

Survey of Local Fauna from Endemic Areas of Northern Queensland, Australia for the Presence of *Mycobacterium ulcerans*

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Abstract

Background: Buruli ulcer (BU), regionally known as the Daintree ulcer or Bairnsdale ulcer is caused by the environmental pathogen *Mycobacterium ulcerans* (MU). This disease is characterized by extensive and painless necrosis of skin and soft tissue with the formation of large ulcers and has been reported in >33 countries worldwide. This organism is geographically restricted and in Australia, the disease has been reported primarily in coastal Victoria and the Mossman–Daintree areas of northern Queensland. Australia is the only country where nonhuman cases of BU have been confirmed. The common ringtail possums and mountain brushtail possums have been suggested as potential animal reservoirs of MU in coastal Victoria, Australia. The exact mode of transmission of this disease remains unknown. **Methods:** In this study, we surveyed local fauna from endemic areas of northern Queensland, Australia, for the presence of MU in scat samples. We collected 140 bandicoot, four white-tailed rats, and two possum scat samples from 56 overnight trapping sessions. Samples were examined for the presence of MU DNA by the polymerase chain reaction. **Results:** Two out of five samples did not contain a sufficient amount of DNA to detect IS2606 and the ketoreductase B (KR) domain of the mycolactone polyketide synthase gene, which is represented by higher cycle threshold (Ct) values for IS2404 shown in table below. Despite of having desired Ct values for IS2404, one IS2404 positive sample possibly contained DNA of closely related *M. ulcerans* subspecies with lower copy number of IS2606 that do not commonly cause disease in human. All three targets: IS2404, IS2606 and KR were detected from the remaining two scat samples. **Conclusion:** We confirm the presence of *M. ulcerans* DNA in the scat samples collected from a Buruli ulcer endemic region of Northern Queensland, Australia.

Keywords: Australia, *Mycobacterium ulcerans*, native mammals, northern Queensland

INTRODUCTION

Buruli ulcer (BU), locally known as Daintree ulcer in northern Queensland, Australia, is a nontuberculous infection of the skin caused by *Mycobacterium ulcerans* (MU). The disease is rarely fatal if diagnosed and treated with appropriate antibiotics in a timely fashion. Any delay in treatment may lead to the requirement for surgical intervention. Till date, the disease has been reported from >33 countries in Africa, the Americas, Asia, and the Western Pacific.^[1] The majority of foci of BU are located in West Africa^[2] with other foci in Australia,^[3,4] Peru,^[5] Papua New Guinea,^[6] and Japan.^[7] Within these locations, the disease is geographically restricted.

Australia is the only developed country where substantial transmission of MU has been recorded. Foci of BU infection have been found in the tropical Far North Queensland,^[8] the Capricorn coast region of central Queensland,^[9] the

Northern Territory,^[10] and temperate coastal Victoria.^[3] In Australia, the cases of BU have also been recorded in animals, including koalas (*Phascolarctos cinereus*),^[11] common ringtail possums (*Pseudocheirus peregrinus*), mountain brushtail possum (*Trichosurus cunninghami*),^[12] horses,^[13] dogs,^[14] an alpaca,^[15] and a cat.^[16] All of these recordings were located in the vicinity of human cases of BU. Unlike Australia, not a single study in Africa has reported the cases in nonhuman species or the presence of MU-positive DNA in animals,

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suggesting that transmission dynamics may be different in Africa and Southern Australia or, alternatively, a host animal is yet to be identified in Africa. A study conducted by Fyfe *et al.*, between 2007 and 2009 in an endemic area of BU in Australia, found 38% of ringtail possums and 24% of brush tail possums with laboratory-confirmed MU lesions DNA.^[12] However, only 1% of possums' samples from nonendemic areas were positive for MU DNA. They suggested terrestrial mammals such as the possums may be potential reservoirs of MU in endemic areas of Victoria, Australia. A similar study conducted in BU endemic villages of Ghana has ruled out the possibility of domestic animals as a reservoir for MU in endemic regions of West Africa.^[17] Another endemic area for MU in Australia is far north Queensland in an area extending from the Daintree River and Forest Creek in the north to Mossman in the south.^[8] Recently, there was a report of the presence MU in two bandicoot (*Isodon macrourus*) scat samples collected in this region.^[18] The isolated detection of MU in a tropical endemic region in Australia highlighted the need to examine a larger sample size to gauge the significance of the role of native terrestrial mammals in the ecology of BU in northern Queensland. The aim of the current study was to survey samples from local fauna from endemic areas of northern Queensland, Australia, for the presence of MU DNA.

METHODS

Study site and sample collection

Geographic Information System mapping of human cases of BU in northern Queensland from 2009 to 2013 was used as the basis for the selection of study sites. For ease of sampling and analysis, BU endemic areas of northern Queensland were allocated into Region-1 covering the areas of Miallo to lower Daintree, including Wonga/Wonga Beach area, Region-2 covering Forest Creek area, and Region-3 covering upper Daintree area. Figure 1 represents the distribution of human cases of BU in northern Queensland from 2009 to 2013 and the sites from which samples were collected.

Trapping and sampling of bandicoots

Animal ethics approval was obtained from the Animal Ethics Committee of JCU (Ethics approval number: A2193). A permit to trap native animals for scientific research and educational purposes were obtained from the Department of Environment and Heritage Protection, Queensland, Australia (Permit number: WISP16539915). Cage traps, especially designed for small native mammals, baited with balls of rolled oats and peanut butter were used for trapping animals. Fifty-six overnight trapping sessions, with each session utilizing at least eight traps, were conducted from March 2016 to February 2018. Of the 56 trapping sessions, 22 were conducted at eight sites within Region-1, 16 at sites within Region-2, and 18 at sites of Region-3. All traps were numbered, flagged, and recorded with the global positioning system coordinates to avoid misplacement. Animal traps were set around 2 h before nightfall in each site and checked at first light for trapped animals. Once captured, animals were transferred into a cloth

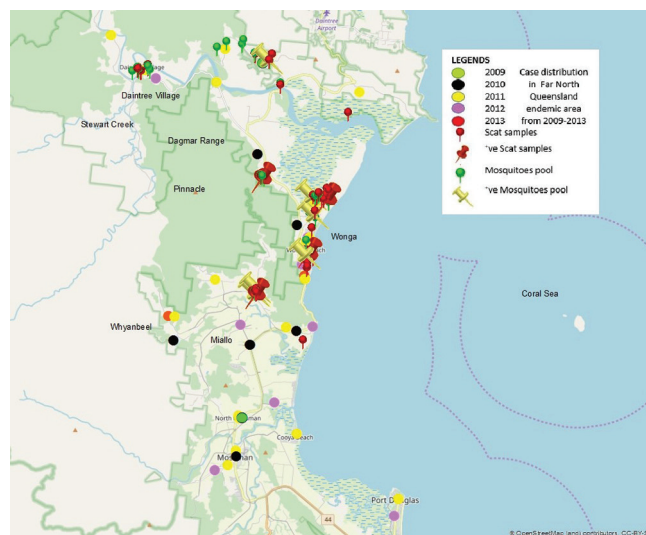


Figure 1: Distribution of human cases of buruli ulcer in buruli ulcer endemic areas of northern Queensland, Australia and location of animal traps. This figure was created using base layer obtained from OpenStreetMap. <https://www.openstreetmap.org>

bag for sample collection. In situations where animals passed scat in the trap, this was collected otherwise a cloacal swab was collected. Trapped animals were examined for external lesions and swabs were obtained from the lesions, if found. The captured animals were released on the same day, and at the same location once the samples and data were collected. To identify any event of recapture, fur clipping at the base of the tail was performed. Surrounding areas were screened for additional scats and collected. Scats were identified by visual identification and with the aid of a scat identification manual.^[19]

DNA extraction

DNA was extracted from samples using the FastPrep Instrument (MP Biomedicals, Solon, OH, USA) as per the manufacturer's instruction with FastDNA SPIN Kit for Soil (MP Biomedicals). Extracted DNA was stored at -20°C .

Detection of *Mycobacterium ulcerans* DNA

Two prevalidated semi-quantitative real-time polymerase chain reaction (PCR) assays targeting the insertion sequences IS2404, IS2606 and a sequence encoding the ketoreductase (KR) B domain, KR were used to assess DNA extracts for the presence of MU DNA.^[20] During each PCR run, three negative controls (double-deionized water and MilliQ) and three positive controls (purified MU DNA obtained from the Victorian Infectious Disease Reference Laboratory) were used to ensure assay validity. All of the extracted DNA samples were initially screened for the MU insertion sequence element IS2404. Samples positive for IS 2404 were reanalyzed by a second quantitative PCR for the detection of two additional regions, namely, IS2606 and KR B domain in the genome of MU. This screening process has been validated for environmental samples by Fyfe *et al.* and differentiates MU from other *Mycobacteria* that encode mycolactone based on the difference in Ct values

between IS2606 and IS2404 (ΔCt [IS 2606– IS 2404]).^[20] The presence of MU DNA in the samples was confirmed if all three targets (IS 2404, IS 2606, and KR) with expected ΔCt values were detected.

RESULTS

Trapping and sampling of bandicoots

From 56 overnight trapping sessions, each session having at least eight traps, a total of 92 animals were trapped. Of these, 86 were identified as bandicoot (*I. macrourmus*), four were white-tailed rats (*Uromys caudimaculatus*), and two common ringtail possums (*P. peregrinus*). Scat samples were collected from all trapped animals. An additional 54 bandicoot scat samples were collected after screening the proximity of the study site, providing a total of 140 bandicoot scat samples, four white-tailed rat scat samples, and two possums scat samples. One bandicoot trapped at Region-1 near (near South Wonga) had a visible ulcer on the back and nose [Figure 2]. Ulcer swabs and scat specimen were collected and tested for the presence of MU. Those samples were negative for MU. The samples were subsequently transported to the MU reference laboratory at the Victorian Infectious Diseases Reference Laboratory for culture and reanalysis. None of the samples from the bandicoot ulcer were positive for MU DNA. No other animals were found to have ulcers.

Out of 146 scat samples, five bandicoot samples were positive for IS2404 [Table 1]. All of the positive scats were from Region-1. Of the five scats positive for IS 2404, three scats did not contain sufficient DNA to detect IS2606 and KR, which



Figure 2: Bandicoot with ulcer trapped from South Wonga

Table 1: Polymerase chain reaction analysis of bandicoot scat for *Mycobacterium ulcerans* collected from endemic areas of northern Queensland, Australia

Samples	qPCR analysis Ct values			
	IS2404	IS2606	IS2606-2404	KR
Bandicoot scat ¹	38.6	ND	ND	ND
Bandicoot scat ²	30.0	ND	ND	ND
Bandicoot scat ³	31.3	32.9	1.6	27.6
Bandicoot scat ⁴	36.1	ND	ND	ND
Bandicoot scat ⁵	31.0	32.3	1.3	32.4

¹ and ²Collected in February 2017, ³Collected in March 2017, ⁴ and ⁵Collected in August 2017. ND: Not detected, qPCR: Quantitative polymerase chain reaction, Ct: Cycle threshold, KR: Ketoreductase

require lower cycle threshold values than IS 2404 [Table 1]. All three targets were detected from the remaining two scats.

DISCUSSION

Identification of transmission pathway (s) and potential environmental reservoirs of MU is essential for effective surveillance and control of BU. The occurrence of disease and the geographical distribution of cases have been clearly linked with the aquatic ecosystems.^[21,22] Nevertheless, the exact mode of transmission of MU still remains unknown. With the previously recorded detection of MU DNA in the scat and laboratory-confirmed MU skin lesions in ringtail and brushtail possums trapped from high- and low-BU endemic regions in Southern Australia^[12,23] and the detection of MU DNA in bandicoot scat in a BU endemic area of northern Queensland,^[18] we conducted an extensive survey of local fauna from the main endemic area of north Queensland, Australia, for the presence of MU.

A survey in Victoria, Australia, has led to the suggestion that MU-infected possums are a potential animal reservoir of MU. These animals may also play a role in the maintenance of the organism in the environment of BU-endemic regions for the onset of human cases of BU.^[12] Fyfe *et al.* found a strong correlation between BU endemicity of a region and detection of MU DNA in possums feces.^[12] An environmental study conducted in Benin has shown a similar correlation, where the proportion of MU DNA in aquatic insects reflected the endemicity of human cases of BU in the same region.^[24] Similarly, recent work conducted by our team in the study site has shown a low level of MU DNA in the mosquito populations (unpublished data). The low levels may reflect the decrease of incidence of human cases of BU in the region at the time of sampling.

There has been a low incidence of human cases of BU in northern Queensland, Australia, since the largest recorded outbreak in 2011–2012, where >60 cases were reported.^[4] The average reported rate over the 15 years period from 2002 to 2016 was 0.2 cases/100,000 population per year.^[25]

The wet season in northern Queensland occurs from November to December and continues up to April, and the dry season starts from May and continues up to October to November. It is well-described that outbreaks of human cases of BU in northern Queensland are linked with heavy rainfall and flooding. The current survey was conducted from March 2016 to February 2018, covering different seasonal conditions with 56 overnight trapping sessions. The rainfall was average during the sampling period. Out of five MU DNA-positive bandicoot scats, three scats were collected during the wet season, and the remaining two were collected in the dry season. Most of the cases of Daintree ulcer in northern Queensland occur at the end of wet season.^[25] The estimated mean incubation period of Daintree ulcer is 4.8 months,^[26] making it more likely that transmission occurs in the wet season and the disease is evident once the wet season ends.

Despite the large-scale trapping of native mammals and testing of bandicoot feces in endemic areas of northern Queensland over both wet and dry seasons, low number of bandicoot feces was found positive for MU DNA. None of the trapped animals had any laboratory confirmed MU lesions. Conversation with local veterinary practices revealed that none had seen any small animals with any sort of suspicious visible ulcer in their practice in this region. All these findings indicate the presence of only a low amount of the pathogen in the environment, which is reflected by the low numbers of human cases of BU in northern Queensland in recent years.^[4] A finding by Roltgen *et al.* in northern Queensland, Australia, of two MU positive bandicoot scats, involved samples that were collected soon after 2011–2012 outbreak, when the transmission was thought to be occurring and the pathogen may have been more prevalent in the environment.^[18]

A study conducted by Steffen and Freeborn reported that most of the cases during the 2011–2012 outbreak in northern Queensland were from Wonga and the Wonga beach area, referred to as Region-1 in the study.^[4] Out of 146 scat samples collected in the current study, five MU positive samples were from this region. In a separate study conducted by our team, we found seven pools of mosquitoes positive for MU DNA collected from the same study site (unpublished data).

Detection of MU DNA in bandicoot scat in northern Queensland in this study supports earlier reports from northern Queensland and Victoria in Australia.^[12,18] Both studies suggest the likelihood of detection of MU positive samples if samples are collected during an epidemic period. Because outbreaks of BU are linked with aquatic ecosystems, we suggest future studies in this region should include sampling of the aquatic environment.

CONCLUSION

This study confirms the presence of MU in the scat samples collected from a BU endemic region of northern Queensland, Australia. We suggest that there is higher possibility of detection of MU positive scats if the samples are collected soon before the peak endemic, when the transmission cycle is occurring and the organisms are maintaining their existence in the environment.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- World Health Organisation. Global Health Observatory (GHO) Data. Available from: https://www.who.int/gho/neglected_diseases/buruli_

- ulcer/en/. [Last accessed on 2018 Dec 13].
- Asiedu K, Scherpbier R, Raviglione M, Mario C; World Health Organization. Buruli ulcer: *Mycobacterium ulcerans* infection. Geneva: World Health Organization; 2000.
- Johnson PD, Aзуolas J, Lavender CJ, Wishart E, Stinear TP, Hayman JA, *et al.* *Mycobacterium ulcerans* in mosquitoes captured during outbreak of buruli ulcer, Southeastern Australia. *Emerg Infect Dis* 2007;13:1653-60.
- Steffen CM, Freeborn H. *Mycobacterium ulcerans* in the daintree 2009-2015 and the mini-epidemic of 2011. *ANZ J Surg* 2018;88:E289-93.
- Guerra H, Palomino JC, Falconi E, Bravo F, Donaires N, Van Marck E, *et al.* *Mycobacterium ulcerans* disease, Peru. *Emerg Infect Dis* 2008;14:373-7.
- Igo JD, Murthy DP. *Mycobacterium ulcerans* infections in Papua New Guinea: Correlation of clinical, histological, and microbiologic features. *Am J Trop Med Hyg* 1988;38:391-2.
- Kazumi Y, Ohtomo K, Takahashi M, Mitarai S, Sugawara I, Izumi J, *et al.* *Mycobacterium shinshuense* isolated from cutaneous ulcer lesion of right lower extremity in a 37-year-old woman. *Kekkaku* 2004;79:437-41.
- Steffen CM, Smith M, McBride WJ. *Mycobacterium ulcerans* infection in North Queensland: The 'daintree ulcer'. *ANZ J Surg* 2010;80:732-6.
- Francis G, Whitby M, Woods M. *Mycobacterium ulcerans* infection: A rediscovered focus in the capricorn coast region of central Queensland. *Med J Aust* 2006;185:179-80.
- Radford AJ. *Mycobacterium ulcerans* in Australia. *Aust N Z J Med* 1975;5:162-9.
- Mitchell PJ, McOrist S, Bilney R. Epidemiology of *Mycobacterium ulcerans* infection in Koalas (*Phascolarctos cinereus*) on Raymond Island, Southeastern Australia. *J Wildl Dis* 1987;23:386-90.
- Fyfe JA, Lavender CJ, Handasyde KA, Legione AR, O'Brien CR, Stinear TP, *et al.* A major role for mammals in the ecology of *Mycobacterium ulcerans*. *PLoS Negl Trop Dis* 2010;4:e791.
- van Zyl A, Daniel J, Wayne J, McCowan C, Malik R, Jelfs P, *et al.* *Mycobacterium ulcerans* infections in two horses in South-Eastern Australia. *Aust Vet J* 2010;88:101-6.
- O'Brien CR, McMillan E, Harris O, O'Brien DP, Lavender CJ, Globan M, *et al.* Localised *Mycobacterium ulcerans* infection in four dogs. *Aust Vet J* 2011;89:506-10.
- O'Brien C, Kuseff G, McMillan E, McCowan C, Lavender C, Globan M, *et al.* *Mycobacterium ulcerans* infection in two alpacas. *Aust Vet J* 2013;91:296-300.
- Elsner L, Wayne J, O'Brien CR, McCowan C, Malik R, Hayman JA, *et al.* Localised *Mycobacterium ulcerans* infection in a cat in Australia. *J Feline Med Surg* 2008;10:407-12.
- Tobias NJ, Ammisah NA, Ahortor EK, Wallace JR, Ablordey A, Stinear TP. Snapshot fecal survey of domestic animals in rural Ghana for *Mycobacterium ulcerans*. *PeerJ* 2016;4:e2065.
- Röltgen K, Pluschke G, Johnson PDR, Fyfe J. *Mycobacterium ulcerans* DNA in bandicoot excreta in buruli ulcer-endemic area, Northern Queensland, Australia. *Emerg Infect Dis* 2017;23:2042-5.
- Triggs B. Tracks, Scats and Other Traces: A Field Guide to Australian Mammals. South Melbourne: Oxford University Press; 2004.
- Fyfe JA, Lavender CJ, Johnson PD, Globan M, Sievers A, Aзуolas J, *et al.* Development and application of two multiplex real-time PCR assays for the detection of *Mycobacterium ulcerans* in clinical and environmental samples. *Appl Environ Microbiol* 2007;73:4733-40.
- Marion E, Landier J, Boisier P, Marsollier L, Fontanet A, Le Gall P, *et al.* Geographic expansion of buruli ulcer disease, Cameroon. *Emerg Infect Dis* 2011;17:551-3.
- Wagner T, Benbow ME, Brenden TO, Qi J, Johnson RC. Buruli ulcer disease prevalence in Benin, West Africa: Associations with land use/cover and the identification of disease clusters. *Int J Health Geogr* 2008;7:25.
- Johnson PD, Lavender CJ. Correlation between buruli ulcer and vector-borne notifiable diseases, Victoria, Australia. *Emerg Infect Dis* 2009;15:614-5.
- Portaels F, Silva MT, Meyers WM. Buruli ulcer. *Clinics in*

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- dermatology 2009;27 (3):291-305.
25. State of Queensland [Queensland Health]. *Mycobacterium ulcerans* in Queensland, 2002-2016. State of Queensland; 2017.
26. Loftus MJ, Trubiano JA, Tay EL, Lavender CJ, Globan M, Fyfe JAM, *et al.* The incubation period of buruli ulcer (*Mycobacterium ulcerans* infection) in victoria, Australia – Remains similar despite changing geographic distribution of disease. PLoS Negl Trop Dis 2018;12:e0006323.