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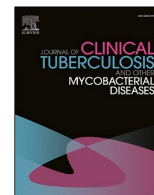

Systematic review: Global host range, case fatality and detection rates of *Mycobacterium ulcerans* in humans and potential environmental sources

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ABSTRACT

Fundamental aspects of the epidemiology and ecology of *Mycobacterium ulcerans* (MU) infections including disease burden, host range, reservoir, intermediate hosts, vector and mode of transmission are poorly understood. Understanding the global distribution and burden of MU infections is a paramount to fight against Buruli ulcer (BU). Four databases were queried from inception through December 2023. After critical review of published resources on BU, 155 articles (645 records) published between 1987 and 2023 from 16 countries were selected for this review. Investigating BU in from old endemic and new emerging foci has allowed detection of MU in humans, animals, plants and various environmental samples with prevalence from 0 % up to 100 % depending of the study design. A case fatality rate between 0.0 % and 50 % was described from BU patients and deaths occurred in Central African Republic, Gabon, Democratic Republic of the Congo, Burkina Faso and Australia. The prevalence of MU in humans was higher in Africa. Nucleic Acid Amplification Tests (NAAT) and non-NAAT were performed in > 38 animal species. MU has been recovered in culture from possum faeces, aquatic bugs and koala. More than 7 plant species and several environmental samples have been tested positive for MU. This review provided a comprehensive set of data on the updates of geographic distribution, the burden of MU infections in humans, and the host range of MU in non-human organisms. Although MU have been found in a wide range of environmental samples, only few of these have revealed the viability of the *mycobacterium* and the replicative non-human reservoirs of MU remain to be explored. These findings should serve as a foundation for further research on the reservoirs, intermediate hosts and transmission routes of MU.

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1. Introduction

Mycobacterium ulcerans (MU) is the causative agent of Buruli ulcer (BU), a neglected necrotizing skin disease. MU is unique due to its ability to produce a lipid toxin called mycolactone, which serves as the main virulence factor of this bacterium [1,2]. Mycolactone plays a significant role in the colonization of both human and invertebrate hosts. In invertebrate hosts, mycolactone exhibits cellular activity that enables MU to invade salivary glands, which serve as the site of MU proliferation [3]. MU structured with an extracellular matrix that contains mycolactone are more potent for colonization in mammalian hosts [4]. Mycolactone is produced by a polyketide synthase that consists of modules that may also contain optional domains responsible for mediating the various types of reduction of the growing polyketide. These optional domains include a ketoreductase (KR) domain that adds hydrogen, a dehydratase domain that removes oxygen and hydrogen, and/or an enoyl reductase (ER) domain that reduces the C = C double bond [5]. Molecular tests targeting 16S rRNA, insertion sequences, and genes encoding the ER/KR have increased the rate of mycobacterium identification in clinical and environmental samples [6].

After tuberculosis and leprosy, BU is the third most common mycobacterial disease in humans [7]. This disease has been reported in more than 30 countries around the world with high incidence in the humid intertropical regions of sub-Saharan Africa, Latin America and the temperate regions of Asia and Australia [8–13]. The bulk of the burden of BU falls particularly on children up to 15 years old from rural areas in West and Central Africa which account for more than half of incident cases, including Côte d'Ivoire, Togo, Ghana, Benin, the Democratic Republic of Congo and Cameroon [14–17]. In West Africa, BU has replaced leprosy as the second mycobacterial disease [18]. Sporadic cases of BU have been described in non-endemic areas with appearance of new emerging areas in Africa (Senegal, Mali) and Asia (Japan) [19–21].

Circumstantial evidence suggests aquatic ecosystems as the primary risk factor for BU [22,23]. In fact, MU molecular markers have been found in various elements of the aquatic environment including animals, plants biofilms, soils and detritus [24,25]. These environmental elements constitute the potential sources and reservoirs of MU in endemic areas. In Africa, agricultural activities near water courses, fishing, and swimming in rivers represent the main risk factors for BU [23,26]. Studies hypothesize that humans may become infected from the environment after insect bites or stings from biotic and abiotic elements [27,28]. The environmental elements such aquatic insects and plant biofilms that are associated with slow flowing or stagnant watercourses may play a significant role in the transmission of BU to humans [4,28–30]. In Australia, aerosols from infected watercourses and animals are incriminated as potential sources of BU transmission to humans [31–33]. Overall, the transmission of MU from the environment to humans is hypothesized to be mediated either by insect bites such as aquatic bugs (e.g. water bugs belonging to the family Naucoridae and Belostomatidae) in Africa [30].

In Australia, mosquitoes could be involved in MU transmission but their role is not yet demonstrated experimentally [34] due to a lack of correlation between BU incidence and locally acquired vectorborne diseases [35]. Through experimental infections in mice, studies demonstrated that a very low dose of MU, delivered beneath the skin through a minor injury caused by a traumatic source such as mosquitoes or an experimental needle puncture, is sufficient to cause Buruli ulcer [36,37]. This suggests that the presence of MU on the skin is a prerequisite before a mosquito bite, which is problematic as this scenario is unlikely to occur frequently. Anthropological activities and natural events such as deforestation, floods, dams, artificial lakes, swimming in rivers, mining, agricultural activities near rivers and swamp extension have been associated with the emergence of BU cases [22,38–44]. Recently, a quantitative correlation was established between the release of MU from possum excrement and the onset of BU in humans [45].

There are several poorly understood aspects of the epidemiology and ecology of MU infections. The presence or incidence of MU infection could vary geographically and may exist in a wide range of animal and plant hosts, as well as environmental matrices. Updates on the geographic distribution, host range, disease burden and mode of transmission of MU infections are essential for better understanding the epidemiology of BU disease and contribute to the development of intervention strategies. Here, we performed a systematic review is to bring up to speed geographic distribution and host range, and to determine detection and case fatality rates of MU infection using Nucleic Acid Amplification Tests (NAAT) and non-NAAT.

2. Methods

2.1. Study design

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist was used gather information for this systematic review (Supplementary Table 1) [46]. Article research was performed by author SK. Articles were independently selected on the basis of title and abstract by authors ST, JETB and SK in the R-ayyan review platform. Each of the remaining articles were screened for eligibility and data retrieved by at least two of the authors of this review. The discussion and consensus were undertaken to resolve any disagreements.

2.2. Literature search

A literature search was performed on November 23, 2020 and October 4, 2023in 4 databases (PubMed, Embase, Web of Science, and Global Index Medicus) to retrieve all the articles on natural MU infections at the global level (Supplementary Table 2). Reference review articles from the bibliography were curated manually to extract any article missing in the list of papers obtained during the literature search strategy.

2.3. Inclusion and exclusion criteria

Research articles included in the review are: 1) those relating to humans (suspected BU cases, suspected BU cases tested positive for MU and presumed healthy), animals, plants and environmental samples; 2) papers describing the presence of MU in biological materials and environmental samples after analysis by NAAT and non-NAAT (culture, microscopy, histopathology and ELISA); 3) manuscripts describing the epidemiology of BU worldwide or at the regional or country level; and 4) cohort studies. Articles that are published in a language other than French and English, those not describing the case fatality rate (CFR) and/or detection rate of MU, and duplicates were not included in this review.

2.4. Data extraction and curation

The *meta*-data retrieved from each article were: name of the first - author, year of publication, study design, sampling method, time of participant recruitment (retrospective/prospective), country, United Nations Statistics Division (UNSD) region, country income level, study period, recruitment site (rural/urban, hospital/community, and endemic/non-endemic area), treatment administered (for human studies only), hospitalization, inclusion criteria for participants, definition of BU case, acknowledgment of potential bias in study data as defined by the assessment tool of Hoy et al. (Supplementary Table 3) [47], study population or material (humans, animals, plants and environmental samples), taxonomy for animals and plants, MU detection method, diagnostic targets and target genes, sample types and number tested, number of samples positive to MU, and the BU case fatality rate (number deaths).

2.5. Data analysis

The proportions with 95 % confidence intervals (95 % CI) were estimated… For studies that detected the same target with several types of assays and/or types of samples, we selected the most specific approach for MU or the one with the highest detection rate. For pooled animal and plant samples tested, we collected the names of the positive species and considered the number of individual insects for the negative groups for the calculation of the detection rate. Where possible, we grouped animals according to their classes, and authors reporting animals above the classes were grouped as unclassified. Where possible, we grouped plants according to their orders and authors reporting plants above the order level were grouped as unclassified. We classified the MU detection techniques into NAAT such as Polymerase Chain Reaction (PCR) and Variable number tandem repeat (VNTR); and non-NAAT. PCRs were reported according to the target genes: 16S rRNA, polyketide synthase (PKS), *IS2404*, *IS2606*, enoyl reductase (ER) and ketoreductase (KR). *IS2404* is multicopy insertion sequence that encodes a 328-amino-acid transposase found in mycobacteria (including *Mycobacterium liflandii, Mycobacterium Pseudoshottsii*, and mycolactone-producing *Mycobacterium marinum* strains) previously thought to be specific to MU [48,49]. *IS2404* PCR is highly specific and sensitive for testing diagnostic specimens from humans, but is less straightforward for environmental samples due to inhibitors and the existence of other mycobacteria containing *IS2404* gene [48,50–53]. Detection of both IS2404 and sequence associated with ER or KR domains from the PKS genes which encode the lactone core of mycolactone is required for identification of MU DNA in environmental samples [54]. Mycolactone is made from PKS that are encoded by the genes mlsA1 (51 kb), mlsA2 (7 kb), and mlsB (42 kb) located on the MU virulence plasmid [55–57]. A positive ER-PCR gives strong confirmation for the existence (more specific) of mycolactone-producing mycobacteria in the *M. marinum* complex but is less sensitive compared to *IS2404* PCR [58]. In order to improve detection performance of MU in both environmental and clinical samples, three independent repeated sequences are targeted in two multiplex Taqman assays. These PCRs comprise two multicopy insertion sequences (*IS2404, IS2606*), and a multicopy sequence encoding the KR B domain (KR-B). The assay allows for the control of PCR inhibitors and the differentiation of *M. ulcerans* from other *IS2404*-containing mycobacteria [6].

We considered the detection results as reported by the authors of included studies regardless of the considered cycle threshold. The laboratory culture of MU and VNTRs respectively constituted the confirmatory assays for non-NAATs and NAATs. Genotyping techniques based on VNTR allow distinction between MU and other mycolactone-producing mycobacteria and provides strong evidence MU. VNTR profiling can be used to follow chains of transmission from the environment to humans [54,59].

The analyses were performed using R software version 4.0.3 [60,61].

3. Results

3.1. Literature search selection

A total of 5,948publications were retrieved from public literature search databases including Embase (n = 2,368), Pubmed (n = 1,602), Web of Science (1,967), and Global Index Medicus (n = 11). Additional 39 articles omitted by the electronic search were also retrieved manually and added to the list (Fig. 1). Overall, 3,043 duplicates were found from the various databases and removed from the final list; the remaining 2,905 publications were selected by article title and abstract. The selection process conducted to 482full texts eligible which were reviewed. After a critical evaluation of each article content, 368articles were also removed and excluded for multiple reasons (Fig. 1). This left 155 unique articles corresponding to 645 MU detection and/or case fatality rates records included in this review [15,24,28,30,33,34,40,54,57,62–120].

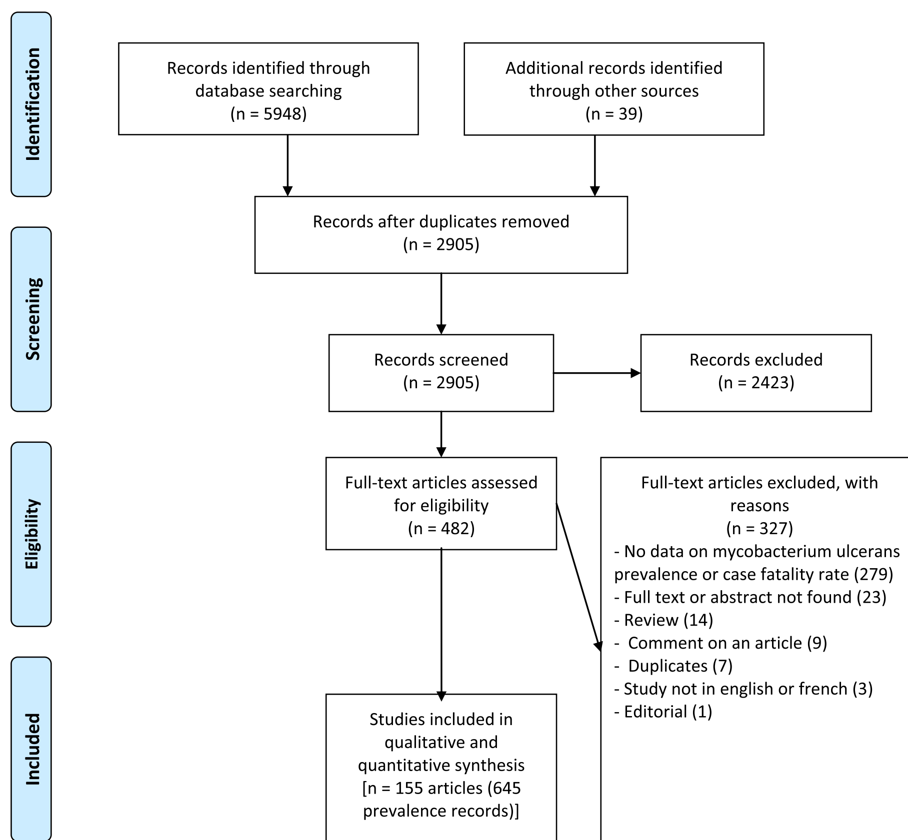

Fig. 1. Flowchart diagram for article selection and processing.

The risk of bias was moderate in 96 out of 155 included articles and low in 59.

3.2. Environmental host-range of Mycobacterium ulcerans

In this systematic review, 173 cross-sectional studies focused on determining the prevalence of MU in environmental samples were incorporated. All studies employed prospective data collection, with the majority (75.14 %) using non-probabilistic sampling. The research was predominantly conducted in Ghana (30.06 %), Ivory Coast (17.92 %), and Togo (16.76 %), largely covering the African World Health Organization (WHO) region (78.61 %). The majority of studies took place in rural settings (39.88 %) or a combination of urban and rural areas (27.75 %). However, the endemicity of Buruli ulcer in the sampled areas was unclear in more than half of the cases (55.49 %). The study populations were diverse, including a wide range of environmental materials such as soil (9.83 %), water samples (8.67 %), detritus (4.62 %), and various types of biofilms (4.05 %). Diagnostic methods were overwhelmingly oriented towards Real-time PCR (73.41 %), although Conventional PCR and Variable Number Tandem Repeat (VNTR) methods were also utilized. Bacterial DNA was the primary target for diagnostic methods, encompassing 92.49 % of the studies. The majority of studies used NAAT (91.33 %) for diagnosis. In terms of molecular targets, IS2404 was the most commonly reported (41.62 %), followed by a combination of IS2404 + IS2606 + KR-B (17.34 %) and other molecular markers. The sample types were diverse but predominantly unreported or unclear, indicating a need for more detailed documentation in future studies. Soil, water filtrands, and various types of faeces and biofilms were among the reported sample types. Table 1 reports environmental detection of MU following diagnostic methods with large prevalence in Africa (Supplementary Table 4. Prevalence estimates of MU infections in the environment).

Table 1
Environmental detection of Mycobacterium ulcerans following diagnostic methods.

MU diagnostic methods	Number of environmtal samples tested	Number tested positive to MU	Range prevalence (%)
NAAT diagnostic methods			
Conventional PCR with target ER	550	218	[3.0–73.5]
Conventional PCR with target IS2404	578	149	[2.2–59.2]
Conventional PCR with target IS2606	86	28	[6.7–54.5]
Conventional PCR with target IS2404 + ER + VNTR	98	36	36.7
Conventional PCR with target Unclear/ Not reported	150	15	10.0
Real time PCR with target ER	77	33	42.9
Real time PCR with target IS2404	10,762	1116	[0.0–50.0]
Real time PCR with target IS2404 + ER	195	36	[6.5–29.8]
Real time PCR with target IS2404 + IS2606	960	42	[0.0–55.6]
Real time PCR with target IS2404 + IS2606 + KR-B	3113	226	[0.0–66.7]
Real time PCR with target IS2404 + KR-B	1114	22	[0.0–33.3]
Real time PCR with target KR-B	460	10	[0.0–3.5]
Real time PCR with target Unclear/ Not reported	377	7	[1.2–2.1]
Variable number tandem repeat (VNTR)	642	63	[0.0–36.2]
Non-NAAT diagnostic methods			
Culture	20	1	5.0
Multiple detection assays: PCR, Culture, microscopy	337	112	[0.0–100]

3.3. Host-range of Mycobacterium ulcerans in plants

This work included 21 cross-sectional studies investigating the prevalence of MU in plants. The majority of the studies employed probabilistic sampling (71.43 %) and were conducted prospectively. The research spanned across Australia and several African countries, predominantly Ghana (61.9 %). Most studies were carried out in lower-middle-income countries (90.48 %) and in urban/rural settings (57.14 %). The study areas were largely reported as endemic (28.57 %) or a mix of endemic and non-endemic (19.05 %). All studies were community-based. The plant orders studied were diverse, with a significant number remaining unclassified (42.86 %). Among the classified, Alismatales, Asterales, Commelinales, Myrtales, Nymphaeales, and Poales were each reported in 9.52 % of the studies. The study populations included various aquatic and terrestrial plants, with a notable focus on plant roots (14.29 %). For diagnostic methods, Real-time PCR was the most used (66.67 %). Bacterial DNA was the sole target diagnostic method. The primary molecular targets were ER (28.57 %) and IS2404 (14.29 %), with some studies using Variable Number Tandem Repeat (VNTR) (28.57 %) as the diagnostic method. MU detection in plants varied from 0.0 % to 78.8 % and mostly in Ghana, Benin and Ivory Coast (Table 2; supplementary table 5).

Table 2
Global detection rate of *Mycobacterium ulcerans* in plants according to assays.

MU diagnostic methods	Number of plants tested	Number tested positive to MU	Range prevalence (%)
Conventional PCR with target Unclear/ Not reported	30	0	0.0
Real time PCR with target ER	100	36	[18.5–53.8]
Real time PCR with target IS2404	175	96	[20.0–78.8]
Real time PCR with target IS2404 + ER	69	36	52.2
Real time PCR with target IS2404 + IS2606	66	45	68.2
Real time PCR with target IS2404 + IS2606 + KR-B	166	54	[4.8–63.6]
Variable number tandem repeat (VNTR)	101	8	[0.0–18.2]

3.4. Global detection rate of Mycobacterium ulcerans infections in animals

Our systematic review encompassed 186 studies examining the prevalence of MU in various animal species. The majority of studies were cross-sectional (89.25 %), predominantly employing non-probabilistic sampling methods (75.27 %), and the data collection was mainly prospective (96.77 %). The research spanned various countries, with the highest number of studies conducted in Benin, Cameroon (combined 20.97 %), Australia (18.28 %), and Ghana (20.97 %). Regionally, the

bulk of the studies were carried out in Africa (77.42 %), followed by the Western Pacific (19.89 %). Lower-middle-income countries accounted for most of the studies (79.03 %). In terms of recruitment settings, rural areas were predominant (41.94 %), and the endemicity status of the study areas was largely reported as unclear or not reported (47.85 %). The studies were primarily community-based (94.09 %). The animal classes studied were diverse, with Mammalia (27.42 %) and Insecta (17.2 %) being the most represented, although a significant number of studies did not clearly report the class (41.4 %). The majority of the study population involved animals suspected of carrying MU (various species with specific mentions). For the diagnostic methods, Real-time PCR was the most utilized (62.9 %), followed by Conventional PCR (19.35 %). The primary molecular target for diagnosis was IS2404 (32.8 %), though ER (12.37 %) and IS2606 (6.45 %) were also commonly targeted. The sample types were varied, with a notable use of swab samples, tissue samples, and faecal samples. Table 3 reports detection rate of MU following diagnostic test.

Except for culture, NAATs and non-NAATs allowed detection of MU targets in several species belonging to *Actinopterygii, Amphibia, Arachnida, Aves, Clitellata, Diplopoda, Gastropoda, Insecta, Mammalia, Ostracoda*, and *Reptilia*. MU positive animals in Australia consisted of mammals (Possums, Koalas and Rattus rattus) while in Africa a wide range of animal classes were positive for MU including *Actinopterygii, Amphibia, Arachnida, Aves, Clitellata, Diplopoda, Gastropoda, Insecta, Mammalia, Ostracoda* and *Reptilia* (Supplementary Table 6. Prevalence estimates of *Mycobacterium ulcerans* infections in animals).

In this systematic review, 13 cross-sectional studies focusing on the prevalence of MU in animals were included where samples from multiple animals were pooled before testing. All studies used non-probabilistic sampling and data collection was prospective. The research covered Australia (30.77 %) and several African countries including Benin, Cameroon, and Ghana (each 23.08 %). The majority of the studies were conducted in lower-middle-income countries (69.23 %) and in rural settings (46.15 %). Most studies reported the study areas as endemic (76.92 %). All studies were community-based. The pooled samples encompassed a wide range of animal classes, with Insecta being the most common (46.15 %). Other classes included Actinopterygii, Amphibia, and Mammalia. The study populations were diverse, ranging from aquatic bugs and various insect species to domestic animals such as dogs, ducks, and goats. For the diagnostic methods, Real-time PCR was predominantly used (92.31 %). Bacterial DNA was the sole target diagnostic method. Nucleic Acid Amplification Tests (NAATs) were used across all studies. Molecular targets were primarily IS2404 (38.46 %) and its combinations with other genes. Pooled prevalence was 4.6 % (95 % CI: 4.2–4.9) and most MU detection was reported in, Cameroon (13.9 %) [92], Ghana [63] and Benin [120] (8.7 % each) (Table 4, supplementary table 7).

Table 3
Global detection of *Mycobacterium ulcerans* in animal hosts.

MU diagnostic methods	Number of animal tested	Number tested positive to MU	Range prevalence (%)
NAAT diagnostic methods			
Conventional PCR with target ER + VNTR	938	1	[0.0–0.3]
Conventional PCR with target ER	1729	146	[2.6–29.6]
Conventional PCR with target IS2404	771	0	0
Conventional PCR with target Unclear/ Not reported	534	0	0
Real time PCR with target KR-B	281	21	[0.0–39.2]
Real time PCR with target IS2404	9436	1098	[0.0–69.2]
Real time PCR with target IS2404 + ER			
Real time PCR with target IS2404 + IS2606	91	7	[3.9–30]
Real time PCR with target IS2404 + IS2606 + KR-B	1985	160	[0.0–100]
Real time PCR with target IS2606 + KR-B	51	2	3.9
Real time PCR with target IS2404 + KR-B	17,653	60	[0.0–17.4]
Real time PCR with target IS2606	528	37	[0.0–100]
Real time PCR with target Unclear/ Not reported	546	63	[0.0–100]
Variable number tandem repeat (VNTR)	1068	12	1.1
Non-NAAT diagnostic methods			
Culture	737	4	[0.0–50]
Microscopy	121	4	[0.0–5.6]
Multiple detection assays: PCR, Culture, histopathology, microscopy	193	35	[0.0–100]

4. Global detection rate of *Mycobacterium ulcerans* infections using nucleic acid amplification tests in plants

4.1. Detection rate of Mycobacterium ulcerans infections in humans

This systematic review analyzed 199 studies to understand the prevalence of BU in humans, primarily focusing on children and adults. The majority of studies were cross-sectional (58.29 %), with most employing non-probabilistic sampling (95.98 %) and conducting data collection prospectively (80.9 %). The research covered a global scope, with the highest number of studies in Ghana (23.62 %), Australia (14.57 %), and Benin (11.06 %). Regionally, the majority of studies were conducted in Africa (71.36 %), followed by the Western Pacific (20.6 %). The studies predominantly involved lower-middle-income countries (54.27 %). A significant portion of the studies included all age groups (54.77 %), with adults and children comprising 12.56 % and 5.03 % respectively. Recruitment settings varied, with 29.65 % in rural areas, 21.11 % in urban, and 14.57 % in combined urban/rural settings. The endemicity of the study areas was unclear in most cases (74.87 %). The settings were predominantly hospital-based (70.35 %). When it came to hospitalization, a considerable number of studies did not clearly report this information (53.27 %), with 31.16 % of studies involving ambulatory patients. The study population primarily consisted of MU suspected cases (92.46 %). Diagnostic methods varied widely across studies, with Real-time PCR being the most used (22.61 %), followed by microscopy (20.78 %) and Conventional PCR (15.58 %). The primary molecular target for diagnosis was IS2404 (24.12 %), followed by unclear/not reported (22.61 %). Tissue samples were the most commonly used

Table 4
Global detection of *Mycobacterium ulcerans* in animal hosts pooled.

MU diagnostic methods	Number of pool animals tested	Number tested positive to MU	Range prevalence (%)
Conventional PCR with target ER	1068	78	7.3
Real time PCR with target IS2404	4171	194	[0.0–8.7]
Real time PCR with target IS2404 + IS2606 + KR-B	1104	80	[0.0–8.0]
Real time PCR with target IS2404 + KR-B	4542	294	[0.0–13.9]
Real time PCR with target Unclear/ Not reported	244	12	4.9

sample type (27.14 %), followed by swab samples (24.62 %).

The prevalence of MU was 32.5 % (95 % CI: 31.3–33.7): by NAAT only, it ranged from 41.6 % (95 % CI: 40.7–42.5) to 25.3 % (95 % CI: 24.4–26.2) for non-NAAT, and 39.3 % (95 % CI; 37.2–41.4) for multiple detection assay (NAAT and non-NAAT). The prevalences of MU determined by culture and microscopy were respectively 20.3 % (95 % CI: 18.5–22.2) and 27.5 % (95 % CI: 26.2–28.8)(Table 5; Supplementary Table S8).

4.2. Case fatality rate of Mycobacterium ulcerans infections in humans

We analyzed 50 records involving patients diagnosed with MU. The majority of these studies (68 %) were case reports, followed by cohort studies (24 %) and cross-sectional studies (8 %). Most studies employed non-probabilistic sampling methods (98 %) and conducted data collection prospectively (84 %). The research spanned across several countries, with the highest number of studies from Australia (30 %) and the Democratic Republic of the Congo (10 %).

Regarding regional distribution, the Western Pacific region accounted for the largest share of studies (48 %), followed by Africa (32 %) and the Americas (14 %). The study populations were primarily from high-income (36 %) and upper-middle-income countries (30 %). Children were predominantly represented in the study populations, with 40 % of studies including all age groups and 34 % focusing on adults. Most studies were conducted in hospital-based settings (94 %), with 36 % in urban areas, and 26 % in rural settings. Notably, 94 % of studies did not report the endemicity of the area.

Among the hospitalized patients, 40 % were treated as outpatients, and 12 % were hospitalized. The rest were either a combination of hospitalized/ambulatory (18 %) or not clearly reported (30 %). Diagnostic methods varied widely across studies, with microscopy being used in 20 % of cases, followed by Real-time PCR (12 %) and Conventional PCR (10 %). The molecular target for diagnosis included IS2404 (14 %), and other targets which were not reported (32 %); non molecular targets accounted for 48 % of all tests. Tissue samples were the most commonly used sample type (58 %), followed by swab samples and tissue samples combined (22 %).

Six studies from different countries have provided consistent data on the case fatality rate (CFR) of MU in humans (Supplementary table 9). Death occurred in 24 MU positive patients in 5 countries including: Democratic Republic of the Congo that accounted for 3.2–19.4 % [15,121], 7.5 % in Gabon [69], 1.2 % in Australia [122], 50 % in case reports in Central African Republic [123] and in Burkina Faso [124] (Supplementary table 9). In Gabon, these patients have a immunocompromised system due to coinfection of BU and HIV. In Australia, four deaths occurred among MU positive patients but only one in the reported article was attributed to MU.

Table 5
Detection rate of *Mycobacterium ulcerans* infections in humans following diagnostic methods.

MU diagnostic methods	Number of people tested	Number tested positive to MU	Range prevalence (%)
NAAT diagnostic methods	6055	1967	[0.0–100]
Conventional PCR with target IS2404	4496	1051	[0.0–95.8]
Conventional PCR with target ER	382	183	47.9
Conventional PCR with target IS2404 + ER	30	7	23.3
Conventional PCR with target Unclear/ Not reported	1147	726	[0.0–100]
Loop mediated isothermal amplification (IS2404)	408	209	[44.1–63.2]
Loop mediated isothermal amplification (Unclear/ Not reported)	816	349	[20.6–64.0]
Real time PCR with target 16S rRNA	1	1	100,0
Real time PCR with target ER	15	14	93.3
Real time PCR with target IS2404	2441	1539	[0.0–100]
Real time PCR with target IS2404 + ER	15	14	93.3
Real time PCR with target IS2404 + IS2606 + KR-B	197	35	[0.0–72.7]
Real time PCR with target IS2404 + IS2606, or IS2404 + KR-B, IS2404 + IS2606 + KR B	9	7	77.8
Real time PCR with target IS2404 + KR-B	18	7	[33.3–44.4]
Real time PCR with target IS2404 + PKS	1	1	100,0
Real time PCR with target IS2606	382	217	56.8
Real time PCR with target Unclear/ Not reported	1094	401	[0.0–100]
Variable number tandem repeat (VNTR)	15	14	93.3
Non-NAAT diagnostic methods			
Culture	1860	377	[0.0–100]
ELISA (IgG)	1933	409	[12.1–32.0]
Fluorescent-thin layer chromatography (Mycolactone detection)	449	122	[26.5–44.4]
Histopathology	327	142	[0.0–100]
Microscopy	4689	1288	[0.0–100]
Multiple detection assays: PCR, Culture, histopathology, microscopy, VNTR, Sequencing	2077	816	[0.0–100]

5. Discussion

This review confirms like previous findings that MU is present in multiple terrestrial and aquatic environments, biotic and abiotic, and in animal and plant species. The environmental samples represented the vast majority of included studies. MU infections prevalence in humans is high in West African countries. In addition to human cases, this review shows that MU is present in a wide range of other host species,; and multiple liquid and solid environmental matrices. The mystery in understanding the epidemiology of BU is not solely based on the transmission route of its etiological agent. It also depends on the diversity between the ecology of this pathogen between the main endemic foci: Australia and Africa. Unlike Africa where MU is found in several animal and plant hosts, MU has been reported in Australia only in mammals.

Different hypotheses have been raised regarding the bioecology and the actual reservoirs of MU. Overall the role of blood-feeding insect (mosquito) through laboratory evidence was established in the MU transmission pathway but it is difficult to define the importance in the field so far [36]. Hypotheses about the existence of multiple transmission routes are advanced. In Australia, animals, including small mammals (possums), are considered reservoirs of MU, while mosquitoes are indexed as vectors [34,79]. The release of MU from possum excrement has recently been found to correlate with the onset of BU in humans[45]. In southeastern Australia, native marsupials-possums have been identified as susceptible hosts of MU, with high numbers of the bacteria shed in the feces of infected animals. Mosquitoes have also been found to harbor MU biomarkers in this region, and a zoonotic model of disease transmission has been proposed involving possums, mosquitoes, and humans [79]. However, experimental field studies to test these hypotheses were not fine-tuned to adequately identify specific modes of transmission [125].

In Africa, aquatic water bugs (Hemiptera) have been suspected as replicative reservoirs of MU. A vector-borne transmission model of BU has also been proposed involving watercourses, water bugs and humans

[28,126], but these insects are not hematophagous. The lack of data on the colonization of mosquitoes and any other terrestrial insect by MU and the absence of an animal reservoir of BU in Africa hinder our understanding of the reservoirs and vectors of BU to correlate the data between Australia and Africa. Moreover, the significance of detecting the molecular signatures of MU in environmental samples remain elusive, complicating the understanding of its routes of transmission from the environment to humans. To investigate whether the distribution pattern of the BU disease can be ascribed through the intermediary of a vector, further studies should rely on advances in environmental and molecular techniques to identify habitats and reservoirs of MU persistence and proliferation. Studies based on the "EcoHealth" concept, which is based on a holistic approach involving humans, animals, and the environment, should be conducted within the human microenvironment to decipher the close relationship(s) between the aquatic and terrestrial ecosystems of MU in endemic areas. Current research activities should determine the impact of humans, animals, and the environment (fauna and flora) on the emergence of the BU disease to decipher the epidemiological links between humans and the environment. Although this study reports most casesof MU in humans in Africa, BU cases have however been reported in other parts of world (Australia, Southeast Asia, China, Japan, Central and South America and the Western Pacific for example) [21,127,128].

Non-NAAT methods, such as culture, are crucial for detecting the viability of MU in the environment. While PCR targeting MU has limitations due to specificity issues, as other mycobacteria carry the same targets, culture provides the highest level of evidence for confirming MU infection or colonization in a given sample [129]. Although NAAT approaches, such as metagenomic and whole-genome sequencing, could serve as an alternative, their implementation could pose a significant challenge with environmental samples. In this review, the prevalence of MU by culture varied between 0.0 % and 100 % depending on the study design in humans. Although various mycobacterial species have already been isolated from the environment [104,130], MU has only been recovered in culture from humans and animals. Various environmental studies have been conducted to recover MU in culture from animal sources [93,104,131], and possum faeces, aquatic bugs and koala have had MU recovered in culture[79,104,132].

Cultivation of MU, however, in addition to requiring specialized laboratories and highly trained personnel, takes several weeks or even months and is therefore not suitable for MU diagnosis before treatment. More than half of human samples tested by histopathological analysis were positive for MU in this review. The histopathological analysis of BU is however not without ambiguity for the MU identification [133–135]. This suggests the possibility of biased results determined by histopathology in this review. In the absence of a field-based rapid diagnosis test for BU diagnosis, the detection of acid-fast bacilli of MU by microscopy initially represented the front-line diagnosis method for BU in poor endemic areas [129]. Studies from this review revealed that quarter of BU patients have been diagnosed by microscopy in the health facilities, following by confirmation with PCR according to WHO requirements. However, in some remote endemic areas of Africa, the treatment of BU has completely been based on microscopy results due to the delay in collecting the sample and delivering it in laboratory that performs PCR (confirmatory assays) [136]. Due to continuous efforts of the WHO to harmonize BU diagnosis protocols, reference laboratories have been set in the major endemic countries and PCR currently constitutes the gold-standard method for diagnosis and confirmation of BU cases. Although this method is rapid and affordable for countries with limited resources, it requires high well-trained specialists for samples collection, staining and visualization under a microscope. Variations in the applicability of microscopy in BU endemic settings has been attributed to the staining method used [137–139]. Although the WHO has recommended the Ziehl-Nielsen staining as the reference method. These variations appeared among the first studies on BU and before standardization of BU diagnosis methods in 2014 by the WHO. Hence, since this time, BU is diagnosed and confirmed by four methods including Ziehl-Nielsen staining, laboratory culture onto LJ solid media, histopathology and PCRs [140].

Even though MU infections are not generally fatal, they cause massive disfiguring ulcers in patients with a substantial social impact. Studies from this review revealed case fatality among BU patients. BU-associated mortalities have mainly been described in BU patients who had a complicated ulcerative form and co-infection with human immunodeficiency virus for example in Africa [15,69]. In the absence of a vaccine, BU disease is controlled by a combination of antimicrobials (Rifampicin/Streptomycin or Rifampicin/Clarithromycin), thus the low mortality [141–143]. However, MU infections can also be the source of disfigurement or permanent disability if treatment is not appropriate or given on time. Although these therapeutic measures exist, the morbidity and burden of the disease are highest in sub-Saharan African areas where health systems are classified among the poorest in the world with a lack of infrastructures and limited access to diagnosis and treatment [144]. This suggests that improving the health systems and conducting early diagnosis and treatment of BU may significantly reduce the case fatality rate attributed to BU in these areas. Efforts of the WHO, non-governmental organizations, and national programs have contributed to a better knowledge of the disease in the communities through educational and sensitization campaigns, owing to the decrease in BU incidence in certain endemic African areas. However, new emerging areas have recently been described, and little is known about how the MU is circulation among the communities and how patients contract this pathogen and develop the BU disease.

A major limitation of this study is that the prevalence or the case fatality ratio obtained in this review are not robustly measured since our findings are based on a subset of the reported cases without reports from national programs (or WHO BU program). Given the focal distribution of BU, the prevalence completely depends on the scale of the study (e.g. carried out nationally vs. in a particular endemic area), which the authors do not take into account. For the case fatality, we average from only 4 studies, of which one had a nearly 20 % fatality rate and the other was done on immunocompromised patients. This introduces very important biases that could invalidate the resulting estimates. The reported results of each study depend completely on the underlying sampling frame used: some sample in endemic areas while others also use control areas; some pool multiple individuals for detection of MU whereas other do not and the timing of the sampling matters. Thus, pooling of detection rates can be hard to interpret. Although VNTR data has been used, this method is not suitable for elucidating the transmission route due to its low sensitivity. Additionally, it cannot distinguish between MU strains from patients and the environment. A more appropriate approach would be whole-genome sequencing [145–147].

Despite the limitations, this systematic review describes a very thorough analysis on data from peer-reviewed papers on various aspects of MU epidemiology in multiple hosts. Overall, this is a thoughtful systematic review which creates further knowledge about global variations in MU CFR and prevalence in humans, animals, plants and environment. Our systematic review of MU CFR/prevalence sets itself apart from others by focussing on four categories: humans, animals, plants, and environment.

Based on our results, we recommend that the fight against MU infections should consider adopting a "One health" approach integrating close collaboration between human, animal, plant and environmental health actors in an attempt to elucidate the reservoirs, intermediate hosts, vectors and mode of transmission of MU. These findings suggest the promotion of leadership to establish or strengthen national integrated BU surveillance programs and foster multisectoral collaboration in all endemic countries. Health workers in hospitals and the community should be better trained on the early recognition and case management of MU infection in sub-Saharan Africa. The measures to be implemented should include rapid referral of suspected cases, early diagnosis, intensification of treatment, improvement of access to

care and the development of rehabilitation centres for individuals already deformed by BU, focusing mainly on the countries of West Africa [68]. As MU infections are prevalent in predominantly poor rural areas, significantly reducing the cost of medical care for BU could reduce the burden of this infection and reduce rates of discontinuation and avoidance of treatment [148]. Even though it is not easy to practise, our results recommend restricting contact with animals, plants and aquatic environments such as rice paddies to prevent the risk of MU infection. Personal protection such as wearing gloves and boots and clothing with long sleeves while farming or handling bushmeat could - help reduce contact with the MU hosts described in this review and hence reduce the risk of transmission [149,150]. Healthcare workers should be trained enough to be able to combine clinical diagnosis with laboratory diagnosis which remains essentially presumptive apart from culture and VNTR. Further research for the development of simple diagnostic tests with better predictive values is strongly encouraged to aid in the diagnosis of MU in peripheral areas of endemic countries [151].

It emerges from this systematic review which highlights a “One health” vision of Buruli ulcer that 1) MU is present in a vast panoply of animal, plant and environmental hosts, 2) MU infections in humans are mainly recorded in Africa and more particularly in West Africa, and 3) MU infections can be sporadically fatal in endemic regions of sub-Saharan Africa and Australia.

CRediT authorship contribution statement

Serges Tchatchouang: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Conceptualization. **Chris Andre Mbongue Mikangue:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Conceptualization. **Sebastien Kenmoe:** Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology, Formal analysis, Data curation. **Arnol Bowo-Ngandji:** Writing – review & editing, Validation, Methodology. **Gadji Mahamat:** . **Jean Thierry Ebogo-Belobo:** Writing – review & editing, Validation, Methodology. **Donatien Serge Mbaga:** Writing – review & editing, Validation, Methodology. **Joseph Rodrigue Foe-Essomba:** Writing – review & editing, Validation, Methodology. **Hycenth Numfor:** Writing – review & editing, Validation, Methodology. **Ginette Irma Kame-Ngasse:** Writing – review & editing, Validation, Methodology. **Inès Nyebe:** Writing – review & editing, Validation, Methodology. **Jean Bosco Taya-Fokou:** Writing – review & editing, Validation, Methodology. **Cromwel Zemnou-Tepap:** Writing – review & editing, Validation, Methodology. **Jacqueline Félicité Yéngué:** Writing – review & editing, Validation, Methodology. **Jeannette Nina Magoudjou-Pekam:** Writing – review & editing, Validation, Methodology. **Larissa Gertrude Djukouo:** Writing – review & editing, Validation, Methodology. **Marie Antoinette Kenmegne Noumbissi:** . **Raoul Kenfack-Momo:** Writing – review & editing, Validation, Methodology. **Sabine Aimee Touangnou-Chamda:** Writing – review & editing, Validation, Methodology. **Alfloditte Flore Feudjio:** Writing – review & editing, Validation, Methodology. **Martin Gael Oyono:** Writing – review & editing, Validation, Methodology. **Cynthia Paola Demeni Emoh:** Writing – review & editing, Validation, Methodology. **Hervé Raoul Tazokong:** . **Francis Zeukeng:** Writing – review & editing, Validation, Methodology. **Cyprien Kengne-Ndé:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Richard Njouom:** Writing – review & editing, Validation, Methodology. **Valerie Flore Donkeng Donfack:** Writing – review & editing, Validation, Methodology. **Sara Eyangoh:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author summary

Mycobacterium ulcerans (MU) infection, or Buruli ulcer (BU), is one of the major human mycobacteriosis in the world. MU infection manifests as necrosis and very disfiguring with serious consequences if neglected. Certain aspects of the epidemiology and ecology of MU infections are poorly understood. This systematic review describes global host range, case fatality and detection rates of MU in humans and potential environmental sources. Our results showed that MU is present in a vast panoply of animal, plant hosts and environmental matrices. In Australia, MU has been documented in some non-human mammals, plants and environmental matrices. In Africa, human MU infection is endemic added to their presence in plants, environment and in several animal classes. MU-associated mortalities occurred mainly in Africa in immunocompromised patients in whom BU is concomitantly found with other illnesses or disabilities. These results should reinforce the understanding and actions to be undertaken in the work on the epidemiology of MU infections

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2024.100457.

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