Leptospira weilii serovar Topaz, a new member of the Tarassovi serogroup isolated from a bovine source in Queensland, Australia

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This paper reports on a *Leptospira* isolate of bovine origin and its identification as belonging to a previously unknown serovar, for which the name Topaz is proposed. The isolate (94-79970/3) was cultured from bovine urine from a north Queensland dairy farm in Australia. Strain 94-79970/3 grew at 30 °C in Ellinghausen McCullough Johnson Harris (EMJH) medium but failed to grow at 13 °C in EMJH medium or in the presence of 8-azaguanine. Serologically, strain 94-79970/3 produced titres against the *Leptospira borgpetersenii* serovar Tarassovi, the reference strain for the Tarassovi serogroup; however, no significant titres to any other serovars within the serogroup were obtained. Using 16S rRNA and DNA gyrase subunit B gene analysis, strain 94-79970/3 was identified as a member of the species *Leptospira weilii*. We propose that the serovar be named Topaz, after the location where the original isolate was obtained. The reference strain for this serovar is 94-79970/3 (=KIT 94-79970/3=LT722).

Leptospirosis is a disease caused by members of the genus *Leptospira* and is considered to be an emerging zoonotic disease of global importance (Bharti *et al.*, 2003). In Australia, there have been 7507 notifications of the disease since 1934 and 57.6% of these notifications have occurred in the state of Queensland (Slack *et al.*, 2006a).

Two streams of taxonomy are concurrently used for *Leptospira*; species level identification based upon DNA–DNA hybridization and serological identification using the similarity of cell-wall antigens as determined by using the cross-agglutinin absorption test (CAAT). Seventeen species have been described (Brenner *et al.*, 1999; Levett *et al.*, 2006; Perolat *et al.*, 1998; Yasuda *et al.*, 1987) and over 200 serovars have been identified worldwide (Levett, 2001). In addition to the aforementioned taxonomy systems, serovars are grouped into serogroups. Although serogroups

have no official taxonomic status, they remain as a practical way of grouping serovars that share common antigens (Kmety & Dikken, 1993).

Ten serovars from within four species have been identified previously from within Australia with the majority of the serovars being isolated in the state of Queensland. These serovars include *Leptospira interrogans* serovars Australis, Zanon, Kremastos, Robinsoni, Broomi, Pomona, Szwajizak, *Leptospira kirschneri* serovar Valbuzzi, *Leptospira weilii* serovar Celledoni and *Leptospira meyeri* serovar Perameles (Kmety & Dikken, 1993).

This study reports the isolation and identification of a new *Leptospira* serovar isolated in Australia, using phenotypic, serological and molecular techniques. We determined that this is a unique serovar belonging to the species *Leptospira weilii*, with serological similarity with members of the Tarassovi serogroup. We propose that the serovar be named Topaz, after the locality of the dairy farm from which the original isolate was obtained.

The isolate, 94-79970/3, was obtained from bovine urine using a culture method as described previously (McClintock *et al.*, 1993). The isolate was forwarded to the WHO/FAO/OIE Collaborating Centre for Reference &

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Abbreviations: CAAT, cross-agglutinin absorption test; MAT, microscopic agglutination test.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of *Leptospira weilii* serovar Topaz strain 94-79970/3 are DQ483058 and DQ483059, respectively.

Research on Leptospirosis, Brisbane, Australia for further identification. Phenotypic characterization using growth at various temperatures and growth in 8-azaguanine was performed as described by Johnson & Rogers (1964) and Perolat *et al.* (1998).

Hyperimmune antiserum against strain 94-79970/3 was produced in rabbits using a method described by Graves & Faine (1970). Serological identification of the isolates was performed using the microscopic agglutination test (MAT) method with serovars representative of the major *Leptospira* serogroups (Faine *et al.*, 1999). The *Leptospira* serogroups tested included: Icterohaemorrhagiae, Javanica, Celledoni, Canicola, Ballum, Pyrogenes, Cynopteri, Autumnalis, Djasiman, Australis, Pomona, Grippotyphosa, Hebdomadis, Mini, Sejroe, Bataviae, Tarassovi, Panama, Shermani and Semeranga. Where a significant titre was found against a serogroup, screening was then done using all available serovars within that serogroup. CAAT was performed to determine whether the unknown isolate was similar to that serovar (Kmety *et al.*, 1970).

DNA extracts were prepared from strain 94-79970/3 and 16S rRNA and DNA gyrase subunit B (*gyrB*) gene amplification/sequencing was performed as described previously (Slack *et al.*, 2006b). Sequences from strain 94-79970/3 and representative sequences from members of the genus *Leptospira* were aligned using CLUSTAL W (Thompson *et al.*, 1994). Estimates of evolutionary divergence between sequences analyses were conducted using the Jukes–Cantor method in MEGA4 (Jukes & Cantor, 1969; Tamura *et al.*, 2007). Codon positions included were 1st+2nd+3rd+non-coding. All positions containing gaps and missing data were eliminated from the dataset. A total of 1290 positions were used in the 16S rRNA gene sequence analysis and a total of 405 positions for the *gyrB* gene sequence analysis. A phylogenetic tree was constructed from the aligned 16S rRNA gene sequences using the neighbour-joining method (Saitou & Nei, 1987), bootstrapped 1000 times and rooted using *Leptonema illini* serovar Habaki strain Habaki as an outgroup.

The isolate, 94-79970/3, was cultured from the urine of a heifer on a dairy farm near the town of Topaz on the Atherton Tableland in Queensland, Australia in August 1994. The heifer had two MATs performed prior to urine collection: a baseline test in April 1994 that was negative for all *Leptospira* serovars and a second test in June 1994, which gave a titre of 1:100 to the serogroup Tarassovi. The herd had been established 12 months prior to the study commencing and the leptospirosis vaccination history of the herd was unknown at the time of the study. This work was part of a University of Queensland, longitudinal study of patterns of *Leptospira* infections in north Queensland dairy herds with an emphasis on zoonotic infections. The laboratory support for the study was provided by Queensland Department of Primary Industries and Fisheries and Queensland Health.

When isolate 94-79970/3 was screened serologically against reference hyperimmune antisera of the common serogroups

found in Australia, titres were produced against the Tarassovi (1:1600) and Bataviae (1:100) serogroups. When the culture and hyperimmune sera for isolate 94-79970/3 were tested against the reference strains of the Tarassovi serogroup (Table 1), there were no significant reactions as compared with the control reaction of 94-79970/3 antisera vs 94-79970/3 culture. To confirm these results, CAAT reactions were performed against 94-79970/3 using *Leptospira borgpetersenii* serovar Tarassovi and *L. weilii* serovar Celledoni, as these two serovars have been previously identified in Australia (Slack *et al.*, 2006a) and gave less than 10% similarity (results not shown).

Phenotypically, strain 94-79970/3 grew at 30 °C, but failed to grow at 13 °C or in the presence of 8-azaguanine. Species identification was performed using 16S rRNA and *gyrB* gene sequencing. The 16S rRNA gene sequence similarity between strain 94-79970/3 and the 17 previously described *Leptospira* was found to be in the range 89–99.69%. *L. weilii* (99.69%), *L. borgpetersenii* (99.46%) and *Leptospira noguchii* (99.2%) showed the highest level of 16S rRNA gene similarity to strain 94-79970/3. As there is a high level of similarity between 16S rRNA genes of *Leptospira*, this result was confirmed using *gyrB* sequence analysis. The range of similarity using *gyrB* gene sequences was much greater than that using the 16S rRNA gene (79.7–100%), with *L. weilii* (100%) showing the highest level of similarity. Phylogenetic analysis using aligned 16S rRNA gene sequences showed that strain 94-79970/3 was placed within the radiation of the genus *Leptospira* and within the clade formed by the pathogenic *Leptospira*, which includes *L. weilii*.

The isolate was forwarded to the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Royal Tropical Institute, Amsterdam, The Netherlands, for confirmation and for storage within their culture collection. These data were presented to and recommended by the subcommittee on the taxonomy of *Leptospiraceae* (Levett & Smythe, 2006).

Short description of *Leptospira weilii* serovar Topaz

L. weilii serovar Topaz strain 94-79970/3 was isolated from a urine culture taken from a heifer in the locality of Topaz in far Northern Queensland, Australia. Phenotypically, *L. weilii* serovar Topaz strain 94-79970/3 grows at 30 °C in Ellinghausen McCullough Johnson Harris (EMJH) medium, but failed to grow at 13 °C in EMJH medium or in the presence of 8-azaguanine. The isolate was found to be a unique serovar as confirmed by the CAAT. Strain 94-79970/3 was identified as a member of the species *L. weilii* by 16S rRNA and *gyrB* gene sequencing. The reference strain for this serovar is 94-79970/3 and is held in the culture collections of the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Royal Tropical Institute, Amsterdam, The Netherlands (KIT 94-79970/3) and WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Western Pacific Region, Brisbane, Australia (94-79970/3=LT722).

Table 1. MAT results for *L. weilii* serovar Topaz strain 94-79970/3 against the cultures and antisera of the Tarassovi serogroup

Leptospira species	Serovar	Strain	Titre	
			94-79970/3 vs reference antisera	Reference culture vs 94-79970/3 antisera
<i>L. borgpetersenii</i>	Tarassovi	Perepelitsin	200	200
<i>L. borgpetersenii</i>	Kisuba	Kisuba	100	800
<i>L. santarosai</i>	Bakeri	LT79	1600	200
<i>L. santarosai</i>	Atlantae	LT81	400	Not performed
<i>L. borgpetersenii</i>	Guidae	RP 29	200	100
<i>L. santarosai</i>	Atchafalaya	LSU 1013	400	200
<i>L. santarosai</i>	Bravo	Bravo	800	800
<i>L. santarosai</i>	Rama	316	100	200
<i>L. borgpetersenii</i>	Tunis	P2/65	400	400
<i>L. inadai</i>	Kaup	LT64-68	<100	Not performed
<i>L. weilii</i>	Vughia	LT89-68	800	400
<i>L. santarosai</i>	Navet	TRVL 109873	200	3200
<i>L. borgpetersenii</i>	Kanana	Kanana	<100	Not performed
<i>L. santarosai</i>	Chagres	1913 K	<100	50
<i>L. santarosai</i>	Darien	637 K	Not performed	400
<i>L. santarosai</i>	Gatuni	1473 K	800	400
Unknown	Banna	A 31	Not performed	800
Unknown	Gengma	M48	1600	400
<i>L. weilii</i>	Langati	M 39090	Not performed	200
<i>L. weilii</i>	Mogdeni	Compton 746	Not performed	800
<i>L. borgpetersenii</i>	Yunxian	L 100	Not performed	800
<i>L. weilii</i>	Topaz	94-79970/3	>6400	>6400

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