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Leptospirosis in Tasmanian Devils (*Sarcophilus harrisii*) in Tasmania, 2008–12

Sarah J. Wynwood,[1,2,6] Mary-Anne Burns,[2] Glenn C. Graham,[1,3] Steven L. Weier,[4] David B. McKay,[1] Sarah Peck,[5] and Scott B. Craig[1,2,4] [1]Faculty of Science, Health and Education, University of the Sunshine Coast, Sippy Downs Drive, Sippy Downs, Queensland 4556, Australia; [2]WHO/OIE/FAO Collaborating Centre for Reference and Research on Leptospirosis, Queensland Health Forensic and Scientific Services, PO Box 594, Archerfield, Queensland 4108, Australia; [3]Chemical Analysis Unit, Queensland Health Forensic and Scientific Services, PO Box 594, Archerfield, Queensland 4108, Australia; [4]School of Biomedical Sciences, Queensland University of Technology, Brisbane 4001, Australia; [5]Department of Primary Industries, Parks, Water and Environment, 3rd Floor, 134 Macquarie Street, Hobart, Tasmania, 7000, GPO Box 44, Hobart 7001, Australia; [6]Corresponding author (email: sarah.wynwood@utas.edu.au)

ABSTRACT: In 2014, we performed a diagnostic study of leptospirosis in Tasmanian devil (*Sarcophilus harrisii*) samples collected between 2008 and 2012 from wild and captive animals. Tasmanian devil populations have been declining because of a facial tumor disease since the 1990s, with ongoing investigations examining potential causative agents. Identifying other causative pathogens that may contribute additively to their decline is important to preserve current and future populations. We tested 81 Tasmanian devil serum samples and two tissue samples using PCR, microscopic agglutination test (MAT), and microsphere immunoassay (MIA). We found evidence of leptospirosis in Tasmanian devil populations across a wide geographic range of Tasmania. Antibodies to serovars in the serogroup Javanica, which are not considered endemic to Australia, were identified in 10 Tasmanian devils using MAT. We also identified serovar Celledoni serologically using the immunoglobulin G MIA and detected *Leptospira* in one sample using PCR.

Key words: Conservation, *Leptospira*, marsupial, microbiology, pathology, Tasmania, wildlife disease.

Leptospirosis is a disease affecting humans and animals worldwide and is caused by organisms belonging to the diverse and serologically complex genus *Leptospira*. *Leptospira* includes 18 recognized species classified on the basis of DNA relatedness, and more than 300 serovars based on agglutinating lipopolysaccharide antigens (Victoriano et al. 2009). Previous studies of *Leptospira borgpetersenii* serovar Hardjobovis in Tasmania suggest that leptospirosis occurs widely throughout the beef and dairy industry (Corbould 1970).

Leptospirosis can range from asymptomatic to acute and fatal in both animals and humans, with severe forms of disease characterized by hepatic involvement, acute renal failure, carditis, and hemorrhagic syndrome (Yersin et al. 2000). In the acute phase, PCR and culture isolation from blood or tissue are the most reliable diagnostic tests because they determine the presence of leptospiral organisms. In the immune phase of the disease, serology is used to detect leptospiral antibodies. The microscopic agglutination test (MAT) is the current gold standard; however, technical difficulties with this test, in particular the reliance on a live antigenic panel and highly skilled operators and the arbitrary nature of the necessarily restricted size of the antigenic panel, have led to the development of the microsphere immunoassay (MIA), which promises improvements over the MAT (Wynwood et al. 2015).

Leptospirosis has been identified in native and nonnative animals in Tasmania. Corbould (1968) isolated serovar Icterohaemorrhagiae from brown rats (*Rattus norvegicus*) in Tasmania and found serologic evidence of the same serovar in greyhound dogs (*Canis lupus familiaris*) in the same area. In a serologic study of Tasmanian wildlife, including Gunn's bandicoots (*Perameles gunnii*), potoroos (*Potorous tridactylus*), wombats (*Vombatus ursinus*), European hares (*Lepus europaeus*), and fallow deer (*Dama dama*), Munday (1972) found evidence of *Leptospira interrogans* serovar Pomona, *Leptospira kirschneri* serovar Grippotyphosa, *L. borgpe-*

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TABLE 1. Leptospiral antigens used in a microscopic agglutination test screening panel to test Tasmanian devil (*Sarcophilus harrisii*) samples collected from Tasmania, 2008–12, and if the antigen is recognized as indigenous to Tasmania or exotic.

Leptospirosis antigen	Indigenous/exotic
Leptospira borgpetersenii serovar Arborea	Indigenous
Leptospira interrogans serovar Australis	Indigenous
L. borgpetersenii serovar Hardjobovis	Indigenous
L. interrogans serovar Zanoni	Indigenous
Leptospira weilii serovar Topaz	Indigenous
L. interrogans serovar Robinsoni	Indigenous
L. borgpetersenii serovar Tarassovi	Indigenous
L. interrogans serovar Kremastos	Indigenous
L. interrogans serovar Medanensis	Indigenous
L. interrogans serovar Szwajizak	Indigenous
L. interrogans serovar Pomona	Indigenous
L. interrogans serovar Copenhageni	Indigenous
L. interrogans serovar Canicola	Indigenous
Leptospira kirschneri serovar Bulgarica	Indigenous
L. kirschneri serovar Grippotyphosa	Indigenous
L. weilii serovar Celledoni	Indigenous
L. interrogans serovar Bataviae	Exotic
L. interrogans serovar Cynopteri	Exotic
L. interrogans serovar Panama	Exotic
L. interrogans serovar Shermani	Exotic
L. interrogans serovar Djasiman	Exotic
L. borgpetersenii serovar Javanica	Exotic

TABLE 2. *Leptospira* serogroup Javanica antigens (all exotic) used for serovar-specific testing of those Tasmanian devil (*Sarcophilus harrisii*) samples that were positive for serogroup Javanica in the original microscopic agglutination screening (Table 1).

Leptospira serogroup Javanica antigens
Leptospira borgpetersenii serovar Javanica
L. borgpetersenii serovar Poi
L. borgpetersenii serovar Sorexjalna
Leptospira weilii serovar Coxi
Leptospira meyeri serovar Sofia
L. borgpetersenii serovar Ceylonica
L. borgpetersenii serovar Menoni
Leptospira santarosai serovar Fluminensi
L. borgpetersenii serovar A85
L. borgpetersenii serovar Dehong
L. weilii serovar Menrun
L. borgpetersenii serovar Yaan
L. weilii serovar Mengma
L. borgpetersenii serovar Zhenkang
L. santarosai serovar Vargonicas
L. santarosai serovar Arenal
L. borgpetersenii serovar Kalimentani

tersenii serovar Hyos (now known as serovar Tarassovi), and *L. interrogans* serovar Icterohaemorrhagiae.

It is of great concern that Tasmanian devils (*Sarcophilus harrisii*), the largest remaining carnivorous marsupial since the extinction of the thylacine (*Thylacinus cynocephalus*), have recently been affected by devil facial tumor disease, which has reduced wild populations throughout Tasmania by more than 80% (McCallum and Jones 2006). To improve the long-term survival of these animals, further research into other potential diseases affecting their populations is needed. Herein we report evidence of leptospiral infections in Tasmanian devils.

Eighty-one serum samples and two kidney tissue samples collected from Tasmanian devils between 2008 and 2014 were provided by the Department of Primary Industries, Parks, Water and Environment, Tasmania. Samples were collected from seven areas (and 81 sites) in Tasmania: South (10 sites), Southeast (28 sites), North (nine sites), Northeast (21 sites), West Coast (one site), East Coast (10 site), and Northwest (two sites), from both wild and captive animals; however, individual living condition (wild vs. captive) for each devil was not provided. The two kidney tissue samples were provided from deceased devils at two sites.

All samples were tested with a quantitative real-time PCR using a TaqMan probe for the detection of pathogenic leptospires (Smythe et al. 2004) to identify acute infection. Results were reported as detected or not detected.

The current gold standard for total antibody, MAT (Faine 1982), was performed on 81 of the 83 samples. We used 22 leptospiral serovar cultures as antigens in a routine MAT screening panel (Table 1). A panel of antigens, all exotic to Australia, in serogroup Javanica was used in further, serovar-specific antibody MAT testing for those samples found positive

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TABLE 3. Titers for *Leptospira* spp. test results for microsphere immunoassay (MIA), microscopic agglutination test (MAT), and PCR for Tasmanian devil samples collected 2008–14 in Tasmania. Titers expressed as reciprocal of the highest dilution with a positive (reactive) result. All samples were sera except sample 82, which was a tissue (kidney) sample. Dashes indicate that no serum sample was available.

Sample no.	Area[a]	Year collected	MIA[b]	Serogroup MAT titer		PCR[c]
				Celledoni	Javanica	
16	NE	2008	NR	<50	50	ND
27	SE	2008	R (Celledoni)	<50	<50	ND
31	S	2008	NR	<50	50	ND
32	NE	2008	NR	<50	50	ND
47	NE	2012	NR	<50	100	ND
49	NE	2012	NR	<50	100	ND
55	SE	2012	NR	<50	100	ND
62	N	2012	NR	<50	400	ND
63	EC	2012	NR	<50	50	ND
65	N	2012	NR	<50	400	ND
82	N	2012	—	—	—	D

[a] NE = Northeast area; SE = Southeast; S = Southern; N = Northern; EC = East Coast.

[b] NR = not reactive; R = reactive.

[c] ND = not detected; D = detected.

for this serogroup during the original screen MAT (Table 2). The MAT is specific for serovars or closely related serovars, and results were reported as a titer with the end point being the final serum dilution with ≥50% leptospire agglutination. Titers ≥50 were deemed reactive, indicating past or present exposure.

We performed an MIA for immunoglobulin G (IgG) antibody only (Wynwood et al. 2015) on serum samples. The MIA uses magnetic, coated polystyrene beads, filled with fluorescent dyes allowing high-throughput multiplexing assays. For this purpose, we used 22 leptospiral antigens (Table 1). Because a Tasmanian devil–specific IgG–R-phycoerythrin (RPE) conjugate was not available, biotinylated protein A was used in place of a secondary antibody, followed by treatment with streptavidin-RPE to enable detection of fluorescence. Sample analysis was performed on the BioRad MagPix (BioRad Laboratories, New South Wales, Australia) multiplex reader. Results were recorded as reactive or nonreactive based on the mean fluorescent intensity (MFI). An MFI ≥1,000 was deemed reactive; results <1,000 were deemed nonreactive. An immunoglobulin M (IgM) antibody MIA was not performed as a Tasmanian devil–specific IgM-RPE conjugate was not available.

Table 3 shows the results of the diagnostic testing. The 10 antibody-reactive samples were from five of the seven regions of Tasmania. These samples had MAT-reactive titers of 50–400 for serovar Javanica, a serovar not previously regarded as endemic to Australia. Sample 27 was also reactive as measured by MIA and MAT and had low reactivity to serovar Celledoni, which has been shown to occur in Australia (Queensland Government 2014), though not shown to be present in Tasmania.

The relatively high MAT titers of some samples with serogroup Javanica prompted further investigation. Because there was an insufficient volume of serum available for further testing of three MAT-reactive samples, seven of the 10 serogroup Javanica MAT reactive samples were tested against the 17 available serovars in serogroup Javanica. There was cross-reactivity between serovars in this group (Table 4).

Leptospira spp. and leptospiral antibodies were detected in Tasmanian devils. Given the significant decrease in the population of Tasmanian devils (Hawkins et al. 2006), the

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TABLE 4. Titers for microscopic agglutination test–reactive (positive) Tasmanian devil serum samples tested against a range of Javanica serovars from the serogroup Javanica. Bold numbers denote results considered positive.

Sample serovar	Strain	Sample no.						
		47	49	55	62	63	65	82
Javanica	Veldrat Bataviae 46	**100**	**100**	**100**	**400**	**50**	**400**	**50**
Poi	Poi	**100**	**200**	**100**	**1,600**	**100**	**1,600**	**400**
Sorexjalna	Sorex Jalna	**100**	**200**	**100**	**1,600**	**100**	**1,600**	**200**
Coxi	Cox	<50	<50	<50	**400**	<50	<50	<50
Sofia	Sofia 874	<50	<50	**100**	**400**	<50	**400**	**100**
Ceylonica	Piyasena	<50	<50	<50	<50	<50	<50	<50
Menoni	Kerala	<50	<50	**50**	**400**	<50	**400**	<50
Fluminensi	Aa 3	<50	<50	<50	**200**	<50	**400**	<50
A85	A 85	<50	<50	<50	**100**	<50	**50**	<50
Dehong	De 10	<50	<50	<50	<50	<50	<50	<50
Menrun	A 102	<50	<50	<50	<50	<50	<50	<50
Yaan	80-27	<50	**200**	<50	<50	<50	<50	**50**
Mengma	S590	<50	<50	**100**	**200**	<50	**400**	<50
Zhenkang	L 82	**50**	**100**	**200**	**800**	<50	**800**	**50**
Vargonicas	24	<50	<50	<50	<50	<50	<50	<50
Arenal	MAVJ401	<50	<50	<50	**400**	<50	**800**	<50
Kalimentani	Amos	<50	<50	<50	<50	<50	<50	<50

history of marsupial extinction in Australia, and that leptospirosis causes clinical illness and death in other animal species in Australia (Miller et al. 2007), it is of concern that leptospirosis is present in devils. Animal disease management is increasingly recognized as an essential tool for the conservation of many species (Hess 1996), even though diseases are often viewed as a natural feature, serving to stabilize populations. However, the presence of additional threats can affect the ability of a population to recover. Because (to our knowledge) this is the first report of leptospirosis in devils, the burden of the disease (if any) is yet to be determined.

The MAT (and MIA) panel of antigens we used is routinely used in MAT testing for animals in Australia. In previous studies involving Tasmanian wildlife, the panel of leptospiral antigens in the MAT consisted of up to six serovars only (Corbould 1970; Munday 1972; Whyte and Burke 1973).

Although the MIA has the potential to distinguish IgG and IgM antibodies, and can therefore, in contrast to MAT, produce information about the temporal status of the infection, antibody class differentiation relies on the availability of secondary antibodies. In our study, the lack of available secondary antibodies meant that we could potentially detect only IgG using Protein A. Protein A is an immunoglobulin-binding protein and has binding sites for the Fc region of mammalian IgG. Although no studies have been performed to specifically determine the reactivity in Tasmanian devil samples, given the relatively broad phylogenetic range at which Protein A functions, we assumed that some reactivity was present. Given that previous work using human samples (Wynwood et al. 2015) indicated that the MIA was more sensitive than the MAT, the paucity of reactive samples in our study points to issues around the efficacy of Protein A as a secondary antibody when testing Tasmanian devil samples. It also suggests further work in relation to the development of the MIA using at least marsupial-specific secondary antibodies would be useful.

Upon initial screening, antibodies to *L. borgpetersenii* serovar Javanica were detected in 10 (12%) of the 81 Tasmanian devil samples

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by MAT. This particular serovar had not been identified as being endemic in Australia and therefore was not included in any previous MAT panel for testing Tasmanian wildlife, highlighting one of the problems with the MAT in using an arbitrary and necessarily restricted panel of antigens.

Originating from Java, Indonesia, serovar Javanica is most commonly associated with disease in rats (*Rattus* spp.) and transmitted to humans and other animals by exposure to rat urine (Natarajaseenivasan et al. 2011). Although serovar Javanica has been found in dairy cattle (*Bos taurus*) in India, as well as rats in Malaysia, there have been no confirmed cases of serovar Javanica in Australian animals (Natarajaseenivasan et al. 2011; Benacer et al. 2013).

Remaining sera from seven of 10 of the serovar Javanica MAT-reactive samples were tested by MAT against all available serovars (n=17) in the Javanica serogroup. Reactions were noted for serovars Poi and Sorexjalna, neither of which is endemic to Australia. These results indicate the presence of these serovars or a related uncharacterized serovar. Further work using isolation and culture would clarify this issue.

Antibodies to *Leptospira weilii* serovar Celledoni were identified by the MIA in only one case. Serovar Celledoni is associated with rats in Australia and has also been found in dairy cattle in Queensland, Australia (Queensland Government 2014). There is currently no evidence of serovar Celledoni being isolated in Tasmania. As the MIA in this study detects IgG, this result may indicate a past infection, cross-reactivity between serogroups, or an early immune response to a recent infection. Without paired-sample testing and IgM testing, it is not possible to determine whether these antibodies represent a recent or past infection.

The PCR-positive tissue sample was from a deceased Tasmanian devil that had clinical signs and symptoms of leptospirosis, including nephritis and kidney failure, prior to death, suggesting leptospirosis as a possible cause of death. The remainder of the devils tested showed no indication of leptospirosis.

One weakness of this study was that we were unable to isolate leptospires from the Tasmanian devil samples because of the age (up to 7 yr) and frozen storage conditions of these samples. There was no information regarding the living conditions of the Tasmanian devils (wild versus captive), so we were unable to determine any differences in disease spread and severity between wild and captive devils. Further research will enable a more comprehensive analysis of the serovars circulating in Tasmanian wildlife and ensure a better detection system to determine the impact of leptospirosis on wild and captive Tasmanian devil populations.

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