

1 *Systematic Review*

2 **Potential Animal Reservoir of *Mycobacterium* 3 *ulcerans*: Systematic Review**

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15 **Abstract:** *Mycobacterium ulcerans* is the causative agent of the Buruli ulcer, also known, in Australia,
16 as Daintree ulcer or Bairnsdale ulcer. This destructive skin disease is characterized by extensive and
17 painless necrosis of the skin and soft tissue with the formation of large ulcers, commonly on the leg
18 or arm. To date, 33 countries with tropical, subtropical and temperate climates in Africa, the
19 Americas, Asia and the Western Pacific have reported cases of Buruli Ulcer. The disease is rarely
20 fatal, although it may lead to permanent disability and/ or disfigurement if not treated appropriately
21 or in time. It is the third most common mycobacterial infection in the world after tuberculosis and
22 leprosy. The precise mode of transmission of *M. ulcerans* is yet to be elucidated. Nevertheless, it is
23 possible that the mode of transmission varies with different geographical areas and epidemiological
24 settings. The knowledge about the possible route of transmission and potential animal reservoir of
25 *M. ulcerans* is poorly understood and still remains patchy.

26

27 We conducted a systematic review with selected key words on PubMed and INFORMIT databases
28 to aggregate available published data on animal reservoirs of *M. ulcerans*. After certain inclusion
and exclusion criteria, a total of 17 studies were included in the review. A variety of animals, e.g.
rodents, shrews, possums (ringtail and brush tail), horses, dogs, alpacas, koalas and Indian flap-
shelled turtles have been recorded as being infected with *M. ulcerans* around the world.

29

30 The majority of studies included in this review identified animal reservoirs, either aquatic or
31 terrestrial, as predisposing for the emergence and reemergence of *M. ulcerans* infection. Taken
32 together, the selected studies in this systematic review and discussed so far, it is clear that exotic
wildlife, aquatic animals and native mammals play a significant role as reservoirs for *M. ulcerans*.

33

34 **Keywords:** keyword 1. *Mycobacterium ulcerans*, 2. Animal reservoir; 3. Transmission

35

36 **1. Introduction**

37 Sir Albert Cook, a British missionary doctor appointed at the Mengo Hospital in Kampala, Uganda
38 first noted the skin ulcer caused by *Mycobacterium ulcerans* in 1896. Later, in the late 1930's, two
39 general practitioners, Dr. J. R. Searl and D. G. Alsop, working in rural Victoria, noticed a group of
40 cases of mysterious skin ulcers around the town of Bairnsdale [1]. The cases were not published in
41 the literature at the time and the causative organism was not identified or characterized. Professor
42 Peter MacCallum and his colleagues first provided the detailed description of the disease in 1948
43 using presentation data of six patients at Bairnsdale district, near Melbourne, Australia. They were
44 the first to isolate *M. ulcerans* as the causative organism of the mysterious skin ulcer [2]. The first large
cluster of *M. ulcerans* infection was identified in the Buruli County of Uganda (now called
Nakasongola District) in 1960's and the disease was termed "Buruli Ulcer" thereafter [3].

45 There have been several known outbreaks of Buruli Ulcer (BU) around the world and each outbreak
46 has its own unique characteristics in terms of epidemiology and the animals reported to be involved
47 in transmission [4, 5]. The World Health Organization (WHO) has classified BU as a neglected
48 tropical disease [6]. Presently, BU has been reported (but not always microbiologically confirmed) in
49 more than 30 countries spread over Africa, the Americas, Asia, and Oceania [7]. Fyfe and colleagues
50 from Australia suggested the terrestrial mammals as reservoirs for *M. ulcerans* [8]. Australia is the
51 only developed country with significant local transmission of BU with foci of infection in tropical Far
52 North Queensland [9, 10], the Capricorn Coast region of central Queensland [11], the Northern
53 Territory [12] and temperate coastal Victoria [11]. Several cases of BU have been described in both
54 native wildlife and domestic mammal species in Australia such as koalas (*Phascolarctos cinereus*) [13,
55 14], common ringtail possums (*Pseudocheirus peregrinus*) [8, 15], a mountain brushtail possum
56 (*Trichosurus cunninghami*) [5, 8, 15], two horses [16], an alpaca [17], four dogs [18] and a cat [19].
57 Recent research in Victoria, Australia, has suggested the transmission of infection by the mosquito
58 and possums with chronic BU as an important environmental reservoir of *M. ulcerans* in Victoria [8].

59

60 2. Materials and Methods

61 The guidelines developed by the Centre for Review Dissemination (CRD) - PRISMA guidelines
62 - was used as the methodology for the systematic review [20]. A review protocol was registered with
63 PROSPERO international prospective register of systematic reviews, which can be viewed online [21].
64 The systematic literature review was conducted using online databases MEDLINE and INFORMIT
65 to aggregate all the published literature. Initially, MEDLINE was used to retrieve all the scientific
66 information concerning the research topic. INFORMIT was searched with same search strategies
67 adopted for Medline. The following key words were chosen after a series of trial searches in order to
68 ensure an adequate number of relevant articles were reviewed: (Buruli OR "Mycobacterium ulcerans")
69 AND (Host OR Vector OR Reservoir OR Animal). The title and abstract of each of the articles were
70 initially scanned to ensure that the included articles met the aim and scope of the systematic review.
71 Articles which were deemed irrelevant to the aim of this systematic review or out of the research
72 scope were excluded. For those articles which were not clear by the title and abstract, the full text was
73 retrieved and further analyzed in order to determine if they met the inclusion and exclusion criteria
74 below. The studies that reported only experimental or laboratory exposure of *M. ulcerans* in animals
75 were excluded. The search strategy exclusively focused on potential animal reservoirs, not the vectors.
76 The detection of the causative agent had to be confirmed by culture of bacteria and/or PCR. To be
77 considered positive a sample needed to be positive for IS 2404 and confirmed by KR and IS 2606.
78 There were no language restrictions. Studies were published between January 1985 and the date the
79 searches were included. Risk of bias was assessed by one reviewer on the basis of independent factors
80 such as sample size, location and nature of infection.

81 3. Results

82 3.1. Results of the literature search and method of inclusion:

83 The total number of discovered articles in MEDLINE database was 301. Two hundred and sixty-five
84 articles were excluded after reading the title and abstracts as they were not relevant to the research
85 question. Full texts of thirty-six studies were retrieved in Portable Document Format (PDF) for further
86 analysis. Of these remaining 36 studies, 19 were excluded as they clearly did not meet inclusion
87 criteria (i.e. were review articles, focused on vector rather than on animal reservoir, pertained to
88 laboratory or experimental exposure). The remaining 17 studies from PubMed database were
89 included for systematic review. There were no additional articles in INFORMIT that did not appear
90 in the initial Medline search results. The flow chart for study selection process is shown in figure 1.

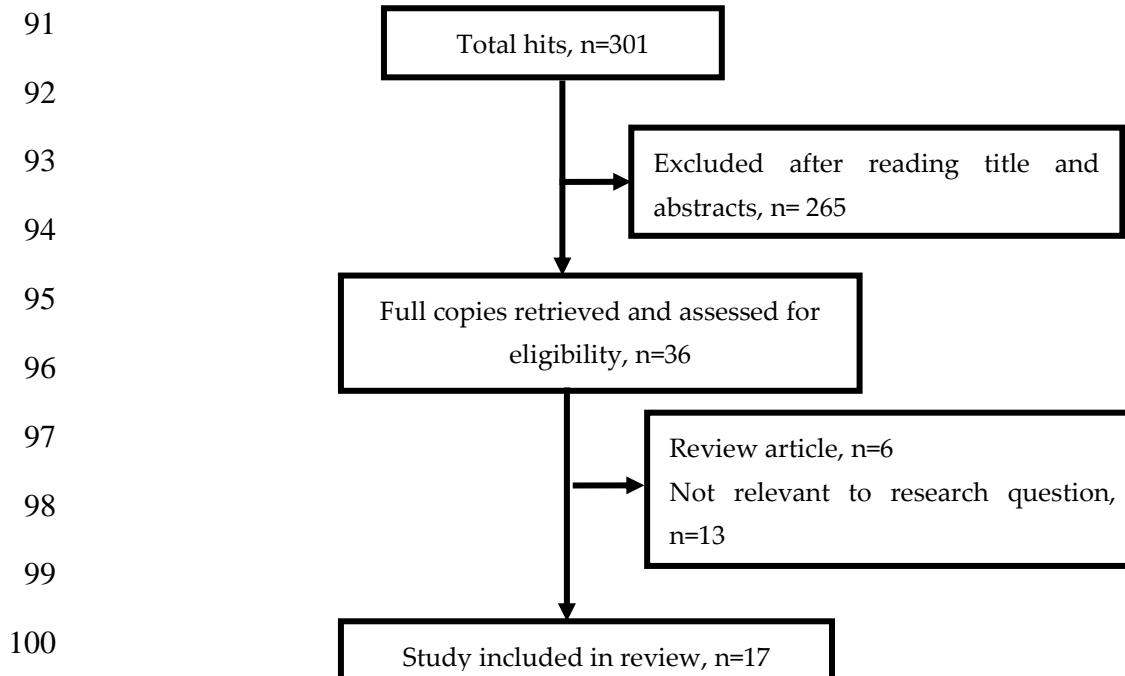


Figure 1. Flow chart of study Selection Process

3.2. Basic characteristics of selected studies:

Out of the 17 included studies, ten were conducted in Australia, two in Ghana and one was conducted in each of Ivory Coast, North America, United States, Benin and Japan. The basic characteristics of selected studies for review are shown in table 1 below.

Author and year	Sample and Sample Size	Collection year, Location and Setting	Detection method, Result Or <i>M. ulcerans</i> positive signal
Roltgen, Pluschke, Johnson, & Fyfe, 2017 [10]	102 environmental samples: 55 from soil /vegetation; 35 from insects or small insects pool and 12 from animal excreta	Sep 2013 Northern Queensland, Australia	RT-PCR IS2404 positive:1 soil specimen: 2 bandicoot feces, one individual mosquito and 1 pool of 2 mosquitoes IS2606 and KR (Ketoreductase) positive: 2 bandicoot feces and pool of two mosquitoes
Tobias et al., 2016 [22]	180 Fecal specimen from dominant domestic animals (Ovine, Porcine, Avian, Reptiles, Canine)	Sep 2013 BU (Buruli Ulcer) 4 endemic and one non-endemic villages of Ghana	RT-PCR IS2404 positive: 2/86 Ovine; 1/69 avian : 1/16 Reptiles IS2606 and KR (Ketoreductase) positive: All negative
Tian, Niamke, Tissot-Dupont, & Drancourt, 2016 [23]	496 environmental samples: 100 from soil (endemic n=50 and non-endemic n=50); 200 from stagnant water (endemic n=100 and non-endemic	Jun-Oct 2014 Ivory Coast, West Africa	RT-PCR 43 samples with at least one positive IS2404 and KR Out of 43, only 10 positive for both IS2404 and KR, IS 2606 not performed: 7 water

	n=100); 100 from plants (endemic n=50 and non-endemic n=50) and 96 animal feces (<i>Thryonomys swinderianus</i> (agouti) stools) (endemic n=48 and non-endemic n=48)		specimen; 2 <i>T. swinderianus</i> (agouti) feces and one soil specimen
Carson et al., 2014 [5]	Fecal Sample: 216 Common ringtail possums and 6 common brushtail possums	South-East Australia, State Victoria	RT-PCR targeting IS2404, IS2606 and KR 20 Common ringtail possums and 4 common brushtail possums
O'Brien et al., 2014 [15]	69 possums (ringtail and brushtail) trapped at Point Lonsdale: Fecal samples: 57; Blood samples: 63; Buccal swab: 67; Urine sample: 16; Pouch swab: 15; Cloacal swab: 20 69 fecal samples from 15 Mountain Brushtail Possums	1998-2011 Victoria, Australia	RT-PCR targeting IS2404, IS2606 and KR Point Lonsdale: Positive: Fecal sample: 12 (25%); Blood sample: 0; buccal swab: 7 (16%); Urine sample: 0; pouch swab: 3 (20%) Bellbird Creek: Positive: 4 Mountain Brushtail Possums (27%)
C. O'Brien et al., 2013 [17]	Case Report: Two alpacas (<i>Vicugna pacos</i>) ulcerated tissue	Case 1: Sept 1997 Case 2: May 2011 Victoria, Australia	RT-PCR targeting IS2404, IS2606 and KR positive
Willson et al., 2013 [24]	587 Fish representing 13 genera and 17 species and 351 Amphibians representing 10 genera: external swab	2008-2009 Ghana, West Africa	RT-PCR targeting IS 2606 and KR not performed. Not confirmed
C. R. O'Brien et al., 2011 [18]	Case report: Case 1: 14 months old female Kelpie Case 2: 3 years old female Kelpie Case 3: 6 years old male Whippet Case 4: 3 years old male Koolie	2011 Victoria, Australia	RT-PCR targeting IS2404, IS2606 and KR All 4 dogs positive for <i>M. ulcerans</i>
Sakaguchi et al., 2011 [25]	Case report; Indian flap-shelled turtle, <i>Lissemys punctata punctata</i>	Imported from India to aquarium in Japan	PCR assays targeting the <i>rpoB</i> gene: unable to differentiate <i>M. ulcerans</i> from mycolactone producing <i>M. marinum</i> (MPMM)
Fyfe et al., 2010 [8]	589 fecal samples from ringtail possums and 250 samples from brushtail possums.	2007-2009 Vicroria, Australia	RT-PCR targeting IS2404, IS2606 and KR <i>M. ulcerans</i> DNA detected in 43% of ringtail possum and

	Life trapping: 42 ringtail possums and 21 brushtail possums		29% of brushtail possum fecal samples. 38% ringtail possum have <i>M. ulcerans</i> lesion and/or positive feces Lower in brushtail possums: 1 with <i>M. ulcerans</i> lesion and/or positive feces and 4 with no lesions and low <i>M. ulcerans</i> DNA in feaces.
Durnez et al., 2010 [26]	565 small mammals: 326 rodents and 222 shrews	2006 Benin	RT-PCR: No <i>M. ulcerans</i> specific DNA detected
Van Zyl et al., 2010 [16]	2 horses: Case report Case 1: 21-year-old Quarterhorse-cross Case 2: 32-year-old Standardbred gelding	Case 1: May 2006 Case 2 : Oct 2006 South-eastern Australia	RT-PCR <i>M. ulcerans</i> specific DNA detected from both horses
Elsner et al., 2008 [19]	A cat: Case report 10-year-old castrated male domestic cat	2006 Victoria, Australia	RT-PCR <i>M. ulcerans</i> specific DNA detected
Appleyard & Clark, 2002 [27]	Case report: three cats Case 1: An 8-year-old spayed female short hair Case 2: 6-year-old spayed female short hair Case 3: 11-year-old domestic longhair cat	2002 North America	PCR Could not differentiate <i>M. ulcerans</i> from other <i>Mycobacterium</i> spp. (a new <i>Mycobacterial</i> spp. namely " <i>Mycobacterium visibilis</i> " suggested)
Heckert, Elankumaran, Milani, & Baya, 2001 [28]	60 wild striped bass: Swab from external ulcerative dermatitis and granulomatous-like lesions in the internal organs	1997 Chesapeake Bay, USA	PCR No <i>M. ulcerans</i> specific DNA detected (a new <i>Mycobacterial</i> spp. suggested)
Mitchell, McOrist, & Bilney, 1987 [14]	36 male and 51 female adult koalas captured	1980-1985 Raymond Island, southeastern Australia	Pathological and bacteriological examination 18 out of 87 captured koalas had skin wound 11 koalas were found positive for <i>M. ulcerans</i>
McOrist, Jerrett, Anderson, & Hayman, 1985 [13]	Case study: 2 koalas: one male and one female Ulcerated tissue	1982 Raymond Island, southeastern Australia	Pathological and bacteriological examination Both koalas suggested positive for <i>M. ulcerans</i>

110 **4. Discussion on possible reservoirs and vectors of *Mycobacterium ulcerans* by country:**111 This systematic review assessed the potential animal reservoir of *M. ulcerans* around the world
112 recorded to date. This is essential for understanding the epidemiology and mode of transmission of
113 the disease, which subsequently aids in prevention, control and elimination strategies.114 **4.1. Australia:**115 Out of 17 studies included in this review, 10 were conducted in Australia. In Australia, the disease is
116 more prevalent in the southeastern state of Victoria and in Far-north Queensland. After the detection
117 of *M. ulcerans* infection in four koalas in 1980 at Raymond island, Australia [14], the entire island was
118 searched for koalas in the following year. Thirty-six male and 51 female koalas were captured and
119 examined. Of these, 18 out of 87 animals had skin wounds and 11 were found positive for *M. ulcerans*.
120 Diagnosis was made on pathological and bacteriological examination; the PCR-based method used
121 for the identification of *M. ulcerans* from clinical and environmental samples was only implemented
122 in 1996 [29]. Non-human cases of *M. ulcerans* in Australia have been reported in marsupial species
123 such as koalas [14], ring tail and brush tail possums [8, 15, 30], horses [16], alpacas [17], dogs [18] and
124 cats [19]. A study conducted by Fyfe and colleagues between 2007-2009, at Point Lonsdale, a small
125 coastal town south east of Melbourne, Australia, which is also endemic for BU, found that 43% of
126 ring tail possums and 29% of brush tail possum faeces samples were positive for *M. ulcerans* DNA
127 [8]. Only 1% of faecal samples from non-endemic area possums were positive for *M. ulcerans* DNA in
128 this study, suggesting terrestrial mammals such as possums are potential reservoirs of *M. ulcerans* in
129 South-east Australia. Several studies have identified possums (both ring tail and brush tail) as
130 potential reservoirs since then [5, 15]. In Australia, other than the southeastern state of Victoria, BU
131 is also prevalent in far north Queensland [9]. Inspired by the evidence of possums as potential
132 reservoirs of *M. ulcerans* in Victoria, a study conducted by Roltgen and colleagues (2013) in Northern
133 Queensland, Australia, detected *M. ulcerans* DNA from two bandicoot faecal samples, suggesting the
134 possibility that bandicoots are a potential reservoir of *M. ulcerans* in far north Queensland [10].135 **4.2. Africa:**136 Out of the 17 studies included in this review, 4 were conducted in West African countries: 2 in
137 Ghana [22, 24], 1 in the Ivory Coast [23] and 1 in Benin [26]. Durnez and colleagues (2006) caught 326
138 rodents and 222 shrews from endemic and non-endemic villages of Benin and tested for *M. ulcerans*
139 but no specific DNA was detected from any of their samples [26]. Despite their result, they suggested
140 the necessity of more intensive research focusing on small mammals in Africa. Willson reported
141 positive PCR with IS2404 only from tadpoles and fishes from Ghana [24]. Similarly, two faecal
142 specimens from *Thryonomys swinderianus* (agouti) were reported positive for *M. ulcerans* in a study
143 conducted by Bi Diangoné Tian and colleagues (2014) from the Ivory Coast [23]. They suggested
144 agouti, which is closely related to Australian possums, could be a potential reservoir of *M. ulcerans*
145 in Africa. However, RT-PCR targeting IS 2606 was not conducted to confirm the case of *M. ulcerans*.
146 A faecal survey of domestic animals in rural Ghana for *M. ulcerans* was conducted by Tobias and
147 associates suggested no evidence of association between domestic animals and *M. ulcerans* in endemic
148 and non-endemic villages in Ghana [22]. Unlike Australia, not a single study in Africa has reported
149 the presence of *M. ulcerans* positive DNA or cases in non-human species, suggesting that transmission
150 dynamics may be different in Africa and Australia or, alternatively, a host animal is yet to be
151 identified in Africa .152 **4.2. Other countries:**153 No study has reported *M. ulcerans* DNA or cases in non-human species in any country other than
154 Australia. A study conducted by Heckert in 1997 at Chesapeake Bay, USA detected a new
155 *Mycobacterium* spp. from Wild Stripped Bass [28]. This new isolate was closely related to *M. marinum*,

156 *M. ulcerans*, and *M. tuberculosis*. Similarly, Sakaguchi and his associates reported an atypical
157 mycobacterial infection in an Indian flap-shelled turtle (*Lissemys punctata punctata*), imported from
158 India to Japan in an aquarium [25]. A PCR assay targeting the *rpoB* gene revealed the isolate had 89-
159 100% homology to *M. ulcerans* and *M. marinum*. Again, this study could not differentiate *M. ulcerans*
160 from mycolactone producing *M. marinum* (MPMM). Appleyard and Clark in 2002 reported a new
161 *Mycobacterial spp.* namely "*Mycobacterium visibilis*" from three cats initially suspected of having *M.*
162 *ulcerans* infection [27].

163 **4. Conclusion:**

164 Human cases of BU have been reported in more than 30 countries from Africa, America, Asia and
165 Oceania. Since the implementation of PCR-based methods for the detection and identification of *M.*
166 *ulcerans* from clinical and environmental samples, there has been a significant increase in overall
167 knowledge of BU. However, there is no record of direct human-to-human transmission of *M. ulcerans*,
168 unlike tuberculosis and leprosy. Australia is the only country where non-human cases of BU have
169 been identified with small mammals, especially possums and, to some extent, bandicoots, being
170 implicated as potential reservoirs of *M. ulcerans*. Despite having several outbreaks in African
171 countries, no non-human cases have been recorded so far and there is no evidence of any other
172 animals acting as a potential reservoir for this organism. This systematic review suggests the need
173 for extensive laboratory and field research focusing on domestic animals and wildlife to elucidate
174 their role in BU endemic countries.

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180 wrote the paper with input from all authors. All authors reviewed the final manuscript.

181 **Conflict of interest:** The authors declare no conflict of interest.

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