

EPIDEMIOLOGY OF *LEPTOSPIRA WEILII* SEROVAR TOPAZ INFECTIONS IN AUSTRALIA

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Abstract

Leptospirosis is a zoonotic disease with a worldwide distribution. *Leptospira weilii* serovar (sv.) Topaz is a newly described serovar first isolated in the far north of Queensland, Australia. The epidemiology of *L. weilii* sv. Topaz infections in Australia was characterised through the use of surveillance questionnaires and molecular studies. There have been 24 human and 2 animal (bovine and bandicoot) *L. weilii* sv. Topaz infections diagnosed since 1991. The majority of these infections have occurred in Far North Queensland, with the remaining infections occurring in South East Queensland and in Western Australia. The majority of patients with *L. weilii* sv. Topaz infections presented with classical leptospirosis symptoms including; fever, headaches, sweats, chills and myalgia. The occupations of human cases of *L. weilii* sv. Topaz infection included banana farming, dairy and beef cattle production and tourist related activities. Fluorescent amplified fragment length polymorphism (FAFLP) was performed on 15 *L. weilii* sv. Topaz isolates including 2 animal isolates. Clustering analysis grouped the 15 isolates into 5 main clades with 13 unique FAFLP profiles. A high level of relatedness was demonstrated between 2 animal and 2 human isolates. *Commun Dis Intell* 2007;31:216–222.

Keywords: epidemiology, *Leptospira weilii*, leptospirosis, zoonoses

Introduction

Leptospirosis is caused by infection with spirochaetes of the genus *Leptospira*.¹ *Leptospira* are motile helical spirochaetes that metabolise long chain fatty acids as their sole carbon source.¹ The genus *Leptospira* contains 17 species as delineated by DNA-DNA hybridisation.^{2–5} *Leptospira* are divided serologically into serovars of which there have been over 200 described. Leptospirosis is one of the world's most widespread zoonotic diseases with outbreaks reported worldwide in both humans and animals.^{6,7,8} The organism enters humans through contact of abrasions or mucus membranes with urine or body fluids from an infected animal. This may occur directly or indirectly through contact with contaminated water or soil. Leptospirosis is more prevalent in tropical countries than temperate countries as the higher humidity, rainfall and temperature promote the survival of the organism in the environment.⁹

Leptospirosis was first reported in Australia in 1933. Since 1991, leptospirosis infections have been notified to the National Notifiable Diseases Surveillance System. From 1991 to 2005, there have been 7,629 cases notified in Australia.¹⁰ The majority of cases have occurred in Queensland situated on the eastern seaboard of Australia.¹¹ Twenty-three *Leptospira* serovars have been identified in Australia including several which were first discovered in Australia.¹¹ *L. weilii* sv. Topaz was first isolated by Corney, et al. from a bovine source near the township of Topaz in Far North Queensland and is the second member of the *L. weilii* species found in Australia.¹² It was found to be a unique serovar by cross agglutinin absorption test (CAAT). The aim of this study is to present the descriptive and molecular epidemiology of *L. weilii* sv. Topaz in Australia.

Materials and methods

Serological identification of *L. weilii* sv. Topaz

Leptospira IgM-specific enzyme linked immunosorbent assay (ELISA, Panbio) was performed as a screening test by the submitting private and hospital laboratories. Positive ELISA samples were forwarded to the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Brisbane (WHO Reference Centre) for confirmation and serovar identification using the microscopic agglutination test (MAT). *L. weilii* sv. Topaz strain 94-79979/3 was used as the reference culture in the MAT for the detection of human antibodies against *L. weilii* sv. Topaz. Patients with a positive IgM ELISA supported by a single MAT titre of greater than or equal to 400, or a demonstrated fourfold rise or fall in MAT titres over paired specimens, were considered to meet the notification criteria for leptospirosis.

Culture identification

Leptospira culture from humans was performed as follows: 2–5 drops of uncoagulated whole blood was inoculated into Ellinghausen McCullough Johnson Harris (EMJH) media containing 0.5% agar. This was performed by the submitting laboratories and forwarded at room temperature to the WHO Reference Centre. Once at the WHO Reference Centre, the cultures were sub-cultured into EMJH media (containing no agarose) and incubated at 30°C for 6 weeks. The cultures would be inspected weekly for the growth of *Leptospira* using dark field

microscopy. Positive cultures were identified to a species level by sequencing of a partial fragment of the DNA gyrase sub-unit B gene (*gyrB*).¹³ Serological identification of the isolates was performed by MAT using reference antisera covering the major serogroups of the genus *Leptospira*. Hyper-immune antiserum was prepared in rabbits using standard techniques.¹⁴ Definitive serovar identification was performed by CAAT¹⁵ using the *L. weilii* sv. Topaz strain 94-7997/3.

Surveillance questionnaires and descriptive epidemiology

Information regarding infection was collected from the patient's doctor using enhanced surveillance questionnaires. This provided information on symptoms, recreational activities, animal contacts, occupational data and hospitalisation. The data were entered into a Microsoft Access database and Microsoft Excel was used to conduct statistical analysis of these data.

Fluorescent amplified fragment length polymorphism

Cultures were prepared for DNA extraction by centrifugation of 1 mL of an actively growing *Leptospira* culture in EMJH at 12,500 g for 5 minutes. Genomic DNA was then extracted from the pellet using the ChargeSwitch gDNA mini bacterial kit (Invitrogen) as per manufacturers' instruction. FAFLP restriction digestion, ligation and amplification reactions was performed as previously described.^{16,17}

Fragment sizing and data analysis

One μL of the 6 selective polymerase chain reaction products was mixed with 18.5 μL of HiDi formamide (Applied Biosystems) and 0.5 μL of GeneFlo-625 (Chimerx) and denatured at 95°C for 5 minutes. The products were then loaded onto the ABI-310 capillary sequencer (Applied Biosystems) and were injected into a 47 cm capillary filled with performance-optimised polymer 4 (Applied Biosystems) at 15 kV for 12 seconds. The fragments were separated at 1.3 kV for 35 minutes. The resulting electropherograms were manipulated using the Genotyper v2.5 software (Applied Biosystems) and the combined allele sizes were exported into an Excel spreadsheet. A previously described Excel macro¹⁸ was used to convert the alleles into a binary sequence suitable for analysis using Bionumerics software (Applied Maths). Each unique FAFLP binary pattern was assigned a letter code (e.g. A, B, C) to allow for easier referencing of the data.

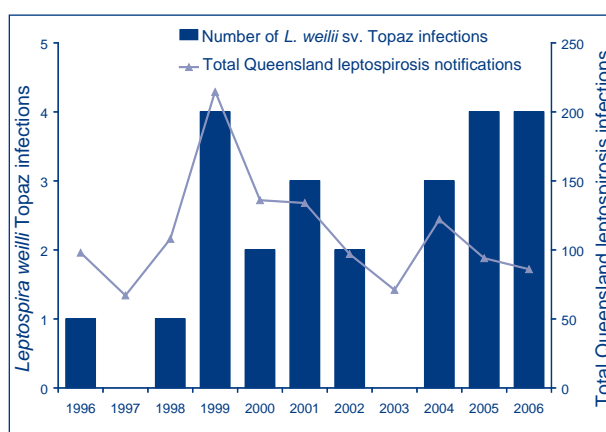
Results

Descriptive epidemiology of *Leptospira weilii* sv. Topaz

Since the initial isolation of *L. weilii* sv. Topaz in 1994,¹² there have been 26 additional cases of this serovar identified: 24 from human and 2 from animal sources (bovine and bandicoot). The first isolation of *L. weilii* sv. Topaz predates the identification of the type strain; 94-79970/3 having been isolated in 1991 and was identified at the time as a member of the Tarrasovi serogroup. Subsequently, examination of the isolate by CAAT using *L. weilii* sv. Topaz strain 94-7997/03, identified the isolate as serovar Topaz. Fifteen cases were diagnosed from *Leptospira* cultures taken from blood (human), urine (bovine) or from kidney tissue (bandicoot). The remaining 11 cases were diagnosed by MAT with titres ranging from 1 in 800 to 1 in 6,400 (Table 1).

Since 1994, *L. weilii* sv. Topaz infections in humans have been reported every year except in 1997 and 2003. Four cases were reported in 1999, 2005 and 2006 (up to August) (Figure 1). The majority of isolations occurred between January and June, with a single case reported in October, this is consistent with the seasonal trend of *Leptospira* infections noted by Slack, et al.¹¹

Figure 1. Distribution of *Leptospira weilii* sv. Topaz cases from 1996 to August 2006 compared with the total Queensland leptospirosis notifications for the same period



The geographical distribution of *L. weilii* sv. Topaz in Australia has been dominantly in the far north of Queensland around the leptospirosis endemic areas extending south from Cairns to Innisfail and Tully, and west onto the eastern side of the Atherton

Table 1. *Leptospira weillii* serovar Topaz cases in Australia, 1991 to 2006

Year	Source	Laboratory details	Age	Sex	Serum MAT titre (against strain 94-79970/3)	Culture
1991	Human	LT 596*	Unknown	F	Not performed	Detected
1994	Bovine	94-79970/3* (type strain)	N/A	N/A	N/A	Detected
1996	Human	LT 762*	30	M	1600	Detected
1998	Human	LT 925*	34	M	Not performed	Detected
1999	Human	LT 952*	22	M	800	Detected
1999	Human	LT 969*	33	M	Not performed	Detected
1999	Human	LT 974*	21	M	Not performed	Detected
1999	Human	LT 981*	39	M	400	Detected
2000	Human	LT 1060*	58	M	1600	Detected
2000	Human	LT 1055*	37	M	Not performed	Detected
2001	Human	LT 1187*	14	M	Not performed	Detected
2001	Human	LT 1188*	33	M	800	Detected
2001	Human	LT 1191*	28	M	1600	Detected
2002	Bandicoot	LT 1412*	N/A	N/A	N/A	Detected
2002	Human	LT 1414*	48	M	Not performed	Detected
2004	Human	TSI 1†	62	M	800	Not performed
2004	Human	TSI 2†	23	M	800	Not performed
2004	Human	TSI 3†	40	M	800	Not performed
2005	Human	TSI 4†	28	M	3200	Not performed
2005	Human	TSI 5†	64	M	1,600	Not performed
2005	Human	TSI 6†	54	M	800	Not performed
2005	Human	TSI 7†	47	M	800	Not performed
2006	Human	TSI 8†	56	F	800	Not performed
2006	Human	TSI 9†	22	M	6400	Not performed
2006	Human	TSI 10†	24	M	800	Not performed
2006	Human	TSI 11†	21	M	1,600	Not performed

* Diagnosis of *Leptospira weillii* sv. Topaz made by identification of the *Leptospira* isolate from the blood culture

† Diagnosis of *Leptospira weillii* sv. Topaz made by serology using the microscopic agglutination test (MAT).

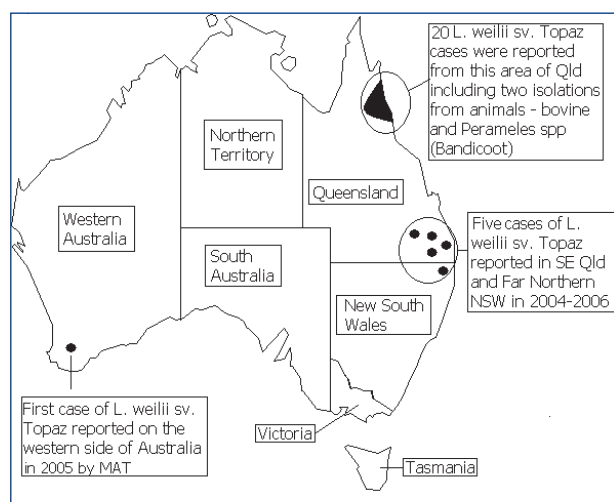
N/A Not applicable.

Tablelands (Figure 2). Twenty cases (77%) of *L. weillii* sv. Topaz have originated from this area; an area that has accounted for approximately 66% of Queensland leptospirosis notifications from 1998 to 2004 (Figure 2).¹¹ Five cases of *L. weillii* sv. Topaz were detected in South East Queensland and 1 case in northern New South Wales. The first detection of *L. weillii* sv. Topaz away from the eastern seaboard of Australia was a case in 2005 from Western Australia in which the patient had no history of recent travel to the eastern seaboard.

Of the 24 *L. weillii* sv. Topaz cases in humans, there was a male:female ratio of 11:1, which is consistent with the sex distribution of leptospirosis in previous

studies.^{11,20} The age of the patients ranged from 14 years to 64 years with a median age of 33 years. Twelve (50%) cases were reported to have been hospitalised with an average stay of 3 days. Patients with *L. weillii* sv. Topaz infections presented with classical symptoms of leptospirosis including; fever, headaches, sweats, chills and myalgia. Two patients presented with the more severe leptospirosis complications of pulmonary haemorrhage and aseptic meningitis (Table 2). The occupations of cases of *L. weillii* sv. Topaz infection included banana farming, dairy and beef cattle production and tourist related activities (Table 2). Contact with animals before infection was reported in the majority of cases, which is consistent with the rural occupa-

Figure 2. Geographical distribution of *Leptospira weilli* sv. Topaz cases, Australia, 1991 to 2006



tions and/or recreational exposure generally associated with *Leptospira* infections. The major animal contacts reported include rats or mice (this included native rodents such *Rattus fuscipes* or *R. sordidus* as well as the imported *R. rattus* and *Mus domesticus*), dogs, beef and dairy cattle (Table 2).

Molecular epidemiology of *Leptospira weilli* sv. Topaz

FALFP was performed on all 15 *L. weilli* sv. Topaz isolates including the 2 animal isolates (94-79970/3 and LT1412). There were 13 unique FAFLP profiles (designated A to M) amongst the 15 isolates tested. Clustering analysis of the FAFLP data was performed using the Jaccard coefficient (>50% mean) and unweighted pair group method with arithmetic mean (UPGMA) algorithm (Figure 3). There were 5 identifiable clades (designated i to v) within the dataset each containing between 2 and 4 isolates. Each clade contained isolates found over multiple years and showed clustering around the geographic area of isolation. For example clade ii and clade v contained isolates from the Cairns or Atherton tableland section of Far North Queensland whilst clade i and iv contained isolates from the more southern areas of Tully and Innisfail. An isolate from a native bandicoot species (LT1412) showed a high level of similarity to the human isolate; LT952. The type strain 94-7997/03 isolated from cattle, was found to share an identical FAFLP pattern with the human isolate; LT974.

Discussion

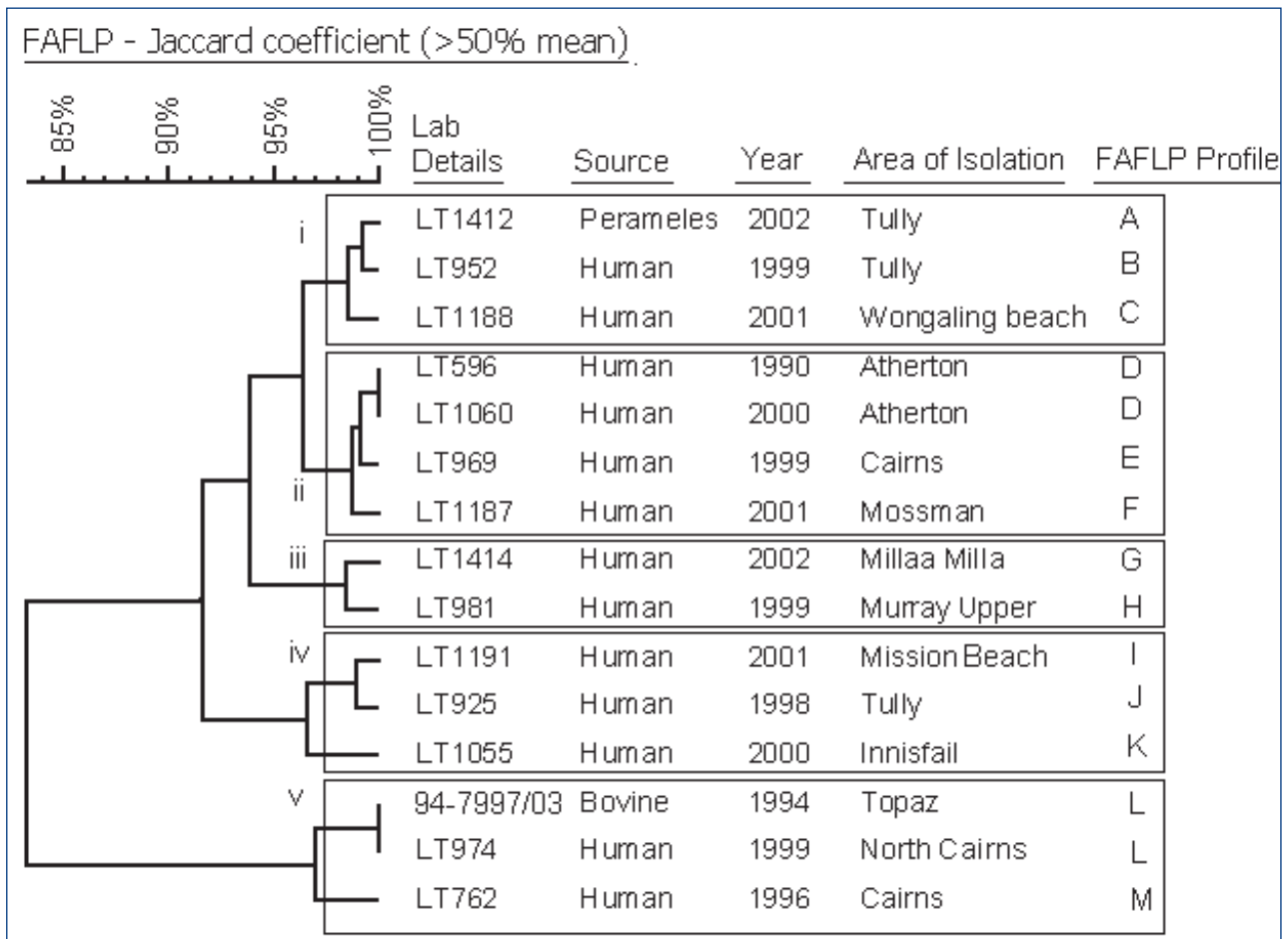
L. weilli sv. Topaz has been identified in both animal and human sources since its initial isolation in 1994 using both culture and serological methods (IgM ELISA combined with MAT) (Table 1). By utilising

Table 2. Clinical and epidemiological characteristics of *Leptospira weilli* sv. Topaz infections

Clinical characteristics	Positive responses n=18	Percentage of positive responses
Fever	14	78
Headache	12	67
Arthralgia	10	56
Sweats	10	56
Chills	9	50
Myalgia	9	50
Nausea	7	39
Vomiting	6	33
Back pain	4	22
Respiratory symptoms	2	11
Asceptic meningitis	1	6
Conjunctival suffusion	1	6
Diarrhoea	1	6
Liver involvement	1	6
Pulmonary haemorrhage	1	6
Rash	1	6
Vision disturbance	1	6
Occupation	Positive responses n=24	Percentage of positive response
Banana farmer	4	17
Tourist operator/tourist	4	17
Dairy	3	13
Grazier	3	13
Labourer/tradesperson	2	8
Veterinarian	1	4
Not reported or unemployed	7	29
Animal	Positive responses n=20	Percentage of positive responses
Rats and mice	9	45
Dogs	8	40
Beef cattle	5	25
Dairy cattle	3	15
Feral pigs	3	15
Cats	2	10
Domestic pigs	1	5
Bandicoot	1	5
Sheep	1	5
No contact	1	5

both methods initially, we were able to validate the specificity of the MAT in the diagnosis of *L. weilli* sv. Topaz infections. Diagnosis by MAT alone has been possible since the inclusion of *L. weilli* sv. Topaz in

Figure 3. Clustering analysis of *Leptospira weilii* sv. Topaz constructed from the FAFLP data using the Bionumerics software package



the WHO Reference Centre MAT panel since 2000. Initially, this testing was conducted by screening samples that had low *L. borgpetersenii* sv. Tarassovi titres (*L. weilii* sv. Topaz produces a serological cross-reaction with members of the Tarassovi serogroup, data not shown) for the presence of *L. weilii* sv. Topaz antibodies and in 2004 was added to the panel as a standalone serovar. Whilst the MAT has been criticised for a lack of specificity when determining the infecting *Leptospira* serovar,¹⁹ we believe that its use in a well-defined leptospirosis environment such as Australia means that it can be sufficiently relied upon to determine the individual serovar involved.

The geographical distribution of *L. weilii* sv. Topaz in Australia suggests that there are 2 distinct pockets of this serovar in Queensland and northern New South Wales. However, the single Western Australia case of *L. weilii* sv. Topaz may indicate that this serovar may be much more widespread in Australia compared to the more geographical isolated *Leptospira* serovars such as *L. interrogans* sv. Zanoni.¹¹

Since *L. weilii* sv. Topaz infection has been isolated from a native animal (bandicoot) this serovar may be indigenous to Australia.¹¹ Several *Leptospira* serovars in Australia are not found elsewhere in the world.¹¹ A recent unpublished study examining leptospirosis in Macropods (kangaroos), found that a significant proportion of the study animals had serological titres that would indicate exposure to *L. weilii* sv. Topaz. (personal communication, M Roberts and L Smythe).¹¹

Overall, *L. weilii* sv. Topaz infections represent only 1.9% of the 1,288 reported leptospirosis infections (based upon Queensland data from 1996 to August 2006), however this figure is likely to be an under-estimate since assays for its identification have only recently (since 2000) been available at the WHO Reference Centre. To assess the geographic distribution of the serovar in Australia, it is recommended that all Australian laboratories performing leptospirosis testing include *L. weilii* sv. Topaz in their MAT panels or alternatively, that the samples with serovar Tarrasovi titres are forwarded to the WHO Reference Centre for confirmatory testing.

The epidemiology of *L. weilii* sv. Topaz infections is similar to that of other leptospirosis infections in Australia.¹¹ There is a higher likelihood of *Leptospira* infection in the period from January to May, as this is the peak rainfall period for Far North Queensland. The high rainfall combined with relatively high ambient temperatures provides ideal survival conditions for *Leptospira* in the environment, translating to a higher risk to humans.

Males of working age (18 to 60) are the most at risk. The median age of 33 years at infection with *L. weilii* sv. Topaz is consistent with other studies of leptospirosis in Australia.¹¹ The majority of *L. weilii* sv. Topaz patients report symptoms classically associated with leptospirosis. Banana farming and dairy/beef cattle farming appear to be the most at risk occupation groups. Both these occupations require contact directly and indirectly with animals and at times require close contact with contaminated soil and water. The other major occupation at risk is the tourist operator/tourist sector, accounting for 4 (17%) of the total number of *L. weilii* sv. Topaz infections (Table 2). A recently study by Slack, et al demonstrated that 17.8% (n=883) of *Leptospira* infections in Queensland were from recreational exposure.¹¹

The isolation of *L. weilii* sv. Topaz from a bovine source may require further risk assessments to determine the risk posed to the domestic animal industries or workers in these industries in Australia. Additionally, further studies are required to determine what are the carriage rates and the level of disease caused by this serovar in Australian animal herds and wildlife.

FAFLP has been successfully used to examine the molecular epidemiology of *Leptospira* isolates.^{16,17} Using FAFLP we were able to demonstrate a high level of relatedness amongst 2 animal and 2 human isolates (LT1414 and LT952, LT722 and LT974). These molecular tools allow links to be made between the carriage of this serovar in animals and human disease.

In conclusion, we have described both the descriptive and molecular epidemiology of *L. weilii* sv. Topaz in Australia. This research provides evidence for the presence of this serovar in native, domesticated animals and humans, however the role and burden of this serovar in animal health and disease needs to be further defined by additional research. Further research is needed to establish the prevalence of this serovar in Australian native fauna and animal industries.

Acknowledgements

The authors would like to thank the staff of the Queensland Health Population Health Units and other submitting laboratories for their assistance in the completion of surveillance questionnaires. The authors also wish to thank Queensland Health and the Queensland Department of Primary Industries and Fisheries for their past and present support of leptospirosis research in Australia.

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