborea. *L. borgpetersenii* sv. Arborea was first isolated in 1955 from a wood mouse (*Apodemus sylvaticus*) in Arborea-Sardegna, Italy.⁴ Subsequently, it has been reported throughout the world in Barbados, the Terceira Islands (Azores), Argentina, and most recently, Australia.^{3,5–7}

The aim of this study was to define the molecular epidemiology of this serovar in Australia utilizing strains isolated from humans and rodents. This was performed using the fluorescent-amplified fragment-length polymorphism (FAFLP) methodology that has been previously used successfully to type *Leptospira* spp.^{8–10}

Leptospira were isolated by culture; briefly, 1 mL of whole blood (human) or kidney tissue (rodent) was inoculated into 3 mL of Ellinghausen–McCullough–Johnson–Harris (EMJH) medium, incubated at 30°C for 6 weeks, and examined weekly by dark-field microscopy. Positive cultures were identified to species level through amplification and sequencing of a 504-bp fragment of the DNA gyrase subunit B (*gyrB*) gene; this was performed as previously described by Slack and others.¹¹ Isolates were then serologically identified using a cross-agglutinin absorption test (CAAT) against the reference strain *L. borgpetersenii* sv. Arborea strain Arborea. FAFLP was performed as previously described using the Microbial FAFLP kit (Applied Biosystems).^{8,9} The fluorescently labeled DNA fragments were separated using the ABI-310 genetic analyzer (Applied Biosystems), and the fragment-sizing data were converted into binaries and analyzed using the Bionumerics

software package (Applied Maths). Clustering analysis was performed using the dice coefficient and the unweighted pair-group method with an arithmetic mean (UPGMA) algorithm (Figure 1).

Molecular typing using FAFLP was performed on 22 *L. borgpetersenii* isolates of serovar Arborea strains and the reference strain for this serovar (strain Arborea). The 22 isolates were composed of 15 rodent isolates and 7 human isolates. These isolates were found over an 8-year period from 1998 to 2005 and were geographically distributed across the state of Queensland (Table 1).

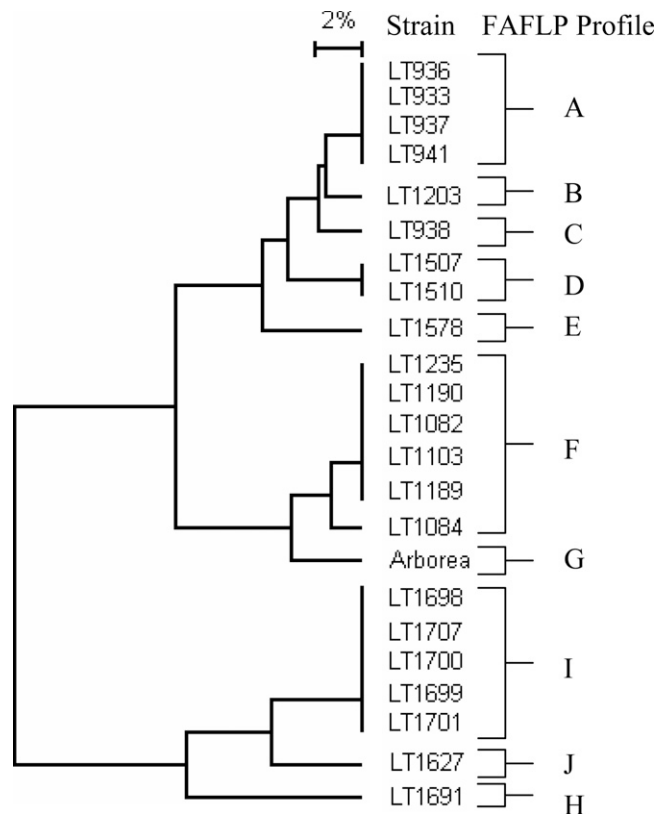


FIGURE 1. Dendrogram of the *L. borgpetersenii* serovar Arborea strains constructed from the FAFLP results using the dice coefficient and the UPGMA algorithm. Bar = percent difference.

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TABLE 1

Details of *L. borgpetersenii* serovar Arborea isolates examined using FAFLP

Strain	Location	Source*	Year	FAFLP profile
LT 936	Brisbane	M	1998	A
LT 933	Brisbane	M	1998	A
LT 937	Brisbane	M	1998	A
LT 941	Brisbane	M	1998	A
LT 1203	Sunshine Coast	H	2001	B
LT 938	Brisbane	R	1998	C
LT 1507	Tully	M	2003	D
LT 1510	Tully	M	2003	D
LT 1578	Innisfail	H	2004	E
LT 1235	Innisfail	H	2002	F
LT 1190	Sunshine Coast	H	2001	F
LT 1082	Brisbane	R	2000	F
LT 1103	Brisbane	R	2000	F
LT 1189	Sunshine Coast	H	2001	F
LT 1084	Brisbane	M	2000	G
Arborea	Arborea-Sardegna	A	1955	H
LT 1698	Sunshine Coast	M	2005	I
LT 1707	Sunshine Coast	M	2005	I
LT 1700	Sunshine Coast	M	2005	I
LT 1699	Sunshine Coast	M	2005	I
LT 1701	Sunshine Coast	M	2005	I
LT 1627	Innisfail	H	2004	J
LT 1697	Innisfail	H	2004	K

* M = *Mus* sp.; H = human; R = *Rattus* sp.; A = *Apodemus sylvaticus*.

When clustering analysis was performed, there were 11 unique FAFLP profiles among the 23 isolates (Figure 1). Within the 11 unique profiles, there were seven individual profiles and four profiles that contained two or more strains. Two profiles were of particular interest. Profile A contained isolates obtained from trapped rodents at an urban area in Brisbane, and Profile I contained isolates taken from trapped rodents at a semirural area on the sunshine coast. Both of these areas were not previously known for endemic leptospirosis and had sustained an increase in the number of *Leptospira* infections since 1999.³ FAFLP Profiles D and F were the only other profiles to contain more than one isolate; Profile D contained two rodent isolates, and Profile F was the only profile containing a mixture of human and rodent isolates from three geographically separate areas ranging from Innisfail in the north of the state to Brisbane in the south of the state, a distance of approximately 1,600 km. The type strain for this serovar, Arborea, was isolated in 1955; it showed a high level of similarity with FAFLP profiles F and G, suggesting some form of relatedness between European and Australian isolates. Given that *L. borgpetersenii* serovar Arborea was not isolated in Australia before 1998,³ the FAFLP studies could suggest that Arborea was possibly imported through rodents aboard cargo ships entering Australian ports.

Using a powerful molecular epidemiological technique such as FAFLP, we have been able to characterize the *L. borgpetersenii* serovar Arborea strains isolated from both humans and rodents. Although there was little evidence to suggest that there was direct infection transmission between rodents and humans, this could be influenced by the fact that the majority

of human infections were diagnosed serologically by microscopic agglutination test (MAT) testing rather than by culture.³ This study shows that there has been clonal transmission of the serovar among rodents at a local and state level. Additionally, it shows the potential of using culture and FAFLP for the epidemiological typing of *Leptospira* isolates from both human and rodent populations.

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