Australia's National
Science Agency

Evaluation of the Evidence of the Recreational Water Quality Guidelines

Section: Free-living organisms

Technical Report

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CSIRO

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Report to the Recreational Water Quality Advisory Committee of the National Health and Medical Research Council

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Foreword

This Technical Report accompanies the associated Evidence Evaluation Report which together comprise a narrative review of evidence for the topic of free-living organisms to inform the update to the NHMRC *Guidelines for Managing Risks in Recreational Water* (2008).

The Evidence Evaluation Report is the primary document for this narrative review and contains the background, purpose of the review, a summary of the methodology and results and the full and complete discussion and conclusions for the primary and secondary questions and supplementary topics for the review.

This Technical Report contains detailed information about the full methodology used, including but not limited to:

- the research questions;
- the search strategy used to identify and retrieve studies;
- the process for selecting studies (i.e. inclusion/exclusion criteria);
- the methodology used to critically appraise the literature and the quality assessment of included studies;
- the methods used for data extraction;
- the methods used to critically appraise and synthesise the data of included studies;
- the methods used to analyse and summarise the results of included studies;
- the methods used for any calculations and explanatory text for any assumptions if used;
- documentation of the declared interest(s) of the author(s) of each paper;

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Acknowledgments

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1 Introduction

1.1 Background and purpose

The National Health and Medical Research Council (NHMRC) is updating the *Guidelines for Managing Risks from Recreational Water* (2008) to ensure that they reflect the best available evidence and are current and relevant for the Australian context. This update of the 2008 Guidelines will enable NHMRC to continue its role of providing advice to jurisdictions on how to manage risks to public health from recreational waters and ensure that recreational water sites are safe to use. The update is being overseen by the Recreational Water Quality Advisory Committee (the Committee).

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) has been engaged to undertake the Narrative Review for the subtopic of Free-living Organisms. This review will be used to inform the update to Section 8.2.6 of the *Guidelines for Managing Risks in Recreational Water (2008)* and any relevant sections throughout the rest of the Guideline.

1.2 Purpose of the Review

The update of the *Guidelines for Managing Risks in Recreational Water* (2008) includes a Risk Management Framework (referred to as the "Framework"). The proposed Framework for the updated Australian Recreational Water Quality Guidelines (the "Guidelines") is a new feature developed by the NHMRC that provides a structured process for identifying, planning for, and managing risks related to recreational water quality.

As such, the Framework is intended as an overarching risk assessment and management framework for recreational water quality. To support this Framework, the Guidelines will provide comprehensive elements including guideline values, technical fact sheets and specific technical guidance along with citing of associated evidence.

The Narrative Review, comprising of Evidence Evaluation and Technical Reports, as part of this project are designed to gather, assess, and contribute to the detailed and up-to-date body of evidence. They will provide the rigour to support the above comprehensive information components contained within the Framework and the Guidelines.

1.3 Approach

Unlike the *Guidelines for Managing Risks in Recreational Water* (2008), the updated Guidelines will cover the public health risks associated with recreational water quality only. This includes human health risks from biological and chemical hazards that affect the quality of recreational water that people might be exposed to. Other risks associated with recreational water use such as physical risks should be considered as part of the risk management planning process while applying the Framework; however, specific guidance on how to manage these risks will not be provided in the updated Guidelines. In addition, the Guidelines will not cover details on rescue, resuscitation or treatment associated with risks from recreational water quality.

The Guidelines should be applied within the broader context of protecting public health and as such are not intended to be prescriptive given the variety of recreational water settings and climates across Australia. The inclusion of the Framework is intended to allow for structured risk assessment and risk management planning across the wide variety of existing and emerging recreational water environments that Australian risk

managers might encounter. This also includes any unique sites that are currently unregulated and may present risks to public health. The risks to be addressed in Framework are as follows:

Included:

- Risks from microorganisms, cyanobacteria and algae, free-living microorganisms, chemical hazards.

Excluded:

- Risks from sun, heat and cold and other physical hazards associated with recreational water (e.g. drowning, animal attacks)
- Risks associated with exposure to foodstuffs collected from recreational water or its surroundings
- Risks associated with ancillary facilities that are not part of the recreational water environment other than risks that may affect water quality (e.g. toilet facilities in adjacent areas are not considered unless these need to be managed to minimise contamination of the recreational water body)
- Adverse health effects that are not caused by recreational water quality (e.g. seasickness, the 'bends')
- Risks from sand/soil around recreational water bodies (unless disturbances of sand/soil affect water quality); however, the risk management framework should include assessment of these risks.

The definitions of recreational water, recreational water use and recreational water users to be applied are:

Recreational water:
Included: Any natural or artificial water bodies without a chlorine disinfectant residual that might be used for recreating including coastal, estuarine, and freshwater environments. Includes public, private, commercial, and non-commercial recreational water sites. Includes unique unregulated sites such as wave pools, ocean- or river-fed swimming pools, artificial lagoons, and water ski parks.
Excluded: Aquatic facilities using chemical disinfection including swimming pools, spas, splash parks, ornamental water sites.

Recreational water use:
Included: Any designated or undesignated activity relating to sport, pleasure and relaxation that involves whole body contact or incidental exposure (through any exposure route) to recreational water (e.g. swimming, diving, boating, fishing).
Excluded: Consuming the catch from fishing or foodstuffs collected from recreational water or its surroundings. Therapeutic uses of waters (e.g. hydrotherapy pools). Occupational exposure.

Recreational water users:
Recreators or users of recreational water bodies including:

- the general public including all relevant life stages, ages and states of health other than persons that are explicitly advised to avoid such activities (e.g. for specific medical conditions)
- tourists
- specialist sporting users (e.g. athletes, anglers, kayakers, divers, surfers)
- any groups that may have high exposures to recreational water.

Target audience for the Guidelines

The Guidelines are intended for end users that will implement the Guidelines (government agencies, local councils, private recreational water managers); however, it is anticipated that there will also be significant public interest. It is anticipated that tailored guidance (e.g. plain English fact sheets or summaries) will be developed for specific groups where necessary.

2 Methodology

2.1 Research Questions

This review was conducted by answering prespecified research questions to inform the update of the NHMRC *Guidelines for Managing Risks in Recreational Water* in relation to the sub-topic of free-living organisms. The research questions that formed the basis of this review were developed by the NHMRC Recreational Water Quality Advisory Committee (the Committee).

Table 2.1 Research Questions for the Narrative Review: Free-living organisms (provided by the Committee)

Research Questions
Primary Question: What is the risk of any adverse health outcome for water users from exposure to *Naegleria fowleri* or *Burkholderia pseudomallei* in recreational water? **Secondary Questions:** 1. What are the indicators/surrogates of this/these risk/s? 2. What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters? 3. What is known about the occurrence of these organisms in natural waters in Australia? 4. What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms? 5. What is known about the exposure pathway for each organism? 6. What is known about the dose-response for each organism? 7. What are the current practices to minimise or manage this/these risk/s?

This review process involved a mixed methodological approach including review of:

- Primary studies, reports and other types of direct evidence/data. Each study was assessed separately against prespecified criteria to evaluate the quality and certainty of the evidence.
- Existing systematic/literature reviews. Each review was assessed against predetermined criteria to determine the trustworthiness of the reviews.
- Existing guidelines/guidance/advice. The processes used by the agency/organisations to develop the guidelines/advice was assessed against set criteria to determine how robust the advice was.

The table below outlines which methods were used for each question.

Table 2.2 Methods to evaluate evidence for each research question

Methods to evaluate evidence for each research question	
Primary Question: What is the risk of any adverse health outcome for water users from exposure to *Naegleria fowleri* or *Burkholderia pseudomallei* in recreational water?	Mixed methods approach – primary studies/reports and any existing review/guidance that contains relevant data to address the question
Secondary Questions: What are the indicators/surrogates of this/these risk/s?	Review of reviews only

Methods to evaluate evidence for each research question	
What is the frequency of occurrence of identified health outcomes in Australia? Is there an association with exposure to recreational waters?	Mixed methods approach
What is known about the occurrence of these organisms in natural waters in Australia?	Mixed methods approach
What are the conditions associated with increased occurrence? What are the conditions associated with absence of these microorganisms?	Mixed methods approach
What is known about the exposure pathway for each organism?	Mixed methods approach
What is known about the dose-response for each organism?	Mixed methods approach
What are the current practices to minimise or manage this/these risk/s?	Review existing guidance only

2.2 Search Strategy and Selection of Evidence

2.2.1 Scientific Literature Databases

The databases searched for this review were PubMed®, Scopus® and Web of Science™. PubMed® was used due to its coverage of biomedical journals and its capacity for advanced searching. Scopus® was used due to its coverage of life sciences, health sciences, physical sciences, social sciences and humanities. Web of Science™ was used to identify publications from organisations.

2.2.2 Keywords

Table 2.3 Literature search key words

Literature search keywords and variants			
Population terms	**Recreational water terms**	**Exposure terms**	**Outcome reporting terms**
human/s	recreation/al water use	free-living microorganisms	primary amoebic meningoencephalitis (PAM)
general population	primary/secondary contact	free-living amoebae	melioidosis
elderly	swimming	surface water pathogens	
children	bathing	pathogen	health
infant/s	wading	amoeba	health effects
pregnant/pregnancy	paddling	ameba	health outcome/s
susceptible/vulnerable	water sports	amoebae	adverse effects
immunocompromised	boating	amebae	waterborne disease/s
athlete/s	sailing/sailboating	thermophilic	recreational water infections
recreational water user/s	body boarding/surfing	*Naegleria fowleri*	disease
recreator/s	wakeboarding	*Burkholderia pseudomallei*	infection
tourists	wind surfing		illness/es
Aboriginal	water/jet skiing	aerosol/s	symptoms
Torres Strait Islander	fishing	sediment/s	gastrointestinal
indigenous	anglers/angling	sand	nausea
(*check Lowitja library terms*)	kayaking	water quality	vomiting
	canoeing	exposure	diarrhea
Study type terms	rowing	oral	diarrhoea
study	snorkelling	ingestion	accidental faecal discharge
review	scuba divers/diving	inhalation	pneumonia-like symptoms
epidemiology	surfers/surfing	dermal	fever
epidemiological	kite boarding/surfing	aural	headache
systematic review	parasailing	ocular	

Literature search keywords and variants			
narrative review	pentathlon	nasal	hay fever-like
literature review	triathlon	aerosols	flu-like
randomised controlled trial		climate change	skin rash/es
cohort	recreational water	storm events	skin irritation
case report	fresh/salt water/marine	water	eye irritation
case-control	beach/es		pruritis
cross-sectional	river/s	**Measurement terms**	dermatologic
diagnostic test study	lake/s	temperature	allergic reaction/s
recreational guidelines	dam/s	monitoring	neurotoxicity
guidelines	hot spring/s	direct pathogen monitoring	neurologic/al
report	reservoir/s	non-microbial indicator/s	hepatotoxicity
jurisdiction/al	catchment/s	sampling	dermal irritation
legislation	coast/al	indicator/s	allergic reaction/s
	estuary	surrogate/s	inhalation-related symptoms
	shoreline	source tracking	induction of asthma
	riverbank	source vulnerability	shortness of breath
	water park/s	dose-response	meningitis
	stormwater	outbreak/s	
	rural	risk/s	
		risk factors (physical, chemical, biological)	
		environmental conditions	
		water quality conditions	
		analytical methods	

2.2.3 Search strings

Table 2.4 Scopus® search string

Category	Strings
Population	(((TITLE-ABS-KEY (adult) OR TITLE-ABS ("young adult")) OR (TITLE-ABS-KEY ("middle age") OR TITLE-ABS-KEY ({middle-age}) OR TITLE-ABS-KEY ({middle-aged})) OR (TITLE-ABS-KEY (adolescen*) OR TITLE-ABS-KEY (teen) OR TITLE-ABS-KEY (teenager) OR TITLE-ABS-KEY (youth) OR TITLE-ABS-KEY ("adolescent female") OR TITLE-ABS-KEY ("adolescent male")) OR (TITLE-ABS-KEY (male) OR TITLE-ABS-KEY (female) OR TITLE-ABS-KEY (m?n) OR TITLE-ABS-KEY (wom?n))) OR ((TITLE-ABS-KEY (child) OR TITLE-ABS-KEY (children) OR TITLE-ABS-KEY (boy) OR TITLE-ABS-KEY (girl)) OR (TITLE-ABS-KEY ("general public") OR TITLE-ABS-KEY ("general populace") OR TITLE-ABS-KEY ("general population") OR TITLE-ABS-KEY (public) OR TITLE-ABS-KEY (population)) OR (TITLE-ABS-KEY (elder*) OR TITLE-ABS-KEY (old*) OR TITLE-ABS-KEY (nonagenarian) OR TITLE-ABS-KEY (octogenarian) OR TITLE-ABS-KEY (centenarian) OR TITLE-ABS-KEY (senior) OR TITLE-ABS-KEY ("senior citizen")) OR (TITLE-ABS-KEY (mature) OR TITLE-ABS-KEY ("mature aged") OR TITLE-ABS-KEY ({mature-aged}) OR TITLE-ABS-KEY ("older adult"))) OR ((TITLE-ABS-KEY (infant) OR TITLE-ABS-KEY (toddler) OR TITLE-ABS-KEY (baby) OR TITLE-ABS-KEY ("pregnant wom?n") OR TITLE-ABS-KEY (pregnan*)) OR (TITLE-ABS-KEY (immunocompromised) OR TITLE-ABS-KEY ("Immunocompromised patient") OR TITLE-ABS-KEY ("Immunocompromised host") OR TITLE-ABS-KEY (immunodeficien*) OR TITLE-ABS-KEY (immunosupres*) OR TITLE-ABS-KEY ("weak* immune system") OR TITLE-ABS-KEY (weak* PRE/ 3 immunity) OR TITLE-ABS-KEY (low* PRE/ 3 immunity)) OR (TITLE-ABS-KEY (athlete) OR TITLE-ABS-KEY (recreator) OR TITLE-ABS-KEY ("recreational water user") OR TITLE-ABS-KEY (tourist)) OR ((TITLE-ABS-KEY (koori) OR TITLE-ABS-KEY ({Australia's

Category	Strings
	First people}) OR TITLE-ABS-KEY ({Australia's First nation}) OR TITLE-ABS-KEY ("Aboriginal and Torres Strait Islander")) OR ((TITLE-ABS-KEY (aborigin*) OR TITLE-ABS-KEY (australoid) OR TITLE-ABS-KEY (indigenous) OR TITLE-ABS-KEY ("Torres Strait Islander") OR TITLE-ABS-KEY ("Oceanic ancestry group")) AND (TITLE-ABS-KEY (austral*) OR TITLE-ABS-KEY (queensland) OR TITLE-ABS-KEY ("New South Wales") OR TITLE-ABS-KEY (victoria) OR TITLE-ABS-KEY ("South Australia") OR TITLE-ABS-KEY ("Australian Capital Territory") OR TITLE-ABS-KEY ("Western Australia") OR TITLE-ABS-KEY ("Northern Territory") OR TITLE-ABS-KEY (qld) OR TITLE-ABS-KEY (nsw) OR TITLE-ABS-KEY (vic) OR TITLE-ABS-KEY (sa) OR TITLE-ABS-KEY (act) OR TITLE-ABS-KEY (wa) OR TITLE-ABS-KEY (nt)))))) OR TITLE-ABS-KEY (human) AND
Recreational Water	((TITLE-ABS-KEY (recreation*) W/15 TITLE-ABS-KEY ("surface water")) OR (TITLE-ABS-KEY (recreation*) W/15 TITLE-ABS-KEY ("fresh water")) OR (TITLE-ABS-KEY (recreation*) W/15 TITLE-ABS-KEY (freshwater)) OR (((TITLE-ABS-KEY ("hot spring") OR TITLE-ABS-KEY (hotspring) OR TITLE-ABS-KEY ("thermal spring") OR TITLE-ABS-KEY (dam) OR TITLE-ABS-KEY ("salt water") OR TITLE-ABS-KEY (saltwater) OR TITLE-ABS-KEY (tributary)) OR (TITLE-ABS-KEY (estuary) OR TITLE-ABS-KEY (coast) OR TITLE-ABS-KEY (coastal) OR TITLE-ABS-KEY (catchment) OR TITLE-ABS-KEY (reservoir)) OR (TITLE-ABS-KEY ("storm water") OR TITLE-ABS-KEY (stormwater) OR TITLE-ABS-KEY ("river bank") OR TITLE-ABS-KEY (shoreline) OR TITLE-ABS-KEY (shore)) OR (TITLE-ABS-KEY (stream) OR TITLE-ABS-KEY (lake) OR TITLE-ABS-KEY ("water cycle") OR TITLE-ABS-KEY ("water supply") OR TITLE-ABS-KEY (beach) OR TITLE-ABS-KEY ("bathing beach") OR TITLE-ABS-KEY (rural)) OR (TITLE-ABS-KEY ("fresh water") OR TITLE-ABS-KEY (freshwater) OR TITLE-ABS-KEY (bay) OR TITLE-ABS-KEY (inlet) OR TITLE-ABS-KEY ("water resource") OR TITLE-ABS-KEY (river)))) OR ((TITLE-ABS-KEY ("water sport") OR TITLE-ABS-KEY ("water surf*") OR TITLE-ABS-KEY ("wave surf*") OR TITLE-ABS-KEY (row*) OR TITLE-ABS-KEY (kayak*)) OR (TITLE-ABS-KEY (boat*) OR TITLE-ABS-KEY (surfboard*) OR TITLE-ABS-KEY ("water ski*")) OR (TITLE-ABS-KEY (recreation*) W/15 TITLE-ABS-KEY ("water exposure")) OR (TITLE-ABS-KEY ("water recreation")) OR (TITLE-ABS-KEY (recreation*) W/15 TITLE-ABS-KEY ("water use")) OR (TITLE-ABS-KEY (recreation*) W/15 TITLE-ABS-KEY ("water user")) OR (TITLE-ABS-KEY ("whole body contact") OR TITLE-ABS-KEY ({whole-body contact}) OR TITLE-ABS-KEY ("incidental contact") OR swim*) OR (TITLE-ABS-KEY (bath*) OR TITLE-ABS-KEY (wading) OR TITLE-ABS-KEY (wade) OR TITLE-ABS-KEY (waded) OR TITLE-ABS-KEY (paddl*) OR TITLE-ABS-KEY (sail*) OR TITLE-ABS-KEY ("sailboat*")) OR (TITLE-ABS-KEY ("bodyboard*") OR TITLE-ABS-KEY ("body surf*") OR TITLE-ABS-KEY (bodysurf*) OR TITLE-ABS-KEY ("wake board*") OR TITLE-ABS-KEY (wakeboard*) OR TITLE-ABS-KEY ("wind surf*") OR TITLE-ABS-KEY (windsurf*)) OR (TITLE-ABS-KEY ("water ski*") OR TITLE-ABS-KEY (waterski*) OR TITLE-ABS-KEY ({water-ski}) OR TITLE-ABS-KEY ({water-skiing}) OR TITLE-ABS-KEY ({water-skied}) OR TITLE-ABS-KEY ({water-skier}) OR TITLE-ABS-KEY ("jet ski*")) OR (TITLE-ABS-KEY (fish) OR TITLE-ABS-KEY (fishing) OR TITLE-ABS-KEY (angler) OR TITLE-ABS-KEY (angling)) OR (TITLE-ABS-KEY (canoe) OR TITLE-ABS-KEY (canoeing) OR TITLE-ABS-KEY (canoeist) OR TITLE-ABS-KEY (canoer)) OR (TITLE-ABS-KEY (snorkel*) OR TITLE-ABS-KEY ("scuba div*") OR TITLE-ABS-KEY (dive) OR TITLE-ABS-KEY (diving) OR TITLE-ABS-KEY (diver)) OR (TITLE-ABS-KEY (surf*) OR TITLE-ABS-KEY ("kiteboard*") OR TITLE-ABS-KEY ("kitesurf*") TITLE-ABS-KEY ("kite surf*") OR TITLE-ABS-KEY (parasail*)) OR (TITLE-ABS-KEY (pentathlon) OR TITLE-ABS-KEY (pentathlete) OR TITLE-ABS-KEY (triathlon) OR TITLE-ABS-KEY (triathlete))))) AND (((TITLE-ABS-KEY (amoeba) OR TITLE-ABS-KEY ("Burkholderia pseudomallei") OR TITLE-ABS-KEY ("Pseudomonas pseudomallei") OR TITLE-ABS-KEY ("*Naegleria fowleri*") OR TITLE-ABS-KEY ("Naegleria fowlerus")) OR (TITLE-ABS-KEY ({free-living amoeba}) OR TITLE-ABS-KEY ({free-living amoebas}) OR TITLE-ABS-KEY ({free-living amoebae}) OR TITLE-ABS-KEY ({free-living ameba}) OR TITLE-ABS-KEY ({free-living amebas}) OR TITLE-ABS-KEY ({free-living amebae})) OR (TITLE-ABS-KEY ({free-living amoebic}) OR TITLE-ABS-KEY ({free-living amebic}) OR TITLE-ABS-KEY ("free living amoebic") OR TITLE-ABS-KEY ("free living amebic")) OR (TITLE-ABS-KEY ({free-living microorganism}) OR TITLE-ABS-KEY ({free-living microorganisms}) OR TITLE-ABS-KEY ("free living microorganism") OR TITLE-ABS-KEY ({free-living micro-organism}) OR TITLE-ABS-KEY ({free-living micro-organisms}) OR TITLE-ABS-KEY ({free

Category	Strings
	living micro-organisms}) OR TITLE-ABS-KEY ({free living micro-organism})) OR (TITLE-ABS-KEY ("free living amoeba") OR TITLE-ABS-KEY ("free living amoebae") OR TITLE-ABS-KEY ("free living ameba") OR TITLE-ABS-KEY ("free living amebae"))) AND
Exposure	((((TITLE-ABS-KEY (increas* OR warm* OR hot* OR high*) W/15 TITLE-ABS-KEY (temperature)) OR (TITLE-ABS-KEY (hot* OR warm*) W/10 TITLE-ABS-KEY (weather)) OR (TITLE-ABS-KEY ("climate change")) OR (TITLE-ABS-KEY (climat*) W/15 TITLE-ABS-KEY (chang* OR tropic*)) OR (TITLE-ABS-KEY ("climate warming")) OR (TITLE-ABS-KEY (climat*) W/15 TITLE-ABS-KEY (warm*))) OR ((TITLE-ABS-KEY ("global warming")) OR (TITLE-ABS-KEY (global) W/15 TITLE-ABS-KEY (warm* OR heat*)) OR (TITLE-ABS-KEY (storm) OR TITLE-ABS-KEY (typhoon) OR TITLE-ABS-KEY (tropic*) OR TITLE-ABS-KEY ("tropical climate") OR TITLE-ABS-KEY (flood*) OR TITLE-ABS-KEY ("tropical storm") OR TITLE-ABS-KEY ("cyclonic storm") OR TITLE-ABS-KEY (cyclone) OR TITLE-ABS-KEY (hurricane)) OR (TITLE-ABS-KEY (tropic* OR cyclon*) W/15 TITLE-ABS-KEY (storm)))) OR (((TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (quality)) OR (TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (microbiology)) OR (TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (microbe)) OR (TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (contaminat*))) OR ((TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (temperature)) OR (TITLE-ABS-KEY (warm) W/15 TITLE-ABS-KEY (water)) OR (TITLE-ABS-KEY (warm) W/15 TITLE-ABS-KEY (freshwater)) OR (TITLE-ABS-KEY (warm) W/15 TITLE-ABS-KEY ("fresh water"))) OR ((TITLE-ABS-KEY (warm) W/15 TITLE-ABS-KEY ("recreational water")) OR (TITLE-ABS-KEY (freshwater) OR TITLE-ABS-KEY ("fresh water") OR TITLE-ABS-KEY ("surface water")) OR (TITLE-ABS-KEY (recreational) W/10 TITLE-ABS-KEY ("surface water" OR water OR "fresh water" OR freshwater)) OR (TITLE-ABS-KEY ("warm water") OR TITLE-ABS-KEY ("warm fresh water") OR TITLE-ABS-KEY ("warm freshwater") OR TITLE-ABS-KEY ("Recreational water"))))) OR (((TITLE-ABS-KEY ("warm water") W/15 TITLE-ABS-KEY (expos*)) OR (TITLE-ABS-KEY ("fresh water") W/15 TITLE-ABS-KEY (expos*)) OR (TITLE-ABS-KEY (freshwater) W/15 TITLE-ABS-KEY (expos*)) OR (TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (expos*))) OR ((TITLE-ABS-KEY ("Recreational water") W/15 TITLE-ABS-KEY (expos*)) OR (TITLE-ABS-KEY ("warm water") W/15 TITLE-ABS-KEY (contaminat*)) OR (TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (contaminat*)) OR (TITLE-ABS-KEY ("fresh water") W/15 TITLE-ABS-KEY (contaminat*))) OR ((TITLE-ABS-KEY (freshwater) W/15 TITLE-ABS-KEY (contaminat*)) OR (TITLE-ABS-KEY ("recreational water") W/15 TITLE-ABS-KEY (contaminat*)) OR (TITLE-ABS-KEY (soil) W/15 TITLE-ABS-KEY (contaminat*)) OR (TITLE-ABS-KEY (soil))) OR ((TITLE-ABS-KEY (expos*) OR TITLE-ABS-KEY (contact) OR TITLE-ABS-KEY (inhal*) OR TITLE-ABS-KEY (breath*) OR TITLE-ABS-KEY (ingest*) OR TITLE-ABS-KEY (swallow*)) OR (TITLE-ABS-KEY (nose) OR TITLE-ABS-KEY (nasal) OR TITLE-ABS-KEY (skin) OR TITLE-ABS-KEY (dermal*) OR TITLE-ABS-KEY (aural) OR TITLE-ABS-KEY (ear) OR TITLE-ABS-KEY (ocular) OR TITLE-ABS-KEY (eye)) OR (TITLE-ABS-KEY ("water droplet")) OR (TITLE-ABS-KEY (water) W/15 TITLE-ABS-KEY (droplet))) OR ((TITLE-ABS-KEY ("liquid droplet")) OR (TITLE-ABS-KEY (liquid) W/15 TITLE-ABS-KEY (droplet)) OR (TITLE-ABS-KEY (aerosol)) OR (TITLE-ABS-KEY (droplet) OR TITLE-ABS-KEY (thermophilic) OR TITLE-ABS-KEY (pathogen) OR TITLE-ABS-KEY ("Surface water pathogen")))))) AND
Health Outcomes	(((((TITLE-ABS-KEY ("Central nervous system protozoal infection") OR TITLE-ABS-KEY ("protozoal infection") OR TITLE-ABS-KEY ("*Naegleria fowleri* Infection") OR TITLE-ABS-KEY ("*Naegleria fowleri* Meningoencephalitis") OR TITLE-ABS-KEY ("*Naegleria fowleri* Meningoencephalitides")) OR (TITLE-ABS-KEY (protozoal) W/15 TITLE-ABS-KEY (infection)) OR (TITLE-ABS-KEY ("*Naegleria fowleri*") W/15 TITLE-ABS-KEY (infection OR meningoencephalitides))) OR ((TITLE-ABS-KEY ("Protozoal Meningoencephalitis") OR TITLE-ABS-KEY ("Protozoal Meningoencephalitides") OR TITLE-ABS-KEY (melioidosis) OR TITLE-ABS-KEY (melioidoses)) OR (TITLE-ABS-KEY ("*Burkholderia pseudomallei* Infection")) OR (TITLE-ABS-KEY ("Burkholderia pseudomallei") W/15 TITLE-ABS-KEY (infection))) OR ((TITLE-ABS-KEY (protozoan) W/15 TITLE-ABS-KEY (infection)) OR (TITLE-ABS-KEY ("protozoan Infection")))) OR (TITLE-ABS-KEY ("Primary Amebic Meningoencephalitis") OR TITLE-ABS-KEY (pam) OR TITLE-ABS-KEY ("Primary Amoebic Meningoencephalitis") OR TITLE-ABS-KEY ("Primary Amebic Meningoencephalitides") OR TITLE-ABS-KEY ("Primary Amoebic Meningoencephalitides"

Category	Strings
	) OR TITLE-ABS-KEY ("Amebic Meningoencephalitis") OR TITLE-ABS-KEY ("Amoebic Meningoencephalitis") OR TITLE-ABS-KEY ("Amebic Meningoencephalitides") OR TITLE-ABS-KEY ("Amoebic Meningoencephalitides")) OR (((TITLE-ABS-KEY (health) W/15 TITLE-ABS-KEY (effect OR outcome OR adverse)) OR (TITLE-ABS-KEY (mortality) OR TITLE-ABS-KEY (morbidity) OR TITLE-ABS-KEY (death) OR TITLE-ABS-KEY (fatal) OR TITLE-ABS-KEY (fatality) OR TITLE-ABS-KEY (die) OR TITLE-ABS-KEY (died)) OR (TITLE-ABS-KEY ("Waterborne disease") OR TITLE-ABS-KEY ({Water-borne disease}) OR TITLE-ABS-KEY ({Water-borne diseases}) OR TITLE-ABS-KEY (disease)) OR (TITLE-ABS-KEY ("Recreational water") W/15 TITLE-ABS-KEY (infection))) OR ((TITLE-ABS-KEY (gastrointestin*) OR TITLE-ABS-KEY (digest*) OR TITLE-ABS-KEY (nausea) OR TITLE-ABS-KEY (nauseous) OR TITLE-ABS-KEY (vomit*) OR TITLE-ABS-KEY (spew*)) OR (TITLE-ABS-KEY (diarrhea) OR TITLE-ABS-KEY ("Accidental fecal discharge") OR TITLE-ABS-KEY ("Accidental faecal discharge") OR TITLE-ABS-KEY ("Accidental bowel leakage")) OR (TITLE-ABS-KEY ("Fecal incontinence") OR TITLE-ABS-KEY ("Faecal incontinence") OR TITLE-ABS-KEY ("Bowel Incontinence") OR TITLE-ABS-KEY ("Bowel leakage") OR TITLE-ABS-KEY ("Fecal soiling") OR TITLE-ABS-KEY ("Faecal soiling") OR TITLE-ABS-KEY ("Fecal discharge") OR TITLE-ABS-KEY ("Faecal discharge")) OR (TITLE-ABS-KEY ({Pneumonia-like}) OR TITLE-ABS-KEY ({Pneumonia-like symptoms}) OR TITLE-ABS-KEY ("Pneumonia like symptoms") OR TITLE-ABS-KEY (pneumonia) OR TITLE-ABS-KEY (pneumonitis) OR TITLE-ABS-KEY (pneumonitides))) OR ((TITLE-ABS-KEY (pulmonary OR lung) W/15 TITLE-ABS-KEY (infection)) OR (TITLE-ABS-KEY (pulmonary OR lung) W/15 TITLE-ABS-KEY (inflam*))))) OR (((TITLE-ABS-KEY (breath) W/8 TITLE-ABS-KEY (shortness)) OR (TITLE-ABS-KEY (breathless) OR TITLE-ABS-KEY (dyspnea) OR TITLE-ABS-KEY (breathlessness)) OR (TITLE-ABS-KEY (difficult* OR trouble) W/15 TITLE-ABS-KEY (breathing)) OR (TITLE-ABS-KEY (inflam* OR sore) W/15 TITLE-ABS-KEY (throat OR pharynx))) OR ((TITLE-ABS-KEY (infect*) OR TITLE-ABS-KEY (symptom)) OR (TITLE-ABS-KEY (signs) W/8 TITLE-ABS-KEY (symptoms)) OR (TITLE-ABS-KEY (illness) OR TITLE-ABS-KEY (ill) OR TITLE-ABS-KEY (sick)) OR (TITLE-ABS-KEY (asthma) OR TITLE-ABS-KEY ("Amebic meningitis") OR TITLE-ABS-KEY ("Amoebic meningitis") OR TITLE-ABS-KEY (meningitis)) OR (TITLE-ABS-KEY (brain) W/15 TITLE-ABS-KEY (inflam* OR infect* OR damag*))) OR ((TITLE-ABS-KEY (fever) OR TITLE-ABS-KEY (pyrexia) OR TITLE-ABS-KEY (pyrexiae) OR TITLE-ABS-KEY (hyperthermia)) OR (TITLE-ABS-KEY (headache) OR TITLE-ABS-KEY ("Head pain") OR TITLE-ABS-KEY (cephalalgia) OR TITLE-ABS-KEY ({Hay fever-like}) OR TITLE-ABS-KEY ("Hay fever like") OR TITLE-ABS-KEY ("Hay fever") OR TITLE-ABS-KEY (hayfever) OR TITLE-ABS-KEY (rhinitis) OR TITLE-ABS-KEY (allergic) OR TITLE-ABS-KEY ("Allergic reaction") OR TITLE-ABS-KEY (hypersensitivity) OR TITLE-ABS-KEY (allerg*)) OR (TITLE-ABS-KEY ({Flu-like}) OR TITLE-ABS-KEY ("Flu like") OR TITLE-ABS-KEY (cough*) OR TITLE-ABS-KEY ("Chest pain") OR TITLE-ABS-KEY ("Sore chest") OR TITLE-ABS-KEY (myalgia)) OR (TITLE-ABS-KEY (muscle) W/15 TITLE-ABS-KEY (sore OR soreness OR tenderness OR pain)) OR (TITLE-ABS-KEY (chest) W/15 TITLE-ABS-KEY (pain* OR sore OR soreness))) OR ((TITLE-ABS-KEY (skin) OR TITLE-ABS-KEY (dermal) OR TITLE-ABS-KEY (dermatology*) OR TITLE-ABS-KEY (eye) OR TITLE-ABS-KEY (ocular) OR TITLE-ABS-KEY (irritation) OR TITLE-ABS-KEY (infection) OR TITLE-ABS-KEY (rash) OR TITLE-ABS-KEY (exanthema) OR TITLE-ABS-KEY (pruritus) OR TITLE-ABS-KEY (itch*)) OR (TITLE-ABS-KEY (neurological) OR TITLE-ABS-KEY (neurologic)) OR (TITLE-ABS-KEY (neurologic*) W/15 TITLE-ABS-KEY (infection OR sign OR symptom)) OR (TITLE-ABS-KEY (seizure) OR TITLE-ABS-KEY (coma) OR TITLE-ABS-KEY ("Central Nervous System") OR TITLE-ABS-KEY (neurotoxic*) OR TITLE-ABS-KEY (neurotoxin*) OR TITLE-ABS-KEY ("nervous system")) OR (TITLE-ABS-KEY ("nervous system") W/15 TITLE-ABS-KEY (damage OR symptom)) OR (TITLE-ABS-KEY (liver) W/15 TITLE-ABS-KEY (damage OR injury)) OR (TITLE-ABS-KEY (hepatotoxicity))))) AND (LIMIT-TO (PUBYEAR , 2022) OR LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008

Category	Strings
	) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMIT-TO (PUBYEAR , 2005) OR LIMIT-TO (PUBYEAR , 2004))

Table 2.5 PubMed® Mesh and Keywords search strings

	((("adult"[MeSH Terms] OR "adolescent"[MeSH Terms] OR "middle aged"[MeSH Terms] OR "young adult"[MeSH Terms] OR "aged"[MeSH Terms] OR "aged, 80 and over"[MeSH Terms] OR "80 and over aged"[All Fields] OR "aged 80 and over"[All Fields] OR "child"[MeSH Terms] OR "infant"[MeSH Terms] OR "pregnant women"[MeSH Terms] OR "immunocompromised host"[MeSH Terms] OR "athletes"[MeSH Terms] OR "oceanic ancestry group"[MeSH Terms]) AND
	("water sports"[MeSH Terms] OR "swimming"[MeSH Terms] OR "diving"[MeSH Terms] OR "fresh water"[MeSH Terms] OR "estuaries"[MeSH Terms] OR "hot springs"[MeSH Terms] OR "bays"[MeSH Terms] OR "water resources"[MeSH Terms] OR "rivers"[MeSH Terms] OR "bathing beaches"[MeSH Terms] OR "water cycle"[MeSH Terms] OR "water supply"[MeSH Terms]) AND
	("amoeba"[MeSH Terms] OR "burkholderia pseudomallei"[MeSH Terms] OR "*Naegleria fowleri*"[MeSH Terms] OR "water quality"[MeSH Terms] OR "water microbiology"[MeSH Terms] OR "environmental exposure"[MeSH Terms] OR "inhalation exposure"[MeSH Terms] OR "climate change"[MeSH Terms] OR "cyclonic storms"[MeSH Terms]) AND
	((("central nervous system protozoal infections"[MeSH Terms] OR "melioidosis"[MeSH Terms] OR "health"[MeSH Terms] OR "waterborne diseases"[MeSH Terms] OR "disease"[MeSH Terms] OR "infections"[MeSH Terms] OR "critical illness"[MeSH Terms] OR "signs and symptoms"[MeSH Terms] OR "signs and symptoms, digestive"[MeSH Terms] OR "nausea"[MeSH Terms] OR "vomiting"[MeSH Terms] OR "diarrhea"[MeSH Terms] OR "fecal incontinence"[MeSH Terms] OR "pneumonia"[MeSH Terms]) AND "abdominal pain"[MeSH Terms]) OR "bronchitis"[MeSH Terms] OR "dyspnea"[MeSH Terms] OR "chest pain"[MeSH Terms] OR "myalgia"[MeSH Terms] OR "arthralgia"[MeSH Terms] OR "fever"[MeSH Terms] OR "headache"[MeSH Terms] OR "sleepiness"[MeSH Terms] OR "pharyngitis"[MeSH Terms] OR "rhinitis, allergic, seasonal"[MeSH Terms] OR "exanthema"[MeSH Terms] OR "pruritus"[MeSH Terms] OR "skin"[MeSH Terms] OR "hypersensitivity"[MeSH Terms] OR "central nervous system"[MeSH Terms] OR "peripheral nervous system"[MeSH Terms] OR "brain"[MeSH Terms] OR "spinal cord"[MeSH Terms] OR "cranial nerves"[MeSH Terms] OR "peripheral nerves"[MeSH Terms] OR "spinal nerve roots"[MeSH Terms] OR "autonomic nervous system"[MeSH Terms] OR "neuromuscular junction"[MeSH Terms] OR "asthma"[MeSH Terms] OR "meningitis"[MeSH Terms]) AND 2004/01/01:2020/12/01[Date - Publication]) OR
Population	(("adults"[Title/Abstract] OR "young adult"[Title/Abstract] OR "young adults"[Title/Abstract] OR "middle age"[Title/Abstract] OR "middle aged"[Title/Abstract] OR "middle aged"[Title/Abstract] OR ("adolescent"[Title/Abstract] OR "adolescents"[Title/Abstract] OR "adolescence"[Title/Abstract] OR "teen*"[Title/Abstract] OR "teen"[Title/Abstract] OR "teenager*"[Title/Abstract] OR "youth*"[Title/Abstract] OR "adolescent females"[Title/Abstract] OR "adolescent female"[Title/Abstract] OR "female"[Title/Abstract] OR "adolescent male"[Title/Abstract] OR "adolescent males"[Title/Abstract] OR "male"[Title/Abstract] OR "males"[Title/Abstract] OR "female"[Title/Abstract] OR "females"[Title/Abstract] OR "man"[Title/Abstract] OR "men"[Title/Abstract] OR "woman"[Title/Abstract] OR "women"[Title/Abstract] OR "child*"[Title/Abstract]) OR ("general public"[Title/Abstract] OR "general populace"[Title/Abstract] OR "general population"[Title/Abstract] OR "public"[Title/Abstract] OR "population*"[Title/Abstract] OR "elderly"[Title/Abstract] OR "old"[Title/Abstract] OR "nonagenarian*"[Title/Abstract] OR "octogenarian*"[Title/Abstract] OR "centenarian*"[Title/Abstract] OR "elder*"[Title/Abstract] OR "senior*"[Title/Abstract] OR "senior citizen"[Title/Abstract] OR "senior citizens"[Title/Abstract]) OR ("matur*"[Title/Abstract] OR "mature aged"[Title/Abstract] OR "older adult"[Title/Abstract] OR "older adults"[Title/Abstract] OR "infant*"[Title/Abstract] OR "pregnant woman"[Title/Abstract] OR "pregnant women"[Title/Abstract] OR "pregnant"[Title/Abstract] OR "pregnanc*"[Title/Abstract] OR ("baby"[Title/Abstract] OR "babies"[Title/Abstract] OR "toddler"[Title/Abstract] OR

	"toddlers"[Title/Abstract])) OR ("immunocompromis*"[Title/Abstract] OR "immunocompromised patient"[Title/Abstract] OR "immunocompromised patients"[Title/Abstract] OR "immunodeficien*"[Title/Abstract] OR "immunosupres*"[Title/Abstract] OR "weaker immune system"[Title/Abstract]) OR ("athlete"[Title/Abstract] OR "athletes"[Title/Abstract] OR "athlete*"[Title/Abstract] OR "recreator*"[Title/Abstract] OR "tourist*"[Title/Abstract]) OR (("aborigin*"[Title/Abstract] OR "australoid"[Title/Abstract] OR "indigenous"[Title/Abstract] OR "torres strait islander"[Title/Abstract]) AND ("austral*"[Title/Abstract] OR "queensland"[Title/Abstract] OR "new south wales"[Title/Abstract] OR "victoria"[Title/Abstract] OR "south australia"[Title/Abstract] OR "australian capital territory"[Title/Abstract] OR "western australia"[Title/Abstract] OR "northern territory"[Title/Abstract] OR "qld"[Title/Abstract] OR "nsw"[Title/Abstract] OR "vic"[Title/Abstract] OR "sa"[Title/Abstract] OR "act"[Title/Abstract] OR "wa"[Title/Abstract] OR "nt"[Title/Abstract])) OR ("aboriginal and torres strait islander"[Title/Abstract] OR "koori"[Title/Abstract]) OR "human"[Title/Abstract] OR "humans"[Title/Abstract]) AND 2004/01/01:2021/12/31[Date - Publication] AND
Exposure	(("free living amoebae"[Title/Abstract] OR "free living amoebae"[Title/Abstract] OR "free living amoeba"[Title/Abstract] OR "free living amoebas"[Title/Abstract] OR "free living amoeba"[Title/Abstract] OR "free living amoebas"[Title/Abstract] OR "free living ameba"[Title/Abstract] OR "free living amebas"[Title/Abstract] OR "free living ameba"[Title/Abstract] OR "free living amebas"[Title/Abstract] OR "free living microorganism"[Title/Abstract] OR "free living microorganisms"[Title/Abstract] OR "free living microorganism"[Title/Abstract] OR "free living microorganisms"[Title/Abstract] OR ("amoeba"[Title/Abstract] OR "amoebas"[Title/Abstract] OR "amebas"[Title/Abstract] OR "amoebae"[Title/Abstract] OR "ameba"[Title/Abstract] OR "burkholderia pseudomallei"[Title/Abstract] OR "pseudomonas pseudomallei"[Title/Abstract] OR "*Naegleria fowleri*"[Title/Abstract])) AND ((("increas*"[Title/Abstract] OR "warm*"[Title/Abstract] OR "hot"[Title/Abstract] OR "hotter"[Title/Abstract] OR "high"[Title/Abstract]) AND ("temperature"[Title/Abstract] OR "temperatures"[Title/Abstract])) OR (("warm*"[Title/Abstract] OR "hot"[Title/Abstract] OR "hotter"[Title/Abstract]) AND ("weather"[Title/Abstract] OR "climate"[Title/Abstract])) OR ("global heating"[Title/Abstract] OR "climatic warming"[Title/Abstract] OR "warming climate"[Title/Abstract] OR "storm"[Title/Abstract] OR "storms"[Title/Abstract] OR "typhoon"[Title/Abstract] OR "typhoons"[Title/Abstract] OR "tropic*"[Title/Abstract] OR "tropical climate"[Title/Abstract] OR "flood*"[Title/Abstract] OR "cyclonic storm"[Title/Abstract] OR "cyclonic storms"[Title/Abstract] OR "cyclone"[Title/Abstract] OR "cyclones"[Title/Abstract] OR "hurricane"[Title/Abstract] OR "hurricanes"[Title/Abstract] OR "tropical storm"[Title/Abstract] OR "tropical storms"[Title/Abstract]) OR (("water"[Title/Abstract] AND ("qualit*"[Title/Abstract] OR "microbiology"[Title/Abstract] OR "microbes"[Title/Abstract] OR "microbe"[Title/Abstract] OR "contamination"[Title/Abstract] OR "temperature*"[Title/Abstract])) OR ("expos*"[Title/Abstract] OR "contact"[Title/Abstract] OR "inhal*"[Title/Abstract] OR "breath*"[Title/Abstract] OR "ingest*"[Title/Abstract] OR "swallow*"[Title/Abstract] OR "nose"[Title/Abstract] OR "nasal"[Title/Abstract] OR "skin"[Title/Abstract] OR "dermal*"[Title/Abstract] OR "aural"[Title/Abstract] OR "ear"[Title/Abstract] OR "ears"[Title/Abstract] OR "ocular"[Title/Abstract] OR "eyes"[Title/Abstract] OR "eye"[Title/Abstract] OR "sediment"[Title/Abstract] OR "sediments"[Title/Abstract] OR "sand"[Title/Abstract] OR "sands"[Title/Abstract] OR "water droplet"[Title/Abstract] OR "water droplets"[Title/Abstract] OR "liquid droplet"[Title/Abstract] OR "liquid droplets"[Title/Abstract] OR "aerosol"[Title/Abstract] OR "aerosols"[Title/Abstract] OR "droplets"[Title/Abstract] OR "climate change"[Title/Abstract] OR "climate warming"[Title/Abstract] OR "global warming"[Title/Abstract] OR "global climate change"[Title/Abstract]) OR ("warm"[Title/Abstract] AND ("water"[Title/Abstract] OR "waters"[Title/Abstract] OR "freshwater"[Title/Abstract] OR "fresh waters"[Title/Abstract] OR "recreational waters"[Title/Abstract]))) OR ("water"[Title/Abstract] OR "waters"[Title/Abstract] OR "surface water"[Title/Abstract] OR "surface waters"[Title/Abstract] OR (("warm water"[Title/Abstract] OR "warm freshwater"[Title/Abstract] OR "warm fresh water"[Title/Abstract] OR "recreational water"[Title/Abstract] OR "water"[Title/Abstract]) AND ("expos*"[Title/Abstract] OR "contaminat*"[Title/Abstract])) OR ("contaminat*"[Title/Abstract] AND "soil"[Title/Abstract])) OR ("thermophilic"[Title/Abstract] OR "pathogen*"[Title/Abstract] OR "surface water pathogen"[Title/Abstract])) AND 2004/01/01:2021/12/31[Date - Publication]) AND

Health Outcomes	(("hepatotoxicity"[Title/Abstract] OR ("liver"[Title/Abstract] AND "injuries"[Title/Abstract]) OR ("liver"[Title/Abstract] AND "injury"[Title/Abstract]) OR (("liver"[Title/Abstract] AND "damage"[Title/Abstract]) OR ("nervous system"[Title/Abstract] AND ("damage"[Title/Abstract] OR "symptom"[Title/Abstract] OR "symptoms"[Title/Abstract])) OR ("neurotoxic*"[Title/Abstract] OR "neurotoxin*"[Title/Abstract] OR "nervous system"[Title/Abstract])) OR ("seizure"[Title/Abstract] OR "seizures"[Title/Abstract] OR "coma"[Title/Abstract] OR "comas"[Title/Abstract] OR "central nervous system"[Title/Abstract] OR ("neurologic"[Title/Abstract] AND ("infection"[Title/Abstract] OR "infections"[Title/Abstract] OR "sign"[Title/Abstract] OR "signs"[Title/Abstract] OR "symptom"[Title/Abstract] OR "symptoms"[Title/Abstract])) OR ("neurological"[Title/Abstract] AND ("infection"[Title/Abstract] OR "infections"[Title/Abstract] OR "sign"[Title/Abstract] OR "signs"[Title/Abstract] OR "symptom"[Title/Abstract] OR "symptoms"[Title/Abstract]))) OR ("neurological"[Title/Abstract] OR "neurologic"[Title/Abstract] OR ((("eye irritation"[Title/Abstract] OR "eye irritations"[Title/Abstract] OR "ocular irritation"[Title/Abstract]) AND "ocular irritations"[Title/Abstract]) OR "ocular infection"[Title/Abstract] OR "ocular infections"[Title/Abstract] OR "eye infection"[Title/Abstract] OR "eye infections"[Title/Abstract]) OR ("dermal irritation"[Title/Abstract] OR "skin infections"[Title/Abstract] OR "skin infection"[Title/Abstract] OR "skin allergy"[Title/Abstract] OR "skin allergies"[Title/Abstract] OR "skin irritation"[Title/Abstract] OR "skin irritations"[Title/Abstract] OR "skin rash"[Title/Abstract] OR "skin rashes"[Title/Abstract] OR "dermatolog*"[Title/Abstract] OR "rash"[Title/Abstract] OR "rashes"[Title/Abstract] OR "exanthema"[Title/Abstract] OR "pruritus"[Title/Abstract] OR "itch*"[Title/Abstract])) OR (("sore"[Title/Abstract] AND ("muscles"[Title/Abstract] OR "muscle"[Title/Abstract])) OR ("flu like"[Title/Abstract] OR "flu like"[Title/Abstract] OR "cough*"[Title/Abstract] OR "chest pain"[Title/Abstract] OR "chest pains"[Title/Abstract] OR "myalgia"[Title/Abstract] OR "muscle pain"[Title/Abstract] OR "muscle pains"[Title/Abstract] OR "muscle soreness"[Title/Abstract] OR "muscle tenderness"[Title/Abstract]) OR ("hyperthermia"[Title/Abstract] OR "hyperthermias"[Title/Abstract] OR "headache"[Title/Abstract] OR "headaches"[Title/Abstract] OR "head pain"[Title/Abstract] OR "head pains"[Title/Abstract] OR "cephalalgia"[Title/Abstract] OR "cephalalgias"[Title/Abstract] OR "hay fever like"[Title/Abstract] OR "hay fever like"[Title/Abstract] OR "hay fever"[Title/Abstract] OR "hayfever"[Title/Abstract] OR "rhinitis"[Title/Abstract] OR "allergic"[Title/Abstract] OR "allergic reaction"[Title/Abstract] OR "allergic reactions"[Title/Abstract] OR "hypersensitivity"[Title/Abstract] OR "hypersensitivities"[Title/Abstract] OR "allergy"[Title/Abstract] OR "allergies"[Title/Abstract]) OR ("fever"[Title/Abstract] OR "fevers"[Title/Abstract] OR "pyrexia"[Title/Abstract] OR "pyrexias"[Title/Abstract] OR "pyrexiae"[Title/Abstract] OR ("brain"[Title/Abstract] AND ("inflammation"[Title/Abstract] OR "infection"[Title/Abstract] OR "damage"[Title/Abstract])) OR ("asthma"[Title/Abstract] OR "amebic meningitis"[Title/Abstract] OR "amoebic meningitis"[Title/Abstract] OR "meningitis"[Title/Abstract])) OR ("disease"[Title/Abstract] OR "diseases"[Title/Abstract] OR "infection"[Title/Abstract] OR "infections"[Title/Abstract] OR "symptom"[Title/Abstract] OR "symptoms"[Title/Abstract] OR "signs and symptoms"[Title/Abstract] OR "symptoms and signs"[Title/Abstract] OR "illness"[Title/Abstract] OR "illnesses"[Title/Abstract] OR "ill"[Title/Abstract] OR "sick*"[Title/Abstract] OR ("sore"[Title/Abstract] AND ("throat"[Title/Abstract] OR "pharynx"[Title/Abstract])) OR ("inflam*"[Title/Abstract] AND ("throat"[Title/Abstract] OR "pharynx"[Title/Abstract]))) OR ("breathless"[Title/Abstract] OR "dyspnea"[Title/Abstract] OR "dyspneas"[Title/Abstract] OR "dyspnoea"[Title/Abstract] OR "dyspnoeas"[Title/Abstract] OR "breathlessness"[Title/Abstract] OR "difficulty breathing"[Title/Abstract] OR "breathing difficulty"[Title/Abstract] OR "trouble breathing"[Title/Abstract] OR ("breath"[Title/Abstract] AND "shortness"[Title/Abstract]) OR (("pulmonary"[Title/Abstract] OR "lung"[Title/Abstract]) AND "inflammation"[Title/Abstract]))) OR ((("pulmonary"[Title/Abstract] OR "lung"[Title/Abstract]) AND "infection"[Title/Abstract]) OR ("pneumonia like"[Title/Abstract] OR "pneumonia like symptoms"[Title/Abstract] OR "pneumonia like symptoms"[Title/Abstract] OR "pneumonia"[Title/Abstract] OR "pneumonias"[Title/Abstract] OR "pneumonitis"[Title/Abstract] OR "pneumonitides"[Title/Abstract]) OR ("gastrointestin*"[Title/Abstract] OR "digest*"[Title/Abstract] OR "nausea"[Title/Abstract] OR "nauseous"[Title/Abstract] OR "vomit*"[Title/Abstract] OR "spew*"[Title/Abstract] OR "diarrhea"[Title/Abstract] OR "accidental bowel leakage"[Title/Abstract] OR "fecal incontinence"[Title/Abstract] OR "faecal incontinence"[Title/Abstract] OR "bowel incontinence"[Title/Abstract] OR "bowel leakage"[Title/Abstract] OR "fecal soiling"[Title/Abstract]

	OR "faecal soiling"[Title/Abstract] OR "fecal discharge"[Title/Abstract] OR "faecal discharge"[Title/Abstract]) OR ("waterborne disease"[Title/Abstract] OR "waterborne diseases"[Title/Abstract] OR "water borne disease"[Title/Abstract] OR "water borne diseases"[Title/Abstract]) OR ("health"[Title/Abstract] OR "health effects"[Title/Abstract] OR "health outcome"[Title/Abstract] OR "health outcomes"[Title/Abstract] OR "adverse effect"[Title/Abstract] OR "adverse effects"[Title/Abstract] OR "mortality"[Title/Abstract] OR "morbidity"[Title/Abstract] OR "death"[Title/Abstract] OR "fatal"[Title/Abstract] OR "fatality"[Title/Abstract] OR "fatalities"[Title/Abstract] OR "death"[Title/Abstract] OR "die"[Title/Abstract] OR "died"[Title/Abstract] OR ("primary amebic meningoencephalitis"[Title/Abstract] OR "primary amoebic meningoencephalitis"[Title/Abstract] OR "amebic meningoencephalitis"[Title/Abstract] OR "amoebic meningoencephalitis"[Title/Abstract] OR "amebic meningoencephalitides"[Title/Abstract]) OR ("protozoan"[Title/Abstract] AND ("infection"[Title/Abstract] OR "infections"[Title/Abstract]))) OR ("protozoal meningoencephalitis"[Title/Abstract] OR "melioidosis"[Title/Abstract] OR "melioidoses"[Title/Abstract] OR "*Burkholderia pseudomallei* infection"[Title/Abstract] OR "*Burkholderia pseudomallei* infections"[Title/Abstract] OR ("central nervous system protozoal infections"[Title/Abstract] OR "protozoal infection"[Title/Abstract] OR "protozoal infections"[Title/Abstract] OR "*Naegleria fowleri* infection"[Title/Abstract] OR "*Naegleria fowleri* infections"[Title/Abstract] OR "*Naegleria fowleri* meningoencephalitis"[Title/Abstract]))))) AND 2004/01/01:2021/12/31[Date - Publication]) AND
Recreational Water	(("fresh water"[Title/Abstract] OR "freshwater"[Title/Abstract] OR "freshwaters"[Title/Abstract] OR "fresh waters"[Title/Abstract] OR "bay"[Title/Abstract] OR "bays"[Title/Abstract] OR "inlet"[Title/Abstract] OR "inlets"[Title/Abstract] OR "water resource"[Title/Abstract] OR "water resources"[Title/Abstract] OR "river"[Title/Abstract] OR "rivers"[Title/Abstract] OR "stream"[Title/Abstract] OR "streams"[Title/Abstract] OR "lake"[Title/Abstract] OR "lakes"[Title/Abstract] OR "water cycle"[Title/Abstract] OR "water cycles"[Title/Abstract] OR "water supply"[Title/Abstract] OR "water supplies"[Title/Abstract] OR ("beach"[Title/Abstract] OR "beaches"[Title/Abstract] OR "bathing beaches"[Title/Abstract] OR "bathing beach"[Title/Abstract] OR "rural"[Title/Abstract] OR "storm water"[Title/Abstract] OR "storm waters"[Title/Abstract] OR "stormwater"[Title/Abstract] OR "stormwaters"[Title/Abstract] OR "river bank"[Title/Abstract] OR "river banks"[Title/Abstract] OR "shoreline"[Title/Abstract] OR "shorelines"[Title/Abstract] OR "shore"[Title/Abstract] OR "shores"[Title/Abstract] OR "estuar*"[Title/Abstract] OR "coast"[Title/Abstract] OR "coasts"[Title/Abstract] OR "coastal"[Title/Abstract]) OR ("catchment*"[Title/Abstract] OR "reservoir*"[Title/Abstract] OR "hot spring"[Title/Abstract] OR "hot springs"[Title/Abstract] OR "hotspring*"[Title/Abstract] OR "thermal spring"[Title/Abstract] OR "thermal springs"[Title/Abstract] OR "dam"[Title/Abstract] OR "dams"[Title/Abstract] OR "salt water"[Title/Abstract] OR "salt waters"[Title/Abstract] OR "tributar*"[Title/Abstract] OR "pentathlon"[Title/Abstract] OR "pentathlons"[Title/Abstract] OR "pentathlete"[Title/Abstract] OR "triathlon"[Title/Abstract] OR "triathlons"[Title/Abstract] OR "triathlete"[Title/Abstract]) OR ("scuba dive"[Title/Abstract] OR "scuba diving"[Title/Abstract] OR "scuba diver"[Title/Abstract] OR "dive"[Title/Abstract] OR "diving"[Title/Abstract] OR "diver"[Title/Abstract] OR "surf*"[Title/Abstract] OR "surfing"[Title/Abstract] OR "surfer"[Title/Abstract] OR "surfers"[Title/Abstract] OR "kite boarding"[Title/Abstract] OR "kite surfing"[Title/Abstract] OR "parasail*"[Title/Abstract] OR "canoe"[Title/Abstract] OR "canoeing"[Title/Abstract] OR "row"[Title/Abstract] OR "rowing"[Title/Abstract] OR "rower"[Title/Abstract] OR "rowed"[Title/Abstract] OR "canoer"[Title/Abstract] OR "canoeist"[Title/Abstract] OR "canoed"[Title/Abstract] OR "snorkel*"[Title/Abstract] OR ("body surfing"[Title/Abstract] OR "wake boarding"[Title/Abstract] OR "wind surfing"[Title/Abstract] OR "water ski"[Title/Abstract] OR "water skiing"[Title/Abstract] OR "jet ski"[Title/Abstract] OR "jet skiing"[Title/Abstract] OR "fish"[Title/Abstract] OR "fishing"[Title/Abstract] OR "angling"[Title/Abstract] OR "angler*"[Title/Abstract] OR "kayak"[Title/Abstract] OR "kayaking"[Title/Abstract] OR "kayaker"[Title/Abstract] OR "kayaked"[Title/Abstract]) OR ("water ski"[Title/Abstract] OR "water skiing"[Title/Abstract] OR "recreation"[Title/Abstract] OR "recreational water exposure"[Title/Abstract] OR "water recreation"[Title/Abstract] OR "recreational water use"[Title/Abstract] OR "recreational water"[Title/Abstract])) OR ("incidental contact"[Title/Abstract] OR "swim*"[Title/Abstract] OR "bath*"[Title/Abstract] OR "wade"[Title/Abstract] OR "waded"[Title/Abstract] OR "wading"[Title/Abstract] OR "paddle"[Title/Abstract] OR "paddling"[Title/Abstract] OR "paddled"[Title/Abstract] OR "sail*"[Title/Abstract] OR "sailboating"[Title/Abstract] OR "sail boat"[Title/Abstract] OR ("water

	sport"[Title/Abstract] OR "water sports"[Title/Abstract] OR "wave surfing"[Title/Abstract] OR "boat*"[Title/Abstract] OR "surfboard*"[Title/Abstract])) AND 2004/01/01:2021/12/31[Date - Publication])) NOT (animals [mh] NOT humans [mh])

2.2.4 Assessing Evidence from Other Sources

Grey literature searches were undertaken of the websites covering a period of 20 years.

Grey literature searches covered the following sources:

- Reports and news articles by searching the ProQuest, and ANZ News stream. Additional news articles from the Conversation.
- Conference papers by searching Scopus database
- Reports by World Health Organisation
- Journal articles, factsheets, reports, publications and statistics from US CDC
- Factsheets and online resources from government health websites- NSW Health, NT Health, Queensland Health
- Articles from journals articles/online publications published by organisations (e.g. Infectious Diseases Society of America, Water Research Australia etc.). Found by searching Web of Science™ and organisational websites.

It was not possible to search the websites of these sources using the search terms and search strings used in the major database searches.

2.2.5 Publication dates and language

Papers and reports published from 2004 until 2021 were considered in this review. The selection of this date ensured the inclusion of relevant studies and reports published since the last review for the *Guidelines for Managing Risks in Recreational Water* (2008). Search results were restricted to English publications only.

2.2.6 Key definitions

Key definitions as outlines in the protocol were used to define the scope of the review.

Table 2.6 Key definitions

Key definitions	
Free-living microorganisms	Microscopic organisms such as amoeba, saprozoic bacteria and protozoa that can exist independently of other organisms and which are generally considered opportunistic pathogens.
Recreational water	*Included*: Any natural or artificial water bodies without a chlorine disinfectant residual that might be used for recreation including coastal, estuarine and freshwater environments. Includes public, private, commercial and non-commercial recreational water sites. Includes unique unregulated sites such as wave pools, ocean- or river-fed swimming pools, artificial lagoons and water ski parks. *Excluded*: Aquatic facilities using chemical disinfection including swimming pools, spas, splash parks, ornamental water sites.
Recreational water use	*Included*: Any designated or undesignated activity relating to sport, pleasure and relaxation that involves whole body contact or incidental exposure (through any exposure route) to recreational water (e.g. swimming, diving, boating, fishing) *Excluded*: Consuming the catch from fishing or foodstuffs collected from recreational water or its surroundings. Therapeutic uses of waters (e.g. hydrotherapy pools). Occupational exposure.
Recreational water users	Recreators or users of recreational water bodies including: • the general public including all relevant life stages, ages and states of health other than persons that are explicitly advised to avoid such activities (e.g. for specific medical conditions) • tourists • specialist sporting users (e.g. athletes, anglers, kayakers, divers, surfers) • any groups that may have high exposures to recreational water.

2.2.7 Population, Exposure, Comparator, Outcome

The following advice has been scoped out and provided by RWQAC to inform the evidence review:

Table 2.7 Population, Exposure, Comparator, Outcome table

Population, Exposure (Comparator), Outcome (PE(C)O) table	
Element	**Criteria**

Population	Population groups that are relevant to the Guidelines:
	• The general population
	• Specific subpopulations:
	  ○ Elderly
	  ○ Infants and children
	  ○ Pregnant women
	  ○ Aboriginal and Torres Strait Islander peoples
	  ○ Any groups that might be exposed more frequently as a result of inequity e.g. geographic location, socioeconomic status or lifestyle/occupation.
	  ○ Subgroups with unusual exposure patterns making them more susceptible (e.g. athletes, people or age-groups practicing energetic water-based activities or using recreational water for cultural ablution purposes) due to larger volumes of water ingested and/or inhaled, different frequency of exposure etc.

Exposure (and comparator)	Free-living microorganisms of interest (through all routes of exposure, compared to no exposure): • *Naegleria fowleri* • *Burkholderia pseudomallei* Include circumstances that lead to elevated exposures (e.g. sediment concentrations and exposure, settings with incidences of thermal pollution)
Outcomes	Relevant human health outcomes of interest: For *Naegleria fowleri:* • primary amoebic meningoencephalitis (PAM) • all other adverse health outcomes For *Burkholderia pseudomallei* • melioidosis • all other adverse health outcomes

2.2.8 Inclusion and exclusion criteria

Key terms including those listed in the key definitions and PECO were used to determine if studies were relevant in the review.

Publications were screen by title and abstract using the defined key terms to determine if they were included or excluded from the review.

When a reviewer was unsure of the inclusion/exclusion of a publication at title and abstract screening, full text publications were screened to determine eligibility.

2.2.9 Documentation of search results

Search results were exported to Microsoft Excel spreadsheets and sorted and filtered based on relevancy and quality. Search results also recorded which publications were excluded and the justification/criteria for exclusion (Section 7).

2.3 Evidence Collection

2.3.1 Classification of evidence

Two broad classes of literature were reviewed, namely (i) guidelines/reports and (ii) primary studies. For the purposes of quality assessment, primary studies were further classified according to type of research involved.

2.3.2 Quality assessment (by types)

Guidelines

The methodological quality of existing guidelines was assessed using administrative and technical criteria in the assessment tool shown in Appendix 1. The criteria listed in the tool were based on common domains that have been evaluated in several existing tools for assessing guidelines and systematic reviews (e.g. AGREE tool: Brouwers, Kerkvliet, et al., 2016; AGREE Next Steps Consortium, 2017). Based on the responses in the form a decision was made on whether that guideline should be included or excluded from the

review. Due to the paucity of material on free living organisms in recreational waters the decision on inclusion/exclusion was weighted towards inclusion.

In addition to this formal quality assessment approach, the close inspection of the full text document indicated that the evidence contained in the document did not satisfactorily contribute to answering the primary and/or secondary research questions in some cases. Where that was the case, the document was classified as “Quality satisfactory but content not relevant (or obsolete)” and excluded on relevance.

Primary Reviews

Definitions used here were provided by NHMRC as follows:

- “Bias refers to factors that can systematically affect the observations and conclusions of a study and cause them to be different from the truth”
- “Risks of bias (RoB) are the likelihood that features of the study design will give misleading results”

Reference: https://www.nhmrc.gov.au/guidelinesforguidelines/develop/assessing-risk-bias

The methodological quality of individual studies was assessed using an adaptation of the OHAT risk of bias tool (Appendix 2) (OHAT, 2019). Studies were evaluated on applicable risk of bias questions based on study design. The rating or answer to each risk of bias question was selected on an outcome basis from four options:

- definitely low risk of bias (++)
- probably low risk of bias (+)
- probably high risk of bias (-)
- definitely high risk of bias (--)

Studies that were determined to have a high risk of bias or serious concerns with study quality were excluded from the review. Their removal was recorded with justification in the PRISMA Flow Diagram.

Conflicts of interest and funding data from the study characteristics tables were considered when assessing whether these might have affected any of the risk of bias domains (e.g. selection of comparators, selective reporting of results). If there were serious overall concerns, these were noted under ‘Other sources of bias’ in Appendix 1. The outcome of the risk of bias assessments are presented in the in Section 4.2 of the Evidence Evaluation Report, together with a discussion of the overall quality of each study.

2.4 Data extraction

Documentation of the attributes of the shortlisted primary research literature in support of responses to the primary and secondary search questions were recorded using the form shown in Appendix 3, which is based loosely on the PRISMA approach (Moher, Liberati, et al., 2009). The form includes bibliographic information e.g. authors, year of publication, year(s) of study period, country of study, study characteristics.

2.5 Process for assessing the Body of Evidence

The evidence collected and appraised for each research question was grouped by study type and outcome where possible and summarised in an Evidence Summary table that assigned the level of certainty (or

confidence) in that body of evidence. Due to the different nature and quality of evidence between guidelines and primary studies different approaches were required to review and evaluate the body of evidence for each type of literature.

3 Literature search results

3.1 Existing guidelines/reports

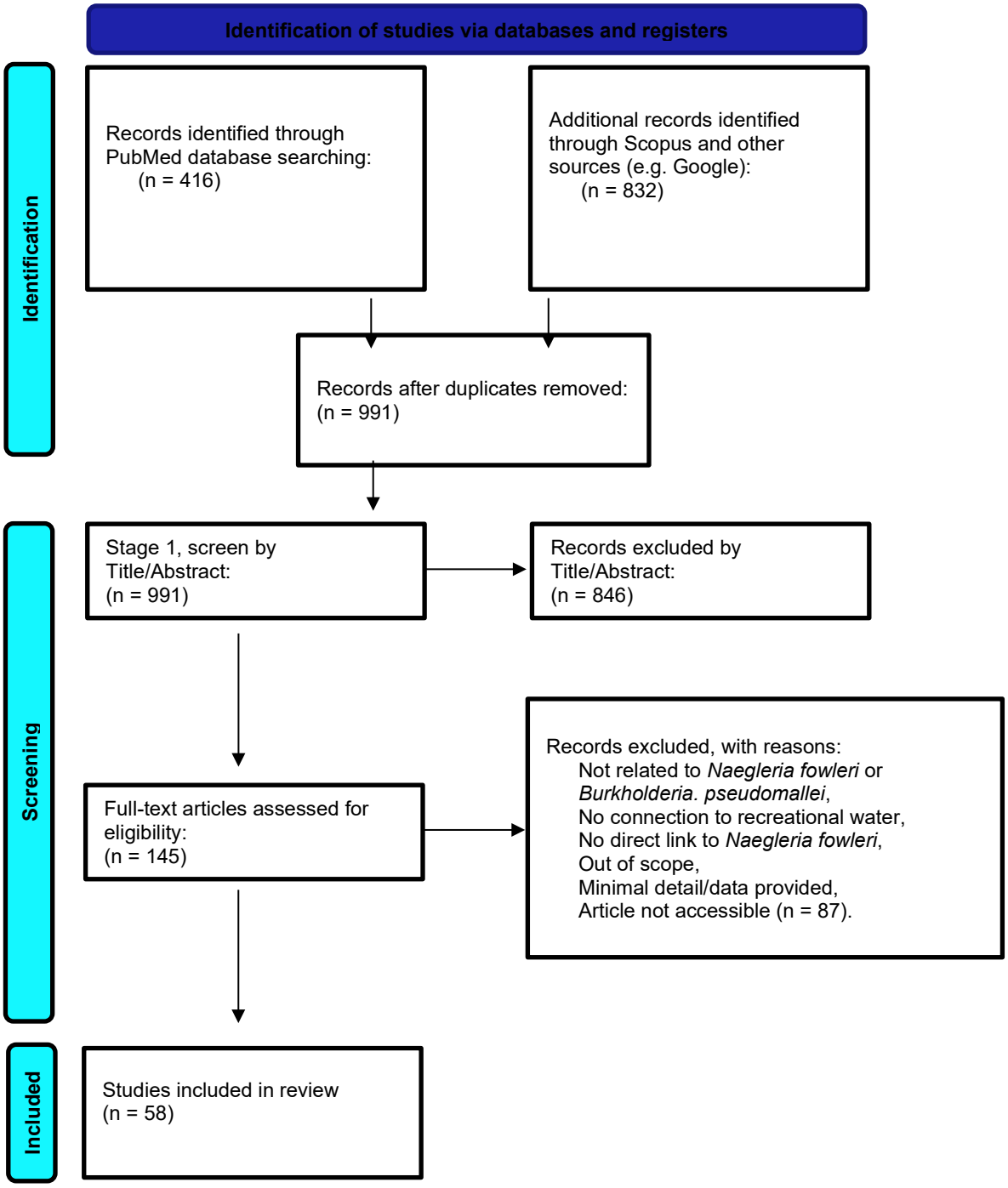
Searches for grey literature (using the method described in Section 2.2.4) identified 144 documents in including, reports and news articles, conference papers, reports by World Health Organisation, journal articles, factsheets, reports, publications and statistics from government health websites, and articles from journals articles/online publications published by organisations. One item was suggested by the committee. Each document was evaluated for its relevance based on the inclusion and exclusion criteria (section 2.2.8) related to the primary and secondary questions and excluded if not relevant. This process identified one additional document for inclusion. The document was quality assessed following the outlined process (Section 2.3 and 2.4) and included in the Table 4.1.

3.2 Primary studies

Searches for primary studies (using the method described in Section 2.2) with the modification of the Keywords to include the term “water” in the list of Exposure terms (Table 2.3). A total of 1104 documents (416 by PubMed search and 688 by Scopus) were identified. An additional two documents were identified searching other sources and included with the primary studies. The primary studies were then combined with the 144 grey literature and duplicate records were removed. a total of 991 documents were evaluated for relevance (inclusion or exclusion) based on the inclusion and exclusion criteria (section 2.2.8) related to the primary and secondary questions. A total of 702 articles were excluded after initial review due to a lack of relevance with 145 subjected to additional scrutiny. A Further 87 articles were excluded with reasons listed (Section 7, Tables 7.1-7.3) after review of the abstract and full text. The remaining 58 documents met the quality criteria for inclusion in the review. The documents were quality assessed following the outlined process (Section 2.3 and 2.4) and included in in the (Section 4, Tables 4.2-4.5).

3.2.1 Screening process and PRISMA diagram

Figure 1 PRISMA diagram



4 Full list of included studies

4.1 Existing guidelines

4.1.1 Naegleria fowleri

Table 4.1 Included Guidelines for *Naegleria fowleri*

Study ID	*Naegleria fowleri*
N42	Department of Health, Western Australia. (2019). *Naegleria* Response Protocol for drinking water supply systems. Retrieved from https://www.health.wa.gov.au/~/media/Files/Corporate/general-documents/water/PDF/Naegleria-Response-Protocol.pdf

4.1.2 Burkholderia pseudomallei

No Guidelines for *Burkholderia pseudomallei* were included in the review.

4.2 Literature reviews

4.2.1 Naegleria fowleri

Table 4.2 Included literature reviews for *Naegleria fowleri*

Study ID	*Naegleria fowleri*
N30	Bright, K.R., Gerba, C.P. Review: Occurrence of the pathogenic amoeba *Naegleria fowleri* in groundwater. *Hydrogeol J* **25**, 953–958 (2017).
N31	Capewell LG, Harris AM, Yoder JS, Cope JR, Eddy BA, Roy SL, Visvesvara GS, Fox LM, Beach MJ. Diagnosis, Clinical Course, and Treatment of Primary Amoebic Meningoencephalitis in the United States, 1937-2013. J Pediatric Infect Dis Soc. 2015 Dec;4(4):e68-75.
N32	Cooper, Amanda Marie PA-C; Aouthmany, Shaza MD; Shah, Kruti MD; Rega, Paul P. MD, FACEP. Killer amoebas: Primary amoebic meningoencephalitis in a changing climate. Journal of the American Academy of Physician Assistants 32(6):p 30-35, June 2019.
N33	Cope JR, Ali IK. Primary Amebic Meningoencephalitis: What Have We Learned in the Last 5 Years? Curr Infect Dis Rep. 2016 Sep;18(10):31. doi: 10.1007/s11908-016-0539-4. PMID: 27614893; PMCID: PMC5100007.
N34	De Jonckheere JF. The impact of man on the occurrence of the pathogenic free-living amoeboflagellate *Naegleria fowleri*. Future Microbiol. 2012 Jan;7(1):5-7.
N36	Grace E, Asbill S, Virga K. *Naegleria fowleri*: pathogenesis, diagnosis, and treatment options. Antimicrob Agents Chemother. 2015 Nov;59(11):6677-81.
N37	Heggie TW. Swimming with death: *Naegleria fowleri* infections in recreational waters. Travel Med Infect Dis. 2010 Jul;8(4):201-6.

Study ID	*Naegleria fowleri*
N38	Stahl LM, Olson JB. Environmental abiotic and biotic factors affecting the distribution and abundance of *Naegleria fowleri*. FEMS Microbiol Ecol. 2021 Jan 1;97(1):fiaa238.
N39	Yoder JS, Eddy BA, Visvesvara GS, Capewell L, Beach MJ. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962-2008. Epidemiol Infect. 2010 Jul;138(7):968-75.

4.2.2 Burkholderia pseudomallei

Table 4.3 Included literature reviews for *Burkholderia pseudomallei*

Study ID	*Burkholderia pseudomallei*
B13	Foong YC, Tan M, Bradbury RS. Melioidosis: a review. Rural Remote Health. 2014;14(4):2763. Epub 2014 Oct 30.
B14	Hsueh PT, Huang WT, Hsueh HK, Chen YL, Chen YS. Transmission Modes of Melioidosis in Taiwan. Trop Med Infect Dis. 2018 Feb 28;3(1):26.
B15	Inglis TJ, Sousa AQ. The public health implications of melioidosis. Braz J Infect Dis. 2009 Feb;13(1):59-66.
B11	Merritt AJ, Inglis TJJ. The Role of Climate in the Epidemiology of Melioidosis. Curr Trop Med Rep. 2017;4(4):185-191.
B12	Stephens DP, Thomas JH, Ward LM, Currie BJ. Melioidosis Causing Critical Illness: A Review of 24 Years of Experience From the Royal Darwin Hospital ICU. Crit Care Med. 2016 Aug;44(8):1500-5.

4.3 Grey literature

4.3.1 Naegleria fowleri

No Grey literature for *Naegleria fowleri* was included in the review.

4.3.2 Burkholderia pseudomallei

No Grey literature for *Burkholderia pseudomallei* was included in the review.

4.4 Primary studies

4.4.1 Naegleria fowleri

Table 4.4 Included primary studies for *Naegleria fowleri*

Study ID	*Naegleria fowleri*
N41	Abrahams-Sandí E, Retana-Moreira L, Castro-Castillo A, Reyes-Batlle M, Lorenzo-Morales J. Fatal meningoencephalitis in child and isolation of *Naegleria fowleri* from hot springs in Costa Rica. Emerg Infect Dis. 2015 Feb;21(2):382-4. doi: 10.3201/eid2102.141576. PMID: 25625800; PMCID: PMC4313663.
N19	Bonilla-Lemus P, Rojas-Hernández S, Ramírez-Flores E, Castillo-Ramírez DA, Monsalvo-Reyes AC, Ramírez-Flores MA, Barrón-Graciano K, Reyes-Batlle M, Lorenzo-Morales J, Carrasco-Yépez MM. Isolation and Identification of *Naegleria* Species in Irrigation Channels for Recreational Use in Mexicali Valley, Mexico. Pathogens. 2020 Oct 7;9(10):820.
N1	Booth PJ, Bodager D, Slade TA, Jett S. Primary Amebic Meningoencephalitis Associated with Hot Spring Exposure During International Travel - Seminole County, Florida, July 2014. MMWR Morb Mortal Wkly Rep. 2015 Nov 6;64(43):1226.
N6	Budge PJ, Lazensky B, Van Zile KW, Elliott KE, Dooyema CA, Visvesvara GS, Beach MJ, Yoder JS. Primary amebic meningoencephalitis in Florida: a case report and epidemiological review of Florida cases. J Environ Health. 2013 Apr;75(8):26-31.
N7	Chen M, Ruan W, Zhang L, Hu B, Yang X. Primary Amebic Meningoencephalitis: A Case Report. Korean J Parasitol. 2019 Jun;57(3):291-294.
N2	Cope JR, Murphy J, Kahler A, Gorbett DG, Ali I, Taylor B, Corbitt L, Roy S, Lee N, Roellig D, Brewer S, Hill VR. Primary Amebic Meningoencephalitis Associated With Rafting on an Artificial Whitewater River: Case Report and Environmental Investigation. Clin Infect Dis. 2018 Feb 1;66(4):548-553. doi: 10.1093/cid/cix810. PMID: 29401275; PMCID: PMC5801760.
N29	Dean K, Weir MH, Mitchell J. Development of a dose-response model for *Naegleria fowleri*. J Water Health. 2019 Feb;17(1):63-71.
N14	Diaz J. Seasonal primary amebic meningoencephalitis (PAM) in the south: summertime is PAM time. J La State Med Soc. 2012 May-Jun;164(3):148-50, 152-5.
N15	Dunn AL, Reed T, Stewart C, Levy RA. *Naegleria fowleri* That Induces Primary Amoebic Meningoencephalitis: Rapid Diagnosis and Rare Case of Survival in a 12-Year-Old Caucasian Girl. Lab Med. 2016 May;47(2):149-54.
N35	Gharpure R, Gleason M, Salah Z, Blackstock AJ, Hess-Homeier D, Yoder JS, Ali IKM, Collier SA, Cope JR. Geographic Range of Recreational Water-Associated Primary Amebic Meningoencephalitis, United States, 1978-2018. Emerg Infect Dis. 2021 Jan;27(1):271-274.
N40	Gharpure R, Bliton J, Goodman A, Ali IKM, Yoder J, Cope JR. Epidemiology and Clinical Characteristics of Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri*: A Global Review. Clin Infect Dis. 2021 Jul 1;73(1):e19-e27.
N24	Goudot S, Herbelin P, Mathieu L, Soreau S, Banas S, Jorand F. Growth dynamic of *Naegleria fowleri* in a microbial freshwater biofilm. Water Res. 2012 Sep 1;46(13):3958-66.
N8	Hamaty E Jr, Faiek S, Nandi M, Stidd D, Trivedi M, Kandukuri H. A Fatal Case of Primary Amoebic Meningoencephalitis from Recreational Waters. Case Rep Crit Care. 2020 May 28;2020:9235794.

Study ID	*Naegleria fowleri*
N16	Heggie TW, Küpper T. Surviving *Naegleria fowleri* infections: A successful case report and novel therapeutic approach. Travel Med Infect Dis. 2017 Mar-Apr;16:49-51.
N20	Jamerson M, Remmers K, Cabral G, Marciano-Cabral F. Survey for the presence of *Naegleria fowleri* amebae in lake water used to cool reactors at a nuclear power generating plant. Parasitol Res. 2009 Apr;104(5):969-78.
N3	Kemble SK, Lynfield R, DeVries AS, Drehner DM, Pomputius WF 3rd, Beach MJ, Visvesvara GS, da Silva AJ, Hill VR, Yoder JS, Xiao L, Smith KE, Danila R. Fatal *Naegleria fowleri* infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism. Clin Infect Dis. 2012 Mar;54(6):805-9.
N25	Lam C, He L, Marciano-Cabral F. The Effect of Different Environmental Conditions on the Viability of *Naegleria fowleri* Amoebae. J Eukaryot Microbiol. 2019 Sep;66(5):752-756.
N17	Linam WM, Ahmed M, Cope JR, Chu C, Visvesvara GS, da Silva AJ, Qvarnstrom Y, Green J. Successful treatment of an adolescent with *Naegleria fowleri* primary amebic meningoencephalitis. Pediatrics. 2015 Mar;135(3):e744-8.
N9	Lopez C, Budge P, Chen J, Bilyeu S, Mirza A, Custodio H, Irazuzta J, Visvesvara G, Sullivan KJ. Primary amebic meningoencephalitis: a case report and literature review. Pediatr Emerg Care. 2012 Mar;28(3):272-6.
N21	Maclean RC, Richardson DJ, LePardo R, Marciano-Cabral F. The identification of *Naegleria fowleri* from water and soil samples by nested PCR. Parasitol Res. 2004 Jun;93(3):211-7.
N13	Matthews, S., D. Ginzl, D. Walsh, K. Sherin, J. Middaugh, R. Hammond, D. Bodager, K. Komatsu, J. Weiss, N. Pascoe, F. Marciano-Cabral, E. Villegas, G. Visvesvara, J. Yoder, B. Eddy, L. Capewell, R. Sriram, K. Bandyopadhyay, Y. Qvarnstrom, A. DaSilva, S. Johnston, L. Xiao, V. Hill, S. Roy and M. J. Beach. Centers for Disease Control and Prevention (CDC). Primary amebic meningoencephalitis--Arizona, Florida, and Texas, 2007. MMWR Morb Mortal Wkly Rep. 2008 May 30;57(21):573-7. PMID: 18509301.
N22	Miller HC, Morgan MJ, Walsh T, Wylie JT, Kaksonen AH, Puzon GJ. Preferential feeding in *Naegleria fowleri*; intracellular bacteria isolated from amoebae in operational drinking water distribution systems. Water Res. 2018 Sep 15;141:126-134.
N26	Morgan MJ, Halstrom S, Wylie JT, Walsh T, Kaksonen AH, Sutton D, Braun K, Puzon GJ. Characterization of a Drinking Water Distribution Pipeline Terminally Colonized by *Naegleria fowleri*. Environ Sci Technol. 2016 Mar 15;50(6):2890-8.
N23	Moussa M, De Jonckheere JF, Guerlotté J, Richard V, Bastaraud A, Romana M, Talarmin A. Survey of *Naegleria fowleri* in geothermal recreational waters of Guadeloupe (French West Indies). PLoS One. 2013;8(1):e54414.
N4	Nicholls CL, Parsonson F, Gray LE, Heyer A, Donohue S, Wiseman G, Norton R. Primary amoebic meningoencephalitis in North Queensland: the paediatric experience. Med J Aust. 2016 Oct 3;205(7):325-8.
N10	Phu NH, Hoang Mai NT, Nghia HD, Chau TT, Loc PP, Thai le H, Phuong TM, Thai CQ, Man DN, Van Vinh Chau N, Nga TV, Campbell J, Baker S, Whitehorn J. Fatal consequences of freshwater pearl diving. Lancet. 2013 Jan 12;381(9861):176.
N27	Puzon GJ, Wylie JT, Walsh T, Braun K, Morgan MJ. Comparison of biofilm ecology supporting growth of individual Naegleria species in a drinking water distribution system. FEMS Microbiol Ecol. 2017 Apr 1;93(4).
N42	Sifuentes LY, Choate BL, Gerba CP, Bright KR. The occurrence of *Naegleria fowleri* in recreational waters in Arizona. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2014 Sep 19;49(11):1322-30.

Study ID	*Naegleria fowleri*
N11	Stowe RC, Pehlivan D, Friederich KE, Lopez MA, DiCarlo SM, Boerwinkle VL. Primary Amebic Meningoencephalitis in Children: A Report of Two Fatal Cases and Review of the Literature. Pediatr Neurol. 2017 May;70:75-79.
N5	Su MY, Lee MS, Shyu LY, Lin WC, Hsiao PC, Wang CP, Ji DD, Chen KM, Lai SC. A fatal case of *Naegleria fowleri* meningoencephalitis in Taiwan. Korean J Parasitol. 2013 Apr;51(2):203-6.
N12	Vareechon C, Tarro T, Polanco C, Anand V, Pannaraj PS, Dien Bard J. Eight-Year-Old Male With Primary Amebic Meningoencephalitis. Open Forum Infect Dis. 2019 Jul 29;6(8):ofz349.
N18	Vargas-Zepeda J, Gómez-Alcalá AV, Vásquez-Morales JA, Licea-Amaya L, De Jonckheere JF, Lares-Villa F. Successful treatment of *Naegleria fowleri* meningoencephalitis by using intravenous amphotericin B, fluconazole and rifampicin. Arch Med Res. 2005 Jan-Feb;36(1):83-6.
N28	Yu Z, Miller HC, Puzon GJ, Clowers BH. Application of untargeted metabolomics for the detection of pathogenic *Naegleria fowleri* in an operational drinking water distribution system. Water Research. 2018 Nov;145:678-686.

4.4.2 Burkholderia pseudomallei

Table 4.5 Included primary studies for *Burkholderia pseudomallei*

Study ID	*Burkholderia pseudomallei*
B1	Alvarez-Hernandez G, Cruz-Loustaunau D, Ibarra JA, Rascon-Alcantar A, Contreras-Soto J, Meza-Radilla G, Torres AG, Estrada-de Los Santos P. Description of two fatal cases of melioidosis in Mexican children with acute pneumonia: case report. BMC Infect Dis. 2021 Feb 23;21(1):204.
B5	Baker AL, Warner JM. *Burkholderia pseudomallei* is frequently detected in groundwater that discharges to major watercourses in northern Australia. Folia Microbiol (Praha). 2016 Jul;61(4):301-5.
B3	Baker A, Tahani D, Gardiner C, Bristow KL, Greenhill AR, Warner J. Groundwater seeps facilitate exposure to Burkholderia pseudomallei. Appl Environ Microbiol. 2011 Oct;77(20):7243-6.
B6	Draper AD, Mayo M, Harrington G, Karp D, Yinfoo D, Ward L, Haslem A, Currie BJ, Kaestli M. Association of the melioidosis agent *Burkholderia pseudomallei* with water parameters in rural water supplies in Northern Australia. Appl Environ Microbiol. 2010 Aug;76(15):5305-7.
B4	Inglis TJ, Foster NF, Gal D, Powell K, Mayo M, Norton R, Currie BJ. Preliminary report on the northern Australian melioidosis environmental surveillance project. Epidemiol Infect. 2004 Oct;132(5):813-20.
B9	Kaestli M, Grist EPM, Ward L, Hill A, Mayo M, Currie BJ. The association of melioidosis with climatic factors in Darwin, Australia: A 23-year time-series analysis. J Infect. 2016 Jun;72(6):687-697.
B7	Kaestli M, O'Donnell M, Rose A, Webb JR, Mayo M, Currie BJ, Gibb K. Opportunistic pathogens and large microbial diversity detected in source-to-distribution drinking water of three remote communities in Northern Australia. PLoS Negl Trop Dis. 2019 Sep 5;13(9):e0007672.

Study ID	*Burkholderia pseudomallei*
B8	Knappik M, Dance DA, Rattanavong S, Pierret A, Ribolzi O, Davong V, Silisouk J, Vongsouvath M, Newton PN, Dittrich S. Evaluation of Molecular Methods To Improve the Detection of *Burkholderia pseudomallei* in Soil and Water Samples from Laos. Appl Environ Microbiol. 2015 Jun;81(11):3722-7.
B10	Liu X, Pang L, Sim SH, Goh KT, Ravikumar S, Win MS, Tan G, Cook AR, Fisher D, Chai LY. Association of melioidosis incidence with rainfall and humidity, Singapore, 2003-2012. Emerg Infect Dis. 2015 Jan;21(1):159-62.
B2	Sharif, Saidatulakma. *Ocular Burkholderia Pseudomallei, a Rare Variant in Presentation – A Case Series*. Pediatria i Medycyna Rodzinna. 2020 October 16(3):329-333.

5 Completed quality assessment and data extraction for the included guidelines and literature reviews

5.1 Assessment of Guidelines for *Naegleria fowleri*

5.1.1 Department of Health, Western Australia 2019 (Study ID – N42)

Table 5.1 Review Assessment for Department of Health, Western Australia 2019 (Study ID – N42)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not known
	Are the administrative processes documented and publicly available?	N/A	Not known
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from expert advisory committee. Conflicts not listed or declared.
	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not known
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	N	Assume article is internally review but not external peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N	This is the most current guidance developed.
	Evidence review parameters		

	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	N	No details listed
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	N	No details listed
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details listed
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	Y	Guidance appears to be modelled on earlier guidance documents and information from the literature
	Can grey literature such as government reports and policy documents be included?	Y	Multiple government documents referenced in the guidance
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided

	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No detail provided
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	Y	Policy lists several levels for managing and reporting the detection of the organism.
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	

	Comments		
	Reviewer's comments		Guidance makes the statement that *Naegleria fowleri* can be found independent of faecal coliforms and *E. coli*, which is important for understanding the secondary question on conditions associated with presence/absence.
	Useful for answering primary research question	N	
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2 Assessment of literature reviews for *Naegleria fowleri*

5.2.1 Bright 2017 (Study ID - N30)

Table 5.2 Review Assessment for Bright 2017 (Study ID – N30)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from experts in the field (researchers) but not an advisory committee. Conflicts not listed or declared.

	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Yes	Review was conducted using current literature, however details of the methods used to gather the literature not listed.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Yes	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details listed
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	Y	Single news report included in review and government advice referenced
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided

	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No detail provided
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	

	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review analysed articles of *Naegleria fowleri* infection related to groundwater and geothermal waters and recreational activity. All articles were referenced and high-level data reported.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.2 Capewell 2015 (Study ID - N31)

Table 5.3 Review Assessment for Capewell 2015 (Study ID – N31)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

Criteria	Y/N/?/NA	Notes
Overall guidance/advice development process		
Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product

	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from experts in the field (researchers) but not an advisory committee. No conflicts.
	Are funding sources declared?	Y	Funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Yes	Review was conducted using a combination of literature and medically reported cases. Information is listed from multiple sources. Some information listed as "shared with CDC" but unclear if publicly available.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Yes	Data included in the review is from peer reviewed journals with references listed and an earlier review article which was referenced.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	Unclear	Data is from CDC registry and no systematic literature search listed.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	Y	Government advice referenced

	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	Y	CDC Free living amoebae Laboratory registry
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N/A	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	Y	1937-2013. No detail provided for selection of dates.
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No detail provided
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	

	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review analysed the medical condition (Diagnosis, Clinical course and treatment) of PAM cause by *Naegleria fowleri* infection. Parameters include age, sex, and geographical locations (USA only) were included. All articles were referenced and medical condition data reported, but no environmental data included.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.3 Cooper 2019 (Study ID – N32)

Table 5.4 Review Assessment for Cooper 2019 (Study ID – N32)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from experts in the field (researchers) but not an advisory committee. No potential conflicts.
	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	No	Review was using literature, but no methodology listed.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided

	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	Y	Government advice and case referenced
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N/A	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided.
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No detail provided
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		

	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review is wide ranging including Life cycle, pathophysiology, history, Morbidity and Mortality, Clinical presentation, diagnosis, treatment, and changing geography and climate . All articles were referenced, some suggestions on potential life-saving treatment, and concern about expanding range due to climate change.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.4 Cope 2016 (Study ID – N33)

Table 5.5 Review Assessment for Cope 2016 (Study ID – N33)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from experts in the field (researchers) but not an advisory committee. No potential conflicts.
	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	No	Review was using literature, but no methodology listed.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided.

	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	N/A	
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N/A	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	Y	1962-2015. No detail provided.
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No detail provided

	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review is wide ranging but includes information in relation to both questions as well as recreational exposure, associated conditions and expanded range . All articles were referenced. Clinical presentation and treatment included.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.5 De Jonckheere 2012 (Study ID – N34)

Table 5.6 Review Assessment for De Jonckheere 2012 (Study ID – N34)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from a single expert in the field (researcher) but not an advisory committee. No potential conflicts.
	Are funding sources declared?	Y	Nuclear Energy fund in Belgium
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	N	Article is an editorial on the topic. No indication of peer-review.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	No	Article is an editorial.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unclear	Few references listed and personal communications cited. Most data presented without corresponding references.

	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	N/A	
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N/A	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided.
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided

	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No detail provided
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		

	Reviewer's comments		Editorial article converse the presence of *Naegleria fowleri* in the Environment, impact of man on *Naegleria fowleri*'s presence and future perspectives. While this article has minimal referencing, it is written by a well-established researcher in the field and highlights issues with current management practices/guidelines.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.6 Grace 2015 (Study ID – N36)

Table 5.7 Review Assessment for Grace 2015 (Study ID – N36)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from academics but not an advisory committee. No potential conflicts.
	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product

	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	N	No detail provided.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No detail provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	N/A	
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided
	Are search terms and/or search strings specified?	N	No detail provided

	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	

	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review is covers multiple areas in brief (Pathogenesis, Diagnosis, Treatment (most extensive) and prevention). All articles were referenced. Survivor cases discussed.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.7 Heggie 2010 (Study ID – N37)

Table 5.8 Review Assessment for Heggie 2010 (Study ID – N37)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from academics but not an advisory committee. No potential conflicts.
	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product

	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	Review was conducted using current literature, however details of the methods used to gather the literature not listed.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No detail provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	Y	News report included.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No detail provided

	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	

	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review is an overview of *Naegleria fowleri* and PAM infections, but cases relate to exposure associated with recreational activity. All articles were referenced. Survivor cases discussed.
	Useful for answering primary research question	Partially	Include to provide supporting information
	Useful for answering secondary research question	Partially	Include to provide supporting information

5.2.8 Stahl and Olson 2021 (Study ID – N38)

Table 5.9 Review Assessment for Stahl and Olson 2021 (Study ID – N38)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from academics researchers but not an advisory committee. No potential conflicts.
	Are funding sources declared?	N	No funding source listed

	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	Review outlines current knowledge (assumed using current literature), however details of the methods used to gather the literature not listed.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No detail provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No detail provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	Y	CDC website included.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No detail provided

	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No detail provided
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	

	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review is a comprehensive overview of the biotic and abiotic factors associated with the presence and abundance of *Naegleria fowleri*. Review is a collection of information which is focused on the secondary questions. All articles were referenced.
	Useful for answering primary research question	N	
	Useful for answering secondary research question	Y	Include to provide supporting information

5.2.9 Yoder 2010 (Study ID – N39)

Table 5.10 Review Assessment for Yoder 2010 (Study ID – N39)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

Criteria		Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product

	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Advice from researchers with expertise in the field but not an advisory committee. No potential conflicts.
	Are funding sources declared?	N	No funding source listed
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	Review article was peer reviewed before publication.
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Y	Review outlines current knowledge (assumed using current literature), however details of the methods used to gather the literature not listed.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Y	Data included in the review is from peer reviewed journals with references listed.
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	Partial	Methods for data collection from multiple sources, including the CDC's, is listed and described in the methods.
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	Y	Case reports included in review if laboratory confirmed detection of *Naegleria fowleri*. Some cases excluded and reasons listed.
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No detail provided
	Can grey literature such as government reports and policy documents be included?	Y	CDC website included.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	

	Evidence search		
	Are databases and other sources of evidence specified?	Y	Multiple databases to identify PAM cases listed.
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No detail provided
	Is it specified what date range the literature search covers? Is there a justification?	Y	1962-2008. No detail provided
	Are search terms and/or search strings specified?	N	No detail provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No detail provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No detail provided
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No detail provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	

	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		Review is focused on *Naegleria fowleri* cases in the USA (1962-2008). This review data is also incorporated into Cope 2016. Review is a collection of information with information for both the primary and the secondary questions. All articles were referenced.
	Useful for answering primary research question	Y	Include to provide supporting information
	Useful for answering secondary research question	Y	Include to provide supporting information

5.3 Guideline data extraction forms for *Naegleria fowleri*

5.3.1 Department of Health, Western Australia (Study ID – N42)

Table 5.11 Data extraction form for Department of Health, Western Australia 2019 (Study ID – N42)

General information	Study ID	WA Gov 2019
	Date template completed	08/12/2023
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Western Australia Government, Department of Health, public and Aboriginal Health Division. 2019.
Study characteristics	Aim/objectives of study	*Naegleria fowleri* response protocol for drinking water supply systems.
	Study type/design	Guidance document.
	Study duration	N/A
	Type of water source/water body	Water supply
Population characteristics	Population/s studied	N/A
	Selection criteria for population	N/A
	Subgroups reported	N/A
	Size of study	
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Four deaths from *Naegleria fowleri* up to 1985. *Naegleria fowleri* ecology noted to be "more complex than that of enteric protozoa". Route is bathing including recreation in swimming pools.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	N/A
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Naegleria fowleri* not associated with faecal contamination and can be detected in the absence of *Escherichia coli*.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	N/A
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	N/A
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. While dealing with drinking water the note on *Naegleria fowleri* not connected with faecal coliforms and *E. coli* is of note for the associated, or lack thereof, microbes/ecology.

5.4 Literature review data extraction forms for *Naegleria fowleri*

5.4.1 Bright 2017 (Study ID – N30)

Table 5.12 Data extraction form for Bright 2017 (Study ID – N30)

General information	Study ID	Bright et al 2017 (N30)
	Date template completed	12/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Kelly R. Bright & Charles P. Gerba, 2017. Review. Peer Reviewed. Arizona, USA. University of Arizona.
Study characteristics	Aim/objectives of study	Review of *Naegleria fowleri* in groundwater and hot springs (geothermal).
	Study type/design	Review of Literature/presence of *Naegleria fowleri* and PAM cases.
	Study duration	N/A
	Type of water source/water body	Ground water and geothermal hot springs.
Population characteristics	Population/s studied	PAM cases; 8-NZ, 1-UK, 3-USA, 1-China, 1-Namibia, 1-AUS, 1-Taiwan, 1-French West Indies, 1-Costa Rica.
	Selection criteria for population	N/A
	Subgroups reported	N/A
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Hot Springs and/or Groundwater. Recreational activity most likely source. Hot springs confirmed to have *Naegleria fowleri.*
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Water Quality measurements included Temperature, pH and turbidity at some but not all sites. Methods for isolation ranged from bulk water (various volumes) to sediment. *Naegleria fowleri* by a variety of PCR methods.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Fatality from PAM and one survivor. No mention of medical methods other that application of antibiotics.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed, but temperature mentioned as not significant, but turbidity was significant in a single study.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* can be present and persistent in some groundwaters and geothermal waters but not others. Unclear what the difference is.

		Groundwaters and geothermal water are not risk free and precautions should be taken.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Identification of *Naegleria fowleri* in multiple groundwaters and surface waters globally. Review does not list water conditions for all sites/samples.

5.4.2 Capewell 2015 (Study ID – N31)

Table 5.13 Data extraction form for Capewell 2015 (Study ID – N31)

General information	Study ID	Capewell et al 2015 (N31)
	Date template completed	9/7/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Capewell LG, Harris AM, Yoder JS, Cope JR, Eddy BA, Roy SL, Visvesvara GS, Fox LM, Beach MJ. 2015. Journal. Peer-reviewed. USA. US Centers for Disease Control and Prevention. No conflict of interest.
Study characteristics	Aim/objectives of study	Review exposure location, clinical signs and symptoms, diagnostic modalities, and treatment from confirmed cases of PAM diagnosed in the USA 1937-2013.
	Study type/design	Review.
	Study duration	1937-2013.
	Type of water source/water body	NA
Population characteristics	Population/s studied	Confirmed PAM cases in USA 1937-2013.
	Selection criteria for population	Confirmed PAM cases in USA 1937-2013.
	Subgroups reported	Early (i.e. flu-like symptoms) and late (i.e. central nervous system signs) groups based on presenting clinical characteristics.
	Size of study	142
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA NA NA NA NA NA NA
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable)	NA Postmortem CSF culture. NA

	Water sampling methods (monitoring, surrogates)	
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Primary amoebic meningoencephalitis (PAM). For evaluating PAM, clinical presentation, Clinical laboratory testing, microscopic diagnosis, radiological imaging. Microscopic diagnosis, wet mount, Wright-Giemsa staining for cerebrospinal fluid (CSF), hematoxylin and eosin, polyclonal antibody staining of brain tissue for *Naegleria fowleri*, postmortem CSF culture, immunofluorescence, real-time PCR. For evaluating outcome of therapeutic management of PAM: death. See above. 142.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Median, range. NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	PAM is a fatal illness with limited treatment success and is expanding into more northern regions of USA.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Ok to include in review.

5.4.3 Cooper 2019 (Study ID – N32)

Table 5.14 Data extraction form for Cooper 2019 (Study ID – N32)

General information	Study ID	Cooper et al 2019 (N32)
	Date template completed	05/08/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Amanda Marie Cooper, Shaza Aouthmany, Kruti Shah, Paul P. Rega. 2019. Review article. Peer Reviewed. University of Toledo, Ohio. No conflicts declared.
Study characteristics	Aim/objectives of study	Brief overview of PAM cases with focus on survivors.
	Study type/design	Review article.
	Study duration	NA
	Type of water source/water body	Freshwater and Tap water.
Population characteristics	Population/s studied	PAM survivors (1-MEX, 2-USA).
	Selection criteria for population	Surviving *Naegleria fowleri* infection.

	Subgroups reported	NA
	Size of study	3
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	None listed but recreational water likely source. NA to others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to all.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Of patients studied; 28% presented with early symptoms, 21% initial misdiagnosed, 72% presented with late-stage symptoms. Review of potential treatments with treatment plan of survivors listed.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Very low survival levels. Most patients present late and only 3 patients survived. Climate change may be driving increased incidences of PAM.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Provides an overview of the drugs used to treat PAM and the treatment plan for the 3 PAM survivors.

5.4.4 Cope 2016 (Study ID – N33)

Table 5.15 Data extraction form for Cope 2016 (Study ID – N33)

General information	Study ID	Cope and Ali 2016 (N33)
	Date template completed	9/7/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Cope JR, Ali IK. 2016. Journal. Peer-reviewed. USA (although examples from Australia, Costa Rica and Pakistan also mentioned). NA No conflict of interest.
Study characteristics	Aim/objectives of study	Discuss lessons learned from last 5 years on primary amebic meningoencephalitis epidemiology, geography, exposure, clinical presentation and treatment, diagnostic testing and advanced molecular techniques

	Study type/design	Review
	Study duration	NA (although title says lessons from the last 5 years).
	Type of water source/water body	Warm freshwater, such as lakes, ponds, reservoirs, rivers, hot springs, pools, untreated drinking water from a geothermal well, tap water, municipal water distribution system, untreated groundwater, untreated rainwater from a cistern.
Population characteristics	Population/s studied	Historic PAM cases.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body	Warm freshwater, such as lakes, ponds, reservoirs, rivers, hot springs, pools, untreated drinking water from a geothermal well, tap water, municipal water distribution system, untreated groundwater, untreated rainwater from a cistern.
	Exposure scenario	Recreational water activities, such as swimming or diving, zip lining, water slide, neti pots for nasal irrigation, backyard slip-n-slide, backyard pools filled with tap water, submerged heads during bathing with tap water, ritual ablution that includes nasal rinsing (Yogic, Ayurvedic and Islamic traditions).
	Exposure pathway	Nose.
	Source of infection/contamination	Water.
	Causal organism/chemical(s)	*Naegleria fowleri.*
	Comparison group(s)	NA
	Confirmed link to Recreational Water	Yes, a number of examples.
Study methods	Water quality measurement used	NA
	Method of microorganism isolation and enumeration (if applicable)	Wet mount of cerebrospinal fluid (CSF), Giemsa or Wright staining of smears, molecular detection (multicopy mitochondrial 5.8S and 18S rRNA genes and internal transcribed spacers (ITS) and single copy genomic DNA for PCR assays, e.g. real-time PCR and loop-mediated isothermal amplification (LAMP) assay, antigen detection serologic tests, genome sequencing, transcriptomic (RNA) and proteomic analyses.
	Water sampling methods (monitoring, surrogates)	NA
Results (for each outcome)	Definition of outcome	Primary amebic meningoencephalitis (PAM).
	How outcome was assessed	

	Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Historic PAM cases reviewed for lessons. NA NA
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	The last 5 years have shown that PAM remains a devastating infection associated with warm freshwater exposure. Clinicians in all regions should consider PAS as the cause for meningitis, particularly in the warm summer months in patients with recent freshwater exposure.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Ok to include

5.4.5 De Jonckheere 2012 (Study ID – N34)

Table 5.16 Data extraction form for De Jonckheere 2012 (Study ID – N34)

General information	Study ID	De Jonckheere 2012 (N34)
	Date template completed	05/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	De Jonckheere 22 Dec 2011 Editorial Unknown Various Scientific Institute of Public Health, B1050 Brussels, Belgium and Research Unit for Tropical Diseases, de Duve Institute, B-1200 Brussels, Belgium NA
Study characteristics	Aim/objectives of study	Describe the impact of man on the occurrence of *Naegleria fowleri*.
	Study type/design	Editorial.
	Study duration	NA
	Type of water source/water body	Surface water/drinking water.
Population characteristics	Population/s studied	global
	Selection criteria for population	Reports of *Naegleria fowleri*.
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Surface water/drinking water. Swimming.

Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA - editorial discussion of cases.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	NA
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Human mediated discharge of warm water can increase the risk of *Naegleria fowleri* infections. Industry should not be allowed to discharge water with high levels of *Naegleria fowleri.*
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This is a relatively short review discussing the potential for human activity to influence the prevalence of *Naegleria fowleri*. Specifically warm water discharge from nuclear power plants and other industrial sources. Global warming is mentioned as a risk factor as well.

5.4.6 Grace 2015 (Study ID – N36)

Table 5.17 Data extraction form for Grace 2015 (Study ID – N36)

General information	Study ID	Grace E. 2015 (N36)
	Date template completed	14/09/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Eddie Grace, Scott Asbill, Kris Virga 2015. MiniReview. Peer Reviewed. Presbyterian College School of Pharmacy, Clinton, South Carolina, USA No conflicts declared.
Study characteristics	Aim/objectives of study	Review of pathogenesis, diagnosis, pharmacotherapy, and prevention of *Naegleria fowleri* infections in humans.
	Study type/design	Review.
	Study duration	NA
	Type of water source/water body	Recreational waters
Population characteristics	Population/s studied	Adults and kids (not specified).
	Selection criteria for population	*Naegleria fowleri* infection.
	Subgroups reported	4 survivors (9 & 12 year old female2, 8 & 10-year old male.
	Size of study	Non-specified.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway	Freshwater. Recreational activities listed (swimming, diving, and water skiing).

	Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA to others. Listed by not specified.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to all.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	PAM Infection. Pathogenesis described. Diagnosis-common symptoms and presence of trophozoites in CSF (microscopy). Treatment with drugs including amphotericin B, rifampin, azithromycin, and fluconazole and miltefosine. Survivors-Drug treatments Prevention-Avoidance of exposure to freshwater, especially during summer, or avoid jumping, splashing of submerging in water.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	None
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Unclear. Information is of a very general nature and no linking between cases and conditions.

5.4.7 Heggie 2010 (Study ID – N37)

Table 5.18 Data extraction form for Heggie 2010 (Study ID – N37)

General information	Study ID	Heggie T. 2010 (N37)
	Date template completed	22/06/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Heggie. T. 2010. Peer Reviewed. USA. University of North Dakota, USA.
Study characteristics	Aim/objectives of study	Review of PAM cases.
	Study type/design	Review.
	Study duration	NA
	Type of water source/water body	River, lake, pond/ditch, and puddles.
Population characteristics	Population/s studied	9-year old male (Italy), 9-year old female (AZ, USA), 11-year old male (FLA, USA), 12-year old male (TX, USA), 22-year old male (TX, USA), 3-year old male (UK).
	Selection criteria for population	NA

	Subgroups reported	NA
	Size of study	6
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Natural water sources (River, lake, pond/ditch, and puddles). Recreational activities Swimming, wakeboarding, splashing water, falling into water while wakeboarding.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Water Temps (29.1C-TX), Air Temps (32.8-FLA) and elevated air Temps (UK). Microscopy. CDC-method for PCR detection of *Naegleria fowleri* from CSF.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Microscopy of CSF-ID of Naegleria-like species followed by PCR. Outcome was death.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Recreational activity in warm water presents a chance for coming in contact with *Naegleria fowleri*. Reduce risk by preventing water entering nose. Most cases in recreational waters ≥ 26 °C.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include with cavate of unknown water conditions but links to multiple recreational activities (swimming, splashing, wakeboarding). Link to mortality/outcome for exposure.

5.4.8 Stahl 2021 (Study ID – N38)

Table 5.19 Data extraction form for Stahl 2021 (Study ID – N38)

General information	Study ID	Stahl and Olson 2021 (N38)
	Date template completed	20/04/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Stahl, L.M., Olson J.B. 2021. Journal. Peer reviewed. Authors from USA (Human PAM case detections in Australia, Pakistan, Check Republic, India, USA, in mammals in Brazil, Argentina, USA). NA NA
Study characteristics	Aim/objectives of study	Outline current knowledge of the environmental abiotic and biotic factors that affect the distribution and abundance of *Naegleria fowleri.*
	Study type/design	Review.
	Study duration	Cited articles span from 1942 to 2020.

	Type of water source/water body	Freshwater lakes, ponds, rivers, drinking water distribution systems, swimming pools, rainwater tank, tap and well water. A PAM case attributed to inhalation of dust-borne cysts.
Population characteristics	Population/s studied	Human cases of PAM most frequently reported from Australia, Pakistan, Czech Republic, India and USA, observed in mammals in Brazil, Argentina, USA.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Freshwater lakes, ponds, rivers, drinking water distribution systems, swimming pools, rainwater tank, tap and well water. Recreational water activities: swimming, diving, when water is forced up the nasal cavity. Intranasal exposure through water or potentially dust particles. Water, dust. *Naegleria fowleri.* NA Typically yes, one case potentially related to inhalation of dust-borne cysts.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Temperature, pH, conductivity, DO, turbidity, iron, salinity Cultivation, morphological examinations, molecular assays with target gene, mouse pathogenicity test, immunological techniques Sample types: water, sediment, soil, algae, rock/soil swabs, biofilm.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Death Since 1966, there have been almost 400 confirmed PAM cases worldwide with only 7 survivors. Literature reviewed. NA NA
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results	*Naegleria fowleri* distribution and abundance is influenced by various abiotic (e.g. temperature, pH, salinity, dissolved oxygen, nutrients (e.g. amino acids, vitamin, guanosine, glucose, salts and metals such as Fe), chemical agents (e.g. chlorine), and water availability) and biotic factors (predators, prey, competition,

		inclusion in biofilms, interactions with other organisms, chemical signalling). *Naegleria fowleri* appears to have a wide pH range (2.0-8.2, optimum 6.5), low salinity tolerance (up to 1.6% salinity for a short duration) and thermophilic preference. *Naegleria fowleri* has been detected in environmental water samples from 16°C to 47°C and was recovered from sediments at 12°C. *Naegleria fowleri* is fairly resistant to inactivation by ultraviolet radiation. *Naegleria fowleri* preferentially feed upon bacteria and are preyed upon by other free-living amoebae.
	Assessment of uncertainty (if any)	NA
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Yes, if environmental factors are of interest without case studies

5.4.9 Yoder 2010 (Study ID – N39)

Table 5.20 Data extraction form for Yoder 2010 (Study ID – N39)

General information	Study ID	Yoder J.S. et al 2009 (N39)
	Date template completed	15 February 2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Yoder, J. S., B. A. Eddy, G. S. Visvesvara, L. Capewell and M. J. Beach. 22 October 2009. Journal article (review). Peer reviewed. USA. NA. No conflicts of interest.
Study characteristics	Aim/objectives of study	Review and assess the epidemiology of PAM in the USA between 1962 - 2008.
	Study type/design	Review.
	Study duration	46 years.
	Type of water source/water body	Lakes, ponds, reservoirs, canals, ditches and puddles, rivers and streams, geothermally heater water and untreated drinking water used for recreational purposes.
Population characteristics	Population/s studied	111 cases, primarily male children Median age was 12 years (range 8 months – 66 years). 62.2% children 13 years or younger 79.3% male. Race and ethnicity only documented for a few of the cases.
	Selection criteria for population	Laboratory confirmed detection of *Naegleria fowleri* or nucleic acid reported in CSF, biopsy, or tissue specimens.

	Subgroups reported	NA
	Size of study	111 individuals from the USA.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s) Comparison group(s)	Lakes, ponds, reservoirs, canals, ditches and puddles, rivers and streams, geothermally heater water and untreated drinking water used for recreational purposes. Swimming, diving, jumping, splashing, watercraft, skiing, tubing, wakeboarding, facial contact with mud puddles, underwater play, and total immersion by baptism. NA NA *Naegleria fowleri.* NA
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Other methods used: Water sampling methods (monitoring, surrogates)	NA Laboratory confirmed detection of *Naegleria fowleri* or nucleic acid reported in CSF, biopsy, or tissue specimens. Review of cases using 5 databases: 1) Waterborne Disease and Outbreak Surveillance System 2) Compressed mortality file – National Vital Statistics System - International Classification of Diseases, Ninth Division 3) Medical literature review of PAM case patients 4) searches of media reports 5) CDC laboratory tests requests and results NA
Results (for each outcome)	Definition of outcome	111 cases included: Median age was 12 years (range 8 months – 66 years) 62.2% children 13 years or younger 79.3% male Lakes, ponds and reservoirs (73.6%) Canals, ditches and puddles (7.7%) Rivers and streams (7.7%) Geothermally heated water (5.5%) Untreated drinking water used for recreational purposes (3.3%) Swimming pools (2.2%). Recreational activities reported = 74 Swimming (n = 61), diving (n = 10), jumping (n = 3), splashing (n = 2), watercraft (n = 3), water skiing and wakeboarding (n = 10), tubing (n = 2), facial contact with mud puddles (n =

	How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	2), underwater play (n = 7), and total immersion by baptism (n = 1). Average length of time from exposure to onset of symptoms = 5 days Average length of time from onset of symptoms to death = 5.3 days Review of case file NA Varied
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* infections are rare and primarily affect younger people who are exposed to warm recreational fresh-water in the southern-tier states during the summer months. Locations of probable water exposure for PAM were untreated or poorly treated bodies of water susceptible to changes in ambient temperature. Nearly all cases had water exposure during the summer and most of that seasonal exposure occurred at lakes or ponds.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This case report should be included in the review to address the primary question as well as several of the secondary questions. 111 PAM cases were identified in the USA between 1962 – 2008. 91 of these cases reported exposure to recreational water or using untreated drinking water for recreational use. The study was comprehensive and produced findings that associate PAM cases to recreational water, warmer regions and recreational activities.

5.5 Assessment of literature reviews for *Burkholderia pseudomallei*

5.5.1 Foong 2014 (Study ID – B13)

Table 5.21 Review Assessment for Foong 2014 (Study ID – B13)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	N	Not an advice/guideline product so not overseen by expert advisory committee; authors have not declared interests.
	Are funding sources declared?	N	Funding sources for the review are not reported
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	It is reasonable to assume that this paper underwent peer review before publication in a journal
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Partially	The review scope is not stated but reviews information on epidemiology, clinical features, diagnosis and treatment of *Burkholderia pseudomallei*. Definitions and other review parameters are not reported.

	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unknown	No details provided
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	The review appears to summarise and synthesise published literature only.
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No details provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No details provided
	Can grey literature such as government reports and policy documents be included?	N	No government reports or policy documents appear to be reported in the bibliography.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No details provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No details provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No details provided
	Are search terms and/or search strings specified?	N	No details provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No details provided
	Critical appraisal methods and tools		

	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No details appear to be provided on domains/tools that are considered in risk of bias assessments or other study quality assessments.
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No details provided on how the information from the included studies was synthesised – narrative summary was provided in the conclusions section.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No details provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		

	Reviewer's comments		The paper is a general clinical overview that summarises the available literature on the epidemiology, clinical features, diagnosis and treatment of *Burkholderia pseudomallei*. The paper provides a summary of previous findings without reporting methods that demonstrate a critical analysis of the papers and reports under consideration or explanation of any data analysis that led to the review conclusions. This is a general review, relevant sections of which could potentially provide some general information to support other review findings regarding the health risks from exposure to *Burkholderia pseudomallei* or case studies about potential transmission routes and risk factors.
	Useful for answering primary research question	Partially	*Include to provide supporting information*
	Useful for answering secondary research question	Partially	

5.5.2 Hsueh 2018 (Study ID – B14)

Table 5.22 Review Assessment for Hsueh 2018 (Study ID – B14)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Partially	Not an advice/guideline product so not overseen by expert advisory committee; authors have declared interests.

	Are funding sources declared?	Y	Grants that funded the work are reported
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	It is reasonable to assume that this paper underwent peer review before publication in a journal
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Partially	The review scope is limited to studies reporting on the incidence of melioidosis in Taiwan that can provide information into the mechanism of transmission. Definitions and other review parameters are not reported.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unknown	No details provided
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	The review appears to summarise and synthesise published literature such as primary studies only.
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No details provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	The review appears to mainly summarise and synthesise published primary studies, although it appears some of the reported studies assessed data collected by the CDC (Taiwan)
	Can grey literature such as government reports and policy documents be included?	N	No government reports or policy documents are reported in the bibliography.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		

	Are databases and other sources of evidence specified?	N	No details provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No details provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No details provided
	Are search terms and/or search strings specified?	N	No details provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No details provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No details appear to be provided on domains/tools that are considered in risk of bias assessments or other study quality assessments.
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No details provided on how the information from the included studies was synthesised – narrative summary was provided in the conclusions section.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No details provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	

	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		The paper is looking at the historical prevalence and detection of *Burkholderia pseudomallei* over time in Taiwan and possible sources and modes of transmission. The paper provides a summary of previous findings without reporting methods that demonstrate a critical analysis of the papers and reports under consideration or explanation of any data analysis that led to the review conclusions. Although not addressing exposure through recreational water use, relevant sections of the review or the papers included in the review could potentially be referenced to provide some general information to support other review findings regarding the health risks from exposure to *Burkholderia pseudomallei* or case studies about potential transmission routes, conditions of increased occurrence and potential associations with different exposures during weather events.
	Useful for answering primary research question	Partially	*Include to provide supporting information*
	Useful for answering secondary research question	Partially	

5.5.3 Inglis 2009 (Study ID – B15)

Table 5.23 Review Assessment for Inglis 2009 (Study ID – B15)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	N	Not an advice/guideline product so not overseen by expert advisory committee; authors have not declared interests.
	Are funding sources declared?	N	Funding sources for the review are not reported
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	It is reasonable to assume that this paper underwent peer review before publication in a journal
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Partially	The review scope is not stated but reviews information on epidemiology, clinical features, diagnosis and treatment of *Burkholderia pseudomallei*. Definitions and other review parameters are not reported.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unknown	No details provided

	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	The review appears to summarise and synthesise published literature only.
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No details provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No details provided
	Can grey literature such as government reports and policy documents be included?	N	No government reports or policy documents appear to be reported in the bibliography.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No details provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No details provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No details provided
	Are search terms and/or search strings specified?	N	No details provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No details provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No details appear to be provided on domains/tools that are considered in risk of bias assessments or other study quality assessments.

	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No details provided on how the information from the included studies was synthesised – narrative summary was provided in the conclusions section.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No details provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		

	Reviewer's comments		This paper is an overview of the risks of *Burkholderia pseudomallei* and the approaches to consider in diagnosis and surveillance of melioidosis. The paper provides a summary of previous findings without reporting methods that demonstrate a critical analysis of the papers and reports under consideration or explanation of any data analysis that led to the review conclusions. This is a general review, relevant sections could potentially provide some general information to support other review findings regarding the health risks from exposure to *Burkholderia pseudomallei* or case studies about potential transmission routes, environmental sources and risk factors.
	Useful for answering primary research question	Partially	*Include to provide supporting information*
	Useful for answering secondary research question	Partially	

5.5.4 Merritt 2017 (Study ID – B11)

Table 5.24 Review Assessment for Merritt 2017 (Study ID – B11)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Y	Not an advice/guideline product so not overseen by expert advisory committee; authors have declared interests.

	Are funding sources declared?	N	Funding sources for the review are not reported
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	It is reasonable to assume that this paper underwent peer review before publication in a journal
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Partially	The review scope is not stated but reviews information relating to the impacts of climate change on indirect and direct human exposure to of *Burkholderia pseudomallei*. Definitions and other review parameters are not reported.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unknown	No details provided
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N	The review appears to summarise and synthesise published literature only.
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No details provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	Unknown	No details provided
	Can grey literature such as government reports and policy documents be included?	Y	There are mention of several international agency/government reports or policy documents reported in the bibliography.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		

	Are databases and other sources of evidence specified?	N	No details provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No details provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No details provided
	Are search terms and/or search strings specified?	N	No details provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No details provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No details appear to be provided on domains/tools that are considered in risk of bias assessments or other study quality assessments.
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No details provided on how the information from the included studies was synthesised – narrative summary was provided in the conclusions section.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No details provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	

	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		
	Reviewer's comments		The paper is a general review that summarises the available literature on the potential impacts of climate change (such as changes in weather patterns and events) on transmission of *Burkholderia pseudomallei*. The paper provides a summary of previous findings without reporting methods that demonstrate a critical analysis of the papers and reports under consideration or explanation of any data analysis that led to the review conclusions. This is a general review, relevant sections of which could potentially provide some general information to support review findings regarding the health risks from exposure to *Burkholderia pseudomallei* or, Australian case studies and potential transmission routes and risk factors.
	Useful for answering primary research question	Partially	*Include to provide supporting information*
	Useful for answering secondary research question	Partially	

5.5.5 Stephens 2016 (Study ID – B12)

Table 5.25 Review Assessment for Stephens 2016 (Study ID – B12)

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?	N/A	Not an advice/guideline product
	Are the administrative processes documented and publicly available?	N/A	Not an advice/guideline product
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	Partially	Not an advice/guideline product so not overseen by expert advisory committee; authors have declared interests.
	Are funding sources declared?	Y	Funding sources for the review are reported
	Was there public consultation on this work? If so, provide details.	N/A	Not an advice/guideline product
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	Y	It is reasonable to assume that this paper underwent peer review before publication in a journal
	Was the guidance/advice developed or updated recently? Provide details.	N/A	Not an advice/guideline product
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?	Partially	The review scope is not stated but reviews information on epidemiology, clinical features, diagnosis and treatment of *Burkholderia pseudomallei*. Definitions and other review parameters are not reported.
	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	Unknown	No details provided

	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	No details provided
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	N/A	The review appears to summarise and synthesise published literature only.
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	No details provided
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	N	No details provided
	Can grey literature such as government reports and policy documents be included?	N	No government reports or policy documents appear to be reported in the bibliography.
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N/A	
	Evidence search		
	Are databases and other sources of evidence specified?	N	No details provided
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	No details provided
	Is it specified what date range the literature search covers? Is there a justification?	N	No details provided
	Are search terms and/or search strings specified?	N	No details provided
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	No details provided
	Critical appraisal methods and tools		
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	No details appear to be provided on domains/tools that are considered in risk of bias assessments or other study quality assessments.

	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	No details provided on how the information from the included studies was synthesised – narrative summary was provided in the conclusions section.
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	No details provided
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?	N/A	
	Are the parameter value assumptions documented and explained?	N/A	
	Are the mathematical workings/algorithms clearly documented and explained?	N/A	
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	N/A	
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	N/A	
	What processes are used when expert judgement is required and applied? Is the process documented and published?	N/A	
	Is dose response modelling (e.g. BMDL) routinely used?	N/A	
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?	N/A	
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	N/A	
	Comments		

	Reviewer's comments		The paper is a general clinical overview that summarises the available literature on the epidemiology, clinical features, diagnosis and treatment of *Burkholderia pseudomallei*. The paper provides a summary of previous findings without reporting methods that demonstrate a critical analysis of the papers and reports under consideration or explanation of any data analysis that led to the review conclusions. This is a general review, relevant sections of which could potentially provide some general information to support other review findings regarding the health risks from exposure to *Burkholderia pseudomallei* or case studies about potential transmission routes and risk factors.
	Useful for answering primary research question	Partially	*Include to provide supporting information*
	Useful for answering secondary research question	Partially	

5.6 Literature review data extraction forms for *Burkholderia pseudomallei*

5.6.1 Foong 2014 (Study ID – B13)

Table 5.26 Data extraction form for Foong 2014 (Study ID – B13)

General information	Study ID	Foong et al 2014
	Date template completed	12/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	YC Foong, M Tan, RS Bradbury 2014 Review Article. Peer Reviewed. Australia. University of Tasmania and Central Queensland University, No funding provided. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Clinical review of Melioidosis.
	Study type/design	Literature review.
	Study duration	NA
	Type of water source/water body	None specified.
Population characteristics	Population/s studied	Evidence supporting larger endemic area globally. Most common cause of fatal community-acquired septicaemia & pneumonia at Royal Darwin Hospital. Study 1989-2003 showed increasing incidence of melioidosis in Top End Australia. Incidence reached record rates (50.2 cases per 100 000 people) over the 2009–2010 wet season.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Organism recovered from wet soils, streams, pools, stagnant water and rice paddy fields in particular. Survival within fungi spores and amoebae. Commonest mode of transmission is via direct inoculation of contaminated soil and surface water through skin abrasions. Melioidosis is highest amongst immunocompromised individuals. No links to recreational waters.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Heavy Rainfall connected to increased incidence. Compared culture based, biochemical, immunological and molecular detection methods.

Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Review of symptoms for subclinical, acute and chronic clinical features. Latent cases (up to 62 years) have been reported. Can be resistant to broad spectrum antibiotics (such as penicillin, ampicillin, gentamicin, streptomycin, and first- and second-generation cephalosporins). Ceftazidime was the treatment of choice for severe melioidosis.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Much has been learned about the disease in the past 100 years but still a lethal disease with considerable mortality and morbidity in hyper-endemic areas.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include for potential sub-questions. Australian study with data on case increases after heavy rains. However on links to recreation or recreational water. No links with biotic or abiotic factors other than increased rain.

5.6.2 Hsueh 2018 (Study ID – B14)

Table 5.27 Data extraction form for Hsueh 2018 (Study ID – B14)

General information	Study ID	Hsueh et al 2018
	Date template completed	13/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Pei-Tan Hsueh, Wei-Tien Huang, Hsu-Kai Hsueh, Ya-Lei Chen, and Yao-Shen Chen 2018 Review Article. Peer Reviewed. Department of Internal Medicine, Kaohsiung Veterans General Hospital Department of Biotechnology, National Kaohsiung Normal University, Taiwan Funding provided, Ministry of Science and Technology, ROC. Authors declare no conflict of interest.
Study characteristics	Aim/objectives of study	Review of transmission modes of Melioidosis in Taiwan.
	Study type/design	Literature review

	Study duration	1984-2004
	Type of water source/water body	Aerosols
Population characteristics	Population/s studied	Er-Ren River Basin of Taiwan.
	Selection criteria for population	Incidence of Melioidosis downstream (122 cases/100,00 people).
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	En-Ren River. Rainfall (6-8 day plus wind >19 m/s) Aerosols. No links to recreational waters.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Sustained Heavy Rainfall connected to increased wind. River water detections reported but not sampling information.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Greater incidence in areas downstream of positive river sites. Increase case downstream of En-Ren River area. Potential links to amoebae protection, via intracellular survival in Acanthamoeba lenticulate, but no results provided.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Melioidosis-clustered cases increased after typhoon events. Melioidosis is airborne and was transmitted from contaminated soils to aerosols and/or to humans.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Potentially include for sub-questions. Links between wet season, wind and melioidosis as well as movement of *Burkholderia pseudomallei* from upstream to downstream. However no links to recreation or recreational water. No links with biotic or abiotic factors other than increased rain and wind.

5.6.3 Inglis 2009 (Study ID – B15)

Table 5.28 Data extraction form for Inglis 2009 (Study ID – B15)

General information	Study ID	Inglis and Sousa 2009
	Date template completed	13/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Timothy J.J. Inglis and Anastácio Q. Sousa 2009 Review Article. Peer Reviewed. School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia São Jose. Hospital and Tropical Medicine Nucleus, Federal University of Ceará; Fortaleza, Ceará, Brazil, Taiwan. No Funding provided. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Review of Melioidosis.
	Study type/design	Literature review
	Study duration	NA
	Type of water source/water body	NA
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Surface waters and moist soil. Rice farming and non-farming interactions. Exposure undetermined, but likely skin abrasions and inhalations. Infections with co-morbidities (Indigenous Australians) but not ethnicity based. Linked to recreation in dam filled with early rains (Brazil).
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA Infections related to extreme weather events, i.e. rainfall. List of Burkholderia environmental sample processing flowchart provided.

Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	No real results listed. General descript of multiple areas.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Melioidosis is a complex bacterial infection that includes a cluster of overlapping disease entities, resulting from exposure to a contaminated environment. While incomplete, knowledge of the epidemiology, biology and ecology of melioidosis can be applied to improving disease surveillance, outbreak identification and environmental control.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include only for reference to the presence of *Burkholderia pseudomallei* in Australia. Also a link to recreational water in Brazil. No method details provided though.

5.6.4 Merritt 2017 (Study ID – B11)

Table 5.29 Data extraction form for Merritt 2017 (Study ID – B11)

General information	Study ID	Merritt and Inglis et al 2017
	Date template completed	16/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Adam J. Merritt & Timothy J. J. Inglis 2017 Review Article. Peer Reviewed. Department of Microbiology, PathWest Laboratory Medicine Western Australia School of Biomedical Sciences, Faculty of Health and Medical Sciences, The University of Western Australia.

		Australia No funding listed. No conflict of interest.
Study characteristics	Aim/objectives of study	Role of climate in the epidemiology of melioidosis in Western Australia.
	Study type/design	Review
	Study duration	NA
	Type of water source/water body	NA
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA NA NA NA No link to recreational water activity.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to water quality measurements. NA to microorganism isolation, enumeration and water sampling. Review of previous links with rainfall, humidity, cloud cover, cyclones, groundwater seepages, and high wind speeds.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Near-term multi-model Intergovernmental Panel on Climate Change (IPCC) predictions in annual rainfall suggest a likely decrease in rainfall between 20 and 40 degrees latitude and general increases outside this zone. This means that the already wet tropics and moist mid-latitudes will likely receive more rain at the expense of the mid-latitude subtropical arid and semi-arid areas. More rain will be delivered to all areas by more intense extreme weather events, increasing the opportunities for *Burkholderia pseudomallei* exposure. Increases in maximum rainfall in the tropics will further increase melioidosis risk while increased rain outside the tropics will expand the *Burkholderia pseudomallei*-receptive regions.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Predicted increases in temperature, changes in global precipitation patterns and an increased incidence of extreme weather events are expected to change melioidosis epidemiology. Further studies of the physical geographic drivers of melioidosis will deepen

		understanding of the impact of climate on melioidosis.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. No links to recreational water activity are provided. However, description of potential changes due to climate modelling for "future increases" in melioidosis.

5.6.5 Stephens 2016 (Study ID – B12)

Table 5.30 Data extraction form for Stephens 2016 (Study ID – B15)

General information	Study ID	Stephens et al 2016
	Date template completed	16/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Dianne P. Stephens, Jane H. Thomas, Linda M. Ward, Bart J. Currie 2016 Review article. Peer Reviewed. Department of Intensive Care, Royal Darwin Hospital, Darwin, NT, Australia. Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, NT, Australia. Department of Infectious Diseases and Northern Territory Medical Program, Royal Darwin Hospital, Darwin, NT, Australia. Australia Funding grant from the Australian National Health and Medical Research Council (NHMRC) No conflict of interest.
Study characteristics	Aim/objectives of study	Review of 24 years of Melioidosis cases in Darwin (demographics, management and outcomes).
	Study type/design	Review study.
	Study duration	1989-2013.
	Type of water source/water body	
Population characteristics	Population/s studied	Darwin NT.
	Selection criteria for population	Melioidosis infection.
	Subgroups reported	Men, age, Indigenous, Urban, Rural.
	Size of study	207 ICU patients
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA NA NA NA No link to recreational water activity.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable)	NA to water quality measurements. Culture confirmed melioidosis (Ashdown media and

	Water sampling methods (monitoring, surrogates)	hemagglutinations assays (no methods listed)). The data for the study were collected prospectively in two separate databases (Menzies school of Health Research Melioidosis database (1989-2003, and the Royal Darwin Hospital ICU Melioidosis database (2001-2013)) and approved by the Human Research Ethics Committee of the NT Department of Health and the Menzies School of Health Research (Human Research Ethics Committee 02/38). The melioidosis year is defined from October 1 to September 30 to capture the seasonal presentation in the tropical wet season (November to April). Chronic comorbidities in the dataset are defined as follows: hazardous alcohol use greater than an average daily consumption of six standard drinks (60 g, alcohol) for men and four (40 g, alcohol) for women; chronic lung disease, chronic renal disease, and septic shock.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	From 1989 to 2013, 207 patients with melioidosis required admission to ICU. Mortality reduced from 92% (1989–1997) to 26% (1998–2013) ($p < 0.001$). The reduced mortality coincided with the introduction of an intensivist-led service, meropenem, and adjuvant granulocyte colony–stimulating factor for confirmed melioidosis sepsis in 1998. Pneumonia was the presenting illness in 155 of 207 (75%). ICU melioidosis patients (2001–2013) had an Acute Physiology and Chronic Health Evaluation II score of 23, median length of stay in the ICU of 7 days, and median ventilation hours of 130 and one third required renal replacement therapy. Northern Australia current overall mortality rates of around 10% and the single most common cause of bacteremic pneumonia and septic shock. More men than women presented with melioidosis, but not significant. Median age 50 years. Indigenous patients, 67%. Urban patients, 53%. Rural patients, 47%. ICU admissions (207 total)-Pneumonia was the principal-presenting illness

		(75%), 87% bacteremic and 74% septic shock. 67% of the ICU melioidosis patients were indigenous compared to 48% of melioidosis patients not admitted to the ICU ($p < 0.001$). indigenous ICU melioidosis patients were overall younger. than the non-Indigenous patients, with mean ages 44 and 59 years, respectively ($p < 0.001$). On multivariate analysis, Indigenous ethnicity, diabetes, hazardous alcohol use, and congestive cardiac failure and/or rheumatic heart disease were each independently associated with admission to the ICU.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. Patient details were analysed using Intercooled Stata version 14.0 (StataCorp LP, College Station, TX). Chi-square or Fisher exact tests were used to assess categorical variables; *p* value less than 0.05 was considered significant, and risk ratios and 95% CIs were then calculated. To identify associations with admission to ICU, we conducted multivariable logistic regression analyses with stepwise backward elimination of patient demographic and risk factor variables, with odds ratios and 95% CIs calculated.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	The mortality for critically ill patients with melioidosis in the Top End of the Northern Territory of Australia has substantially reduced over the past 24 years. The reduction in mortality coincided with the introduction of an intensivist-led model of care, the empiric use of meropenem, and adjunctive treatment with granulocyte colony-stimulating factor in 1998. The Indigenous population of the Top End of the NT account for 30% but were 67% of the ICU melioidosis population. This overrepresentation can be accounted for by the comorbidities that predispose to developing critical illness from melioidosis infection that occur at an increased rate in the Indigenous population
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. No link to recreational water activity. However data on difference in infection and outcomes between Indigenous and non-Indigenous populations.

6 Completed quality assessment and data extraction for the included primary studies

6.1 *Naegleria fowleri* Risk of Bias (RoB) assessments

6.1.1 Abrahams-Sandi 2015 (Study ID – N41)

Table 6.1 Risk-of-bias assessment tool for Abraham-Sandi 2015 (Study ID- N41) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Abrahams-Sandi et al 2015 (N41) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Water collection sites Pathogenicity test Amoebae culturing - Controls for PCR & sequencing	No	Water samples were collected from nine sites in the Mexicali Valley. Samples were collected in triplicate at each site using sterile containers. Water measurements instruments listed and measurements conducted using modified standard methods (reference provided). Mice type/gender/age listed. Naegleria cell concentration and inoculum volume listed. Negative controls included. Cultivation methods including food source (E. aerogenes) listed DNA extractions using kit based systems (Zymo Research). No mention of DNA extraction controls included. Negative and positive controls were included in each PCR experiment. PCR primers, condition and references listed. Probably low risk of bias.	+

	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. There is reference to the use of sterile bottles used for sampling. Selection of irrigation channels for the study appears to be drive by previous fatalities (reference provided) and the use of the channels for recreational purposes. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Sample collection appear to be uniform and performed in a single month, but no mention of possible weather changes/impacts (rain, cold, heat, etc…). The lab work and analysis was the same for all samples Identical experimental conditions. Probably low risk of bias.	+
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study was to detect the presence of amoebae in water channels and identify which Naegleria species were present. It is unlikely that any bias would be introduced by not blinding to researchers. Low risk of bias	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All data is listed in the text and sequence accession numbers are listed for accessing the full DNA sequences. Potential issue with samples being collected in triplicate but only a single detection mentioned, not a per sample detection. Low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Sterile methods used for collection. Samples process and cultivated identically. PCR, sequencing and sequence analysis methods listed and references provided. Positive and Negative controls included for PCR. No DNA extraction control included. Low risk of bias	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the presence of *Naegleria fowleri* in waters. No real linkage to the other environmental factors. Noted temperatures were < 20 °C for *Naegleria fowleri* positive sites. Low risk of bias	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured data was reported. Low risk of bias	-
	Other Sources of Bias			

11.	Potential impacts on sampling	No	Unclear if samples were collected on the same day or within the same month. Potential impacts of weather inputs, e.g. rain, changing the dynamics of some of the study sites.	-
	Overall risk of bias rating:	No	Some risk of bias introduced when reporting exact sampling timeframe, but overall probably a low risk of bias.	+

Risk of bias rating:

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.2 Bonilla-Lemus 2020 (Study ID – N19)

Table 6.2 Risk-of-bias assessment tool for Bonilla -Lemus 2020 (Study ID- N19) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Bonnilla-Lemus 2020 (N19) **Study Type:** Observational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Water collection sites Pathogenicity test Amoebae culturing - Controls for PCR & sequencing	No	Water samples were collected from nine sites in the Mexicali Valley. Samples were collected in triplicate at each site using sterile containers. Water measurements instruments listed and measurements conducted using modified standard methods (reference provided). Mice type/gender/age listed. Naegleria cell concentration and inoculum volume listed. Negative controls included. Cultivation methods including food source (E. aerogenes) listed DNA extractions using kit based systems (Zymo Research). No mention of DNA extraction controls included. Negative and positive controls were included in each PCR experiment. PCR primers, condition and references listed. Probably low risk of bias.	**+**
	Cofounding bias			

4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. There is reference to the use of sterile bottles used for sampling. Selection of irrigation channels for the study appears to be drive by previous fatalities (reference provided) and the use of the channels for recreational purposes. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Sample collection appear to be uniform and performed in a single month, but no mention of possible weather changes/impacts (rain, cold, heat, etc…). The lab work and analysis was the same for all samples Identical experimental conditions. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study was to detect the presence of amoebae in water channels and identify which Naegleria species were present. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All data is listed in the text and sequence accession numbers are listed for accessing the full DNA sequences. Potential issue with samples being collected in triplicate but only a single detection mentioned, not a per sample detection. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Sterile methods used for collection. Samples process and cultivated identically. PCR, sequencing and sequence analysis methods listed and references provided. Positive and Negative controls included for PCR. No DNA extraction control included. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the presence of *Naegleria fowleri* in waters. No real linkage to the other environmental factors. Noted temperatures were < 20 °C for *Naegleria fowleri* positive sites. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured data was reported. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	Unclear if samples were collected on the same day or within the same month. Potential impacts of weather inputs, e.g. rain, changing the dynamics of some of the study sites. Probably high risk of bias.	-

	Overall risk of bias rating:	No	Some risk of bias introduced when reporting exact sampling timeframe, but overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.3 Booth 2015 (Study ID – N1)

Table 6.3 Risk-of-bias assessment tool for Booth 2015 (Study ID – N1) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Booth et al 2015 (N1) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Single infected individual (Boy aged 11). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. Post-fatality interview identified swimming and recreational water use in hot springs prior to disease onset. *Naegleria fowleri* detected at exposure site (reference listed)	+
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported	Yes	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. However, no methods are listed for *Naegleria fowleri* confirmation by CDC or environmental detection. Potential high risk of bias.	-

	and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)			
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	Sample collection methods not listed for environmental sample (reference provided). No details of experimental conditions for clinical sample provided. Potential high risk of bias.	**-**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the source of infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	No data provided in the text. Potential high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	No characterisation of samples attempted. Low risk of bias	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the link between the positive infection/fatality and the presence of *Naegleria fowleri* in the recreational water body/exposure site. Suggestion of risk due to recreational water exposure, physician diagnosis and treatment. Low risk of bias	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient fatality reported. No other measured data was reported. Low risk of bias	+
	Other Sources of Bias			
11.	Potential impacts on sampling	No	It is presumed exposure was due to recreational swimming at site based on post fatality interview, but no confirmation by comparison of clinical and environmental samples. Probably low risk of bias	+
	Overall risk of bias rating:	No	Some risk of bias introduced due to lack of data, but connection between infection, recreation activity and site exposure support links. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.4 Budge 2013 (Study ID – N6)

Table 6.4 Risk-of-bias assessment tool for Budge 2013 (Study ID – N6) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Budge et al 2013 (N6) **Study Type:** Case Study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes:**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Sample sites/sources - Detection methods Exposure	No	New single fatal case and review of Floridian cases (1962-2010). Authors are CDC and Florida Dept of Health. Case review identified 32 cases (27 recreational). Detection of *Naegleria fowleri* post-mortem by CDC and method referenced but not listed. Some environmental data (Temperature, Turbidity, E coli) included in text but methods not listed. Authors are highly experienced researchers and hence Probably low risk of bias. Interview with parents confirmed recreation water use (swimming and water slide noted). Clinical testing on additional family members for exposure but none detected.	+
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the	No	No confounding bias identified. Definitely low risk of bias.	++

	observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)			
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	No experimental information provided on historical cases but references. Probably high risk of bias.	-
6.	**Blinding of researchers during study?**	No	Researchers were blinded to the different studies. Probably low risk of bias.	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Summary data (fatality, *Naegleria fowleri* confirmation and environmental) included. Probably low risk of bias	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	Characterisation of environmental samples for *Naegleria fowleri* not attempted. Methods for other measurements not described. Probably high risk of bias.	-
9.	**Outcome assessment** - Exposure characterisation - Outcome assessment.	Yes	Recreational water exposure was likely route. No measurement of *Naegleria fowleri* in recreational water attempted. Source appears to be assumed. Potential high risk of bias. Fatality due to *Naegleria fowleri* confirmed.	-
	Selective Reporting Bias			
10.	Outcome reporting	No	*Naegleria fowleri* confirmed in fatality but not in environment. However no other water interactions recorded. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	No mention of sampling techniques. Probably high risk of bias.	-
	Overall risk of bias rating:	No	*Naegleria fowleri* infection related recreation in environmental water. Review of Florida PAM fatalities identify recreational water as being the main route of infection. Probably low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.5 Chen 2019 (Study ID – N7)

Table 6.5 Risk-of-bias assessment tool for Chen 2019 (Study ID – N7) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Chen et al 2019 (N7) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	Yes	Single infected individual (Male aged 43). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. Diagnosis by CSF microscopy and PCR (no method listed or referenced) Family interview identified recreation at a water park 5 days before onset of symptoms. No environmental survey of water park attempted. Unclear if water park used any water treatment processes. Probably high risk of bias	-
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	Yes	No methods are listed for *Naegleria fowleri* confirmation PCR or DNA sequencing or environmental detection. Definitely high risk of bias.	--

	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	No details of experimental conditions for clinical sample provided. Potential high risk of bias.	-
6.	**Blinding of researchers during study?**	N/A		
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Outcome was a patient fatality. Potential low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	No characterisation of environmental samples attempted. Methods for characterisation of clinical samples not listed. No controls listed and no confirmation of positive *Naegleria fowleri* via DNA sequencing. Definitely high risk of bias	--
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on *Naegleria fowleri* infections and difficulty to treat and detect in the environment. Suggestion of risk due to recreational water exposure, physician diagnosis and treatment. Low risk of bias	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient fatality reported and PCR data reported (without mention of methods/references used). No environmental data was reported. Low risk of bias	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	No details on the PCR methods used and controls. No sequencing of PCR fragment to confirm *Naegleria fowleri*. No environmental sample collected to analyse for *Naegleria fowleri*. Definitely high risk of bias.	--
	Overall risk of bias rating:	Yes	Lack of methods, controls and confirmation of *Naegleria fowleri* via sequencing. Overall Definitely high risk of bias.	--

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.6 Cope 2018 (Study ID – N2)

Table 6.6 Risk-of-bias assessment tool for Cope 2018 (Study ID – N2) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Cope et al 2018 (N2) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Single infected individual (Female aged 18). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. *Naegleria fowleri* exposure suspected at artificial whitewater river (occasionally chlorinated). Family interview for freshwater exposure and confirmed whitewater site was only freshwater interaction.. Epidemiologic and environmental investigation conducted at site and *Naegleria fowleri* confirmed in water source.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. Sampling methods described in text along with amounts collected. Methods for analysis described in text and reference listed. Turbidity and chlorine measurement instruments not specified. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Sample collection methods were listed for all environmental samples. All laboratory methods mentioned and referenced. Probably low risk of bias.	**+**

6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the source of infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All data provided in the text. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	All samples were analysis identically in the same laboratory with well published methods. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the challenges of engineered recreational water sites and the risks posed as this case confirmed a positive infection/fatality and the presence of *Naegleria fowleri* in the recreational water body/exposure site. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient fatality reported and presence of *Naegleria fowleri* at exposure site confirmed.	++
	Other Sources of Bias			
11.	Potential impacts on sampling	No	Source of exposure confirmed in recreational water and biofilm. Probably low risk of bias	+
	Overall risk of bias rating:	No	*Naegleria fowleri* confirmed in both source water and patient. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.7 Dean 2019 (Study ID – N29)

Table 6.7 Risk-of-bias assessment tool for Dean 2019 (Study ID – N29) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Dean et al 2019 (N29)	**RoB:** **Yes/No**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating**

Study Type: Case study		**Unknown** **N/A**		**(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Dose response model for N fowleri virulence using mice. Compared dose response for intranasal exposure and swimming. Measured response was death of mice. Treatments infective resulting in death. Exposure types were direct inhalation and swimming.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	*Naegleria fowleri* Lee strain used with dose range from 1,00 to 1,000,000 amoebae per mouse. Male and female mice used (10 each), but only make mice data reported. Exposure timed (2.5, 5, 10 and 20 minutes). Death recorded up to 28 days post exposure. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Methods described and statistical analysis and modelling methods listed. Some calculations (exposure dose) are listed in supplementary methods. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the lethal dose of *Naegleria fowleri* for mice. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	No reporting of female mice. Definitely high risk of bias.	--
	Detection Bias			

8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	N/A	.	+
9.	**Outcome assessment** - *Naegleria fowleri* fatality	No	Male mice deaths were recorded after 28 days and reported. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	Yes	No data on female mice in experiments and no statements as to why. Definitely high risk of bias.	--
	Other Sources of Bias			
11.	Potential impacts on experiments	Yes	Only half of the data reported (male mice) and no comparison between male and female made. Missing female data might contradict the male data. Probably high risk of bias	-
	Overall risk of bias rating:	Yes	Lethal dose prediction made for mice (swimming and intranasal exposure). However only half of the experimental data was used for the statistical analysis and modelling. Potential outcome difference for female mice not addressed. Overall probably a high risk of bias.	-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.8 Diaz 2012 (Study ID – N14)

Table 6.8 Risk-of-bias assessment tool for Diaz 2012 (Study ID – N14) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Diaz 2012 (N14) **Study Type:** Review Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			

1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Author is medical doctor with presumed knowledge of *Naegleria fowleri* and Primary amoebic meningoencephalitis (PAM). Review of 6 PAM cases in 2007 and 121 PAM cases between 1937-2007, and statistical analysis of risk factors. All cases were confirmed by the CDC laboratory. Probably low risk of bias. Summertime recreational freshwater activities listed as exposure route. No further details except 3 cases relating to wakeboarding.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Study used only USA cases and all cases were confirmed by the CDC laboratory. CDC also reviewed all methods used by medical professionals for the initial diagnosis. Source of historic case files listed. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Methods described. Detection by qPCR of CSF, brain biopsy, or brain fixed tissue specimens. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of the study. Cases were selected from known reports with confirmation from CDC. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Data listed in both text and figures. Definitely low risk of bias.	++
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Samples only characterised by gender, age and location. No environmental data. Probably low risk of bias.	+
9.	**Outcome assessment** - *Naegleria fowleri* fatality	No	All cases reported as fatal. Probably low risk of bias.	+

	Selective Reporting Bias			
10.	Outcome reporting	No	All analyzed data presented in text. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on experiments	No	Methods for data collection and analysis were appropriate. Probably low risk of bias	+
	Overall risk of bias rating:	No	All data reported and analysed. Statical analysis we completed for all investigated variable and results presented in text. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.9 Dunn 2016 (Study ID – N15)

Table 6.9 Risk-of-bias assessment tool for Dunn 2016 (Study ID – N15) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Dunn et al 2016 (N15) **Study Type:** Case study	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	One case infection (caucasian female aged 12) who survived. Authors is medical professionals with presumed knowledge of *Naegleria fowleri* and Primary amoebic meningoencephalitis (PAM). Rapid diagnosis (CSF stained cells/microbiology) and treatments (flow chat listed in table 1). Probably low risk of bias. No mention of exposure route in text.	+

	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Study listed the methodology used for the rapid detection. No confounding information. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Methods and steps described in detail. Detection by staining of CSF and microscopic identification. Samples further confirmed *Naegleria fowleri* in CSF by CDC (presumed standard CDC method used as none listed). Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of the study. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	N/A		
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	N/A		
9.	**Outcome assessment** - PAM survival	No	Patient survived infection. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All analyzed data presented in text. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on experiments	No	No potential threats to validity of the study. Probably low risk of bias	+
	Overall risk of bias rating:	No	All methods and data for the case were reported. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.10 Gharpure et al. Jan (2021) (Study ID – N35)

Table 6.10 Risk-of-bias assessment tool for Gharpure et al. Jan(2021) (Study ID – N35) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Gharpure et al 2021 (N35) **Study Type:** Review article		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate	No	Geographical range recreational water-associated of PAM caused by *Naegleria fowleri*. Authors are in the waterborne disease field at the Center for Disease Control and Prevention (USA) and assumed to be knowledgeable of the topic. Overview of topic lists USA occurrence (1978-2018) with known of suspected recreational water exposure (lake, pond, reservoir, river, stream, or outdoor aquatic venue). PAM cases identified cases from CDC laboratory records. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Exposures categorised into quartiles by case year. Methods for statistical analysis and comparison listed. Atmospheric temperatures obtained from NOAA's closest weather station. Air temperature used as a proxy for water temperature as water temperature not recorded at exposure sites. 120 PAM cases reported in time-frame with 85 cases linked to recreational water. 35 patients with exposure to canals, puddles, ditches, geothermal water, tap water or unknown/multiple locations excluded, but unclear why. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection	No	No description of molecular methods used or referenced. Statistical methods and analysis listed in text. Probably low risk of bias	**+**

	- Lab work up and analysis			
6.	**Blinding of researchers during study?**	No	Researchers were not blinded to the study group, subset of cases selected for inclusion in analysis of expansion. Probably low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	No confirmation of *Naegleria fowleri* in water sources listed for cases. No mention about parameters (Physical, chemical or biological) to relate to *Naegleria fowleri* in the environment. Air temperature measured but not water temperatures. Probably high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	N/A		
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Comparison of air temperature, exposure location and time enabled a broad picture of geographical changes in PAM cases over time. However no confirmation of *Naegleria fowleri* in water sources listed and hence difficult to relate to an increased presence/distribution of *Naegleria fowleri* in the environment. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured outcomes were reported. Probably low risk of bias	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	Air temperature used instead of water temperature which could impact the what the real exposure temperature water. Probable high risk of bias.	-
	Overall risk of bias rating:	No	Presentation of USA recreational water exposure PAM case presented. Analysis of temperature, time and geographical measurements support the outcome of northern expansion of PAM cases, but not *Naegleria fowleri* presence. Overall Probably low risk of bias.	+

Risk of bias rating:

Definitely low risk of bias (--)	++	Probably low risk of bias (-)	+	Probably high risk of bias (+)	-	Definitely high risk of bias (++)	--

6.1.11 Gharpure et al. Jul (2021) (Study ID – N40)

Table 6.11 Risk-of-bias assessment tool for Gharpure et al. Jul (2021) (Study ID – N40) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Gharpure et al 2021 (N40) **Study Type:** Review article		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate	No	Geographical range recreational water-associated of PAM caused by *Naegleria fowleri*. Authors are in the waterborne disease field at the Center for Disease Control and Prevention (USA) and assumed to be knowledgeable of the topic. Overview of topic lists USA occurrence (1978-2018) with known of suspected recreational water exposure (lake, pond, reservoir, river, stream, or outdoor aquatic venue). PAM cases identified cases from CDC laboratory records. Probably low risk of bias.	+
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Exposures categorised into quartiles by case year. Methods for statistical analysis and comparison listed. Atmospheric temperatures obtained from NOAA's closest weather station. Air temperature used as a proxy for water temperature as water temperature not recorded at exposure sites. 120 PAM cases reported in time-frame with 85 cases linked to recreational water. 35 patients with exposure to canals, puddles, ditches, geothermal water, tap water or unknown/multiple locations excluded, but unclear why. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	No description of molecular methods used or referenced. Statistical methods and analysis listed in text. Probably low risk of bias	**+**

6.	**Blinding of researchers during study?**	No	Researchers were not blinded to the study group, subset of cases selected for inclusion in analysis of expansion. Probably low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	No confirmation of *Naegleria fowleri* in water sources listed for cases. No mention about parameters (Physical, chemical or biological) to relate to *Naegleria fowleri* in the environment. Air temperature measured but not water temperatures. Probably high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	N/A		
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Comparison of air temperature, exposure location and time enabled a broad picture of geographical changes in PAM cases over time. However, no confirmation of *Naegleria fowleri* in water sources listed and hence difficult to relate to an increased presence/distribution of *Naegleria fowleri* in the environment. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured outcomes were reported. Probably low risk of bias	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	Air temperature used instead of water temperature which could impact the what the real exposure temperature water. Probable high risk of bias.	-
	Overall risk of bias rating:	No	Presentation of USA recreational water exposure PAM case presented. Analysis of temperature, time and geographical measurements support the outcome of northern expansion of PAM cases, but not *Naegleria fowleri* presence. Overall Probably low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.12 Goudot 2012 (Study ID – N24)

Table 6.12 Risk-of-bias assessment tool for Goudot 2012 (Study ID – N24) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Goudot et al 2012 (N24) **Study Type:** Diagnostic/quantitative observational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Growth of *Naegleria fowleri* - Comparison of Temperature and nutrient levels	**No**	The study investigated the growth of *Naegleria fowleri* on freshwater biofilms and how that growth changed with different conditions. Experiments were all laboratory based under controlled conditions. Definitely low risk of bias	**++**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Researchers have performed work in freshwater microbiology and amoebae previously, it can be assumed that standard aseptic technique/procedures to mitigate risk of introducing other organisms were used. Authors listed a total of 10 experiments were run in duplicate. For each run, 11 samples from 3 coupons were randomly and regularly collected at listed intervals. The replication adds a layer of certainty in their sampling methods as well as quality control samples utilised. The alignment of the samples was based on the pooled quality control sample. *Naegleria fowleri* from a listed culture collection and preparation methods listed in text. Thermophilic amoebae in surface water used in experiment were not removed or characterised, hence potential interactions (positive or negative) could have affected the results. Methods used to measure physical and chemical conditions not listed. Probably low risk of bias	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	**No**	Experimental sampling methods were identical for all experiments. Methods are listed and described in text along with reagent preparation and sterilisation. Biofilm reactor set up and operation listed in text and diagram provided in Figure 1.	**+**

			Methods for *Naegleria fowleri* detection listed in test. No assessment of other thermophilic amoebae in source water used for experiments. Methods used to measure physical and chemical conditions not listed. Probably low risk of bias.	
6.	**Blinding of researchers during study?**	**No**	Blinding of researchers is not applicable to the nature of this study. The introduction of bias is not of concern at this point given the aim of the study/experiment type. Probably Low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	Characterisation of other thermophilic amoebae. Probably high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of biofilm colonies – sampling/sequencing/ measurement/analysis methods - Confirming presence of FLAs in biofilm.	**No**	The authors describe most experimental methods and instrument design used or reference previously published work. However, methods used to measure physical and chemical conditions not listed and characterisation of other thermophilic amoebae not attempted or listed. Probably Low risk of bias	+
9.	**Outcome assessment** - Impact of Temperature and nutrients on *Naegleria fowleri*	**Yes**	The study is looking at the impact of water temperature and nutrient levels on the presence and concentration of *Naegleria fowleri* in freshwater biofilms. The overall data and results support this but potential competition from other thermophilic amoebae present in the experiment is not address and hence the impact is unknown. Probably high risk of bias.	-
	Selective Reporting Bias			
10.	Outcome reporting • Data from exposure site	**No**	All data except other thermophilic amoebae is listed. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	**No**	Person test and software used is listed. Probably low risk of bias.	+
	Overall risk of bias rating:	**No**	Design, replication and operation of the biofilm system and the growth of *Naegleria fowleri* based on different temperature and nutrients is well analysed in the paper. Improved understanding of the impact of the other thermophilic amoebae could further clarify the results, but overall learning are worthwhile. Probably low risk of bias	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.13 Hamaty 2020 (Study ID – N8)

Table 6.13 Risk-of-bias assessment tool for Hamaty 2020 (Study ID – N8) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Hamaty et al 2020 (N8) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Single infected individual (Male aged 29). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. *Naegleria fowleri* exposure suspected at recreational water park (surfing). Family interview for freshwater exposure and confirmed water park was only freshwater interaction. Medical symptoms and treatments listed in the manuscript. Epidemiologic and environmental investigation not conducted at site to confirm *Naegleria fowleri* presence in the water source. Later manuscripts from CDC attempt to confirm the source. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. CSF samples were submitted to CDC to confirm *Naegleria fowleri* presence (no method listed).Patient treatments methods described in text. Probably low risk of bias.	+

	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Lab methods for parasite smear test mention but not listed. Confirmation of *Naegleria fowleri* by CDC. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the infection, treatment and case outcome (fatality). It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All data provided in the text. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	Sample characterisation methods were not described in any detail in the text nor were references provided. Probably high risk of bias.	-
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion highlights the link between recreational water activity and *Naegleria fowleri* exposure, challenges of diagnosis, and the need for increased awareness. Discussion did not mention links to engineered recreation water facilities, such as the inland surf park site. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient fatality reported and clinical presence of *Naegleria fowleri* confirmed. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	N/A	No sampling at exposure site done.	
	Overall risk of bias rating:	No	*Naegleria fowleri* confirmed in and patient and exposure site likely a recreational water park. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.14 Heggie 2017 (Study ID – N16)

Table 6.14 Risk-of-bias assessment tool for Heggie 2017 (Study ID – N16) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Heggie 2017 (N16) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Review of PAM survivor (Female 12 years old) and novel treatment. PAM symptoms described in text and treatments for infection listed and performed by medical professionals. Treatments were effective resulting in survival. *Naegleria fowleri* suspected at recreational water sites during recreational activity (swimming) at a waterpark a few days prior to PAM symptoms, but no confirmation with environmental samples. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	All patient cases listed in text and referenced. Patient treatments methods described in text. No listing of environmental parameters, but reference to a waterpark where a previous case at the same waterpark (no reference). Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	Hospital microbiology laboratory identified *Naegleria fowleri* in CSF in text but no methods listed. Waterpark listed as having a previous case and *Naegleria fowleri* detected in water (inadequately chlorinated) but no details or methods provided. Probably high risk of bias.	-

6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the infection, treatment and case outcome (survival) of a *Naegleria fowleri* recreational water exposure case. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	All clinical data provided in the text. No data or references provided for the *Naegleria fowleri* detection methods for the environmental samples. Probably high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	Sample characterisation methods for clinical diagnosis and treatment listed. No methods listed for confirmation of *Naegleria fowleri* in waterpark samples. Probably high risk of bias.	-
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion of early treatment and inclusion of the drug Miltefosine in PAM cases. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient treatment and survival reported and clinical presence of *Naegleria fowleri* confirmed. Patient epidemiology associated with recreational water activity. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	No description of environmental sampling and methods that confirm *Naegleria fowleri* at exposure site. Probably high risk of bias.	-
	Overall risk of bias rating:	No	Successful treatment of a recreational waters associated PAM case. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.15 Jamerson 2009 (Study ID – N20)

Table 6.15 Risk-of-bias assessment tool for Jamerson 2009 (Study ID – N20) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Jamerson 2009 (N20)	**RoB:**	**Notes**	

		Yes/No Unknown N/A		Risk of bias rating (--/-/+/++)
Study Type: Observational study				
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Water collection sites Amoebae culturing - Controls for PCR & sequencing	No	Investigation of *Naegleria fowleri* presence in an industrial thermally heated recreational lake. Sixteen water samples collect from two sectors (warm and cool) of a lake. Triplicate water samples collected from sites on 3 different sampling occasions. Note authors state not all 16 sites were collected on the three sampling times and listed them in tables. Sediments only collected at 3 sites. Details of culturing method listed and referenced and controls (no added food source) included. PCR amplification methods described in detail with referenced provided for methods. Definitely low risk of bias.	**++**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. There is reference to the use of sterile centrifuge tubes used for sample collection. Potential changes in sample take on different dates due to climate changes. Controls have been included in the PCR methods. Instrument for collection of physical and chemical conditions (Ph, DO Conductivity, and Temperature) identified as well as depth within the surface water analysed. Distance from thermal input water recorded. Definitely low risk of bias.	++
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Sample collection appear to be uniform and performed in a single month, but no mention of possible weather changes/impacts (rain, cold, heat, etc…). The lab work and analysis was the same for all samples. Identical experimental conditions. Methods for correlation analysis missing. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study was to detect the presence of *Naegleria fowleri* different locations of the lake water. It is unlikely that any bias would be introduced by not blinding to researchers. Low risk of bias	**+**
	Attrition/Exclusion Bias			

7.	**Missing outcome data**	Yes	All data is listed in the text. No record of sequence accession numbers. No record of correlation analysis results or P-values for correlations. Possible high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Sterile methods used for collection. Samples process and cultivated identically. PCR, sequencing and sequence analysis methods listed and references provided. Positive and Negative controls included for PCR. Probably low risk of bias	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the presence of *Naegleria fowleri* in the thermally impacted recreational waters. No correlation to measured environmental factors found. *Naegleria fowleri* more frequently detected on "warm" side of the lake. Probably low risk of bias	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured data was reported. Probably Low risk of bias	+
	Other Sources of Bias			
11.	Potential impacts on sampling	No	Potential impacts of weather inputs between sampling not noted, however study occurred in late summer and potentially no storms occurred. Probably low risk of bias	+
	Overall risk of bias rating:	No	Methods, data controls used for all aspects of the study. Only exclusion was the method used to identify the correlation between the data. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.16 Kemble 2012 (Study ID – N3)

Table 6.16 Risk-of-bias assessment tool for Kemble 2012 (Study ID – N3) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Kemble et al 2012 (N3)	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Study Type: Case study			

Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Single infected individual (Female aged 7). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. Family interview for freshwater exposure and confirmed freshwater site interaction (swimming in lake). Clinical methods listed. Epidemiologic and environmental investigation conducted at exposure site 1-2 weeks after symptoms. Environmental samples (water and sediment) collected from exposure site. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. Sampling methods described in text along with amounts collected and replication of sampling in sterile bottles. Methods for named in text but not all referenced. PCR methods for environmental and clinical samples referenced. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Sample collection methods were listed for all environmental samples. All laboratory methods mentioned and referenced. Genotyping methods used the same section of DNA (rRNA gene) and the method referenced. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the source of infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All data provided in the text. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation**	No	All samples were analysis identically in the same laboratory with well published methods. Probably low risk of bias.	+

	- Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods			
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion of the mostly northerly case of PAM in the USA, approximately 550 miles north of Missouri case. Potential increase exposure risk due to warming climate. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient fatality reported and presence of *Naegleria fowleri* at exposure site confirmed. Both clinical and environmental samples from exposure site were the same genotype. Definitely low risk of bias.	++
	Other Sources of Bias			
11.	Potential impacts on sampling	No	Sample analysis was comprehensive with the same methods applied at both the exposure site and the patients clinical sample. Probably low risk of bias.	+
	Overall risk of bias rating:	No	Same *Naegleria fowleri* genotype confirmed in both source water and patient. Water temperatures of positive environmental site were between 21-24 °C. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.17 Lam 2019 (Study ID – N25)

Table 6.17 Risk-of-bias assessment tool for Lam 2019 (Study ID – N25) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Lam 2019 (N25) **Study Type:** Diagnostic/quantitative observational study	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1.	**Randomization**	N/A	Randomization: not applicable	

2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Growth of *Naegleria fowleri* - Comparison of environmental conditions on growth.	**No**	Study investigated conditions effecting the viability of *Naegleria fowleri* in a controlled environment. Individual conditions (pH, salinity and temperature) were tested to identify the individual impacts. Experiments were all laboratory based under controlled conditions. Definitely low risk of bias.	**++**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Researchers have performed work in freshwater microbiology and amoebae previously, it can be assumed that standard aseptic technique/procedures to mitigate risk of introducing other organisms were used. Authors used a known *Naegleria fowleri* species for culture collection centre (ATCC 30894). Salinity ranges and pH treatment methods listed in text. Temperature treatment methods listed also. All cultures examined by light microscopy. Viability assessed by growth media methods listed in text. No replication of experiments listed. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Lab work up and analysis	**No**	Experimental sampling methods were identical for all experiments. Axenic culture methods listed. Methods are listed and described in text along with reagent preparation and sterilisation. Methods for *Naegleria fowleri* viability listed as microscopy to identify viable *Naegleria fowleri* on new growth media. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	**No**	Blinding of researchers is not applicable to the nature of this study. The introduction of bias is not of concern at this point given the aim of the study/experiment type. Probably low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	No missing outcome data. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of biofilm colonies – sampling/sequencing/ measurement/analysis methods - Confirming presence of FLAs in biofilm.	**No**	The authors describe all experimental methods used for testing conditions and analysing viability. Probably low risk of bias	+

9.	**Outcome assessment** - Impact of pH, salinity and temperature on *Naegleria fowleri*	**No**	The study looked at the impact of pH, salinity and temperature ranges on the viability of *Naegleria fowleri*. The overall data and results support the outcome. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting • Data from exposure site	**No**	All data is listed. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	**N/A**		
	Overall risk of bias rating:	**No**	Study gives an improved understanding of the environmental conditions in which *Naegleria fowleri* can remain viable . Probably low risk of bias	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.18 Linam 2015 (Study ID – N17)

Table 6.18 Risk-of-bias assessment tool for Linam 2015 (Study ID – N17) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Linam 2015 (N17) **Study Type:** Case study	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1. Randomization	**N/A**	Randomization: not applicable	
2. Allocation concealment	**N/A**	Allocation concealment: not applicable	

3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Review of PAM survivor (Female 12 years old) and novel treatment. Clinical PAM symptoms described in text and treatments for infection listed and performed by medical professionals. Treatments were effective resulting in survival. *Naegleria fowleri* suspected at recreational water sites during recreational activity (swimming) at a waterpark a few days prior to PAM symptoms. , but no confirmation with environmental samples. Probably low risk of bias.	+
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	All patient cases listed in text and referenced. Patient treatments methods described in text. No listing of environmental parameters, but reference to a waterpark. Epidemiologic investigations by State health department identified water park as likely source and detected *Naegleria fowleri* in water samples from the lake (no methods or references). Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	Hospital microbiology laboratory identified *Naegleria fowleri* in CSF in text using Giemsa-Wright stain of CSF. CSF specimen grew *Naegleria fowleri* on culture and was PCR positive (no methods or references listed). Waterpark listed as likely source as *Naegleria fowleri* detected in water but no details or methods provided. Probably low risk of bias.	+
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the infection, treatment and case outcome (survival) of a *Naegleria fowleri* recreational water exposure case. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	All clinical data provided in the text. No data or references provided for the *Naegleria fowleri* detection methods for the environmental samples. Probably high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	Sample characterisation methods for clinical diagnosis and treatment listed. No methods listed for confirmation of *Naegleria fowleri* in waterpark samples. Probably high risk of bias.	-
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion of early treatment and inclusion of the drug Miltefosine in PAM cases. Probably low risk of bias.	+

	Selective Reporting Bias			
10.	Outcome reporting	No	Patient treatment and survival reported and clinical presence of *Naegleria fowleri* confirmed. Patient epidemiology associated with recreational water activity. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	No description of environmental sampling and methods which detected *Naegleria fowleri* at exposure site. Probably high risk of bias.	-
	Overall risk of bias rating:	No	Successful treatment of a recreational waters associated PAM case. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.19 Lopez 2012 (Study ID – N9)

Table 6.19 Risk-of-bias assessment tool for Lopez 2012 (Study ID – N9) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Lopez et al 2012 (N9) **Study Type:** Case study and review	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1. Randomization	N/A	Randomization: not applicable	
2. Allocation concealment	N/A	Allocation concealment: not applicable	
3. Comparison groups appropriate - Individual patient and treatments. Exposure	Yes	Single infected individual (Male aged 13). Case epidemiology, pathophysiology, diagnosis, treatment and outcome of treatment reviewed.	**+**

			Detection of *Naegleria fowleri* by PCR post-mortem in the CSF but method but not listed. . Some environmental data (Temperature, Turbidity, E coli) included in text but methods not listed. Authors are highly experienced researchers and hence Probably low risk of bias. Patient's recent history confirmed recreation water use (swimming and water slide noted). Clinical testing on additional family members for exposure but none detected.	
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	Yes	*Naegleria fowleri* confirmation by PCR and immunofluorescence staining, but methods not listed. Authors are CDC and presumably used in house method. Review of literature covers epidemiology, pathophysiology, diagnosis, treatment and outcome of treatment for USA cases. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	No details of experimental conditions for clinical sample provided. Probably high risk of bias.	-
6.	**Blinding of researchers during study?**	N/A		
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Outcome was a patient fatality. Potential low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	No characterisation of environmental samples attempted. Methods for characterisation of clinical samples via PCR but no methods provided or referenced. Review of previous cases covers general areas epidemiology, pathophysiology and treatment. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on *Naegleria fowleri* infections and difficulty to treat and detect in the environment. Suggestion of risk due to recreational water exposure, physician diagnosis and treatment. Low risk of bias.	+
	Selective Reporting Bias			

10.	Outcome reporting	No	Patient fatality was reported. Low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	N/A		
	Overall risk of bias rating:	No	Case report focuses on fatality without much details. Team conducting the sampling and analysis is very experienced in N .fowleri identification. Probably low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.20 Maclean 2004 (Study ID – N21)

Table 6.20 Risk-of-bias assessment tool for Maclean 2004 (Study ID – N21) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Maclean 2004 (N21) **Study Type:** Observational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Water collection sites Pathogenicity test Amoebae culturing - Controls for PCR & sequencing	No	Water samples were collected from sites in the American states of Virginia (1-site-5 samples) and Connecticut (3 sites-86 samples). Authors have skillset in working with Naegleria and environmental sampling. Samples collected in summer months (July and August) using 15mL (Conn) and 50mL (Va) centrifuge tubes and biofilm samples collected by swabbing of rocks and soil (assumed to be sterile techniques used but no listed). Climatological data obtained from local weather reporting station. No mention of sample replication. Cultivation methods including food source (E. coli) listed and referenced. No mention of DNA extraction technique used.	+

			Multiple known Naegleria and amoebae species used as controls. Negative and positive controls were included in each PCR experiment. PCR primers and cycle condition listed. Probably low risk of bias.	
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	Yes	Selection of sites based on previous Naegleria detection/Pam case and investigation of new recreational water site not previously. Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. However, no reference to the use of sterile bottles used for sampling included but assumed. Low chance of *Naegleria fowleri* contamination. Different volumes taken at the two different locations (15mL vs 50 mL) which could impact detection. No mention of DNA extraction technique and might rely on direct lysis of cells. No mention of the mice assay in methods. Probably high risk of bias.	-
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Sample collection appear to be uniform and performed in during the summer months, but different volumes collected. Impact from possible weather changes/impacts (rain, cold, heat, etc…) recorded in climate data. The lab work methods and analysis were the same for all samples tested by site. Identical experimental conditions. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study was to detect the presence of *Naegleria fowleri* in recreational waters in Connecticut and Virginia. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All data is listed in the text and tables. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Sterile methods no listed for collection of samples. Samples process and cultivated identically. PCR methods listed. Positive and Negative controls included for PCR. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the presence of *Naegleria fowleri* in waters and the use of nested-PCR for detection. Compared pre-cultured detection and direct detection for a subset of samples. Long storage of Connecticut samples may have contributed to competitor overgrowth and lower *Naegleria fowleri* detection. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured data was reported. Probably low risk of bias.	+

	Other Sources of Bias			
11.	Potential impacts on sampling	No	Sampling appears to be uniform at sites. Probably low risk of bias.	+
	Overall risk of bias rating:	No	Method investigated new molecular detection methods for *Naegleria fowleri*. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.21 Matthews 2008 (Study ID – N13)

Table 6.21 Risk-of-bias assessment tool for Matthews 2008 (Study ID – N13) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Matthews et al 2008 (N13) **Study Type:** Case Reports	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Six *Naegleria fowleri* PAM cases in USA (Arizona-1, Florida-3 and Texas-2) in 2007. Overview of cases and review of USA case 1937-2007. Brief clinical symptoms and treatment listed. All *Naegleria fowleri* detections were from clinical CSF samples. All patients died. All cases were presumably linked to recreational water sport activity. Probably low risk of bias.	**+**
	Cofounding bias		
4.	Confounding (design/analysis)	No	Authors are in the medical professionals and researchers at the Center for Disease Control and Prevention (USA) and assumed to be knowledgeable of the topic.	+

	- Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)		Clinical detection of *Naegleria fowleri* in CSF but no methods provided (assumed CDC methods). Water temperature or air temperature measured at all but one presumed exposure site. No sampling for *Naegleria fowleri* at sites listed. Probably low risk of bias.	
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	No mention of methods used for *Naegleria fowleri* confirmation. No description of any methods used at presumed exposure site for water temperatures (near shore or depth) or where air temperature was recorded. Probably high risk of bias	**-**
6.	**Blinding of researchers during study?**	No	Authors were not blinded to the case studies but this would not have impacted the report. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	All listed data was reported in text. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	No mention about methods to detect *Naegleria fowleri* in the CSF. Probably high risk of bias.	-
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Conclusion focused *Naegleria fowleri* PAM cases with associated recreation water activity exposure. Also discussed increase in number of confirmed N .fowleri cases in a single year . Low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured outcomes were reported. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	No details listed on methods for environmental temperature measurements (depth or location) for comparison across sites. Probable high risk of bias.	-
	Overall risk of bias rating:	No	Presentation of multiple cases with reference to recreational water activity exposure. Clinical diagnosis and treatment methods covered in brief.. Overall Probably low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.22 Miller 2018 (Study ID – N22)

Table 6.22 Risk-of-bias assessment tool for Miller 2018 (Study ID – N22) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Miller 2018 (N22) **Study Type:** Quantitative ecological correlational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Biofilm collection sites - Controls for sequencing	No	Biofilm samples were collected from two DWDS sites with low chlorine residual known to harbour *Naegleria fowleri* and other amoebae that were supplied with surface water, and a pre-treatment metropolitan DWDS known to harbour *Naegleria lovaniensis* with no chlorine residual supplied with ground water. Comparison groups surface vs ground water, low chlorine vs no chlorine. Only known/positive amoebae samples collected, no samples with negative amoebae collected. There is mention that for the diversity analyses normalised samples were used. For food source testing a negative control (RNase-free H20) was run with each reaction and positive controls (target DNA) and negative RNase-free H20 and DNA extraction blanks) were included in each qPCR experiment. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally	No	Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. There is reference to the use of sterile solutions and loops. The authors note environmental factors such as the chlorine residual, temperature, seasons and turbidity which may cause variation in the studies. Unsure to what extent this was compared or adjusted for in the analysis or if there was a need for this given the purpose of the study. Probably low risk of bias.	+

	during collection, transfer to lab or during experiment setup?)			
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	The methods to collect and prepare the samples was the same, noting that the conditions varied in the studies to compare different environmental factors (different water temps, time of year). The lab work and analysis was the same for all samples. Identical experimental conditions for different samples in the lab (definitely low risk of bias).	**++**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment, however given that the aim of the study was to uncover potential food sources for *Naegleria fowleri* therefore it is unlikely that any bias would be introduced by not blinding to researchers. Low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	Unsure if all raw data is provided in the supplementary documents. Samples taken between August and October but paper only reports August data for the reader to be able to interpret the results. There is not much consideration of how temperature has impacted the results especially that this is mentioned in the introduction and then it is discussed at the end of the article. Possibly a high risk of bias.	--
	Detection Bias			
8.	**Sample characterisation** - Characterisation of biofilm colonies – sampling/sequencing/ measurement/analysis methods - Confirming presence of FLAs in water supply at point of collection	No	Assuming methods used for collection and preparation of biofilm samples, sequencing, measurement and analysis were all standard and previously reported methods for this type of work and organisms. Replicates, validation across panel decreases RoB. Assuming methods for water sampling and analysis were undertaken using standard methods for different water characteristics measured.	++
9.	**Outcome assessment** - Causality (linking different bacteria/fungi/FLAs	No	Discussion critically analyses findings and acknowledges uncertainty in results and areas for further research.	+
	Selective Reporting Bias			
10.	Outcome reporting • Data from exposure site	Yes	It doesn't appear that the study reports that full analysis not undertaken on all datasets, this could contribute to selective reporting bias. Probably high risk of bias.	-
	Other Sources of Bias			

11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	No	It doesn't appear that any other threats to do with statistical methods would have introduced any further bias.	+
	Overall risk of bias rating:	No	Some risk of bias introduced when reporting on outcomes and selection of sites, but overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.23 Morgan 2016 (Study ID – N26)

Table 6.23 Risk-of-bias assessment tool for Morgan 2016 (Study ID – N26) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Morgan 2016 (N26) **Study Type:** Quantitative observational/ correlational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Biofilm collection sites - Controls for sequencing	No	The authors used a DWDS pipeline that is known to be colonized by *Naegleria fowleri*. Bulk-water samples and triplicate biofilm samples were collected at different sites along the DWDS (with decreasing chlorine residuals) four times over the year to correspond with seasons. Triplicate analysis of bulk water or biofilm were compared to freshly produced ATP standard curves at each sample time which is likely appropriate. Assume that controls are appropriate for sequencing – probably low risk of bias.	+
	Cofounding bias			

4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	The authors mentioned that the sample points were heat-sterilised and flushed under constant flow for 5 minutes before sample collection. A previously published method was used which is likely to limit the introduction of other microorganisms. Water temperature, chlorine residuals and turbidity were all measured which helps to identity other factors that may have impacted the findings. Definitely low risk of bias.	++
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Separate samples were taken from six sample points and were collected on four separate occasions to correspond with the seasons. Identical sample methods were performed at both the sampling and in the lab dependant on the sample type (bulk water or biofilm) and were done using previously described or by manufacture's protocol. Identical experimental conditions for different samples in the lab (low risk of bias).	**++**
6.	**Blinding of researchers during study?**	No	Blinding of researchers is not applicable to the nature of this study. The introduction of bias is not of concern at this point given the aim of the study/experiment type. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	The authors provided details of all samples. There doesn't appear to be any characterisation missing.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of biofilm colonies – sampling/sequencing/ measurement/analysis methods - Confirming presence of FLAs in water supply at point of collection	No	The authors mention that most methods used were either previously published or done in accordance to the manufacture's protocol. Where this isn't specified it can be assumed these are standard and not novel methods.	++
9.	**Outcome assessment** - Causality (linking different bacteria/fungi/FLAs		Discussion critically analyses findings and acknowledges uncertainty in results and areas for further research. The authors discuss that there is uncertainty whether the correlations between increased bacterial richness or abundance of specific groups is due to a causal relationship to the presence of *Naegleria fowleri* or a due to similar underling environmental conditions that promote both microbial groups. These effects cannot be separated by the current data, and future studies are needed to clarify this relationship.	+
	Selective Reporting Bias			
10.	Outcome reporting • Data from exposure site		There doesn't appear to be any issues with outcome reporting.	++

	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol		There doesn't appear to be any issues with the way the data was analysed.	++
	Overall risk of bias rating:		Low risk of bias in this study	++

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.24 Moussa 2013 (Study ID – N23)

Table 6.24 Risk-of-bias assessment tool for Moussa 2013 (Study ID – N23) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Moussa 2010 (N23) **Study Type:** Observational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Water collection sites Amoebae culturing - Controls for PCR & sequencing	No	Investigation of *Naegleria fowleri* presence in a geothermal recreational waters associated with previous fatal case. Water samples (73), sediments samples (48) and swab samples (54) collected from 6 sample points for the study. One to four water samples (500mL) collected using sterile containers dipped below surface. Swabs were 10cm^2. Sediment collected in 15 mL sterile tubes. from sites on 3 different sampling occasions. Samples collected in 2011 and 2012. Details of amoebae isolation and culturing method listed and referenced and controls (no added food source) included.	++

			PCR amplification methods described in detail with referenced provided for methods. Definitely low risk of bias.	
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. There is reference to the use of sterile centrifuge tubes used for sample collection. Temperature and pH measured at time of sampling. Additional water sample analysed at accredited lab. DNA extraction methods described and kit listed. PCR methods listed and referenced. DNA sequencing methods listed and sequenced deposited in GenBank. Statistical analysis tools and methods described. Positive and negative controls listed for PCR experiments. Definitely low risk of bias.	++
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	All methods described in detail, references and applied across all samples. Positive and negative controls listed for PCR experiments. Definitely low risk of bias.	++
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study was to detect the presence of *Naegleria fowleri* different locations of the geothermal waters. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	All data is listed in the text. Sequence accession numbers listed in text. No record of correlation analysis results or P-values for correlations. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Methods for samples characterisation listed and referenced. Positive and negative controls listed for PCR experiments. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Assessment focused on the presence of *Naegleria fowleri* in the geothermal recreational waters and noted that *Naegleria fowleri* was not transient at the geothermal sites. Correlation of turbidity and amoebae was found. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured data was reported. Probably Low risk of bias.	+
	Other Sources of Bias			

11.	Potential impacts on sampling	No	No other threats to internal validity. Probably low risk of bias.	+
	Overall risk of bias rating:	No	Methods, data and controls used for all aspects of the study. Study presents suite of environmental data at N .fowleri positive and other amoeba positive sites. . Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.25 Nicholls 2016 (Study ID – N4)

Table 6.25 Risk-of-bias assessment tool for Nicholls 2016 (Study ID – N4) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Nicholls et al 2016 (N4) **Study Type:** Case study and review	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1. Randomization	N/A	Randomization: not applicable	
2. Allocation concealment	N/A	Allocation concealment: not applicable	
3. Comparison groups appropriate - Individual patient and treatments. Exposure	No	Australian cases, North Queensland. Infected individual (Female aged 18 months old and Male aged 12 months). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. Naegleria like amoebae noted in CSF of female case and *Naegleria fowleri* confirmed postmortem in the CSF by PCR in the male case. *Naegleria fowleri* exposures potentially from untreated and unfiltered domestic water during waterplay or bathing on rural property.	+

			Review of literature for *Naegleria fowleri* Pathophysiology, Epidemiology, clinical challenges (distance between remote towns and hospital noted), diagnostic challenges (PCR method by CDC mentioned and referenced), treatment.	
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	Yes	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. Clinical sampling methods only described. CSF used for detection by microscopy and additionally PCR in the male case. Method used for PCR in Queensland is the CDC method as stated in the text. Public health investigation detected *Naegleria fowleri* at patient's home, but no sampling methods listed. Source water was noted as bore water stored in a surface dam before piping hundreds of meters to the house. Probably high risk of bias.	-
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	Yes	Clinical samples were taken from patient's CSF. No description of how environmental samples were collected and the volumes of samples or is biofilm was also collected. PCR method referenced but not described. Probably high risk of bias.	-
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the PAM infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Microscopy images of amoebae and PCR data described in text. Clinical analysis in table. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	Details of clinical samples provided. No characterisation of water samples at the homestead where *Naegleria fowleri* was detected. Probably high risk of bias.	-
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Assessment confirmed the presence of *Naegleria fowleri* in both patient and water at property. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	Yes	No description of where within homestead *Naegleria fowleri* was detected or not detected to connect to route of infection. Probably high risk of bias.	--

	Other Sources of Bias			
11.	Potential impacts on sampling	N/A		
	Overall risk of bias rating:	No	*Naegleria fowleri* confirmed in both source water and patient. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.26 Phu 2013 (Study ID – N10)

Table 6.26 Risk-of-bias assessment tool for Phu 2013 (Study ID – N10) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Phu et al 2013 (N10) **Study Type:** Case study	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q			
	Selection bias		
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Infected individual (Male aged 25 years). Treatments for infection listed and performed by medical professionals. Treatments infective resulting in death. Naegleria like amoebae noted in CSF and confirmed postmortem in the CSF by PCR and DNA sequencing. *Naegleria fowleri* exposure from recreational water activity in a freshwater lake (pearl diving).	+
	Cofounding bias		

4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used for clinical work. Clinical sampling methods described and used microscopy of CSF to detect amoebae. PCR of 18S gene (CDC method referenced) listed as target and DNA sequencing to confirm N fowleri. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Clinical samples were taken from patient's CSF. PCR method referenced and sequencing to confirm. No mention of method used to confirm sequence just match of 100% homology. No environmental water samples collected or analysed. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the PAM infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Data described in text. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Details of case study provided in text for patient. No water characterisation was conducted. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	PCR method referenced and sequencing to confirm *Naegleria fowleri*. No mention of method used to confirm sequence just match of 100% homology. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Fatality confirmed as caused by *Naegleria fowleri*. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	N/A		
	Overall risk of bias rating:	No	Exposure of *Naegleria fowleri* via recreational water activity. *Naegleria fowleri* confirmed in patient. Overall, probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.27 Puzon 2017 (Study ID – N27)

Table 6.27 Risk-of-bias assessment tool for Puzon 2017 (Study ID – N27) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Puzon 2017 (N27) **Study Type:** Quantitative observational/ correlational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Biofilm collection sites - Controls for sequencing	Maybe	Selection of positive sample sites appropriate (known zones with *Naegleria fowleri* outbreaks) but were sites with definitely no FLA (negative control) also measured or set up with biofilms? Could introduce some bias by not comparing to negative control. Study design focuses on positive sites to compare similarities and differences in biodiversity. Assume that controls are appropriate for sequencing – low RoB.	-
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Study design wants to examine biodiversity and has an open ended design regarding possible findings. Possibility of confounding of measured results with sample transfer/preparation through leaking and contamination – noted and checked to confirm that this did not occur. Water sampling conducted at site to measure other factors that might impact findings (e.g. chlorine residual, microbial, chemical, temperature, turbidity).	+

	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	 No No	 Two separate samples taken from each different site in distribution network with different water conditions (but methods for sample collection and preparation the same). Study is designed to assess biodiversity of different sites (probably low risk of bias). Identical experimental conditions for different samples in the lab (definitely low risk of bias).	**+**
6.	**Blinding of researchers during study?**	No	Blinding of researchers not applicable during sample collection. Not reported during sample prep and lab work up/ analysis of results but also not expected to introduce bias given the open-ended nature of experiment.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Unsure	Full characterisation not completed for several samples – noted in the results section.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of biofilm colonies – sampling/sequencing/ measurement/analysis methods - Confirming presence of FLAs in water supply at point of collection	No	Assuming methods used for collection and preparation of biofilm samples, sequencing, measurement and analysis were all standard and previously reported methods for this type of work and organisms. Replicates, validation across panel decreases RoB. Assuming methods for water sampling and analysis were undertaken using standard methods for different water characteristics measured.	++
9.	**Outcome assessment** - Causality (linking different bacteria/fungi/FLAs	No	Discussion critically analyses findings and acknowledges uncertainty in results and areas for further research.	+
	Selective Reporting Bias			
10.	Outcome reporting • Data from exposure site	Yes	 Study reports that full analysis not undertaken on all datasets.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	No	Sampling and sequencing biases addressed through diversity analyses and other standard methods to validate datasets.	+

	Overall risk of bias rating:	No	Some risk of bias regarding site selection and some uncertainty noted in outcomes but lab based work is definitely low risk of bias. Overall, probably low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.28 Sifuentes 2014 (Study ID – N42)

Table 6.28 Risk-of-bias assessment tool for Sifuentes 2014 (Study ID – N42) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Sifuentes et al 2014 (N42) **Study Type:** Observational study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Water collection sites Pathogenicity test Amoebae culturing - Controls for PCR & sequencing	No	Personnel are highly skilled in collecting samples and analysing samples for *Naegleria fowleri*. Water samples collected from known recreational surface water sites. Samples were collected in replicate at each site using sterile containers. Water quality measurements listed and references. Filtration and cultivation methods including incubation temperature and food source (*E. coli*) listed. DNA extraction method referenced but not listed. No mention of DNA extraction controls included. Positive and negative controls were included in each PCR experiment. PCR primers, *Naegleria fowleri* control strain and method referenced. No DNA sequencing done.	++

			Definitely low risk of bias.	
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and lab conditions, it is assumed that aseptic technique would have been used. There are references to the methods used for the cultivation and molecular work. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Identical location of sampling sites by using GPS coordinates. Samples volumes identical. Laboratory processing and analysis of samples done with identical techniques and included both positive and negative controls. Transport of samples on ice, which is not ideal for thermophilic amoeba. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study was to detect the presence of *Naegleria fowleri* at multiple recreational water sites, including two locations with *Naegleria fowleri*-linked death. It is unlikely that any bias would be introduced by not blinding to researchers. Low risk of bias	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	No DNA sequencing of samples, but target sequence is specific to *Naegleria fowleri* and both positive and negative controls included in all PCR assays. Low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Sterile methods used for collection. Samples process and cultivated identically. PCR, and statistical analysis methods listed and references provided. Positive and negative controls included for PCR. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion focused on the seasonal presence of *Naegleria fowleri* in waters and analysis of associated factors. Low risk of bias	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All measured data was reported. Low risk of bias	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	Potential impact on viable *Naegleria fowleri* due to transport on ice. Probably high risk of bias.	-

	Overall risk of bias rating:	No	Techniques and methods listed in detail will all data provided. Both positive and negative controls included in the study with replication of sampling and use of identical sample locations on a seasonal basis. Potential issue with sample transport conditions. Probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.29 Stowe 2017 (Study ID – N11)

Table 6.29 Risk-of-bias assessment tool for Stowe 2017 (Study ID – N11) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Stowe et al 2017 (N11) **Study Type:** Case study and review		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Infected individual (Male aged 4, and Male aged 14). Treatments for infection listed and performed by medical professionals. Clinical symptoms listed. Miltefosine used in treatment. Infection resulted in death. Amoebae noted in wet mount of CSF. *Naegleria fowleri* confirmed postmortem in the CSF by PCR by the CDC Both patients swam in a lake 8 days prior to symptoms developing. *Naegleria fowleri* exposures from swimming in a lake. Brief review of cases globally. Probably low risk of bias	+

	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. Clinical sampling methods only described. CSF used for detecting by microscopy and additionally PCR detection by CDC methods. Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Clinical samples were taken from patient's CSF. PCR method referenced as CDC but not described. No sampling of environmental water sources to confirm exposure site. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the PAM infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Microscopy images of amoebae and PCR confirmation described in text. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Details of clinical samples provided. No attempts to characterise environmental samples. Miltefosine used in treatment but case still fatal. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Assessment confirmed the presence of *Naegleria fowleri* in both patients. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	No missing clinical data or corresponding *Naegleria fowleri* detection Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	N/A		
	Overall risk of bias rating:	No	*Naegleria fowleri* confirmed in both patients. Both patients noted to have recreational water interaction prior to onset of symptoms. Overall, probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.30 Su 2013 (Study ID – N5)

Table 6.30 Risk-of-bias assessment tool for Su 2013 (Study ID – N5) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Su et al 2013 (N5) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Infected individual (Male aged 75). Diagnosis and treatment for infection listed and performed by medical professionals. Clinical symptoms listed. Amphotericin B used in treatment. Infection resulted in death. Amoebae noted in wet mount of CSF. *Naegleria fowleri* confirmed postmortem in the CSF by PCR and DNA sequencing. Patient used hot springs prior to symptoms developing. *Naegleria fowleri* confirmed in hot spring. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. Clinical sampling methods only described. CSF used for detecting by microscopy and additionally PCR detection methods listed in the text along with primers and PCR conditions (referenced). Inclusion of controls listed. Environmental sample detection was confirmed *Naegleria fowleri* presence in hot springs but no methods listed (assumed PCR identical) but no sequence comparison Probably low risk of bias.	+

	during collection, transfer to lab or during experiment setup?)			
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Clinical samples were taken from patient's CSF. PCR method described in detail, referenced and sequences compared to database to confirm. No sampling of environmental water sources to confirm exposure site. Probably low risk of bias.	**+**
6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the PAM infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	**+**
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	Yes	Microscopy images of amoebae and PCR confirmation described in text and referenced. No methods or data presented for the corresponding hot spring sampling and analysis. Probably high risk of bias.	-
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	Yes	Details of clinical samples provided. No attempts to characterise environmental conditions even though samples were also *Naegleria fowleri* positive. No gel image of environmental *Naegleria fowleri*. Amphotericin B used in treatment but case still fatal. Probably high risk of bias.	-
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Assessment confirmed the presence of *Naegleria fowleri* in patients. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	Yes	Missing environmental data or corresponding *Naegleria fowleri* detection Probably high risk of bias.	-
	Other Sources of Bias			
11.	Potential impacts on sampling	N/A		
	Overall risk of bias rating:	No	*Naegleria fowleri* confirmed in both patient and environment. Patient noted to have recreational water interaction at hot spring prior to onset of symptoms. Overall probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.31 Vareechon 2019 (Study ID – N12)

Table 6.31 Risk-of-bias assessment tool for Vareechon 2019 (Study ID – N12) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Vareechon et al 2019 (N12) **Study Type:** Case study		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	**Risk of bias rating** **(--/-/+/++)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Individual patient and treatments. Exposure	No	Infected individual (Male aged 8). Diagnosis and treatment for infection listed and performed by medical professionals. Clinical symptoms and medical treatment listed. Infection resulted in death. Amoebae noted in wet mount of CSF. *Naegleria fowleri* confirmed postmortem in the CSF by PCR and DNA sequencing. Patient swam and submerged head in hot springs days prior to symptoms developing. Probably low risk of bias.	**+**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Given the expertise of the authors and medical professional, it is assumed that aseptic technique would have been used. Clinical sampling methods only described. CSF used for detecting by microscopy and additionally PCR listed in the text as method to detect *Naegleria fowleri* (no reference). Probably low risk of bias.	+
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	No	Clinical samples were taken from patient's CSF. PCR stated as confirmation method but no method listed or referenced. No sampling of environmental water sources to confirm exposure site. Probably low risk of bias.	**+**

6.	**Blinding of researchers during study?**	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the PAM infection and fatality. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	+
	Attrition/Exclusion Bias			
7.	**Missing outcome data**	No	Microscopy images of amoebae and PCR confirmation stated. No environmental data provided for hot spring. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Details of clinical treatments methods provided in text. No attempts to sample hot spring presumed was source of infection. Wet mount stain of amoeba but PCR/sequence data of positive *Naegleria fowleri* included. Amphotericin B used in treatment but case still fatal. Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Assessment confirmed the presence of *Naegleria fowleri* in patients. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	Yes	Missing PCR data. Probably high risk of bias.	-
	Other Sources of Bias			
11.	Potential impacts on sampling	N/A		
	Overall risk of bias rating:	No	*Naegleria fowleri* confirmed in patient. Patient noted to have recreational water interaction at hot spring prior to onset of symptoms. Overall, probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.32 Vargas-Zepeda 2005 (Study ID – N18)

Table 6.32 Risk-of-bias assessment tool for Vargas-Zepeda 2005 (Study ID – N18) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Vargas-Zepeda 2005 (N18)	**RoB:**	**Notes: Article is a brief Notes from the Field with minimal supporting details.**	

	Yes/No Unknown N/A		Risk of bias rating (--/-/+/++)
Study Type: Case study			
Q			
	Selection bias		
1. Randomization	N/A	Randomization: not applicable	
2. Allocation concealment	N/A	Allocation concealment: not applicable	
3. Comparison groups appropriate - Individual patient and treatments. Exposure	No	Review of PAM survivor (Male 10 years old). Clinical PAM symptoms described in text and treatments for infection listed and performed by medical professionals. Treatments were effective resulting in survival. Amoebae present in the CSF identified and confirmed as *Naegleria fowleri*. (Culture and PCR). PAM symptoms onset one week after swimming in irrigation canal. Probably low risk of bias.	+
	Cofounding bias		
4. Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	No	Clinical methods for patient and treatments methods described in text. Amoebae present in wet mount of CSF. CSF samples were cultured for amoebae. Immunofluorescence and PCR/sequencing (methods referenced) used to confirm *Naegleria fowleri*. Probably low risk of bias.	+
	Performance Bias		
5. Identical experimental conditions - Sample collection - Lab work up and analysis	No	Hospital microbiology laboratory identified *Naegleria fowleri* in CSF. CSF specimen grew *Naegleria fowleri* on culture and was PCR positive (references listed). Probably low risk of bias.	+
6. Blinding of researchers during study?	No	The researchers were not blinded during any part of this experiment. The aim of the study to report the infection, treatment and case outcome (survival) of a *Naegleria fowleri* recreational water exposure case. It is unlikely that any bias would be introduced by not blinding to researchers. Probably low risk of bias.	+
	Attrition/Exclusion Bias		
7. Missing outcome data	No	All clinical data provided in the text. *Naegleria fowleri* detection methods referenced. Probably low risk of bias.	+

	Detection Bias			
8.	**Sample characterisation** - Characterisation of water samples.– sampling/sequencing/ measurement/analysis methods	No	Sample characterisation methods for clinical diagnosis and treatment listed. *Naegleria fowleri* in patient confirmed and previous studies identified *Naegleria fowleri* present in the same canals used for swimming (referenced). Probably low risk of bias.	+
9.	**Outcome assessment** - Presence of *Naegleria fowleri*	No	Discussion of early treatment based on early identification of amoebae in CSF. Probably low risk of bias.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	Patient treatment and survival reported and clinical presence of *Naegleria fowleri* confirmed. Patient epidemiology associated with recreational water activity. Probably low risk of bias.	+
	Other Sources of Bias			
11.	Potential impacts on sampling	Yes	No follow up environmental sampling to detected/confirm *Naegleria fowleri* at exposure site. Probably high risk of bias.	-
	Overall risk of bias rating:	No	Successful treatment of a recreational waters associated PAM case. Overall, probably a low risk of bias.	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.1.33 Yu 2018 (Study ID – N28)

Table 6.33 Risk-of-bias assessment tool for Yu 2018 (Study ID – N28) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Yu 2018 (N28)	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(--/-/+/++)**
Study Type: Diagnostic or quantitative observational study			
Q			

	Selection bias			
1.	Randomization	N/A	Randomization: not applicable	
2.	Allocation concealment	N/A	Allocation concealment: not applicable	
3.	Comparison groups appropriate - Biofilm collection sites - Metabolomic database	**Yes**	This study was to test the capacity of a new metabolomics technique to rapidly classify amoebae present in different field samples by comparing them to a database developed in previous studies using lab-cultured strains of amoebae. A similar protocol was followed as was in previous studies where field sites were selected based on a history of detection of *Naegleria fowleri* and *Naegleria lovaniensis* and samples collected throughout the year to allow analysis of impacts of different seasonal and environmental factors. It appears that authors also used the conventional method of culturing on non-nutrient agar *E.coli* plates and were compared to standard curves generated from pure cultures of *Naegleria fowleri* and *Naegleria lovaniensis.* This is likely to be appropriate given the aim is to assesses the reliability of the new technique compared to known biomarkers.	**++**
	Cofounding bias			
4.	Confounding (design/analysis) - Anything that could possibly be perceived to cause or impact the observed results should be reported and controlled (e.g. any other organisms introduced accidentally during collection, transfer to lab or during experiment setup?)	**Yes**	Given that this team of researchers have performed this same sampling method previously, it can be assumed that standard aseptic technique/procedures to mitigate risk of introducing other organisms were used. The authors mentioned that all samples were run in duplicates which adds a layer of certainty in their sampling methods as well as quality control samples utilised. The alignment of the samples was based on the pooled quality control sample. Water temperature, chlorine residuals and turbidity were all measured which helps to identity other factors that may have impacted the findings.	++
	Performance Bias			
5.	**Identical experimental conditions** - Sample collection - Lab work up and analysis	**Yes** **Yes**	It appears that 28 samples were collected, spanning from May 2014 to March 2015 to account for the different seasons. Methods of sampling were referenced to previously published papers which shows sampling methods are consistent. Lab work for the new technique is referenced to be the same as in vitro studies and other lab work was done were done using previously described methods or by manufacture's protocol.	**++**
6.	**Blinding of researchers during study?**	**No**	Blinding of researchers is not applicable to the nature of this study. The introduction of bias is not of concern at this point given the aim of the study/experiment type. Low risk of bias	**+**

	Attrition/Exclusion Bias			
7.	**Missing outcome data**	**Yes**	The authors mention that PCA was conducted to evaluate the metabolome similarity among different sample groups and were grouped together based on whether the samples were positive or negative. For example samples 1-3 were *Naegleria fowleri* positive and samples 4-12 negative, so these were analysed together. It doesn't seem that there is missing outcome data although it is noted that only significant features were taken through for further analysis. Probably low risk of bias.	+
	Detection Bias			
8.	**Sample characterisation** - Characterisation of biofilm colonies – sampling/sequencing/ measurement/analysis methods - Confirming presence of FLAs in water supply at point of collection	**Yes**	The authors mention that most methods used were either previously published or done in accordance to the manufacture's protocol. Where this isn't specified it can be assumed these are standard and novel methods were in line with those carried out in the previous in vitro studies.	++
9.	**Outcome assessment** - Causality (linking different bacteria/fungi/FLAs)	**N/A**	This study isn't looking at causality but piloting a new method of identifying the presence of amoebae in the field. It seems that adequate effort and steps were taken to compare field vs lab samples. The authors acknowledge several areas of uncertainty and note that further work is needed to standardise the procedure and verify the findings before they use them. Probably high risk of bias until further data is collected.	-
	Selective Reporting Bias			
10.	Outcome reporting • Data from exposure site	**Yes**	There doesn't appear to be any issues with outcome reporting.	++
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	**Yes**	Different types of statistical analysis were carried out on raw LCMS data and during the matching process. There doesn't appear to be any issues with the way the data was analysed.	++
	Overall risk of bias rating:		No major issues with this study. This is a first attempt at using this method in a real-world sample, so assumed this will be tested further prior to this method replacing conventional methods. Probably low risk of bias	+

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.2 *Naegleria fowleri* data extraction forms

6.2.1 Abrahams-Sandi et al. (2015)

Table 6.34 Data extraction form for Abrahams-Sandi 2015 (Study ID – N42)

General information	Study ID	Abrahams-Sandi et al 2015 (N42)
	Date template completed	09/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Elizabeth Abrahams-Sandí, Lissette Retana-Moreira, Alfredo Castro-Castillo, María Reyes-Batlle, Jacob Lorenzo-Morales. 2015. Letter to editor. Costa Rica. University of Costa Rica.
Study characteristics	Aim/objectives of study	Assessment of suspected *Naegleria fowleri* source water link to PAM fatality.
	Study type/design	Detection of *Naegleria fowleri*
	Study duration	N/A
	Type of water source/water body	Hot Springs
Population characteristics	Population/s studied	Male age 11
	Selection criteria for population	N/A
	Subgroups reported	N/A
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Swimming pool, river pond and resort hot springs-Costa Rica. Recreational activity most likely source. Water samples confirmed to have *Naegleria fowleri.*
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	N/A-Water Quality N fowleri isolated by growth on NNA-plates and identified by PCR-DNA sequencing.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Viable *Naegleria fowleri* confirmed in source water for PAM case. Confirmed by viability, PCR and DNA sequencing.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	N/A
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* in Costa Rica water sources visited by fatal PAM case. Monitoring and public awareness of *Naegleria fowleri* in any warm water is crucial.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Further connection between infection and source water (hot spring and river pond) both having *Naegleria fowleri*.

6.2.2 Bonilla-Lemus 2020 (Study ID – N19)

Table 6.35 Data extraction form for Bonilla-Lemus 2020 (Study ID – N19)

General information	Study ID	Bonilla-Lemus et al 2020 (N19)
	Date template completed	09/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Patricia Bonilla-Lemus, Saúl Rojas-Hernández, Elizabeth Ramírez-Flores, Diego A. Castillo-Ramírez, Alejandro Cruz Monsalvo-Reyes, Miguel A. Ramírez-Flores, Karla Barrón-Graciano, María Reyes-Batlle, Jacob Lorenzo-Morales and María Maricela Carrasco-Yépez. 2020. Research article. Peer Reviewed. Mexico (Mexicali Valley). UNAM. Funded by UNAM, RICET and FEDER.
Study characteristics	Aim/objectives of study	Detection of *Naegleria fowleri* in recreational waters.
	Study type/design	Research article-detection in recreational waters.
	Study duration	N/A
	Type of water source/water body	Recreational waters-irrigation canals.
Population characteristics	Population/s studied	N/A
	Selection criteria for population	N/A
	Subgroups reported	N/A
	Size of study	N/A- people, 9-sampling locations.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Irrigation canals (Mexicali Valley) used for recreational swimming. N/A for exposure and other parameters.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Water Quality- pH, Water Temperature, Dissolved oxygen and conductivity. *Naegleria fowleri* isolation- viable testing (NNA-plates) with microscopy, flagellate transformation, mouse pathogenicity test, PCR and DNA sequencing. Water samples-250mL water (in triplicate).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Identification of pathogenic *Naegleria fowleri* in waters and associated environmental conditions. N/A participants.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	N/A

Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Viable pathogenic *Naegleria fowleri* was present in waters during cold months in the Mexicali valley.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Physicochemical water quality included along with Naegleria viability, pathogenicity (mouse tests) and molecular classification.

6.2.3 Booth 2015 (Study ID – N1)

Table 6.36 Data extraction form for Booth 2015 (Study ID – N1)

General information	Study ID	Booth et al 2015 (N1)
	Date template completed	09/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Peggy J. Booth1; Dean Bodager MPA2; Tania A. Slade, MPH1; Swannie Jett, 2015. Case notes. Peer Reviewed. Florida USA. Florida Dept of Health.
Study characteristics	Aim/objectives of study	Report of PAM fatality
	Study type/design	Report of *Naegleria fowleri*/PAM death
	Study duration	N/A
	Type of water source/water body	Hot Springs
Population characteristics	Population/s studied	Male age 11
	Selection criteria for population	N/A
	Subgroups reported	N/A
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Hot Springs-Costa Rica. Swimming and water slide. Recreational activity most likely source. Hot springs confirmed to have *Naegleria fowleri.*
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	N/A-Water Quality. *Naegleria fowleri* in CSF confirmed by qPCR (CDC-method).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Fatality from PAM. Preliminary medical assessments for viral meningitis, initial CSF negative for amoeba, second CSF positive for amoeba.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	N/A
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Early diagnosis and public awareness of *Naegleria fowleri* in any warm water is crucial.
Reviewer comments	Results included/excluded in review (if applicable)	Include. Connection between infection and source water both

	Notes on study quality e.g. gaps, methods	having *Naegleria fowleri*. Brief overview of fatal case.

6.2.4 Budge 2013 (Study ID – N6)

Table 6.37 Data extraction form for Budge 2013 (Study ID – N6)

General information	Study ID	Budge et al 2013 (N6)
	Date template completed	12/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Philip J. Budge, Becky Lazensky, Karen E Elliott, Carrie A. Dooyema, Govinda S. Visvesvara. 2013 Case Report. Peer Reviewed. Georgia and Florida USA. Florida Dept of Health and Centers for Disease Control and Prevention.
Study characteristics	Aim/objectives of study	PAM case report and epidemiological review (Florida USA cases only).
	Study type/design	Case report and review.
	Study duration	Cases between 1962-2010.
	Type of water source/water body	Recreational waters.
Population characteristics	Population/s studied	PAM case plus family members.
	Selection criteria for population	Camped at site with fatal case.
	Subgroups reported	N/A
	Size of study	18
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Recreational waters (freshwater swimming lake) filled from deep well and not thermally polluted. Exposure possibly through “rough water” play/waterslide. *Naegleria fowleri.* Comparison to other Florida cases (1962-2010). No attempt to detect *Naegleria fowleri* in lake was done.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	CDC/FDOH investigation of suspected recreational waters (freshwater swimming lake). Faecal coliforms, Temperature and turbidity measured.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Fatality from PAM. Outcome assessed post-mortem. List of PAM symptoms and attempted interventions (antibiotics). *Naegleria fowleri* confirmed in CSF by PCR, CDC-method. 18 family members surveyed for water interactions/use and symptoms. 9 family members, fatal case and 1 park employee tested for anti-

		Naegleria fowleri antibody titers. (no repose). Historical cases listed by exposure site, age and month of infection.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	14-year exposure estimate listed (referenced paper).
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Freshwater recreational activities in Florida should always assume a low level of *Naegleria fowleri* exposure. Risk reduction avoidance during particular condition (high temps-low water). Avoid disturbing sediment.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Review identified PAM case likely through recreational water exposure. However, no attempt to detect *Naegleria fowleri* in water source was done. Other measurements and historical review of case criteria was listed.

6.2.5 Chen 2019 (Study ID – N7)

Table 6.38 Data extraction form for Chen 2019 (Study ID – N7)

General information	Study ID	Chen M. 2019 (N7)
	Date template completed	17 February 2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Chen, M., W. Ruan, L. Zhang, B. Hu and X. Yang. June 2019. Journal article. Peer-reviewed. China. Health Department of Zehjiang Province – General Project Funds (grant no. 2015KYA018). NA
Study characteristics	Aim/objectives of study	Case report of a 43-year-old male who died of PAM in China after exposure to warm freshwater.
	Study type/design	Case report.
	Study duration	NA
	Type of water source/water body	Recreational water park.
Population characteristics	Population/s studied	Single 43-year-old male.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	Single case.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s) Comparison group(s)	Recreational water park – warm freshwater. NA NA NA *Naegleria fowleri.* NA
Study methods	Water quality measurement used	NA

	Method of microorganism isolation and enumeration (if applicable) Other methods used: Water sampling methods (monitoring, surrogates)	*Naegleria fowleri* identified in CSF stained with Wright-Giemsa and positive amplicons obtained from *Naegleria* spp. and *Naegleria fowleri* specific qPCR. NA
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Patient diagnosed with PAM following identification of *Naegleria fowleri* in CSF using staining and qPCR. 1 NA
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Although PAM might be cured it treated early and effectively, most clinicians might never have encountered it before. Therefore, it is imperative to increase the clinical awareness of PAM in every case of purulent meningitis, especially in patient with recent freshwater exposure.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This article should be included in the review to address the primary question. This case-report details a middle-aged male who died from PAM after contracting *Naegleria fowleri* when exposed to warm freshwater at a recreational waterpark. Gaps in this study include missing information about the kind of recreational water park, the activity being performed as well as testing the water for the presence of *Naegleria fowleri.*

6.2.6 Cope 2018 (Study ID – N2)

Table 6.39 Data extraction form for Cope 2018 (Study ID – N2)

General information	Study ID	Cope et al., 2018 (N2)
	Date template completed	9/7/2021
	Authors	Cope JR, Murphy J, Kahler A, Gorbett DG, Ali I, Taylor B, Corbitt L, Roy S, Lee N, Roellig D, Brewer S, Hill VR.
	Publication date	2018.
	Publication type	Journal.
	Peer reviewed	Peer-reviewed.

	Country of origin Source of funding Possible conflicts of interest	USA. NA No conflict of interest.
Study characteristics	Aim/objectives of study	Epidemiologic and environmental investigation on a fatal PAM case to determine water exposure that led to the death of the patient.
	Study type/design	Case report
	Study duration	2016
	Type of water source/water body	Artificial whitewater river.
Population characteristics	Population/s studied	18-year-old woman who died of PAM.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	1 person
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Artificial whitewater river. Rafting. Nose while submerged under water after being through out of the raft. Artificial whitewater river water. *Naegleria fowleri.* NA Yes
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Total chlorine residual, free chlorine residual, turbidity, temperature. *Naegleria fowleri* real-time PCR and culture assay, cultured organisms genotyped by sequencing 5.8S rRNA gene and internal transcribed spacers I and 2 (ITS1 and ITS2). Water, facility filter backwash, submerged plant material and surface swab samples from channels and upper and lower ponds of the USNWC. Water, sediment and surface swab samples from near adjacent Flatwater Dock in the Catawba River.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Death. Cardiac death. Wet mount of cerebrospinal fluid, real-time PCR test. 1 exposed.
Statistics	Statistical methods used	NA

	Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	The case report documents a novel exposure to an artificial whitewater river as the likely exposure causing PAM in the case. Conditions in the whitewater facility (warm, turbid water with little chlorine and heavy algal growth) rendered water treatment ineffective and provided an ideal environment for *Naegleria fowleri* to thrive.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Ok to include.

6.2.7 Dean 2019 (Study ID – N29)

Table 6.40 Data extraction form for Dean 2019 (Study ID – N29)

General information	Study ID	Dean et al 2019 (N29)
	Date template completed	05/07/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Kara Dean, Mark H. Weir and Jade Mitchell. 1 February 2019. Research Article. Peer-reviewed. USA. NA NA
Study characteristics	Aim/objectives of study	To develop a dose response model for *Naegleria fowleri*.
	Study type/design	Statistical analysis of previous work to develop a dose response model.
	Study duration	28 days post experimental exposure.
	Type of water source/water body	Surface water/drinking water.
Population characteristics	Population/s studied	global
	Selection criteria for population	Reports of Naegleria
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Referenced studies used direct nasal exposure and swimming exposure in mice.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement	Death. By death. Dead or alive.

	Number participants (exposed/non-exposed, missing/excluded) (if applicable)	170 – all exposed to either different nasal concentrations of *Naegleria fowleri* or to different concentrations and duration of swimming.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Developed a dose response. Data quality check 1) three or more graded doses were administered in the experiments; (2) at least three animals were tested in each dosing group; and (3) the data had a statistically significant trend by the Cochran–Armitage test. Dose response assessment Exponential dose–response model and the approximate form of the beta-Poisson dose–response model were fit to the data. Goodness of fit calculated by Chi2. Confidence intervals detected by bootstrapping resampling. Exposure per mouse was calculated based off nasal surface area and breaths. Beta Poisson was the best fit. Dose response curve of amoeba per mL calculated.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	LD50 of 13257 amoeba.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This seems like an interesting way to tackle the issue of dose response. Very hard to see if these results will actually be useful as an LD50 is not necessarily a good measure for a human health issue. It would be more interesting to report the reliability of the lower doses in predicting mortality as 13257 per ml seems like an unrealistic concentration.

6.2.8 Diaz 2012 (Study ID – N14)

Table 6.41 Data extraction form for Diaz 2012 (Study ID – N14)

General information	Study ID	Diaz J. 2012 (N14)
	Date template completed	13/09/2021
	Authors Publication date Publication type Peer reviewed	James Diaz 2012 Review article. Peer Reviewed. Louisiana State University, USA.

	Country of origin Source of funding Possible conflicts of interest	No conflicts declared.
Study characteristics	Aim/objectives of study	Review of US cases (1937-2007) with statistical analysis of risk factors for PAM.
	Study type/design	Review article.
	Study duration	NA
	Type of water source/water body	Freshwater.
Population characteristics	Population/s studied	General population-121 cases.
	Selection criteria for population	*Naegleria fowleri* infection.
	Subgroups reported	NA
	Size of study	121
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	None listed but recreational water likely source. NA to others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to all
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Infection by *Naegleria fowleri*. Confirmed detection by CDC methods DNA-based detection. 121.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. Yates-corrected, chi-square analysis. 78%-male. 97%-cases in 15 southern states. 85% July-September infections. More cases post 1977, (X^2=13.827, P = 0.001. Recreational Freshwater Exposure X2=105.875 P = 0.0001) (Note 3 cases associated with Wakeboarding in 2007). Case frequency 0-3 cases per year (1937-2007).
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Increased infections due to increased recreational freshwater activities (no data). Avoidance of Recreational water activities in warm freshwater bodies
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Provides statistical analysis of Gender, timeframe and locations.

6.2.9 Dunn 2016 (Study ID – N15)

Table 6.42 Data extraction form for Dunn 2016 (Study ID – N15)

General information	Study ID	Dunn A. 2016 (N15)
	Date template completed	13/09/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Andrew L. Dunn, Tameika Reed, Charlotte Stewart, Rebecca A. Levy 2016 Case Report. Peer Reviewed. University of Arkansas for Medical Sciences, USA. No conflicts declared.
Study characteristics	Aim/objectives of study	Case study of 12-year-old PAM survivor.
	Study type/design	Case Study.
	Study duration	NA
	Type of water source/water body	Freshwater Park.
Population characteristics	Population/s studied	One (12-year old girl).
	Selection criteria for population	*Naegleria fowleri* infection.
	Subgroups reported	NA
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Freshwater park. Suspected Recreational/swimming reported. NA to others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	None. CSF microscopy of *Naegleria fowleri*. No water samples.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Survival. Early detection of infection by Naegleria species by microscopy. Aggressively treated with drugs including amphotericin, rifampin, azithromycin, and fluconazole and miltefosine. *Naegleria fowleri* later confirmed by CDC methods, DNA-based detection. 1 person.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Early detection (1hr 15 min upon CSF draw) of PAM along with aggressive treatments resulted in a successful recovery after 52 days in hospital. Full recovery.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Successful treatment of PAM potentially due to rapid diagnosis.

6.2.10 Gharpure et al. Jan (2021) (Study ID – N36)

Table 6.43 Data extraction form for Gharpure Jul 2021 (Study ID – N41)

General information	Study ID	Gharpure et al. 2021
	Date template completed	19/04/2021
	Authors	Gharpure R, Gleason M, Salah Z, Blackstock AJ, Hess-Homeier D, Yoder JS, Ali IKM, Collier SA, Cope JR.
	Publication date	2021.
	Publication type	Journal.
	Peer reviewed	Peer reviewed.
	Country of origin	USA.
	Source of funding	Not known.
	Possible conflicts of interest	NA
Study characteristics	Aim/objectives of study	Analysis of the trends in recreational water exposures associated with PAM cases reported during 1978-2018 in USA.
	Study type/design	Review.
	Study duration	1978-2018.
	Type of water source/water body	Recreational water: lakes, ponds, reservoirs, rivers, streams and outdoor aquatic venues.
Population characteristics	Population/s studied	Reported PAM cases in USA between 1978-2018.
	Selection criteria for population	Cases with a single known exposure site or multiple sites within an 80 km radius. In temperature analysis included patients with a known or imputed date of exposure.
	Subgroups reported	Years 1978-1989 (20 cases); 1990-1999 (15 cases); 2000-2009 (26 cases); 2010-2018 (24 cases).
	Size of study	Among 120 PAM cases reported 85 were included in and 35 were excluded from the study.
Exposure and setting	Type of water source/water body	69 patients exposed to lake/pond/reservoir, 14 river/stream, 2 outdoor aquatic venue. Excluded 35 patients exposed at canals/puddles/ditches/ geothermally heated water/tap water at unknow locations or at multiple locations >80 km apart.
	Exposure scenario	NA
	Exposure pathway	NA
	Source of infection/contamination	Recreational water.
	Causal organism/chemical(s)	*Naegleria fowleri.*
	Comparison group(s)	NA
	Confirmed link to Recreational Water	Yes for 85 cases.
Study methods	Water quality measurement used	NA
	Method of microorganism isolation and enumeration (if applicable)	NA

	Water sampling methods (monitoring, surrogates)	NA
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Maximum latitude pf PAM cases shifted 0.12 decimal degrees (i.e. approximately 13.3 km) northward per annum. No change was observed in minimum latitude. On average, daily air temperatures were higher in the 2 weeks before exposure than the 20-year average for that date and location.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Negative binomial regression to assess trends in annual PAM incidence. Evaluation of the latitudes of exposure locations using Kruskal-Wallis tests for overall comparisons and Dwass-Steel-Critchlow-Fligner test for pairwise comparisons. Linear regression to examine trends in annual maximum and minimum latitudes of exposures. Sensitivity analysis to determine the effect of excluding years with single cases and excluding outliers on the basis of leverage, Cook's distance, and studentized residual values. Generalized estimating equation models to compare temperatures, with autoregressive correlation structure using quasi-likelihood under independence model criterion.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	The rise in cases in the Midwest region after 2010 and increases in maximum and median latitudes of PAM case exposures suggest a northward expansion of *Naegleria fowleri* exposures associated with lakes, ponds, reservoirs, rivers, streams and outdoor aquatic venues in USA. Limitations: 1) PAM is probably under-recognised and underreported in USA, so the study might not fully capture trends in incidence and exposure characteristics 2) Temperature data were not collected simultaneously with exposure, and thus might differ from actual exposure conditions 3) Analysis included years with single cases, which could bias the results of the regression analyses of latitude.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Yes, include

6.2.11 Gharpure et al. Jul (2021) (Study ID – N41)

Table 6.44 Data extraction form for Gharpure Jul 2021 (Study ID – N41)

General information	Study ID	Gharpure R. et al 2021 (N41)
	Date template completed	23/06/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Gharpure R., Bliton J., Goodman A., Ali I., Yoder J., Cope J. 2021. Global Review of *Naegleria fowleri* . Peer Reviewed. USA. CDC, Georgia, USA.
Study characteristics	Aim/objectives of study	Global Review of PAM cases
	Study type/design	Literature Review
	Study duration	1937-2018
	Type of water source/water body	River, lake, pond/ditch, and puddles.
Population characteristics	Population/s studied	Global study of all literature-reported and direct CDC-reported PAM cases. 381 total cases.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	381
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Natural water sources (River, lake, reservoir, pond/ditch, canal and puddles). Recreational activities (247 cases) Swimming/diving, wakeboarding, jet skiing, waterskiing, splashing water.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Elevated air Temps-summer months. Microscopy. CDC-method for PCR detection of *Naegleria fowleri* from CSF.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Microscopy of CSF-ID of Naegleria-like species, some followed by PCR. Outcome was death with 7 confirmed survivors (treated with antibiotics). 381 cases reviewed.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Negative binomial regression analysis, Wilcoxon-Mann Whitney tests, Person $\chi 2$ tests (SAS 9.4)
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Recreational activity was most commonly associated with PAM. Males more prominent to be infected. Swimming/diving most associated in reported cases. Need for better/early diagnosis and treatment.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Global review with links and stats to recreational water use linked to reported PAM cases. Results should a significant link, however no

		mention of confirmed detection of *Naegleria fowleri* in source waters.

6.2.12 Goudot 2012 (Study ID – N24)

Table 6.45 Data extraction form for Goudot 2012 (Study ID – N24)

General information	Study ID	Goudot et al 2012 (N24)
	Date template completed	05/08/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Sebastien Goudot, Pascaline Herbelin, Laurence Mathieu, Sylvie Soreau, Sandrine Banas, Frederic Jorand 2012 Research Paper. Peer Reviewed. Universite´ de Lor, France. No conflicts declared.
Study characteristics	Aim/objectives of study	Lab study of *Naegleria fowleri* growth conditions.
	Study type/design	Research.
	Study duration	NA
	Type of water source/water body	Freshwater/Biofilms
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA to all. Study is laboratory based on growth conditions of *Naegleria fowleri*.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Temperature, bacterial density (Epifluorescence microscopy), free-living amoebae (MPN-Pougnard et al 2002), and pathogenic *Naegleria fowleri* (immunosorbent assay-Reveiller et al-2003).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Total bacterial cells measured in biofilm. Total *Naegleria fowleri* measured in biofilm. *Naegleria fowleri* cell density measured as a function of nutrient level (bacteria) and temperature.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. Pearson test at a 95% confidence level. Performed on XLSTAT Version 2010.1.01 software.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* growth was affected by both temperature and nutrient levels. At 32°C *Naegleria fowleri* density remained constant (1-10 cells/cm^2). At 42°C *Naegleria fowleri* density increased (30-900 cells/cm^2). Minimum of 10^4 bacterial/amoeba

		required for growth with an optimal 10^6-10^7 bacteria/amoeba.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Manuscript provides details of the microbial/nutrient levels needed to promote *Naegleria fowleri* growth in freshwater samples.

6.2.13 Hamaty 2020 (Study ID – N8)

Table 6.46 Data extraction form for Hamaty 2020 (Study ID – N8)

General information	Study ID	Hamaty E. 2020 (N8)
	Date template completed	24/11/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Edward Hamaty Jr., Saif Faiek, Minesh Nandi , David Stidd, Manish Trivedi, and Hari Kandukuri 2020. Case Report. Peer Reviewed. Department of Medicine, AtlantiCare Regional Medical Center, Atlantic City, NJ, USA, USA. No conflicts declared.
Study characteristics	Aim/objectives of study	Report of PAM fatality from Recreation Water.
	Study type/design	Case Report
	Study duration	NA
	Type of water source/water body	Recreational water park
Population characteristics	Population/s studied	1 male adult-29 years old
	Selection criteria for population	*Naegleria fowleri* infection
	Subgroups reported	NA
	Size of study	One adult male
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Freshwater Recreational activities at a surf park in Waco, Texas Exact activity not listed. NA to others Exposure at water park assumed as the only link, but noy confirmed.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to all
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	PAM Infection. Pathogenesis described. Diagnosis-common symptoms and presence of trophozoites in CSF (microscopy and CDC). Treatment with drugs including vancomycin, acyclovir, amphotericin B, azithromycin, and fluconazole. Survivors-Drug treatments. Prevention-Avoidance of exposure to freshwater, especially during summer,

		or avoid jumping, splashing of submerging in water.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	"Given the rarity of this case and its very high mortality rate, it is crucial to diagnose primary amoebic meningoencephalitis accurately as its presentation can mimic bacterial meningitis. It is vital to obtain a careful and thorough history, as it can aid in prompt diagnosis and treatment. "
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Case report links *Naegleria fowleri* exposure and PAM fatality to recreational water activity at a Water Park. Gaps are no water quality data and no confirmation of *Naegleria fowleri* in water sources.

6.2.14 Heggie 2017 (Study ID – N16)

Table 6.47 Data extraction form for Heggie 2017 (Study ID – N16)

General information	Study ID	Heggie and Kupper 2017 (N16)
	Date template completed	08/12/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Travis W. Heggie and Thomas Küpper Case Report. Peer Reviewed. Bowling Green State University, School of Human Movement, Sport and Leisure Studies, Bowling Green, OH 43403, USA. School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville, Qld, Australia. Institute of Occupational and Social Medicine, RWTH Aachen Technical University, Pauwelsstr. 30, D-52074 Aachen, Germany. No conflicts declared.
Study characteristics	Aim/objectives of study	Report on PAM survivor and new drug therapy.
	Study type/design	Case Report.
	Study duration	NA
	Type of water source/water body	Recreational waterpark.
Population characteristics	Population/s studied	1 female child-12 years old.
	Selection criteria for population	*Naegleria fowleri* survivor.
	Subgroups reported	NA
	Size of study	One female child.

Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Freshwater lake (manmade). Recreational activities at a waterpark in Arkansas Swimming in lake. *Naegleria fowleri* confirmed in waterpark lake (no methods listed).
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Waterpark reportedly chlorinated (no concentration listed), but lots of organic matter present (no concentration listed) to remove chlorine. Water temperature was "elevated" but no measurements listed. NA to all other water quality measurements. List of medical/drug treatments provided in detail (Drug/Dose/Route/Duration).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	PAM Infection and survival/full recovery. Pathogenesis described. Diagnosis-common symptoms and presence of trophozoites in CSF (Diagnosis of *Naegleria fowleri* by hospital microbiology lab, but not methods listed). Treatment with drugs including Amphotericin B, Azithromycin, Fluconazole, Rifampin, Miltefosine (administered within 36-hours of diagnosis), and Dexamethasone. Catheter placed in patient's brain to reduce swelling. Survivors-Drug treatments.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Prompt diagnosis, lowering of body temperature and early treatment were key to survival. Miltefosine a potentially important therapy for treating *Naegleria fowleri*. Prevention-Avoidance of exposure to freshwater, especially during summer, or avoid jumping, splashing of submerging in water is key.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include, but note that chlorine may have been used. *Naegleria fowleri* linked to both waterpark lake and patient. Case report links *Naegleria fowleri* exposure and PAM fatality to recreational water activity at a Water Park.

		Gaps are no water quality data provided and no methods of *Naegleria fowleri* detection listed.

6.2.15 Jamerson 2009 (Study ID – N20)

Table 6.48 Data extraction form for Jamerson 2009 (Study ID – N20)

General information	Study ID	Jamerson M. et al 2009 (N20)
	Date template completed	01/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Melissa Jamerson & Kenneth Remmers & Guy Cabral & Francine Marciano-Cabral Research paper. Peer Reviewed. Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0678, USA. No statement on conflicts listed.
Study characteristics	Aim/objectives of study	Survey of *Naegleria fowleri* in recreational lake water impacted by industry.
	Study type/design	Research paper.
	Study duration	Summer 2007 (June-September).
	Type of water source/water body	Freshwater lake.
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Recreational freshwater lake thermally impacted by industry. NA scenario NA-pathway NA infection.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	16 water samples collected from two sectors of the lake (9 main reservoir and 7 cooling lagoons). Sediment also collected (3 sites). Water quality measurements (pH, dissolved oxygen, temperature and conductance). Direct amoebae counts (microscopy) Detection of *Naegleria fowleri* by nested PCR (Reveiller method) and sequenced. Coliforms detection by growth on agar plates.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	PCR Positive *Naegleria fowleri* samples collected on both sectors of lake (cool and warm). No correlation between *Naegleria fowleri* presence and distance from

		thermal pollution site. Lake positive for 9 of 16 samples collected. Positive samples were not associated with highest temperatures. Factors in addition to temperature contribute to *Naegleria fowleri* presence. No correlation to pH, DO and conductivity found. Average Coliforms detected (25cfu/mL).
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* detected in the lake at low levels with distribution greater in warmer areas. DO, pH, conductivity and coliform counts not linked to *Naegleria fowleri* Potential microbial predation of *Naegleria fowleri* by other microbes.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Lake ware is consistently warm during the study and *Naegleria fowleri* can be found in both warm and cool sections. No apparent links to other physical measurements and coliforms.

6.2.16 Kemble 2012 (Study ID – N3)

Table 6.49 Data extraction form for Kemble 2012 (Study ID – N3)

General information	Study ID	Kemble S. et al 2012 (N3)
	Date template completed	15/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Sarah K. Kemble, Ruth Lynfield, Aaron S. DeVries, Dennis M. Drehner, William F. Pomputius III, Michael J. Beach, Govinda S. Visvesvara, Alexandre J. da Silva, Vincent R. Hill, Jonathan S. Yoder, Lihua Xiao, Kirk E. Smith, and Richard Danila. Case and field Report. Peer Reviewed. Minnesota Department of Health and Centers for Disease Control and Prevention USA. No statement on conflicts listed.
Study characteristics	Aim/objectives of study	Analysis of suspected site of PAM infection.
	Study type/design	Case and Field Report
	Study duration	August 2010
	Type of water source/water body	Freshwater lake and sediment samples.
Population characteristics	Population/s studied	2 adults, 3 kids (2 male-1 female).
	Selection criteria for population	Family all swam in lake.

	Subgroups reported	1 Female youth age 7.
	Size of study	1.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Recreational freshwater lakes(A and B) and river. Swimming, handstands under water, water up nose, swallowed and aspirated water multiple times by fatality. NA scenario. NA-pathway NA infection.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Water(3x 150mL) and sediment(4x 100mL) sampling (1-2 weeks post illness onset) at location used by patient, ambient local temperature measured, Water Temperature, water clarity, presence of algal blooms, organic matter and storm water drainage in Lake A. Microscopy of CSF = amoeba Amoeba culturing at 44 °C PCR (CDC method) and genotyping
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Positive *Naegleria fowleri* detection in Lake A water and sediment and genotype confirmed by PCR. Surface water temperatures (22.1 °C and 24.5 °C). Algal blooms notes and water clarity described as poor. Mean air temperature (25 °C) was 3.6 °C above normal for August.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	First reported *Naegleria fowleri* (PAM) case in Minnesota. Case was 550 mile north of previously reported northernmost case in the Americas. Local weather patterns and long-term climate change could impact PAM frequency.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Confirmed *Naegleria fowleri* in lake and CSF was same genotype- Direct link to lake. Environmental variables assessed. Expansion of geographical range in USA, potential links to climate change. Lack of clarity on instruments used for water quality analysis. Methods referenced for PCR and genotyping. Risk factors with recreational water.

6.2.17 Lam 2019 (Study ID – N25)

Table 6.50 Data extraction form for Lam 2019 (Study ID – N25)

General information	Study ID	Lam C. et al 2019 (N25)
	Date template completed	16/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Charlton Lam , Li He & Francine Marciano-Cabral. 2019 Research paper. Peer Reviewed. Department of Microbiology & Immunology, Virginia Commonwealth University, Richmond, Virginia USA. No statement on conflicts listed.
Study characteristics	Aim/objectives of study	Analysis of the effect of different environmental conditions on *Naegleria fowleri* viability.
	Study type/design	Laboratory Research
	Study duration	NA
	Type of water source/water body	NA
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	*Naegleria fowleri* used in the laboratory study was originally isolated from a fatal case of PAM. NA scenario NA-pathway NA infection.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	*Naegleria fowleri* cultured on axenic media (Ref provided) and passaged in mice. Viability parameters tested include, Salinity(0.208% to 3.6%), pH (1-14), and Temperature (43-52 °C). Viability determined by movement via microscopy.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Viable *Naegleria fowleri* detected at; Salinity range (0.208%-1.4%) nonviable above 1.6%. pH range (3-12), nonviable at Ph1-2 and above pH12. Temperature range (44-49 °C), nonviable at > 50 °C.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* salinity range is broader than thought (half of seawater and 3-4 time > than saltwater pools). Viable pH range is

		broad. Temperature tolerance is up to 48 °C for 72 hours.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Manuscript provides detailed information on the viability of *Naegleria fowleri* in multiple conditions typically encountered in the environment and recreational waters.

6.2.18 Linam 2015 (Study ID – N17)

Table 6.51 Data extraction form for Linam 2015 (Study ID – N17)

General information	Study ID	Linam W. et al 2015 (N17)
	Date template completed	16/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	W. Matthew Linam, Mubbasheer Ahmed, Jennifer R. Cope, Craig Chu, Govinda S. Visvesvara, Alexandre J. da Silva, Yvonne Qvarnstrom, and Jerril Green. 2015 Case study. Peer Reviewed. Arkansas Children's Hospital, Little Rock, Arkansas and Center for Disease Control and Prevention, Atlanta, Georgia, USA. No conflict of interest.
Study characteristics	Aim/objectives of study	Successful treatment of Adolescent with *Naegleria fowleri* PAM.
	Study type/design	Case Study
	Study duration	Monthly for 1 year (Nov 2007-Oct 2008)
	Type of water source/water body	Outdoor water park
Population characteristics	Population/s studied	Female youth-12 years old
	Selection criteria for population	*N fowleri* infection
	Subgroups reported	Survivor
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Outdoor water park. Swimming No water parameters listed. Water samples from site tested positive to *Naegleria fowleri*. (Confirmed link).
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Viable *Naegleria fowleri* confirmed in patient's CSF by PCR (CDC labs but no method referenced). Early diagnosis of infection and treatment with antimicrobials including miltefosine and management of patient brain trauma.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Patient survived. *Naegleria fowleri* was confirmed by PCR in both patient CSF and water park samples. (CDC- labs but no method referenced)

Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Successful treatment of patient infected with *Naegleria fowleri*. Treatment success due to early diagnosis of infection and treatment with antimicrobials including miltefosine and management of patient brain trauma.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Direct connection between recreational water activity and *Naegleria fowleri* infection. Potential medical treatment options. Gaps. No methods for PCR listed and no water quality parameters included.

6.2.19 Lopez 2012 (Study ID – N9)

Table 6.52 Data extraction form for Lopez 2012 (Study ID – N9)

General information	Study ID	Lopez C. et al 2012 (N9)
	Date template completed	17/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Christina Lopez, Phillip Budge, Jimmy Chen, Suzanne Bilyeu, Ayesha Mirza, Haidee Custodio, MD, Jose Irazuzta, Govinda Visvesvara, and Kevin J. Sullivan. 2012 Case Report. Peer Reviewed. Arkansas University of Florida and Center for Disease Control and Prevention, Atlanta, Georgia, USA. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Fatal case report of adolescent with *Naegleria fowleri*-PAM.
	Study type/design	Case Study and Review of Treatment.
	Study duration	NA
	Type of water source/water body	Suspected recreation lake (Northern Florida).
Population characteristics	Population/s studied	Male youth-13 years old.
	Selection criteria for population	*N fowleri* infection.
	Subgroups reported	
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Suspected recreation lake (Northern Florida). Suspected swimming. No water parameters listed. No water samples collected from suspected site to test for *Naegleria fowleri.*
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable)	*Naegleria fowleri* confirmed in patient's CSF by PCR (no method referenced). Medical treatment regime listed.

	Water sampling methods (monitoring, surrogates)	No water quality/environmental parameters measured or listed.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Patient died. *Naegleria fowleri* confirmed in patient's CSF by PCR (no method referenced). Mention that 80% of USA cases occur between July-September (summer-autumn). Review of Pathophysiology, Clinical manifestations, Diagnosis, and Treatment options.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* is found globally and most infections are fatal. Health care providers need to be aware of the potential of infection for more rapid identification of PAM.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include but only as a fatal outcome case related to recreational activity. Gaps; No methods for PCR listed and no water quality parameters included, no confirmation of *Naegleria fowleri* in water samples.

6.2.20 Maclean 2004 (Study ID – N21)

Table 6.53 Data extraction form for Maclean 2004 (Study ID – N21)

General information	Study ID	Maclean R. et al 2004 (N21)
	Date template completed	17/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Rebecca C. Maclean Æ Dennis J. Richardson Robin LePardo Æ Francine Marciano-Cabral. 2004 Research paper. Peer Reviewed. Virginia Commonwealth University, Richmond, VA 23298, USA. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Detection of *Naegleria fowleri* In water and soil samples.
	Study type/design	Research paper.
	Study duration	July-August 2000.
	Type of water source/water body	Natural and recreational lakes (Virginia and Connecticut, USA).
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway	Natural and Recreational water samples collected from the

	Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	environment in Virginia and Connecticut (15 mL samples). No human infection involved.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	*Naegleria fowleri* detected by viable and nested PCR tests (Reveiller methods). Coliforms and *E. coli* measured on plates. Air Temperature recorded.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Naegleria fowleri* detected in samples. No significant correlations noted in number of thermotolerant amoebae and air temperature, water temperature (20-28 °C), coliforms of *E. coli*.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* detected in water and sediment samples. Water temperature and presence of coliform bacteria are not the only factors to influence the distribution of *Naegleria fowleri*.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Detection of *Naegleria fowleri* in environmental samples. Gaps; No methods statistical analysis for *Naegleria fowleri* to other variables. Also, no mention of when water temperature was recorded or instrument used.

6.2.21 Matthews 2008 (Study ID – N13)

Table 6.54 Data extraction form for Matthews 2008 (Study ID – N13)

General information	Study ID	Matthews S. et al 2008 (N13)
	Date template completed	17/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	S. Matthews, D Ginzl, D Walsh, K Sherin, MD, J Middaugh, MD, R Hammond, D Bodager, K Komatsu, J Weiss, PhD, N Pascoe, F Marciano-Cabral, E Villegas, G Visvesvara, J Yoder, B Eddy, L Capewell, R Sriram, K Bandyopadhyay, Y. Qvarnstrom, A DaSilva, S Johnston, L Xiao, V Hill, S Roy, MJ Beach. 2008 Case Reports. Peer Reviewed. Multiple institutions, USA. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Summary of 2007 *Naegleria fowleri* deaths.

	Study type/design	Case Reports and case review.
	Study duration	June-September 2007; Review (1937-2007).
	Type of water source/water body	Natural recreational lakes (Arizona, Texas and Florida, USA).
Population characteristics	Population/s studied	USA.
	Selection criteria for population	*Naegleria fowleri* fatalities.
	Subgroups reported	Males aged 10-22.
	Size of study	6
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Recreational waters (lakes) Swimming and Wake boarding Nasal (assumed) and ruptured ear drum NA all others
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	*Naegleria fowleri* detected in CSF(no method listed). Water and air temperatures.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Naegleria fowleri* deaths in all cases *Naegleria fowleri* detected in CSF(no method listed). Some water and air temperatures measured. Average case numbers per year (1937-2007) are 0-8 cases/year.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	None listed
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Analysis of data still being conducted. Case range per year is 0-8. Exposure occurred in warm untreated freshwater lakes in 15 southern tier states.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Deaths related to fresh water recreational activities. Gaps. Incomplete temperature data, no corroboration of *Naegleria fowleri* in recreational water sources and no other data or methods provided.

6.2.22 Miller 2018 (Study ID – N22)

Table 6.55 Data extraction form for Miller 2018 (Study ID – N22)

General information	Study ID	Miller H. C. 2018 (N22)
	Date template completed	24 February 2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding	Miller, H. C., M. J. Morgan, T. Walsh, J. T. Wylie, A. H. Kaksonen and G. J. Puzon 6 May 2018 Journal article Peer-reviewed. Australia.

	Possible conflicts of interest	CSIRO Land and Water. No conflicts of interest
Study characteristics	Aim/objectives of study	Uncover preferential food sources for *Naegleria fowleri* in order to predict colonisation events and enable pre-emptive management actions.
	Study type/design	Quantitative environmental investigation of *Naegleria fowleri*, other amoebae and bacteria.
	Study duration	August – October 2015.
	Type of water source/water body	2 DWDS sites supplied with surface water and 1 pre-treatment metropolitan DWDS site supplied with ground water.
Population characteristics	Population/s studied	No human population studied.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	Triplicate biofilm samples from each site.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s) Comparison group(s)	Artificial whitewater river supplied with municipal water and on-site wells. Patient fell and was submerged in the water while rafting. Intranasal water exposure. Artificial whitewater river. *Naegleria fowleri.* NA
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Other methods used: Water sampling methods (monitoring, surrogates)	Temperature, turbidity as well as free and total chlorine residuals. Direct qPCR on environmental samples, culture to assess viability and sequencing to ascertain the genotype. Water sampling included: Bulk water (0.75 – 50 L), facility filter backwash (0.75 L), submerged plant material (4" X 4") and surface swabs (4" X 4") of the channels and upper and lower ponds. The adjacent Flatwater Dock in the Catawba River was also sampled. Patient samples were assessed using CSF wet mount which showed motile trophozoites and qPCR revealed the present of *Naegleria fowleri* in the CSF.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	100% (11/11) samples from the artificial whitewater facility tested positive for *Naegleria fowleri* both viability and molecularly and sequencing revealed genotype 1. Of the 5 samples collected from the adjacent river, only 1 sediment sample

		tested positive for *Naegleria fowleri* viably.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Novel exposure of *Naegleria fowleri* in an artificial whitewater river which led to PAM and subsequent death of an 18-year-old female. The conditions within the facility were ideal for *Naegleria fowleri* growth and included warm, turbid water with little chlorine and heavy algal growth.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This article should be included in the review to address the primary question as well as the secondary questions pertaining to risk indicators and conditions associated with increased occurrence of *Naegleria fowleri* and PAM. This case-report and environmental investigation detail a PAM case where the investigators assessed the potential exposure location for the presence of *Naegleria fowleri* and determined that the recreational water park (artificial whitewater river) was the most likely source of infection after all 11 samples tested positive for *Naegleria fowleri* both molecularly and viably. The study also details concerns regarding how *Naegleria fowleri* made it into the water in the first place given that multiple barriers were put in place to prevent natural water and soil contamination.

6.2.23 Morgan 2016 (Study ID – N26)

Table 6.56 Data extraction form for Morgan 2016 (Study ID – N26)

General information	Study ID	Morgan 2016 (N26)
	Date template completed	29 July 2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Morgan M.J., Halstrom S., Wylie J.T., Walsh T., Kaksonen A.H., Sutton D., Braun K, and Puzon G.J. 6 February 2016 Journal Article. Yes. Australia. Water Corporation of Western Australia and CSIRO Land and Water are acknowledged for funding.

		The authors declare no competing financial interest.
Study characteristics	Aim/objectives of study	To measure changes in the environmental conditions and ecology in bulk water and biofilm collected from drinking water distribution systems (DWDS) with confirmed *Naegleria fowleri*.
	Study type/design	Quantitative observational study
	Study duration	One year, seasonal sampling
	Type of water source/water body	5 Sample sites from drinking water distribution system pipeline in rural Western Australia, post-treatment.
Population characteristics	Population/s studied	No human populations. Viable amoeba, eukaryotic and bacterial communities of biofilms studied.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	Duplicate samples of biofilms and bulk water collected from different sites
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s)	As above. Water sampling: first sample point 10 km post chlorination, remaining sample points placed at 5 km intervals. Seasonal collection. Biofilm sampling: 2 Kiwa biofilm monitors directly connected to the pipeline at 25 and 40 km post chlorination. Identifying amoeba, eukaryotic and bacterial communities in biofilms and bulk water NA Comparative study of samples against environmental variables
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Measured: chlorine residuals, temperature, turbidity Total microbial cell concentrations of biofilm and bulk water were enumerated using a previously published method on a Quanta flow cytometer. Viable amoeba detection was conducted on all samples using methods described previously. Extracted DNA was then used in quantitative PCR melt-curve analysis (qPCR) for the detection and identification of amoebae using general primers and for *Naegleria fowleri* using specific primers. Variations in microbial composition along the length of the DWDS pipeline were assessed by amplicon pyrosequencing. Denoised sequences were analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline software.

		Water sampling methods undertaken using standard equipment and protocols.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants Water quality results Microbial analysis	Comparison of environmental variables against water and biofilm samples and presence or absence of *Naegleria fowleri.* Bacterial and eukaryotic richness and β-diversity within each bulk water and biofilm sample was assessed and compared with water temperature, chlorine residual (free and total), total cell counts, ATP concentrations, and water turbidity at each site. Methods described above. NA Chlorine concentration (both free and total) decreased along the DWDS pipeline with distance from the chlorinator, seasonal impacts observed. Water turbidity 0.4 - 0.6 NTU, increased readings >1.0 NTU at terminal site. Water temperature was influenced by seasonal factors, highest temperature recorded 41 °C. Total cell numbers and microbial activity in the bulk water generally increased with increasing distance from chlorination. Distance from chlorination tank was significantly associated with chlorine concentration in both bulk water and biofilm samples. Biofilm and water samples with confirmed *Naegleria fowleri* had significantly higher bacterial richness and lower free and total chlorine concentrations than those without.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	A number of standard statistical methods were used for the various analyses undertaken and to assess significant differences. Further details provided in paper.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Environmental variables that associated significantly with the presence of viable *Naegleria fowleri* in the bulk water included distance from the last chlorine treatment point, chlorine residual and high bacterial community richness (noting that the last could be attributed to decreased chlorine residual but could factor into preferred food sources for *Naegleria fowleri*). Only site distance from the treatment plant could be significantly linked to viable *Naegleria fowleri* presence in biofilms (likely due to reduction of the chlorine residual in

		the pipeline). Eukaryotic richness, turbidity, water temperature, total cell count, and ATP concentration were poor indicators of *Naegleria fowleri* presence or absence. Authors have noted uncertainty in the causes for the observed patterns and correlations and to further determine the predictive value of specific bacterial taxa for *Naegleria fowleri* management.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Good quality study investigating *Naegleria fowleri* in DWDS in rural WA, relevant to review questions relating to potential indicators/surrogates. The reviewers will need to consider the applicability of this study to recreational water scenarios that are not chemically disinfected, and the studies implications to the water conditions and indicators for *Naegleria fowleri*.

6.2.24 Moussa 2013 (Study ID – N23)

Table 6.57 Data extraction form for Moussa 2013 (Study ID – N23)

General information	Study ID	Moussa M. et al 2013 (N23)
	Date template completed	17/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Mirna Moussa, Johan F. De Jonckheere, Jerome Guerlotte´, Vincent Richard, Alexandra Bastaraud, Marc Romana, Antoine Talarmin. 2013 Research Paper. Peer Reviewed. Unite´ Environnement-Sante´ , Institut Pasteur de la Guadeloupe, Les Abymes, Guadeloupe, France No conflict of interest.
Study characteristics	Aim/objectives of study	Survey of *Naegleria fowleri* in geothermal recreational waters
	Study type/design	Research paper
	Study duration	June 2011 – July 2012
	Type of water source/water body	Geothermal recreational waters in Guadeloupe (French West Indies).
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination	Geothermally feed Recreational waters (lakes) with previous PAM death. Swimming and bathing

	Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA all others
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Water samples (73), sediment samples (48) and swab samples (54) were collected from 6 sample points over a year. Temperature and pH measured on site. Some additional chemical parameters (Turbidity, alkalinity, hardness, K, Ca, Mg, Na, SO_4, Cl, SiO_2, TOC Permanganate, NH_4 NO_3 HCO_3) measured in an accredited lab. Amoebae isolation by filtration Viability on NNA-E. coli at 44 °C Amoeba counting by plaques on NNA-E. coli plates. PCR (De Jonckheere method) and sequencing.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Naegleria fowleri* most frequent thermophilic amoeba detected (38.3% water samples, 10.4% sediment samples and 0% swab samples). *Naegleria fowleri* concentration 0-22 amoebae per litre. *Naegleria fowleri* found in sediments upstream of baths. No significant difference in *Naegleria fowleri* presence based on Temp or pH. No correlation between *Naegleria fowleri* and chemical parameters except turbidity. *Naegleria fowleri* more frequently encountered at sites tested compared to *Naegleria lovaniensis.* *Naegleria fowleri* detected below French standard of 100 amoebae/litre.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Fisher's exact test, Kruskall Wallis test and Spearman test (R-software).
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Naegleria fowleri* detected below French standard of 100 amoebae/litre. Human infection could occur at concentrations below 100 amoebae/litre. *Naegleria fowleri* was not a transient organism and thrived in most hot springs.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Good comparison of physical, chemical, and other amoebae present. Also included an enumeration of *Naegleria fowleri*/litre in water sources. Study indicates continual presence of *Naegleria*

		fowleri. Gaps. Not clear in multiple amoebae are detected at the same time in the same samples.

6.2.25 Nicholls 2016 (Study ID – N4)

Table 6.58 Data extraction form for Nicholls 2016 (Study ID – N4)

General information	Study ID	Nicholls et al 2016 (N4)
	Date template completed	22/06/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Nicholls C., Parsonson F., Gray L., Heyer A., Donohue S., Wiseman G., and Norton R. Narrative Review. Peer Reviewed. Australia. Townsville Hospital (Western QLD).
Study characteristics	Aim/objectives of study	Report of PAM fatality.
	Study type/design	Narrative Report.
	Study duration	NA
	Type of water source/water body	Domestic water.
Population characteristics	Population/s studied	Female 18-months old, Male 12-months old.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	2
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Domestic Water (non-scheme, chlorine or filtration) used for play as well as bathing. Bathing or Garden hose/toys potential but no swimming. Geothermal bore water (60C), cooled in open surface dams and piped into house (Water Temps kept high).
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Water Temperature (60C). Water clarity and taste (subjective) CDC-method for PCR detection of *Naegleria fowleri* from CSF.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Microscopy of CSF-ID of Naegleria-like species followed by PCR. Outcome was death.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Results-Potential elevated risk of *Naegleria fowleri* in rural properties with non-scheme water lacking treatment. Avoid putting water up nose in any manner. Bore water or hot temps should be considered ideal environments for *Naegleria fowleri*. Difficulty in accurate diagnosis.

Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include with cavate of unknown water conditions. Link only to mortality/outcome for exposure.

6.2.26 Phu 2013 (Study ID – N10)

Table 6.59 Data extraction form for Phu 2013 (Study ID – N10)

General information	Study ID	Phu N. et al 2013 (N10)
	Date template completed	22/03/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Nguyen Hoan Phu, Nguyen Thi Hoang Mai, Ho Dang Trung Nghia, Tran Thi Hong Chau, Pham Phu Loc, Le Hong Thai, Tran My Phuong, Cao Quang Thai , Dinh Nguyen Huy Man, Nguyen Van Vinh Chau, Tran Vu Thieu Nga, James Campbell, Stephen Baker, James Whitehorn. 2013 Case Report. Peer Reviewed. University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Case report of fatal *Naegleria fowleri* infection.
	Study type/design	Case Report.
	Study duration	July 2012
	Type of water source/water body	Pearl diving in Vietnam.
Population characteristics	Population/s studied	Male 25 years old.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Freshwater lake. Pearl diving. NA all others. No confirmed link to recreational water.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	*Naegleria fowleri* detection by microscopy in CSF and confirmed by PCR (CDC method). Listed medical treatment steps along with Amphotericin B and rifampicin (*Naegleria fowleri* drug treatment).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Naegleria fowleri* fatality.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA

Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	First reported case of *Naegleria fowleri* infection in Vietnam and linked to recreational water activity of pearl diving. *Naegleria fowleri* was not a transient organism and thrived in most hot springs.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. *Naegleria fowleri* death due to recreational activity. Gaps. No confirmation of *Naegleria fowleri* in recreational water and no water measurements.

6.2.27 Puzon 2017 (Study ID – N27)

Table 6.60 Data extraction form for Puzon 2017 (Study ID – N27)

General information	Study ID	Puzon 2017 (N27)
	Date template completed	9 July 2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Puzon G.J., Wylie J.T., Walsh T., Braun K., Morgan M.J. 2017. Journal Article. Peer reviewed. Australia. Water Corporation Australia and CSIRO Land and Water. None declared.
Study characteristics	Aim/objectives of study	To identify and compare the biofilm ecology conditions promoting free-living amoebae colonisation of biofilms in drinking water distribution systems (DWDS).
	Study type/design	Quantitative observational study of free-living organisms and biofilms in DWDSs
	Study duration	The biofilm monitors were connected and operational at each site for >1 year before samples were collected. Biofilm samples were collected in May 2010.
	Type of water source/water body	DWDS pipelines at five different locations in rural Western Australia at sites known to be colonised by amoebae, including *Naegleria*
Population characteristics	Population/s studied	No human populations. Viable amoeba, eukaryotic and bacterial communities of biofilms studied.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	Duplicate samples of biofilms collected from 5 different sites
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s)	Treated drinking water in DWDS at five different locations in rural WA. NA NA

	Comparison group(s)	Identifying amoeba, eukaryotic and bacterial communities in biofilms. NA Biofilm samples collected from sites with different water conditions for comparison. No biofilm controls.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Measured at time of sampling: chlorine residuals, temperature, turbidity. Measured: total microbial counts and viable amoeba species. Total microbial cell concentrations of biofilm were enumerated using a previously published method (Miller *et al.* 2015) on a Quanta flow cytometer. Viable amoeba detection was conducted on all individual samples using methods described previously (Puzon *et al.* 2009; Miller *et al.* 2015) using qPCR assays. Water sampling methods undertaken using standard equipment and protocols.
Results (for each outcome)	Definition of outcome (NA to human health outcomes) How outcome was assessed Method of measurement Number participants Water quality results Biofilm analysis	Viable amoeba detection compared with bacterial community in biofilm and water conditions at site. Density and identification compared to water conditions and eukaryote and bacterial community diversity, composition and structure. As per study methods above. NA Disinfectant residuals at all sites below effective levels for *Naegleria* disinfection (0–0.12 mg/L chlorine/monochloramine). Water temperature elevated (>20◦C). Turbidity between 0.5 and >2.0 NTU. All biofilm samples were positive for viable amoeba: *Naegleria fowleri* (2 sites), *Naegleria lovaniensis* (2 sites) and *Vermamoeba* (1 site). Biofilm total microbial cells counts in the range of 105–107 cells/cm^2. Results showed that eukaryote communities in *Naegleria*-positive biofilms are neither richer nor more phylogenetically diverse than *Naegleria*-free biofilms, but harbour phylogenetically distinct and potentially diagnostic higher taxa.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Statistical methods used in this study: • one-way analysis of variance (ANOVA) • *post hoc* Tukey's honest significant difference test

		• abundance weighted phylogeny-based weighted Unifrac metric • Principal coordinates analysis (PCoA) of weighted unifrac distance matrices, • permutational multivariate analysis of variance (PERMANOVA) • linear discriminant analysis (LDA) Effect Size Method implemented in LEfSe.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Key finding is that eukaryote community composition of biofilms supporting *Naegleria* is highly distinctive at the sample. locations, and is associated with specific amoeba detected. in these DWDS biofilms samples. Study has confirmed the identity of potential indicator taxa for the ecological conditions under which *Naegleria fowleri* can proliferate. Study acknowledges areas of uncertainty regarding generality and reliability of the diversity and community composition patterns correlating to occurrence of *Naegleria* and the need to test if any are preferential food sources.
Reviewer comments	Results included/excluded in review. Notes on study quality e.g. gaps, methods.	Good quality study that will be relevant to secondary research question on potential indicator/surrogates. Need to consider the applicability to recreational water scenarios, water conditions and indicators for *Naegleria*.

6.2.28 Sifuentes 2014 (Study ID – N42)

Table 6.61 Data extraction form for Sifuentes 2014 (Study ID – N42)

General information	Study ID	Sifuentes et al 2014 (N42).
	Date template completed	19/12/2023
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Laura Y. Sifuentes, Brittany L. Choate, Charles P. Gerba and Kelly R. Bright. 2014. Observational study. Peer Reviewed. University of Arizona, Tucson, Arizona, USA. Funded by University of Arizona's Technology and Research Initiative Fund. No conflict of interest statement provided.

Study characteristics	Aim/objectives of study	Detection of *Naegleria fowleri* in recreational waters in Arizona.
	Study type/design	Research.
	Study duration	N/A
	Type of water source/water body	Recreational waters (creeks, rivers and lakes) in Arizona.
Population characteristics	Population/s studied	N/A
	Selection criteria for population	N/A
	Subgroups reported	N/A
	Size of study	33 recreational lakes sampled. 103 samples collected in total.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	33 recreational lakes. Two sites have been associated with previous *Naegleria fowleri* linked deaths. All sites known for recreational use. N/A to all others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Seasonal sampling of 33 lakes for a total of 103 samples. Each sample consisted of 3 x 1L grab samples (1L for total DNA, 1L Viability, 1L-Physical-chemical measurements). Transport on ice to laboratory. GPS coordinates taken for repeating the seasonal sampling. Molecular detection using nested PCR (referenced) and methods described in detail, along with positive and negative controls. Viability test methods listed. Water quality methods listed and referenced to Standard Methods.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Naegleria fowleri* not significantly associated with total coliforms or *Escherichia coli*. *Naegleria fowleri* occurrence appeared to be seasonal, with eight of 40 (20.0%) samples positive in the winter and spring combined, yet only 5 of 63 (7.9%) samples were positive in the summer and fall. In addition, 61.5% of those samples testing positive for *Naegleria fowleri* (8 of 13) were collected from waters with temperatures below 20°C.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Statistical methods listed. Pearson's test used to determine if any correlations (positive or negative) existed between any of the physical, chemical, or microbial water quality parameters and the presence of viable thermophilic amoebae and or *Naegleria fowleri*. Two-tailed Student's t-test was used to compare the results for individual parameters (e.g., the temperature and the level of heterotrophic

		bacteria) between different recreational bodies of water.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Seasonality was observed, with *Naegleria fowleri* and thermophilic amoebae (20% and 30%, respectively) being detected more often in the winter and spring combined than in the summer and fall combined (7.9% and 9.5%, respectively). The spring and fall both had an average temperature of 18°C, yet had different occurrence data (18.2% versus 5.9% for *Naegleria fowleri*, respectively; 27.3% versus 0% for viable amoebae, respectively). These results are in stark contrast to previous studies in which *Naegleria fowleri* has been found almost exclusively during warmer months. Over the two-year study, *Naegleria fowleri* was detected in six and thermophilic amoebae in eight of the 33 recreational water bodies. Five of these were lakes near Phoenix that tested positive for *Naegleria fowleri* and thermophilic amoebae over multiple seasons. These lakes differed significantly ($P \leq 0.05$) from the other 28 surface waters, with a lower average temperature in the spring, a higher temperature in the fall, a higher pH and turbidity in the summer, and a lower electro-conductivity in the spring. They also had lower *Escherichia coli* and heterotrophic bacteria levels during colder months.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Addresses multiple secondary questions. Recreational water detections of *Naegleria fowleri* seasonally at multiple lakes over 2 years. *Naegleria fowleri* found to be present seasonally, but with higher detections in the winter and spring. *Naegleria fowleri* not significantly associated with total coliforms and *E. coli.* Gap in sampling method was the transport on ice which could affect *Naegleria fowleri* presence.

6.2.29 Stowe 2017 (Study ID – N11)

Table 6.62 Data extraction form for Stowe 2017 (Study ID – N11)

General information	Study ID	Stowe R.C. et al 2017 (N11)
	Date template completed	11 February 2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Stowe, R. C., D. Pehlivan, K. E. Friederich, M. A. Lopez, S. M. DiCarlo and V. L. Boerwinkle. 08 February 2017. Journal article. Peer reviewed. USA. NA NA
Study characteristics	Aim/objectives of study	Report 2 fatal paediatric PAM cases and compare their findings with 13 previously reported PAM survivors.
	Study type/design	Case report.
	Study duration	NA
	Type of water source/water body	Lake.
Population characteristics	Population/s studied	4-year-old male and 14-year-old male.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	Individual samples from each patient and historical treatment data.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s) Comparison group(s)	Lake. Swimming. NA NA *Naegleria fowleri.* NA
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Other methods used: Water sampling methods (monitoring, surrogates)	NA Wet mount of CSF and post-mortem. PCR. NA
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	2 patients diagnosed with PAM where treatment was not successful. *Naegleria fowleri* detected in wet mounts of patient CSF. Observation. 2 current patients and 13 past patients with PAM. There is no clear distinction between the treatments of cases that survived versus cases that died.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Authors recommend that fulminant PAM be considered in the differential diagnosis of individuals with meningitis

		in the warmer months in endemic areas. They also recommend that a clear exposure history be taken in individuals with presumed bacterial meningitis. The study highlights the difficult in the early identification and treatment of PAM.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This case report should be included in the review to address the primary question. *Naegleria fowleri* was identified in the CSF of the 2 patients however both patients died despite medical intervention. The patients had swum in a freshwater lake 8 days prior to the presentation of symptoms. This indicates that the lakes are the likely cause of the infection. It would have been good to test the lakes for *Naegleria fowleri*.

6.2.30 Su 2013 (Study ID – N5)

Table 6.63 Data extraction form for Su 2013 (Study ID – N5)

General information	Study ID	Su M. Y. et al 2013 (N5)
	Date template completed	16 February 2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Su, M. Y., M. S. Lee, L. Y. Shyu, W. C. Lin, P. C. Hsiao, C. P. Wang, D. D. Ji, K. M. Chen and S. C. Lai April 2013 Journal article Peer reviewed. Taiwan NA NA
Study characteristics	Aim/objectives of study	Case report of a 75-year-old male who dies of PAM after pathing in a hot spring in Taiwan.
	Study type/design	Case report
	Study duration	NA
	Type of water source/water body	Hot spring
Population characteristics	Population/s studied	Single individual
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s) Comparison group(s)	Hot spring Bathing NA Presumed to be hot spring *Naegleria fowleri* NA
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Other methods used:	NA Microscopy of wet mounts of patient CSF

	Water sampling methods (monitoring, surrogates)	PCR and DNA sequencing (not specified which sequencing method used) NA
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Patient diagnosed with PAM from *Naegleria fowleri*. Microscopy of wet mounts of patient CSF as well as qPCR and DNA sequencing (not specified which sequencing method used). *Naegleria fowleri* detected in the hot spring that the individual bathed in however methodology was not included. NA 1
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Early diagnosis is essential in order to initiate appropriate therapy before amoebae do extensive damage. The presence of *Naegleria fowleri* in hot springs pose a threat to human health.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This article should be included in the review to answer the primary question. The patient was diagnosed with PAM after identification of *Naegleria fowleri* in the CSF. The patient had a history of bathing in a hot spring the week prior to symptoms and testing of the hot spring revealed it was positive for *Naegleria fowleri*. It would have been good to have more information about how the hot spring was tested for *Naegleria fowleri* as the article does not address this.

6.2.31 Vareechon 2019 (Study ID – N12)

Table 6.64 Data extraction form for Vareechon 2019 (Study ID – N12)

General information	Study ID	Vareechon et al 2019 (N12)
	Date template completed	22/06/2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Vareechon ., Tarro T., Polanco C., Anand V., Pannaraj P., Bard J. Brief Report. Peer Reviewed. USA. Children's Hospital Los Angeles/University of Southern California.
	Aim/objectives of study	Report of PAM fatality

Study characteristics	Study type/design	Narrative Report
	Study duration	NA
	Type of water source/water body	Hot Spring.
Population characteristics	Population/s studied	8-year old Male
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	1
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Swimming in hot spring 12-days before onset of symptoms.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA for Water conditions CDC-method for PCR detection of *Naegleria fowleri* from CSF.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Microscopy of CSF-ID of Naegleria-like species followed by PCR. Outcome was death.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Rapid and destructive features of *Naegleria fowleri*-PAM. Prompt ID of causative agent is paramount.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include with cavate of unknown water conditions. Link only to mortality/outcome for exposure.

6.2.32 Vargas-Zepeda 2005 (Study ID – N18)

Table 6.65 Data extraction form for Vargas-Zepeda 2005 (Study ID – N18)

General information	Study ID	Vargas-Zepeda J. et al 2005 (N18)
	Date template completed	15 February 2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Vargas-Zepeda, J., A. V. Gómez-Alcalá, J. A. Vásquez-Morales, L. Licea-Amaya, J. F. De Jonckheere and F. Lares-Villa22 October 22 October 2005 Journal article. Peer reviewed. Mexico. NA NA
Study characteristics	Aim/objectives of study	Early treatment of PAM case leading to complete recovery of patient.
	Study type/design	Case-report.
	Study duration	NA
	Type of water source/water body	Irrigation canal.
Population characteristics	Population/s studied	10-year-old boy.
	Selection criteria for population	NA

	Subgroups reported	NA
	Size of study	Single individual
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/ contamination Causal organism/chemical(s) Comparison group(s)	Irrigation canal. Swimming. NA Irrigation canal. *Naegleria fowleri.* NA
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Other methods used: Water sampling methods (monitoring, surrogates)	NA Microscopy of wet mounts of patient CSF. Growth of *Naegleria fowleri* on NNA - *E. coli* plates from patient CSF, flagellation tests, indirect immunofluorescence with LESS antibody, and genotype determined by PCR and sequencing of ITS. Another study had previously identified *Naegleria fowleri* in this irrigation canal.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Detection of *Naegleria fowleri* in patient CSF Microscopy of wet mounts of patient CSF, growth of *Naegleria fowleri* on NNA - *E. coli* plates from patient CSF, flagellation tests, indirect immunofluorescence with LESS antibody, and genotype determined by PCR and sequencing of ITS. Successful treatment of PAM in patient with no sequelae. Early treatment by intravenous administration of amphotericin B and fluconazole, and oral administration of rifampicin. Single case
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA NA NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	The use of a triple drug treatment and the early start to the regime contributed to the patient's full recovery. The *Naegleria fowleri* genotype isolated belonged to a genotype that is commonly found in American PAM and thus recovery was not due to low pathogenicity of the *Naegleria fowleri*.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	This case report should be included in the review to address the primary question. It is believed that the patient

		was exposed to *Naegleria fowleri* while swimming in an irrigation canal and this is supported by a previous study which detected *Naegleria fowleri* in the canal. The study did a comprehensive examination of the *Naegleria fowleri* isolated from the patient and was able to attribute the genotype to similar PAM infections in America. More case report studies should include the genotyping of the patient isolated *Naegleria fowleri* to get a greater understanding of the pathogenicity and infectivity between genotypes found in recreational water.

6.2.33 Yu 2018 (Study ID – N28)

Table 6.66 Data extraction form for Yu 2018 (Study ID – N28)

General information	Study ID	Yu 2018 (N28)
	Date template completed	30 July 2021
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Yu Z., Miller H.C., Puzon G.J., Clowers B.H. 4 September 2018. Journal Article. Peer reviewed es Australia (Western Australia [WA]). CSIRO Land and Water (support from Water Corporation of WA). None declared.
Study characteristics	Aim/objectives of study	To apply a previous lab-based approach using untargeted metabolomics to detect pathogenic *Naegleria fowleri* in drinking water distribution systems (DWDSs).
	Study type/design	Quantitative observational study of metabolomics for detection of *Naegleria fowleri* in DWDS.
	Study duration	1 year (May 2014 to May 2015)
	Type of water source/water body	DWDS in rural WA. Field sites were selected based on a history of detection for *Naegleria fowleri* and *Naegleria lovaniensis*, as well as a low free chlorine residual (less than 0.1 mg/L).
Population characteristics	Population/s studied	No human populations. Viable amoeba and metabolites in DWDSs.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	28 samples collected from 4 different sites.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination	Treated drinking water from DWDS in rural WA. NA

	Causal organism/chemical(s) Comparison group(s)	NA Samples taken from sites known to have *Naegleria* infection. NA Biofilm samples collected from sites with different water conditions for comparison.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Measured at time of sampling: free and total chlorine or chloramine residuals, temperature, turbidity Measured: total cell counts and viable amoeba using methods previously described (Puzon et al. 2009), with standard curves generated from pure cultures of *Naegleria fowleri* or *Naegleria lovaniensis*. Metabolite separations, mass analysis, and tandem mass spectrometry were conducted using a liquid chromatography quadrupole time of flight system. Water sampling methods undertaken using standard equipment and protocols.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants Biofilm analysis	Metabolite measurements confirm positive or negative for viable amoeba from samples taken from DWDS. Metabolite measurements from field samples compared to metabolome of lab-cultured samples of *Naegleria fowleri* or *Naegleria lovaniensis*. As per study methods above. NA. Biofilm samples from each site were positive for viable amoeba with seasonal variability: *Naegleria fowleri* (3 sites), *Naegleria lovaniensis* (1 site). Total *Naegleria* cells in viable samples 2 - >600 cells/cm^2. Viable amoebae were only detected when the disinfection residual was below 0.1 mg/L at all four sites. Analysis of the metabolite pools of the collected samples revealed that a total of 60 features are potentially able to discriminate the samples collected from *Naegleria fowleri* positive sites from those coming from *Naegleria fowleri* negative or *Naegleria lovaniensis* positive field sites. A total of 10 common features were found when comparing the 60 significant features found in the current field study with the diagnostic metabolites reported in prior lab-cultured study.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Statistical analysis performed on raw mass spectrometry data. Further statistical analysis performed on

		significant features identified from experimental data when comparing and matching to metabolome databank of lab-cultured samples. Additional details on methods used for statistical analysis provided in Methods section.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	10 diagnostic features have a strong potential to separate *Naegleria fowleri* positive samples from *Naegleria fowleri* negative and *Naegleria lovaniensis* positive. Authors note that further work is needed to: -expand the diagnostic metabolite pool to increase the prediction confidence and to lower the false positive/negative percentage of the prediction results -understand the full impact of environmental and ecological factors on the metabolite profile of *Naegleria fowleri* in DWDS biofilms-standardise and optimise the workflow ranging from sample collection, preparation, and data analysis.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Good quality study that will be relevant to secondary research question on potential indicator/surrogates. Need to consider the applicability of this study to recreational water scenarios that are not chemically disinfected, and the studies implications to the water conditions and indicators for *Naegleria fowleri*.

6.3 Risk of Bias (RoB) Assessments for *Burkholderia pseudomallei*

6.3.1 Alvarez-Hernandez 2021 (Study ID – B1)

Table 6.67 Risk-of-bias assessment tool for Alvarez-Hernandez 2021 (Study ID – B1) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Alvarez-Hernand-2021 (B1) **Study Type: Case report**		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this is a case study of 2 patients who likely acquired their infection in recreational water**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	Yes	While the cases are compared to ones in other locations, no comparison group used as this is a case report of 2 fatalities from *Burkholderia pseudomallei*	--
	Confounding bias			
4.	Confounding (design/analysis)	Yes	This is a case report, outlining the clinical and environmental case for 2 patients, association with exposure to a known swimming hole is reported but limited to samples collected from the one site where exposure may have occurred and no other potential sources/pathways of exposure to the bacteria	-
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Comprehensive clinical data from the patients is presented. Results of bacterial culture of patient and environmental samples provided. Results of molecular testing of bacterial isolates provided.	+

	Detection Bias			
8.	Exposure characterisation - Environmental confirmation	Yes	Case history was taken and testing of environmental samples was performed to identify the possible source of infection. The case report is reporting on only 2 cases and environmental testing of one potential exposure site.	-
9.	Outcome assessment	No	The outcome was assessed post-mortem and with resulting environmental testing conducted based on case history. Laboratory testing was conducted using verified methods and international databases.	+
	Selective Reporting Bias			
10.	Outcome reporting	No	All outcome measures identified were reported in the report.	+
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	N/A		
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.2 Baker 2011 (Study ID – B3)

Table 6.68 Risk-of-bias assessment tool for Baker 2011 (Study ID – B3) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Baker 2011 (B3)	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this study is on the presence of *Burkholderia pseudomallei* in natural ground water seeps**	**Risk of bias rating** **(++/+/-/--)**
Study Type: Epidemiological and environmental study (observational)			

Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	Yes	Control soil sampling was conducted in an area where 267 samples had previously tested negative for *Burkholderia pseudomallei* to determine the sensitivity of the molecular assay. Control samples were not collected in parallel to the testing being conducted by this study.	-
	Confounding bias			
4.	Confounding (design/analysis)	Yes	Testing was conducted on soil and water samples to try and determine the source of the bacteria. Testing was conducted on the soil during wet and dry seasons to help determine if the bacteria is present in the soil or the water. Genomic linking of samples with clinical isolates doesn't account for potential exposure of patients to other sources of *Burkholderia pseudomallei*. Researchers state that further work is needed to determine if the bacteria is surviving in the soil or water sources in the area.	-
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Results are presented and confidence intervals are reported for testing.	+
	Detection Bias			
8.	Exposure characterisation	Yes	Genotyping of environmental isolates of *Burkholderia pseudomallei* were compared with isolates from patients in the local hospital. Of the isolated 8 were directly matched to patient samples. Paper concludes that *Burkholderia pseudomallei* is present in groundwater seeps and that due to molecular matching it may contribute to the case cluster in the area.	-
9.	Outcome assessment - Controls	No	Testing was conducted using verified laboratory methods and test controls. Sensitivity testing was conducted to determine the lowest level of detection of the molecular assay.	+

	Selective Reporting Bias			
10.	Outcome reporting	No	Testing results reported demonstrate the presence of *Burkholderia pseudomallei* in natural ground water seeps and matched to several clinical isolates from the area.	+
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	N/A		
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.3 Baker 2016 (Study ID – B5)

Table 6.69 Risk-of-bias assessment tool for Baker 2016 (Study ID – B5) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Baker 2016 (B5) **Study Type: Environmental study (observational)**		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this study is about the presence of *Burkholderia pseudomallei* in groundwater discharge**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	**Randomization**	**N/A**	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	**Allocation concealment**	**N/A**	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	Yes	No testing of comparison sites known to have shown negative results was conducted in this research. Sampling was undertaken after a single heavy rainfall event and did not compare other events or seasonal conditions.	--

	Confounding bias			
4.	Confounding (design/analysis)	Yes	Testing was conducted at one time point only after a heavy rain event. Testing was conducted on water samples only and did not include soil or sediments that can also contribute to exposure in humans.	-
	Performance Bias			
5.	**Identical experimental conditions**	**N/A**	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	**Blinding of researchers during study?**	**N/A**	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Culture and DNA testing results were presented in the paper.	+
	Detection Bias			
8.	Exposure characterisation	Yes	Researchers conclude that the link between environmental contamination after heavy rainfall and the increased contamination of local waterways with *Burkholderia pseudomallei* needs further research. Researchers highlight that public health warnings should be considered after heavy rainfall events, while the study was mostly unsuccessful in recovering viable organisms from the samples. The link between viable organisms and heavy rainfall events needs to be established.	-
9.	Outcome assessment	Yes	Duplicate samples were collected and tested. Laboratory testing was conducted using verified methods. Controls were tested in duplicate for DNA testing. Viable bacteria were only detected in one sample. Further testing needs to be conducted to determine the significance of the detection of the bacteria in water samples and its effect on public health.	-
	Selective Reporting Bias			
10.	Outcome reporting	Yes	Bacteria detected via DNA testing in water samples, bacterial culture only isolated the organism in one sample. Researchers concluded that this shows the improved sensitivity of DNA testing methods. Researchers highlight that there may be a risk of ground water seeps draining into local waterways after heavy rainfall and the possible health implications to at risk individuals. The evidence on the extent of this seepage or the risk it poses to the public was not demonstrated by the researchers and more research is required in this area.	-
	Other Sources of Bias			

11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	N/A		
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.4 Draper 2010 (Study ID – B6)

Table 6.70 Risk-of-bias assessment tool for Draper 2010 (Study ID – B6) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Draper 2010 (B6) **Study Type: Environmental study (observational)**		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this study is related to the presence of *Burkholderia pseudomallei* in bores**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	Yes	Dry season testing was conducted on 47 bores with no controls. Samples were collected from a variety of different aquifer sources, some for comparison in dry and wet seasons, with multiple samples collected from each bore to reflect the different sections of the bore system. Wet season testing was conducted on the 12 bores that tested positive during the dry season and then on 14 matched bores (matched by aquifer type and location) that tested negative during the dry season.	-
	Confounding bias			
4.	Confounding (design/analysis)	Yes	Environmental and physical water characteristics were used to determine if there was an association with certain characteristics and the presence of *Burkholderia pseudomallei*.	-

			Long term assessment of the bores was not conducted to determine if the characteristics favourable to *Burkholderia pseudomallei* resulted in long term isolation from the bore or if isolation was sporadic or a result of weather changes.	
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	Yes	Median and summarised results presented for water characteristics across positive and negative bores shown by wet and dry season.	-
	Detection Bias			
8.	Exposure characterisation	Yes	Study is looking at the impact of bore water characteristics on the presence of *Burkholderia pseudomallei* and the potential of these to be used as indicators. Further work is needed to determine if the water characteristics identified are essential to the presence of *Burkholderia pseudomallei* in the bores.	-
9.	Outcome assessment	Yes	Sample collection and testing was conducted using verified methods. Bacterial isolates were compared to isolated identified in soil and clinical samples with a 50-km radius. Further research is needed to determine if changes to the water characteristics could impact the presence of *Burkholderia pseudomallei* in the water sources.	-
	Selective Reporting Bias			
10.	Outcome reporting	Yes	Water characteristics and bacterial isolation was analysed to determine associations. Associations determined by the study were compared to other research showing similar associations. Samples were only collected at 2 time-points, ongoing sampling is needed to determine if water characteristics are associated with the isolation of *Burkholderia pseudomallei*.	-
	Other Sources of Bias			

11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	N/A		
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.5 Inglis 2004 (Study ID – B4)

Table 6.71 Risk-of-bias assessment tool for Inglis 2004 (Study ID – B4) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Inglis 2004 (B4) **Study Type: Environmental surveillance (observational)**		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this study is related to the presence of *Burkholderia pseudomallei* in drinking water and soil.**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	No	Environmental testing was conducted in communities of confirmed culture positive cases of *Burkholderia pseudomallei*. Control locations were chosen from surrounding communities and were sampled in quick succession to allow comparison. Samples were collected from a range of sources at each site including potable water, surface and rhizosphere soil.	**+**
	Confounding bias			
4.	Confounding (design/analysis)		Dry weather conditions could have resulted in lower than average culture-confirmed cases.	-
	Performance Bias			

5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Detailed results from testing and location of positive results is provided in the paper.	+
	Detection Bias			
8.	Exposure characterisation	Yes	Comparison of clinical and environmental isolates was used to show the possibility that the water was a source of the infection. The study does not allow the distinction between exposure from soil or water in the cases linked to positive environmental cultures. The study was unable to determine if soil or water were the source of original contamination or if both were contaminated with bacteria at the same time. A structured prospective study has been established to follow up on the outbreak investigations.	-
9.	Outcome assessment	No	Testing was conducted by 3 different centres which had different methods. A proportion of samples from NT and Qld were collected in duplicate for analysis by the WA centre to assess consistency of results. Testing was conducted using verified methods.	+
	Selective Reporting Bias			
10.	Outcome reporting	Yes	In the ongoing study, measures are being taken to ensure that conventional water quality data and geological factors can be taken into account and used for understanding the ecology and distribution of *Burkholderia pseudomallei*.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol	N/A		
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.6 Kaestli 2016 (Study ID – B9)

Table 6.72 Risk-of-bias assessment tool for Kaestli 2016 (Study ID – B9) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Kaestli 2016 (B9) Study Type: Case series		RoB: Yes/No Unknown N/A	Notes	Risk of bias rating (++/+/-/--)
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	N/A	Not applicable to case series	
	Confounding bias			
4.	Confounding (design/analysis)	Yes	The paper is correlating weather patterns to case presentations, patient activity leading to exposure during these times is not considered. The paper notes that while a radius of 10km was considered, there could have been variation in weather within this area.	--
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	N/A	Not applicable to case series	
	Detection Bias			
8.	Exposure characterisation	Yes	This paper works at developing the hypothesis that weather conditions and groundwater fluctuations are related to infection but the paper notes that there is uncertainty about the source of infection.	--
9.	Outcome assessment	Yes	The paper notes that while the modelling can reasonably predict infection, it did not account for all infection, suggesting that there are other factors involved.	-

	Selective Reporting Bias			
10.	Outcome reporting	Yes	Results from the predictive models have been reported on, noting that they have not accounted for all variables.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol			
	Overall risk of bias rating:			--

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.7 Kaestli 2019 (Study ID – B7)

Table 6.73 Risk-of-bias assessment tool for Kaestli 2019 (Study ID – B7) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Kaestli 2019 (B7) **Study Type: Scoping study (observational)**	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – note this paper is on the presence of pathogens in drinking water**	**Risk of bias rating** **(++/+/-/--)**
Q			
	Selection bias		
1. Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2. Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	

3.	Comparison groups appropriate	Yes	This study is looking at a snapshot of microbiota in three drinking water supplies in remote communities. No control group is used; however, the authors compare different natural iron levels (high, medium, low). The five chosen sampling sites at each community were also selected to represent different parts of each water system.	--
	Confounding bias			
4.	Confounding (design/analysis)	Yes	This is a descriptive study, conducted as a snapshot, longer term data is required to confirm results. The study design also measured a number of water quality parameters to examine impacts on microbiota.	-
	Performance Bias			
5.	**Identical experimental conditions**	**N/A**	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	**Blinding of researchers during study?**	**N/A**	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Data from chemical and microbiological assessment has been presented	+
	Detection Bias			
8.	Exposure characterisation	Yes	This is a descriptive study, conducted to assess the microbial levels and potential water quality indicators in the water sources to inform future studies to improve management guidelines. The paper does not address the risks of exposure.	-
9.	Outcome assessment	Yes	This paper does not address health outcomes.	-
	Selective Reporting Bias			
10.	Outcome reporting	Yes	This paper reports on chemical and microbial data that can be used to try and draw associations between water quality parameters and microbiota in water samples and biofilm. It does not report on health outcomes.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol			
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.8 Knappik 2015 (Study ID – B8)

Table 6.74 Risk-of-bias assessment tool for Knappik 2015 (Study ID – B8) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Knappik 2015 (B8) **Study Type: Methods evaluation (observational)**		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this paper evaluates laboratory methods to improve detection of *Burkholderia pseudomallei* in surface water and soil.**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate	Yes	Comparison of methods is undertaken in the study. The use of controls in the comparison is not clear in any of the methods compared. Comparison groups include multiple samples of soil and water taken at different points/depths of the chosen locations.	-
	Confounding bias			
4.	Confounding (design/analysis)	No	Collection and testing methods were consistent across samples and the collection methods aimed to minimised cross-contamination that might lead to confounding across sample points.	+
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Results from testing has been presented.	+
	Detection Bias			
8.	Exposure characterisation	No	The methods use for testing are verified laboratory methods.	+

9.	Outcome assessment	Yes	The paper notes that the variation in results in the soil samples, may be related to an uneven distribution of the organism in the samples.	-
	Selective Reporting Bias			
10.	Outcome reporting	Yes	The paper notes that the inclusion of extraction controls should be considered for future research to increase the quality of the data.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol			
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.9 Liu 2015 (Study ID – B10)

Table 6.75 Risk-of-bias assessment tool for Liu 2015 (Study ID – B10) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT, 2019))

Study ID: Liu 2015 (B10) **Study Type: Epidemiological study (observational)**	**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – this study looks at the impact of rainfall and humidity on exposure to *Burkholderia pseudomallei*.**	**Risk of bias rating** **(++/+/-/--)**
Q			
	Selection bias		
1. Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2. Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	

3.	Comparison groups appropriate	Yes	This study used modelling to assess the association between melioidosis cases and weather factors. No comparison groups were used.	--
	Confounding bias			
4.	Confounding (design/analysis)	Yes	The study identified that many participants did not have exposure to soil and that water may be the vehicle of transmission.	-
	Performance Bias			
5.	**Identical experimental conditions**	**N/A**	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	**Blinding of researchers during study?**	**N/A**	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	No	Results of weather analysis are reported.	+
	Detection Bias			
8.	Exposure characterisation	Yes	Techniques to analyse weather patterns are validated. Testing of water and soil samples was not undertaken to confirm the source of infections.	-
9.	Outcome assessment	Yes	Modelling showed association between rainfall and humidity with the number of cases. This paper does not undertake testing of water or soil samples to identify the presence of *Burkholderia pseudomallei*.	-
	Selective Reporting Bias			
10.	Outcome reporting	Yes	The paper draws on another case control study to conclude that exposure to rain and water inhalation could be considered potential risk factors. This result is based on association and further research to determine the source of infection is needed.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol			
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.3.10 Shariff 2020 (Study ID – B2)

Table 6.76 Risk-of-bias assessment tool for Shariff 2020 (Study ID – B2) adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT,2019))

Study ID: Shariff 2020 (B2) **Study Type: Case series**		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes – case series of 3 patients with ocular *Burkholderia pseudomallei*.**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Case series	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Case series	
3.	Comparison groups appropriate	N/A	Not applicable to case series.	
	Confounding bias			
4.	Confounding (design/analysis)	Yes	This is a retrospective case series, outlining the cases of 3 patients with ocular *Burkholderia pseudomallei*. Diagnosis was based on clinical presentation and positive serology results following various diagnostic tests to rule out other causes. The authors note that cases of ocular melioidosis are often initially misdiagnosed, which would be due to confounders.	-
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			
7.	Missing outcome data	N/A	No applicable to case series.	-
	Detection Bias			
8.	Exposure characterisation	Yes	Diagnosis of *Burkholderia pseudomallei* is based on clinical presentation and positive serology results. Determination of the source of infection is only hypothesised in one of the cases (swimming in a river).	-

9.	Outcome assessment	Yes	These cases due to the nature of the infection did not confirm the diagnosis in culture. Serology results presented provide the diagnosis. Treatment provided vary in the 3 cases and clinical outcomes are reported to different degrees across the cases.	-
	Selective Reporting Bias			
10.	Outcome reporting	Yes	Due to the retrospective nature of the study, the outcomes reported vary across the 3 cases.	-
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol			
	Overall risk of bias rating:			-

Key: Risk of bias rating

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

6.4 Data extraction forms for *Burkholderia pseudomallei*

6.4.1 Alvarez-Hernandez 2021 (Study ID – B1)

Table 6.77 Data extraction form for Alvarez-Hernandez 2021 (Study ID – B1)

General information	Study ID	Alvarez-Hernandez G. et al 2021 (B1)
	Date template completed	08/06/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Gerardo Alvarez-Hernandez, Denica Cruz-Loustaunau, J. Antonio Ibarra, Adela Rascon-Alcantar, Jesús Contreras-Soto, Georgina Meza-Radilla, Alfredo G. Torres and Paulina Estrada-de los Santos. 2021 Case Report. Peer Reviewed. Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas. Mexico. No conflict of interest.
Study characteristics	Aim/objectives of study	Case report of fatal melioidosis in two Mexican children.
	Study type/design	Case Report.
	Study duration	September 2018.
	Type of water source/water body	
Population characteristics	Population/s studied	Children with fatal cases.
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	2
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Natural pool of rainwater. Swimming in water. None listed. Water. *Burkholderia pseudomallei.* None. Confirmed *Burkholderia pseudomallei* in recreational water.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	No water quality measurements taken. Environmental samples taken. Soil (100g) and surface water (50ml) Culturing of organisms-Ashdown medium plates 37C for 3 days. DNA isolation from colonies, 16S gene amplification and DNA sequencing. 99.93% sequence similarity to *Burkholderia pseudomallei*.

Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Death of patients. Both patients presented with intense headache, fever, abdominal and chest pain, nausea, and diarrhea. Non-specific medication was prescribed in addition to rest at home. Readmitted to hospital with severe leukopenia, respiratory distress syndrome and septic shock. Drug treatment attempted. Death 7h after hospital admission. Postmortem detection-A single colony morphology microorganism was preferentially isolated and identified with the VITE K2 System. The results identified the microorganisms HLCR2, HLCR3 and HLCR7 as *Burkholderia pseudomallei*, the causative agent of melioidosis. BOX PCR analysis of environmental and clinical samples gave identical results indicating same clonal group.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	First case with evidence of *Burkholderia pseudomallei* in human and environmental samples. Mexico should be considered as an endemic region for *Burkholderia pseudomallei.*
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Direct link of *Burkholderia pseudomallei* in both environmental and clinical samples, resulting in infection and fatality. Study did not provide details of how the molecular method (BOX PCR) was done, only referenced a manuscript.

6.4.2 Baker 2011 (Study ID – B3)

Table 6.78 Data extraction form for Baker 2011 (Study ID – B3)

General information	Study ID	Baker et al 2011 (B3)
	Date template completed	09/06/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Anthony Baker, Donald Tahani, Christopher Gardiner, Keith L. Bristow, Andrew R. Greenhill, and Jeffrey Warner. 2011. Research paper. Peer Reviewed. Australia. Environmental and Public Health Microbiology Research Group, School of Veterinary and Biomedical Sciences, James Cook University, Townsville, Queensland, CSIRO Land and Water, Townsville, Australia, PNG

		Institute of Medical Research, Goroka, Papua New Guinea No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	To determine the extent of *Burkholderia pseudomallei* in seasonal groundwater seeps (soil and groundwater).
	Study type/design	Research paper.
	Study duration	Early March 2010.
	Type of water source/water body	Natural groundwater seeps and soil post intense rainfall event.
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	40 soil and 40 groundwater samples at Castle Hill (early March 2010), plus 16 residential samples (late March 2010). 40 soil samples retaken during dry season (August 2010).
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Groundwater seeps around Castle Hill (Townsville, QLD). Samples were also taken from groundwater connected tributaries in residential areas in late March (post intense rainfall) and dry season soil samples collected. NA to all others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	No water quality conditions listed. Only noted seasonality, weather event and soil moisture content. Pre-enrichment of samples on Ashdown isolation media (broth and plates) (methods listed). DNA extracted from plate or neat samples and use in probe based qPCR for molecular detection (methods listed) then applied. Molecular epidemiology via BOX-PCR and multi-locus sequence typing (MLST) used to compare *Burkholderia pseudomallei* isolates (methods listed).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	*Burkholderia pseudomallei* DNA was detected by qPCR in 7 of 40 (17.5%) of the soil samples collected during the dry season, 26 of 40 (65%) of the soil samples collected during the wet season, and 37 of 40 (92.5%) of the water samples from seasonal groundwater seeps at the base of Castle Hill (Fig. 1). Analysis with Fisher's exact test calculated a significant difference between all three proportions (P _ 0.005), while the independent t test determined that mean soil water content between seasons was significantly different between the wet season and the dry season.

		Burkholderia pseudomallei DNA was detected in 14 of 16 (88.2% [95% CI, 72.9 to 100]) of the roadside water samples collected from Castle Hill. BOX-PCR and MLST match 8 environmental isolates (groundwater runoff adjacent to residential properties) to clinical isolates from Townsville hospital patients.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Statistical comparison of *Burkholderia pseudomallei* prevalences was performed by OpenEpi software using Fisher's exact test. Confidence intervals included in text.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Comparison of isolates using multi-locus sequence typing demonstrated clinical matches and close associations between environmental isolates and isolates derived from clinical samples from patients in Townsville. The study demonstrated that waterborne *Burkholderia pseudomallei* from groundwater seeps around Castle Hill may facilitate exposure to *Burkholderia pseudomallei* and contribute to the clinical clustering at this site.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Australian study. Linking of *Burkholderia pseudomallei* in environmental systems to clinical isolates. Higher detection rate in soil in wet, versus dry season. However, no recreational activity included or compared and no physical or chemical measurements recorded.

6.4.3 Baker 2016 (Study ID – B5)

Table 6.79 Data extraction form for Baker 2016 (Study ID – B5)

General information	Study ID	Baker and Warner 2016 (B5)
	Date template completed	09/06/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Anthony L. Baker & Jeffrey M. Warner 2016 Research paper. Peer Reviewed. Environmental and Public Health Microbiology Research Group, School of Veterinary and Biomedical Sciences, James Cook University,

		Townsville, Queensland, Australia Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Tasmania, Australia. No conflict of interest.
Study characteristics	Aim/objectives of study	To determine the extent of *Burkholderia pseudomallei* in more diverse natural groundwater seeps in northern Queensland.
	Study type/design	Research paper.
	Study duration	3 days in January 2013.
	Type of water source/water body	Natural groundwater seeps post intense rainfall event.
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	26 groundwater samples.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Groundwater seeps in Townsville, QLD region. NA to all others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Environmental samples (26 samples in total) collected during monsoon season following an intense rainfall event. Pre-enrichment of samples on Ashdown isolation media (methods listed). DNA extracted and use in probe based qPCR for molecular detection (methods listed) then applied. Viable methods on Ashdown agar from pre-enriched samples and non-enriched samples.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Detection of *Burkholderia pseudomallei* in 18 of 26 samples (69.2%) using qPCR. Detection of *Burkholderia pseudomallei* in 1 of 26 samples (3.8%) using viable culture methods.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Sensitivity of molecular techniques far exceeds culture-based detection

		methods for *Burkholderia pseudomallei.* The study suggests that a higher incidence of melioidosis following monsoonal rains may be partially the result of exposure to groundwater sources carrying *Burkholderia pseudomallei.* Many of the studied groundwater seeps flow into major rivers and recreational swimming holes. Study findings indicate that predictive models of melioidosis risk should include an element of topography and surface hydrology.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Australian study detecting *Burkholderia pseudomallei* in natural water post intense rain events. Improved method of detection. However, no comparison to pre-rain presence or concentration of *Burkholderia pseudomallei*. No water conditions reported either.

6.4.4 Draper 2010 (Study ID – B6)

Table 6.80 Data extraction form for Draper 2010 (Study ID – B6)

General information	Study ID	Draper et al 2010 (B6)
	Date template completed	06/07/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	A. D. K. Draper, M. Mayo, G. Harrington, D. Karp, D. Yinfoo, L. Ward,1 A. Haslem, B. J. Currie, and M. Kaestli. 2010. Research paper. Peer Reviewed. Australia. Charles Darwin University, Department of Natural Resources, Environment and the Arts, Northern Territory Government. Funded in part by Australian National Health and Medical Research Council Project grant 383504 (to B.J.C. and M.M.), by the Swiss National Science Foundation (M.K.), and by project grant U01AI075568 from the National Institutes of Health. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Analysed water parameters and the occurrence of the melioidosis agent *Burkholderia pseudomallei* in 47water bores in Northern Australia.
	Study type/design	Research paper
	Study duration	Early March 2010

	Type of water source/water body	Bore water from Darwin properties.
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	188 samples from 47 properties (dry season 2008). Resampling event, 103 samples from 26 properties (wet season).
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Residential groundwater bores (Darwin, NT). NA to all others.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	1L water collected after 1 min, 30 min, 60 min pumping (represent bore head, shaft and aquifer). Water samples were tested for pH, temperature, and electroconductivity (Aqua-CP; TPS); total nitrates, total iron, and phosphates (HI3874, HI3834, and HI3833, respectively; Hanna Instruments, Australia); and total hardness (microtest TH 10; Aquaspex, Australia). Water samples were cultured for total coliform counts. Samples collected from tanks linked to bore. Water samples filtered (reference) and cultured in modified Ashdown broth and TSA plates (methods listed). DNA extracted from plate or neat samples and use in probe-based qPCR for molecular detection (no methods listed). Positive colonies confirmed as *Burkholderia pseudomallei* by latex agglutination and PCR typing (no methods listed).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Dry season, 12 of 47 bores (26%) tested positive for *Burkholderia pseudomallei*. Wet season resampling was 58% tested positive (lower total number tested). Analysis link some bore isolates to clinical isolates (method details not reported). No significant variation in bore characteristics was evident between *Burkholderia pseudomallei*-positive and -negative bores. Significant association was found between the occurrence of *Burkholderia pseudomallei* and more acidic water, low hardness, i.e., soft water and low salinity, higher

		coliforms, turbidity and strongly associated with high iron levels Water parameters in *Burkholderia pseudomallei*-positive bores were even more favourable for *Burkholderia pseudomallei* in the wet season.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Odds ratios (OR) for *Burkholderia pseudomallei*-positive bores were calculated using multivariable logistic regression clustered by bore and allowing standard errors for intragroup correlation and including season. The model was specified correctly as tested by a linktest. Mann-Whitney test unless otherwise noted. Odds ratio for interaction of pH and salinity. Fisher's exact test.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	*Burkholderia pseudomallei* was associated with soft, acidic bore water of low salinity but high iron levels. Results indicates that the occurrence of *Burkholderia pseudomallei* in bores is not only the result of an initial contamination event but also depends on water conditions favourable for *Burkholderia pseudomallei*.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include for sub-questions. Australian study. Link between abiotic factors and presence of *Burkholderia pseudomallei* in the environmental. However, no recreational activity, detection is growth based not molecular. Methods do not contain much detail so potential for bias.

6.4.5 Inglis 2004 (Study ID – B4)

Table 6.81 Data extraction form for Inglis 2004 (Study ID – B4)

General information	Study ID	Inglis et al 2004 (B4)
	Date template completed	21/12/2022

	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Inglis, T. J. J., N. F. Foster, D. Gal, K. Powell, M. Mayo, R. Norton and B. J. Currie. 2004. Research Article. Peer Reviewed. Western Australian Centre for Pathology and Medical Research, Western Australia, Department of Microbiology, The University of Western Australia, Western Australia, Menzies School of Health Research, Northern Territory, School of Medicine, James Cook University, Queensland, QHPS, Townsville Hospital, Queensland, Northern Territory Clinical School, Flinders University, Royal Darwin Hospital, Northern Territory. Australia. NHMRC project grant. No conflict of interest statement provided.
Study characteristics	Aim/objectives of study	Investigation of Water Supplies (WA, NT, QLD) as sources of *Burkholderia pseudomallei*.
	Study type/design	Detection in water supplies.
	Study duration	2001-2002.
	Type of water source/water body	Drinking water supplies.
Population characteristics	Population/s studied	WA, NT, QLD communities and adjacent locations (not listed) with one or more cases of melioidosis.
	Selection criteria for population	Areas of Melioidosis.
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Potable water, surface water and rhizosphere soil sampled. Potential exposure via direct contact, ingestions, inoculation or inhalation. Exposure to potable water. *Burkholderia pseudomallei.* NA. No link to recreational water.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Temperature, pH, residual chlorine and free-living amoeba (FLA) (partial method listed). Water samples (250 mL to 2L) taken. Pipes pre-sterilised (how?) and flushed for 5 min. Surface water sampling via submersion (10 cm) below surface then opened and filled. Multiple soil samples taken. Samples taken in duplicate. One set analysed at local lab and second set analysed in WA. but methods not reported. Culture (Ashdown media and BPSA media) with molecular methods to type the viable *Burkholderia pseudomallei.* Oxidase test, Gram

		staining, substrate utilization (API20NE strips) used as well as PCR and ribotyping.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	A total of 745 environmental samples were collected across northern Australia during the study period, 52% of which were water samples and 48% soil samples. Positive detection in creek (1), Bore (6), bore-water holding tank (2), irrigation pipe (2), bore filter (2), and bore-water head tap (2). FLA tests (315 total) gave positive results in WA (22%), 44 (NT%) and QLD (4%), with Hartmannella (60%) and Acanthamoeba (39%). Environmental detections in NT were closely associated with human, animal infections. No *Burkholderia pseudomallei* isolated from amoebic lysates. None of the positive water supplies were chlorinated.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Fisher's exact test of Duplicate sample results.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Results add to previous observations of water-supply contamination by *Burkholderia pseudomallei*. The number of water-related melioidosis cases were too small to allow useful analysis of water quality, hydrological and geological data. *Burkholderia pseudomallei* in a potable water specimen is uncommon and has potential public health significance. More work needs to be done.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include but only for basis of presence of *Burkholderia pseudomallei* in natural waters in Australia. All drinking water sources for exposure to *Burkholderia pseudomallei* was source of infection and no link to recreational waters. Study samples too small to correlate to the physical and chemical conditions, so no connections drawn.

6.4.6 Kaestli 2016 (Study ID – B9)

Table 6.82 Data extraction form for Kaestli 2016 (Study ID – B9)

General information	Study ID	Kaestli et al 2016 (B9)
	Date template completed	15/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Mirjam Kaestli, Eric P.M. Grist, Linda Ward, Audrey Hill, Mark Mayo , Bart J. Currie. 2016. Research Article. Peer Reviewed. Menzies School of Health Research, Charles Darwin University, PO Box 41096, Casuarina, NT 0811, Australia Centre for Clinical Vaccinology and Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom Australia. Funding provided, National Health and Medical Research Council of Australia (grant number 1046812). No conflict of interest.
Study characteristics	Aim/objectives of study	Analysis of weather and climate factors preceding new melioidosis cases in Darwin to generate a predictive model.
	Study type/design	Research study.
	Study duration	1990-2013.
	Type of water source/water body	surface water.
Population characteristics	Population/s studied	Urban Darwin area (136,200).
	Selection criteria for population	Potential Melioidosis.
	Subgroups reported	Positive Melioidosis (culture-confirmed).
	Size of study	383 positive cases.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA NA NA NA No link to recreational water activity.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA Microbiological as culture-positive statement, but no details provided. NA Daily weather observations from Darwin airport weather station (BOM) (data provided includes rainfall, mean dew point, humidity, mean cloud cover, Temperature (min & max), mean wind speed & direction, max gust and monthly relative sea surface temperature). Groundwater data from NT government at Darwin bore.

Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	429 patients – 35 chronic - 11reactivated = 383 cases for inclusion in study. Rise in the dew point, cloud cover, rainfall, maximum temperature and groundwater to be associated with an increased risk to acquire melioidosis. A shorter 'putative' incubation period was evident after severe rainfall events. Rainfall occurring early in the wet season was linked to more cases as was an increase in the local sea surface temperature reflecting local weather dynamics and precipitation. Positive association between annual total cases and mean cloud cover (Spearman's rho 0.66, P = 0.005) as well as mean annual groundwater levels although to a lesser degree (Spearman's rho 0.44, P = 0.033). For the monthly averaged data, a strong association was evident between cases and rainfall (Spearman's rho 0.89, P < 0.001) and cloud cover (Spearman's rho 0.94, P < 0.001). A negative binomial model structure on two weekly binned data was chosen to associate the melioidosis incidence rate with weather- and climate-related predictors and proved superior to a Poisson (P < 0.01) and zero-inflated negative binomial model (Vuong test, P Z 0.5).
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. Draftsman plots, nonmetric multidimensional scaling of normalized data using Stata/IC 14 and Primer-E7. Partial cross correlations (PXC) were obtained in MatLab (http://au.mathworks.com) ("partialcorr" function with Pearson correlations). Poisson-based boosted regression tree (BRT) was used to link preceding weather events with case admissions. BRT model was applied to two weekly binned data using the gbm library and additional BRT functions in R 3.0.0 (www.r-project.org). Data provided in supplementary materials.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	While an association between melioidosis and rainfall has long been established, to our knowledge this is the first study to report an association of the melioidosis incidence rate with cloud cover, the dew point and

		rainfall connected with high maximum temperature such as is encountered early in the wet season before the arrival of the monsoon. In addition, we showed a positive association exists between the case incidence rate and groundwater fluctuations. We also found that a surge in cases coincided with two strong La Nina events with unusually high sea surface temperature for tropical Australia.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. While no links to recreational water activity are provided, study is Australian based and contributes to the secondary questions providing potential environmental factors which contribute to the presence of *Burkholderia pseudomallei.*

6.4.7 Kaestli 2019 (Study ID – B7)

Table 6.83 Data extraction form for Kaestli 2019 (Study ID – B7)

General information	Study ID	Kaestli et al 2019 (B7)
	Date template completed	15/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Mirjam Kaestli, Michelle O'Donnell, Alea Rose, Jessica R. Webb, Mark Mayo, Bart J. Currie, Karen Gibb. 2019. Research Article. Peer Reviewed. Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, Northern Territory, Australia, Global and Tropical Health, Menzies School of Health Research, Darwin, Northern Territory, Australia, Power and Water Corporation, Darwin, Northern Territory, Australia Australia. Funding provided, by the Power and Water Corporation, Northern Territory, Australia and also supported by the Australian National Health and Medical Research Council through grants 1098337 (The Darwin Prospective Melioidosis Study) and 1131932 (The HOT NORTH initiative). No conflict of interest.
Study characteristics	Aim/objectives of study	Detection of *Burkholderia pseudomallei* in source-to-distribution drinking water systems in Northern Australia.
	Study type/design	Research study/Water & Biofilm.

	Study duration	NA
	Type of water source/water body	Groundwater and drinking water.
Population characteristics	Population/s studied	Water supplies rural Indigenous communities.
	Selection criteria for population	Communities had past cases of melioidosis.
	Subgroups reported	NA
	Size of study	3 community drinking water distribution systems.
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Groundwater and connect DWDS. NA NA NA No link to recreational water activity.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Sample collection March 2017 (2 sites) and May 2017 (1 site) from 5 points along the DWDS three were unchlorinated (bores and tanks) and two from the chlorinated reticulation system. Collected 1L for DNA extraction and 500mL for culturing of *Burkholderia pseudomallei*, 2x 200mL for heterotroph and amoebae culture, and 100 mL for elemental analysis. Bores purged 5 minutes prior to sample collection. Biofilm collected by sterile swab method. YSI meter used to measure pH, salinity, temperature, turbidity. oxidation reduction potential, and dissolved oxygen. Colorimeter used to measure free chlorine. Microbiological culturing in NATA accredited labs NT Government Dept. of Primary Industry and Resources laboratory and the Australian Water Quality Centre (AWQC) (AS/NZS methods listed, but no amoebae method listed). *Burkholderia pseudomallei* culturing on Ashdown broth followed by plating on Ashdown agar. Whole genome sequencing at AGRF for 6 *Burkholderia pseudomallei* isolates and analysis methods listed. Elemental and nutrient analysis listed but no methods provided for nutrients. DNA extraction and amplicon sequencing and sequencing analysis

		methods listed along with 5 DNA extraction negative controls.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	Water in HF community had highest level of metals, nutrients and salts, MF community lower and LF community lowest. pH decreased from HF>MF>LF. All samples negative for *E. coli*. HF and MF bore water and MF biofilm positive for *Burkholderia pseudomallei* (plus *P. aeruginosa* and *Hartmannella*). HF community had *P. aeruginosa*, *Hartmannella* and *Naegleria lovaniensis*. MF and LF had more bacterial sequences present than LF water. Samples clustered based on chlorination status. Significant association of *Burkholderia pseudomallei* culture positive samples with genus *Nitrospira*. *Burkholderia pseudomallei* detected in bores (HF and MF) with "scarce" heterotrophic growth (HPC).
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. Bray Curtis (Primer-E v7. PERMANOVA) used to test bacterial composition differences. multivariate dispersions (PermDISP), canonical analysis of principal coordinates (CAP), distance linear model and distance-based redundancy analysis (dbRDA),. Results was considered significant if $P<0.05$.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Increase HPC did not match presence *Burkholderia pseudomallei.* Nitrate producing *Nitrospiraceae* were associated with *Burkholderia pseudomallei* positive samples. Interesting as Chlorination successfully contained *Burkholderia pseudomallei* is a denitrifier under anaerobic conditions. Chlorination successfully contained *Burkholderia pseudomallei.* *Burkholderia pseudomallei* was cultured from a bore accessing a deeper aquifer and future investigations across seasons will determine whether *Burkholderia pseudomallei* indeed occurs in deeper confined aquifers or is mainly linked to surface or shallow aquifer water intrusions during the wet season.
Reviewer comments	Results included/excluded in review (if applicable)	Include. While no links to recreational water activity are provided, study is

	Notes on study quality e.g. gaps, methods	Australian based and contributes to the secondary questions providing potential physical (low nutrients) and biological (*Nitrospira* genus) factors associated with the presence of *Burkholderia pseudomallei.*

6.4.8 Knappik 2015 (Study ID – B8)

Table 6.84 Data extraction form for Knappik 2015 (Study ID – B8)

General information	Study ID	Knappik et al 2015 (B8)
	Date template completed	15/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Michael Knappik, David A. B. Dance, Sayaphet Rattanavong, Alain Pierret, Olivier Ribolzi, Viengmon Davong, Joy Silisouk, Manivanh Vongsouvath, Paul N. Newton, Sabine Dittrich. 2015. Research Article. Peer Reviewed. Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic; Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, England, United Kingdom; Institute of Ecology and Environmental Science—Paris, Institut de Recherche pour le Développement (IRD), Vientiane, Lao People's Democratic Republic; Géosciences Environnement Toulouse (GET), UMR 5563, IRD, Université de Toulouse, UPS (OMP), CNRS, Toulouse, France. Laos. Funding provided, Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit funded by the Wellcome Trust of Great Britain. Additional funding was provided by a seed award (Lee Ka Shing Foundation) of the University of Oxford (grant SM40) and the Institut de Recherche pour le Développement (IRD) through the regional pilot program Soils, Waters, Coastal Zones and Societies in Southern and Southeast Asia (SELTAR-RPP). No conflict of interest statement included.

Study characteristics	Aim/objectives of study	Compare molecular and culture-based detection of *Burkholderia pseudomallei* in soil and water.
	Study type/design	Research study.
	Study duration	NA
	Type of water source/water body	Soil and River water.
Population characteristics	Population/s studied	NA
	Selection criteria for population	NA
	Subgroups reported	NA
	Size of study	NA
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Soil and river water. NA NA NA No link to recreational water activity.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	Turbidity, temperature and pH recorded. Sterile collection of soil and water samples. Water samples = 20 x 600mL samples Water samples filtered 0.2 µm and 3.0 µm. Culture by Ashdown's agar. Preculture on Ashdown's broth (details listed). DNA extraction by PowerSoil DNA kit. qPCR target (TTS1 gene) and methods listed. Controls were included.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	qPCR highly specific for *Burkholderia pseudomallei* test strains, with local detection limit of 8 GE/µL. Culture based soil detection 44%, 6% direct soil qPCR, enrichment soil qPCR 84% positive. Culture based water detection 65%, enrichment 50%, direct water qPCR 55%, enriched water qPCR 75% positive.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. Statistical analysis was performed using Stata/IC (v10) software (StataCorp, College Station, TX, USA). Comparisons were made by the use of McNemar's test (paired samples) or the Mann-Whitney U test, as appropriate. Significance was set at a *P* value of <0.05.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	This report represents the first description of the use of molecular

		methods to detect *Burkholderia pseudomallei* in surface water samples. Water sampling campaigns might represent a promising alternative to large-scale soil sampling campaigns, for example, by using river water as an initial screen to determine whether *Burkholderia pseudomallei* is present in the relevant catchment area. molecular detection methods using an additional initial enrichment step have proven to be sensitive, specific, and reliable approaches for the detection of *Burkholderia pseudomallei* in environmental samples.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. While no links to recreational water activity are provided, study offers a detection method that may aid in managing the presence of *Burkholderia pseudomallei* (secondary question).

6.4.9 Liu 2015 (Study ID – B10)

Table 6.85 Data extraction form for Liu 2015 (Study ID – B10)

General information	Study ID	Liu et al 2015 (B10)
	Date template completed	16/12/2022
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Xiang Liu, Long Pang, Siew Hoon Sim, Kee Tai Goh, Sharada Ravikumar, Mar Soe Win, Gladys Tan, Alex Richard Cook, Dale Fisher, and Louis Yi Ann Chai. 2015. Research Article. Peer Reviewed. National University Health System University Medicine Cluster, Singapore; National University of Singapore and National University Health System Saw Swee Hock School of Public Health, Singapore; Defence Medical and Environmental Research Institute, Singapore; Ministry of Health, Singapore ; National University of Singapore Yale-NUS College, Singapore; and National University of Singapore Yong Loo Lin School of Medicine, Singapore. Singapore. No funding listed. No conflict of interest statement included.

Study characteristics	Aim/objectives of study	Association of Melioidosis incidence with rainfall and climate in an urban setting.
	Study type/design	Research Study.
	Study duration	2003-2012.
	Type of water source/water body	Soil and/or water.
Population characteristics	Population/s studied	Singapore.
	Selection criteria for population	Potential exposure.
	Subgroups reported	Positive Melioidosis cases.
	Size of study	550
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	NA NA NA NA No link to recreational water activity.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to water quality measurements. NA to microorganism isolation, enumeration and water sampling. Weekly case numbers provided by Ministry of Health, Singapore. Data on patient sex, age and race included. Monthly and weekly rainfall, humidity and temperature (Singapore Meteorological Service, Ministry of Environment and Water Resources, and Weather Underground website).
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	550 cases of melioidosis (range 31–96 cases per year). 84.1% of patients were male. 1.1 cases per 100,000 population mortality rate from the disease was 19.0%. Average total monthly rainfall for the period was 192.5 mm ± 121.6 mm (range 6.3–765.9 mm), and the average humidity and temperature were 83.7 mm ± 2.5% (range 77.3%–88.5%) and 27.7°C ± 0.7°C (range 26.3°C–29.2°C), respectively. Significant correlation between the number of melioidosis cases and the volume of rainfall in the 1-week period before disease onset, with a hazard ratio (HR) of 1.40 per 100 mm increase in rain (95% CI 1.03–1.90; p = 0.03) (Table 2). The humidity level 2 weeks before disease onset was more modestly associated with the number of cases (HR 1.03 per 1% increase in humidity, 95% CI 1.00–1.05; p = 0.04), but this value did not have an independent association beyond that of rainfall in multivariable analysis; rainfall and humidity shared a positive correlation at a 1-week lag interval (R = 0.45; p<0.001).

		No association between temperature and the number of melioidosis cases was found.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	Yes. quasi-Poisson distribution regression model, Wald tests, Statistical significance was set at $p<0.05$. Statistical analyses were performed by using R Statistical Software version 3.0.1.
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	We found a significant correlation of melioidosis cases in Singapore with higher rainfall totals and, to a lesser degree, to higher humidity levels. This finding indicates that water, rather than soil, may be the central vehicle for transmission and acquisition of this disease. most (82.0%) patients with melioidosis in Singapore did not report occupational or recreational exposure to soil. Our findings strengthen support for a possible link between melioidosis transmission and water by demonstrating a strong association between melioidosis case numbers and rainfall amounts 1 week before disease onset and humidity levels 2 weeks before disease onset.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. No links to recreational water activity are provided. However, the manuscript addresses the secondary question about conditions associated with the disease occurrence without soil interaction.

6.4.10 Shariff 2020 (Study ID – B2)

Table 6.86 Data extraction form for Shariff 2020 (Study ID – B2)

General information	Study ID	Shariff et al 2020 (B2)
	Date template completed	16/12/2022

	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	Saidatulakma Shariff, Muhammad Ikmal Mohamad Kamil, Wan Norliza Wan Muda, Akmal Haliza Zamli, Khairy Shamel Sonny Teo, Liza Sharmini Ahmad Tajudin. 2020. Case study. Peer Reviewed. Department of Ophthalmology, Hospital Tengku Ampuan Afzan, Jalan Air Puteh, Kuantan, Pahang, Malaysia Department of Ophthalmology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, Kota Bharu, Kelantan, Malaysia Department of Surgical Based Discipline, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. No funding listed. No conflict of interest.
Study characteristics	Aim/objectives of study	Ocular B. pseudo melioidosis infection.
	Study type/design	Case study
	Study duration	NA
	Type of water source/water body	Potential river water
Population characteristics	Population/s studied	Malaysian
	Selection criteria for population	Ocular infection
	Subgroups reported	males
	Size of study	3
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s) Confirmed link to Recreational Water	Case 1 linked to river water. Swimming. Eye infection. NA Potential link to recreational water activity, swimming, in a river.
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	NA to water quality measurements. NA to microorganism isolation, enumeration and water sampling. Study was a retrospective case series of patients with positive melioidosis serology. The patients presented to the Ophthalmology Clinic, Hospital Tengku Ampuan Afzan, Kuantan, Pahang, in 2018, and were diagnosed with ocular melioidosis. Patients' demographic data, clinical presentations, examination results, imaging findings, anterior segment photos, and fundus photos were analysed.
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	32-year-old male presented with a sudden onset of painless reduction of vision in his left eye. Two weeks prior to the onset of the symptoms, the patient went swimming in a river.

		Treated empirically with intravenous ceftazidime 1 g bid for 2 weeks followed by oral trimethoprim-sulfa-methoxazole (Bactrim) for another 6 weeks. 14-year-old male complained of painless decrease in vision in his left eye. Treated with intravenous ceftazidime and oral prednisolone were empirically commenced, and followed by oral Bactrim. 10-year-old boy presented with a sudden onset of painless blurring of vision in his left eye. Empirically treated with intravenous ceftazidime followed by oral azithromycin. All patients fully recovered.
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	NA
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	Ocular melioidosis comes with various presentations causing multiple incapacitating organ and ocular complications. Therefore, a high index of suspicion is required in order to initiate early and prompt treatment resulting in an excellent visual outcome.
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	Include. Potential link to recreational water activity for one of the cases. However, no description of *Burkholderia pseudomallei* isolation and identification. No water conditions or sampling recorded.

7 Excluded studies at full text screening

7.1 Reports

Table 7.1 List of excluded reports

Naegleria fowleri	
Citation	Reason for exclusion
Gracia, Daniel & Cope, Jennifer & Roberts, Virginia & Cikesh, Bryanna & Kahler, Amy & Vigar, Marissa & Hilborn, Elizabeth & Wade, Timothy & Backer, Lorraine & Montgomery, Susan & Secor, W & Hill, Vincent & Beach, Michael & Fullerton, Kathleen & Yoder, Jonathan & Hlavsa, Michele. (2018). Outbreaks Associated with Untreated Recreational Water - United States, 2000-2014. MMWR. Morbidity and mortality weekly report. 67. 701-706. 10.15585/mmwr.mm6725a1.	Not related to *Naegleria fowleri*
Johnson RO, Cope JR, Moskowitz M, Kahler A, Hill V, Behrendt K, Molina L, Fullerton KE, Beach MJ. Notes from the Field: Primary Amebic Meningoencephalitis Associated with Exposure to Swimming Pool Water Supplied by an Overland Pipe - Inyo County, California, 2015. MMWR Morb Mortal Wkly Rep. 2016 Apr 29;65(16):424.	Chlorinated water study

7.2 Primary studies

Table 7.2 List of excluded studies for *Naegleria fowleri*

Naegleria fowleri	
Citation	Reason for exclusion
Al-Herrawy AZ, Gad MA. Isolation and molecular identification of *Naegleria fowleri* from Nile river, Egypt. J Egypt Public Health Assoc. 2015 Dec;90(4):161-5.	Article not accessible
Baig, A. M. (January 6, 2016). "Primary Amoebic Meningoencephalitis Preventive Nose Plugs: Prophylaxis Against *Naegleria fowleri*." ASME. *J. Med. Devices*. March 2016; 10(1): 014501.	Data does not support conclusions
Beshearse E, Bruce BB, Nane GF, Cooke RM, Aspinall W, Hald T, Crim SM, Griffin PM, Fullerton KE, Collier SA, Benedict KM, Beach MJ, Hall AJ, Havelaar AH. Attribution of Illnesses Transmitted by Food and Water to Comprehensive Transmission Pathways Using Structured Expert Judgment, United States. Emerg Infect Dis. 2021 Jan;27(1):182-195.	Not related to *Naegleria fowleri*
Boukassa, Léon, Ngackosso, O.B., Ekouele, M.H.B., Bambino, S.B., Mpelle, Fils Landry, and Bobili, B.. (2017). Primitive amoebic meningoencephalitis in a young adult after accident of diving. African Journal of Neurological Sciences. 36.	Data does not support conclusions
Celik Y, Arslankoylu AE. A Newborn with Brain-Eating Ameba Infection. J Trop Pediatr. 2021 Jan 29;67(1):fmaa100.	No connection to a recreational water
De Jonckheere JF. What do we know by now about the genus Naegleria? Exp Parasitol. 2014 Nov;145 Suppl:S2-9. doi: 10.1016/j.exppara.2014.07.011. Epub 2014 Aug 6.	Manuscript out of scope

De Jonckheere JF. Origin and evolution of the worldwide distributed pathogenic amoeboflagellate *Naegleria fowleri*. Infect Genet Evol. 2011 Oct;11(7):1520-8.	Manuscript out of scope
Gautam PL, Sharma S, Puri S, Kumar R, Midha V, Bansal R. A rare case of survival from primary amebic meningoencephalitis. Indian J Crit Care Med. 2012 Jan;16(1):34-6.	No connection to a recreational water
Ghanchi NK, Jamil B, Khan E, Ansar Z, Samreen A, Zafar A, Hasan Z. Case Series of *Naegleria fowleri* Primary Amoebic Meningoencephalitis from Karachi, Pakistan. Am J Trop Med Hyg. 2017 Nov;97(5):1600-1602.	No connection to a recreational water
Ghanchi NK, Khan E, Khan A, Muhammad W, Malik FR, Zafar A. *Naegleria fowleri* Meningoencephalitis Associated with Public Water Supply, Pakistan, 2014. Emerg Infect Dis. 2016 Oct;22(10):1835-7.	No connection to a recreational water
Hebbar S, Bairy I, Bhaskaranand N, Upadhyaya S, Sarma MS, Shetty AK. Fatal case of *Naegleria fowleri* meningoencephalitis in an infant: case report. Ann Trop Paediatr. 2005 Sep;25(3):223-6.	Data does not support conclusions
Jain R, Tilak V. Primary amoebic meningoencephalitis due to *Naegleria fowleri*. J Indian Med Assoc. 2011 Jul;109(7):500-1.	Data does not support conclusions.
Kao PM, Hsu BM, Hsu TK, Chiu YC, Chang CL, Ji WT, Huang SW, Fan CW. Application of TaqMan qPCR for the detection and monitoring of Naegleria species in reservoirs used as a source for drinking water. Parasitol Res. 2014 Oct;113(10):3765-71.	Not related to *Naegleria fowleri*
Kao PM, Tung MC, Hsu BM, Chou MY, Yang HW, She CY, Shen SM. Quantitative detection and identification of Naegleria spp. in various environmental water samples using real-time quantitative PCR assay. Parasitol Res. 2013 Apr;112(4):1467-74.	Not related to *Naegleria fowleri*
Karim AM, Yasir M, Ullah I, Lee JH, Lee SH. Important factors causing high fatal cases of *Naegleria fowleri* primary amoebic meningoencephalitis in Pakistan. Int J Infect Dis. 2020 Aug;97:230-232.	No connection to a recreational water
Ghanchi NK, Jamil B, Khan E, Ansar Z, Samreen A, Zafar A, Hasan Z. Case Series of *Naegleria fowleri* Primary Ameobic Meningoencephalitis from Karachi, Pakistan. Am J Trop Med Hyg. 2017 Nov;97(5):1600-1602.	Data does not support conclusions
Mushtaq MZ, Mahmood SBZ, Aziz A. A Fatal Case of Primary Amoebic Meningoencephalitis (PAM) Complicated with Diabetes Insipidus (DI): A Case Report and Review of the Literature. Case Rep Infect Dis. 2020 Jul 24;2020:4925819.	No connection to a recreational water
Rasheduzzaman M, Singh R, Haas CN, Tolofari D, Yassaghi H, Hamilton KA, Yang Z, Gurian PL. Reverse QMRA as a Decision Support Tool: Setting Acceptable Concentration Limits for Pseudomonas aeruginosa and *Naegleria fowleri*. Water. 2019; 11(9):1850.	Manuscript retracted
Sazzad HMS, Luby SP, Sejvar J, Rahman M, Gurley ES, Hill V, Murphy JL, Roy S, Cope JR, Ali IKM. A case of primary amebic meningoencephalitis caused by *Naegleria fowleri* in Bangladesh. Parasitol Res. 2020 Jan;119(1):339-344.	No connection to a recreational water
Shariq A, Afridi FI, Farooqi BJ, Ahmed S, Hussain A. Fatal primary meningoencephalitis caused by *Naegleria fowleri*. J Coll Physicians Surg Pak. 2014 Jul;24(7):523-5.	No connection to a recreational water
Siddiqui R, Ali IKM, Cope JR, Khan NA. Biology and pathogenesis of *Naegleria fowleri*. Acta Trop. 2016 Dec;164:375-394.	Data does not support conclusions
Sood A, Chauhan S, Chandel L, Jaryal SC. Prompt diagnosis and extraordinary survival from *Naegleria fowleri* meningitis: a rare case report. Indian J Med Microbiol. 2014 Apr-Jun;32(2):193-6.	Data does not support conclusions
Stubhaug TT, Reiakvam OM, Stensvold CR, Hermansen NO, Holberg-Petersen M, Antal EA, Gaustad K, Førde IS, Heger B. Fatal primary amoebic meningoencephalitis in a Norwegian tourist returning from Thailand. JMM Case Rep. 2016 Jun 25;3(3):e005042.	No connection to a recreational water

Tiewcharoen S., Junnu, V., Roongruangchai, Kosol, Angkanasinsiri, A., and Rabablert, Jundee. (2018). Molecular identification of *Naegleria fowleri* and pathogenic acanthamoeba spp. in Chao Phraya river and canals around Siriraj hospital, Thailand. Journal of the Medical Association of Thailand. 101. 1303-1309.	Data does not support conclusions
Xue J, Caton K, Sherchan SP. Comparison of next-generation droplet digital PCR with quantitative PCR for enumeration of *Naegleria fowleri* in environmental water and clinical samples. Lett Appl Microbiol. 2018 Oct;67(4):322-328.	Manuscript out of scope
Yoder JS, Blackburn BG, Craun GF, Hill V, Levy DA, Chen N, Lee SH, Calderon RL, Beach MJ. Surveillance for waterborne-disease outbreaks associated with recreational water--United States, 2001-2002. MMWR Surveill Summ. 2004 Oct 22;53(8):1-22.	Minimal details/data provided
Jahangeer M, Mahmood Z, Munir N, Waraich UE, Tahir IM, Akram M, Ali Shah SM, Zulfqar A, Zainab R. *Naegleria fowleri*: Sources of infection, pathophysiology, diagnosis, and management; a review. Clin Exp Pharmacol Physiol. 2020 Feb;47(2):199-212.	Data does not support conclusions
Kaushal V, Chhina DK, Ram S, Singh G, Kaushal RK, Kumar R. Primary amoebic meningoencephalitis due to *Naegleria fowleri*. J Assoc Physicians India. 2008 Jun;56:459-62.	Data does not support conclusions
Lares-Villa F, Hernández-Peña C. Concentration of *Naegleria fowleri* in natural waters used for recreational purposes in Sonora, Mexico (November 2007-October 2008). Exp Parasitol. 2010 Sep;126(1):33-6.	Data does not support conclusions
Moussa M, Tissot O, Guerlotté J, De Jonckheere JF, Talarmin A. Soil is the origin for the presence of *Naegleria fowleri* in the thermal recreational waters. Parasitol Res. 2015 Jan;114(1):311-5.	Data does not support conclusions
Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int J Parasitol. 2004 Aug;34(9):1001-27.	Minimal details/data provided
Zbikowska E, Kletkiewicz H, Walczak M, Burkowska A. Coexistence of Legionella pneumophila Bacteria and Free-Living Amoebae in Lakes Serving as a Cooling System of a Power Plant. Water Air Soil Pollut. 2014;225(8):2066. doi: 10.1007/s11270-014-2066-y. Epub 2014 Jul 29.	Not related to *Naegleria fowleri*

Table 7.3 List of excluded studies for *Burkholderia pseudomallei*

Burkholderia pseudomallei	
Citation	Reason for exclusion
Boonbumrung K, Wuthiekanun V, Rengpipat S, Day NP, Peacock SJ. In vitro motility of a population of clinical *Burkholderia pseudomallei* isolates. J Med Assoc Thai. 2006 Sep;89(9):1506-10.	Manuscript out of scope
Bourque DL, Vinetz JM. Illnesses Associated with Freshwater Recreation During International Travel. Curr Infect Dis Rep. 2018 May 22;20(7):19.	Minimal details/data provided
Chierakul W, Winothai W, Wattanawaitunechai C, Wuthiekanun V, Rugtaengan T, Rattanalertnavee J, Jitpratoom P, Chaowagul W, Singhasivanon P, White NJ, Day NP, Peacock SJ. Melioidosis in 6 tsunami survivors in southern Thailand. Clin Infect Dis. 2005 Oct 1;41(7):982-90.	No connection to a recreational water
Chuah CJ, Tan EKH, Sermswan RW, Ziegler AD. Hydrological connectivity and *Burkholderia pseudomallei* prevalence in wetland environments: investigating rice-farming community's risk of exposure to melioidosis in North-East Thailand. Environ Monit Assess. 2017 Jun;189(6):287.	No connection to a recreational water

Corea EM, de Silva AD, Thevanesam V. Melioidosis in Sri Lanka. Trop Med Infect Dis. 2018 Feb 21;3(1):22.	No connection to a recreational water
Corea EM, Merritt AJ, Ler YH, Thevanesam V, Inglis TJ. Sri Lankan National Melioidosis Surveillance Program Uncovers a Nationwide Distribution of Invasive Melioidosis. Am J Trop Med Hyg. 2016 Feb;94(2):292-8. doi: 10.4269/ajtmh.15-0567. Epub 2015 Nov 30.	No connection to a recreational water
Dance DAB, Luangraj M, Rattanavong S, Sithivong N, Vongnalaysane O, Vongsouvath M, Newton PN. Melioidosis in the Lao People's Democratic Republic. Trop Med Infect Dis. 2018 Feb 19;3(1):21. doi: 10.3390/tropicalmed3010021.	No connection to a recreational water..
Diefenbach-Elstob TR, Graves PM, Burgess GW, Pelowa DB, Warner JM. Seroepidemiology of melioidosis in children from a remote region of Papua New Guinea. Int Health. 2015 Sep;7(5):332-8. doi: 10.1093/inthealth/ihu088. Epub 2014 Dec 8.	No connection to a recreational water
Douglas MW, Lum G, Roy J, Fisher DA, Anstey NM, Currie BJ. Epidemiology of community-acquired and nosocomial bloodstream infections in tropical Australia: a 12-month prospective study. Trop Med Int Health. 2004 Jul;9(7):795-804.	No connection to a recreational water
Douglas NM, Hennessy JN, Currie BJ, Baird RW. Trends in Bacteremia Over 2 Decades in the Top End of the Northern Territory of Australia. Open Forum Infect Dis. 2020 Oct 17;7(11):ofaa472.	No connection to a recreational water
Egilmez HI, Morozov AY, Clokie MRJ, Shan J, Letarov A, Galyov EE. Temperature-dependent virus lifecycle choices may reveal and predict facets of the biology of opportunistic pathogenic bacteria. Sci Rep. 2018 Jun 25;8(1):9642.	No connection to a recreational water
Goodrick I, Todd G, Stewart J. Soil characteristics influencing the spatial distribution of melioidosis in Far North Queensland, Australia. Epidemiol Infect. 2018 Sep;146(12):1602-1607.	No connection to a recreational water
Hamdoon S, Wilson I, Smith S, Gericke C. Melioidosis of the nervous system: atypical presentation of a rare disease in a 48-year-old man. BMJ Case Rep. 2020 Nov 3;13(11):e233498.	No connection to a recreational water.
Hassan, M.R., Pani, S.P., Peng, N.P. *et al.* Incidence, risk factors and clinical epidemiology of melioidosis: a complex socio-ecological emerging infectious disease in the Alor Setar region of Kedah, Malaysia. *BMC Infect Dis* **10**, 302 (2010).	No connection to a recreational water
Hii SFF, Kee CC, Ahmad N. Melioidosis: Overview of seropositivity in Malaysia. Trop Biomed. 2016 Dec 1;33(4):697-701.	No connection to a recreational water
Hinjoy S, Hantrakun V, Kongyu S, Kaewrakmuk J, Wangrangsimakul T, Jitsuronk S, Saengchun W, Bhengsri S, Akarachotpong T, Thamthitiwat S, Sangwichian O, Anunnatsiri S, Sermswan RW, Lertmemongkolchai G, Tharinjaroen CS, Preechasuth K, Udpaun R, Chuensombut P, Waranyasirikul N, Anudit C, Narenpitak S, Jutrakul Y, Teparrukkul P, Teerawattanasook N, Thanvisej K, Suphan A, Sukbut P, Ploddi K, Sirichotirat P, Chiewchanyon B, Rukseree K, Hongsuwan M, Wongsuwan G, Sunthornsut P, Wuthiekanun V, Sachaphimukh S, Wannapinij P, Chierakul W, Chewapreecha C, Thaipadungpanit J, Chantratita N, Korbsrisate S, Taunyok A, Dunachie S, Palittapongarnpim P, Sirisinha S, Kitphati R, Iamsirithaworn S, Chaowagul W, Chetchotisak P, Whistler T, Wongratanacheewin S, Limmathurotsakul D. Melioidosis in Thailand: Present and Future. Trop Med Infect Dis. 2018;3(2):38.	No connection to a recreational water.
Inglis TJ, O'Reilly L, Merritt AJ, Levy A, Heath CH. The aftermath of the Western Australian melioidosis outbreak. Am J Trop Med Hyg. 2011 Jun;84(6):851-7. doi: 10.4269/ajtmh.2011.10-0480. Erratum in: Am J Trop Med Hyg. 2011 Jul;85(1):191. Heath, Christopher [corrected to Heath, Christopher H].	No connection to a recreational water
Inglis TJ, Rolim DB, Sousa Ade Q. Melioidosis in the Americas. Am J Trop Med Hyg. 2006 Nov;75(5):947-54.	No connection to a recreational water

Ismail A, Buckley A, Dubrey SW. Melioidosis in a returning traveller. BMJ Case Rep. 2013 Apr 18;2013:bcr2013009655.	No connection to a recreational water
Jatapai A, Gregory CJ, Thamthitiwat S, Tanwisaid K, Bhengsri S, Baggett HC, Sangwichian O, Jorakate P, MacArthur JR. Hospitalized Bacteremic Melioidosis in Rural Thailand: 2009-2013. Am J Trop Med Hyg. 2018 Jun;98(6):1585-1591.	No connection to a recreational water
Jilani MS, Robayet JA, Mohiuddin M, Hasan MR, Ahsan CR, Haq JA. Burkholderia pseudomallei: Its Detection in Soil and Seroprevalence in Bangladesh. PLoS Negl Trop Dis. 2016 Jan 15;10(1):e0004301.	No connection to a recreational water
Kaestli M, Mayo M, Harrington G, Watt F, Hill J, Gal D, Currie BJ. Sensitive and specific molecular detection of Burkholderia pseudomallei, the causative agent of melioidosis, in the soil of tropical northern Australia. Appl Environ Microbiol. 2007 Nov;73(21):6891-7.	No connection to a recreational water
Krishnan P, Fernandes S, Savio J, Ross CR, Pradeep R, Choudhary R, Shet AS, Pais P. Melioidosis. J Assoc Physicians India. 2008 Aug;56:636-9. PMID: 19051712.	No connection to a recreational water
Lazar Adler NR, Govan B, Cullinane M, Harper M, Adler B, Boyce JD. The molecular and cellular basis of pathogenesis in melioidosis: how does *Burkholderia pseudomallei* cause disease? FEMS Microbiol Rev. 2009 Nov;33(6):1079-99.	No connection to a recreational water
Li XY, Ke BX, Chen CN, Xiao HL, Liu MZ, Xiong YC, Bai R, Chen JD, Ke CW. First co-infection case of melioidosis and Japanese encephalitis in China. BMC Infect Dis. 2018 Sep 4;18(1):452.	No connection to a recreational water
Lu PL, Tseng SH. Fatal septicemic melioidosis in a young military person possibly co-infected with Leptospira interrogans and Orientia tsutsugamushi. Kaohsiung J Med Sci. 2005 Apr;21(4):173-8.	No connection to a recreational water
Mahikul W, White LJ, Poovorawan K, Soonthornworasiri N, Sukontamarn P, Chanthavilay P, Medley GF, Pan-Ngum W. Modelling population dynamics and seasonal movement to assess and predict the burden of melioidosis. PLoS Negl Trop Dis. 2019 May 9;13(5):e0007380.	No connection to a recreational water
Majumder MI, Haque MM, Ahmed MW, Alam MN, Rahman MW, Akter F, Basher A, Maude RJ, Faiz MA. Melioidosis in an adult male. Mymensingh Med J. 2013 Apr;22(2):413-6.	No connection to a recreational water
Miralles IS, Maciel Mdo C, Angelo MR, Gondini MM, Frota LH, dos Reis CM, Hofer E. Burkholderia pseudomallei: a case report of a human infection in Ceará, Brazil. Rev Inst Med Trop Sao Paulo. 2004 Jan-Feb;46(1):51-4.	No connection to a recreational water
Musk AW, James AL, Palmer LJ, Ryan GF, Lake F, Golledge CL, De Klerk NH. Respiratory infections and lung function in an Australian Aboriginal community. Respirology. 2008 Mar;13(2):257-62.	No connection to a *Burkholderia pseudomallei* or recreational water
Nandagopal B, Sankar S, Lingesan K, Appu K, Sridharan G, Gopinathan A. Application of polymerase chain reaction to detect *Burkholderia pseudomallei* and Brucella species in buffy coat from patients with febrile illness among rural and peri-urban population. J Glob Infect Dis. 2012 Jan;4(1):31-7.	No connection to a recreational water
Peddayelachagiri BV, Paul S, Nagaraj S, Gogoi M, Sripathy MH, Batra HV. Prevalence and Identification of *Burkholderia pseudomallei* and Near-Neighbor Species in the Malabar Coastal Region of India. PLoS Negl Trop Dis. 2016 Sep 15;10(9):e0004956.	No connection to a recreational water
Rachlin A, Luangraj M, Kaestli M, Rattanavong S, Phoumin P, Webb JR, Mayo M, Currie BJ, Dance DAB. Using Land Runoff to Survey the Distribution and Genetic Diversity of *Burkholderia pseudomallei* in Vientiane, Laos. Appl Environ Microbiol. 2020 Nov 30;87(4):e02112-20.	No connection to a recreational water

Rhodes J, Jorakate P, Makprasert S, Sangwichian O, Kaewpan A, Akarachotpong T, Srisaengchai P, Thamthitiwat S, Khemla S, Yuenprakhon S, Paveenkittiporn W, Kerdsin A, Whistler T, Baggett HC, Gregory CJ. Population-based bloodstream infection surveillance in rural Thailand, 2007-2014. BMC Public Health. 2019 May 10;19(Suppl 3):521.	No connection to a recreational water
Ribolzi O, Rochelle-Newall E, Dittrich S, Auda Y, Newton PN, Rattanavong S, Knappik M, Soulileuth B, Sengtaheuanghoung O, Dance DA, Pierret A. Land use and soil type determine the presence of the pathogen *Burkholderia pseudomallei* in tropical rivers. Environ Sci Pollut Res Int. 2016 Apr;23(8):7828-39.	No connection to a recreational water
Sapian M, Khair MT, How SH, Rajalingam R, Sahhir K, Norazah A, Khebir V, Jamalludin AR. Outbreak of melioidosis and leptospirosis co-infection following a rescue operation. Med J Malaysia. 2012 Jun;67(3):293-7.	No connection to a recreational water
Sarovich DS, Chapple SNJ, Price EP, Mayo M, Holden MTG, Peacock SJ, Currie BJ. Whole-genome sequencing to investigate a non-clonal melioidosis cluster on a remote Australian island. Microb Genom. 2017 Jun 13;3(8):e000117.	Manuscript out of scope
Seng R, Saiprom N, Phunpang R, Baltazar CJ, Boontawee S, Thodthasri T, Silakun W, Chantratita N. Prevalence and genetic diversity of *Burkholderia pseudomallei* isolates in the environment near a patient's residence in Northeast Thailand. PLoS Negl Trop Dis. 2019 Apr 19;13(4):e0007348.	No connection to a recreational water
Su HP, Yang HW, Chen YL, Ferng TL, Chou YL, Chung TC, Chen CH, Chiang CS, Kuan MM, Lin HH, Chen YS. Prevalence of melioidosis in the Er-Ren River Basin, Taiwan: implications for transmission. J Clin Microbiol. 2007 Aug;45(8):2599-603.	No connection to a recreational water
Valade E, Thibault FM, Biot FV, Vidal DR. La mélioïdose: une maladie tropicale en quête de reconnaissance [Melioidosis: an emerging tropical disease]. Med Trop (Mars). 2009 Oct;69(5):437-45. (French)	No connection to a recreational water
Vandana KE, Mukhopadhyay C, Tellapragada C, Kamath A, Tipre M, Bhat V, Sathiakumar N. Seroprevalence of *Burkholderia pseudomallei* among Adults in Coastal Areas in Southwestern India. PLoS Negl Trop Dis. 2016 Apr 14;10(4):e0004610.	No connection to a recreational water
Warner JM, Currie BJ. Melioidosis in Papua New Guinea and Oceania. Trop Med Infect Dis. 2018 Mar 15;3(1):34. doi: 10.3390/tropicalmed3010034.	No connection to a recreational water
Warner JM, Pelowa DB, Currie BJ, Hirst RG. Melioidosis in a rural community of Western Province, Papua New Guinea. Trans R Soc Trop Med Hyg. 2007 Aug;101(8):809-13.	No connection to a recreational water
Warner JM, Pelowa DB, Gal D, Rai G, Mayo M, Currie BJ, Govan B, Skerratt LF, Hirst RG. The epidemiology of melioidosis in the Balimo region of Papua New Guinea. Epidemiol Infect. 2008 Jul;136(7):965-71.	No connection to a recreational water
Wijewickrama PSA, Weerakoon R. Acute disseminated melioidosis giving rise to pneumonia and renal abscesses complicated with thrombotic thrombocytopenic purpura in a post-partum woman: a case report. BMC Res Notes. 2017 Nov 29;10(1):653.	No connection to a recreational water
Yip CH, Ghazali AK, Nathan S. *Burkholderia pseudomallei* pathogenesis and survival in different niches. Biochem Soc Trans. 2020 Apr 29;48(2):569-579.	No connection to a recreational water
Zheng X, Xia Q, Xia L, Li W. Endemic Melioidosis in Southern China: Past and Present. Trop Med Infect Dis. 2019 Feb 25;4(1):39.	No connection to a recreational water

8 Declared Interests

The Authors of this review have the following declared interests:

Geoffrey J Puzon	
Interest	**Interest Details**
Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA. The reviewer also participates in an active research collaboration focused on *Naegleria fowleri* in natural and engineered water sources/systems with Montana State University and United State Geological Survey. The researcher is an active member of the American Water Works Association Standard Methods committee for *Naegleria fowleri*, the Australian Water Association, Water Research Australia and the International Water Association. The reviewer has ongoing discussions with national and international individuals/organisations (CDC-USA, University of Georgia, Baylor University, and Texas Commission on Environmental Quality).
Anna Kaksonen	
Interest	**Interest Details**
Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA, mining companies, waste sector and NSW Environmental Trust. The researcher is an active member of the Australian Water Association and Water Research Australia. The reviewer also serves on editorial boards for the Nature Publishing Group, MDPI and is an Advisory Board member of Western Australian Minerals Research Institute.
Natalia Malinowski	
Interest	**Interest Details**
	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA. The reviewer also participates in an active research collaboration focused on *Naegleria fowleri* in natural and engineered water sources/systems with Montana State University and United State Geological Survey.
Tom Walsh	
Interest	**Interest Details**
Funded water research and non-funded research activities	The reviewer is currently engaged at CSIRO and conducts funded research project with Water Corporation WA. The reviewer also participates in an active research collaboration focused on *Naegleria fowleri* in natural and engineered water sources/systems with Montana State University and United State Geological Survey.

9 References

AGREE Next Steps Consortium (2017) The AGREE II Instrument [Electronic version]., [online] http://www.agreetrust.org.

Brouwers, M. C., Kerkvliet, K., Spithoff, K., and AGREE Next Steps Consortium (2016) The AGREE Reporting Checklist: a tool to improve reporting of clinical practice guidelines. BMJ, i1152.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., and The PRISMA Group (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Medicine, 6(7), e1000097.

10 Appendices

10.1 Appendix 1 – Form for assessing existing guidance or reviews.

Table 10.1 Guideline and Literature Review assessment template

Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'

	Criteria	Y/N/?/NA	Notes
	Overall guidance/advice development process		
	Are the key stages of the organisation's advice development processes compatible with Australian processes?		
	Are the administrative processes documented and publicly available?		
	Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?		
	Are funding sources declared?		
	Was there public consultation on this work? If so, provide details.		
	Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?		
	Was the guidance/advice developed or updated recently? Provide details.		
	Evidence review parameters		
	Are decisions about scope, definitions and evidence review parameters documented and publicly available?		

	Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?		
	Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?		
	If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?		
	Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?		
	Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?		
	Can grey literature such as government reports and policy documents be included?		
	Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?		
	Evidence search		
	Are databases and other sources of evidence specified?		
	Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?		
	Is it specified what date range the literature search covers? Is there a justification?		
	Are search terms and/or search strings specified?		
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?		
	Critical appraisal methods and tools		

	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?		
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.		
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.		
	Derivation of health-based guideline values		
	Is there justification for the choice of uncertainty and safety factors?		
	Are the parameter value assumptions documented and explained?		
	Are the mathematical workings/algorithms clearly documented and explained?		
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?		
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?		
	What processes are used when expert judgement is required and applied? Is the process documented and published?		
	Is dose response modelling (e.g. BMDL) routinely used?		
	What is the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans? Has the policy been articulated and recorded?		
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?		
	Comments		

	Reviewer's comments		
	Useful for answering primary research question		
	Useful for answering secondary research question		

10.2 Appendix 2 – Modified OHAT assessment template

Table 10.2 Risk-of-bias assessment tool adapted from OHAT RoB tool (Table 5 in OHAT Handbook (OHAT,2019))

Study ID: **Study Type:** Case report (diagnosis)		**RoB:** **Yes/No** **Unknown** **N/A**	**Notes**	**Risk of bias rating** **(++/+/-/--)**
Q				
	Selection bias			
1.	Randomization	N/A	Randomization: not applicable to Cohort, Case studies and Observational studies	
2.	Allocation concealment	N/A	Allocation concealment: not applicable to Cohort, Case studies and Observational studies	
3.	Comparison groups appropriate			
	Cofounding bias			
4.	Confounding (design/analysis)			
	Performance Bias			
5.	Identical experimental conditions	N/A	Identical experimental conditions: not applicable to Cohort, Case studies and Observational studies	
6.	Blinding of researchers during study?	N/A	Blinding of researchers during study?: not applicable to Cohort, Case studies and Observational studies	
	Attrition/Exclusion Bias			

7.	Missing outcome data			
	Detection Bias			
8.	Exposure characterisation - Characterisation of infection - Characterisation of exposure to the organism (nature of organism, exposure pathway and whether it was from recreational water) - Confirming organism in rec water			
9.	Outcome assessment - Symptoms - Causality			
	Selective Reporting Bias			
10.	Outcome reporting • Data from patient • Data from exposure site			
	Other Sources of Bias			
11.	Other threats (e.g. statistical methods appropriate; researchers adhered to the study protocol			
	Overall risk of bias rating:			

Definitely low risk of bias (++)	++	Probably low risk of bias (+)	+	Probably high risk of bias (-)	-	Definitely high risk of bias (--)	--

10.3 Appendix 2 – Data extraction template

Table 10.3 Data extraction template

General information	Study ID	
	Date template completed	
	Authors Publication date Publication type Peer reviewed Country of origin Source of funding Possible conflicts of interest	
Study characteristics	Aim/objectives of study	
	Study type/design	
	Study duration	
	Type of water source/water body	
Population characteristics	Population/s studied	
	Selection criteria for population	
	Subgroups reported	
	Size of study	
Exposure and setting	Type of water source/water body Exposure scenario Exposure pathway Source of infection/contamination Causal organism/chemical(s) Comparison group(s)	
Study methods	Water quality measurement used Method of microorganism isolation and enumeration (if applicable) Water sampling methods (monitoring, surrogates)	
Results (for each outcome)	Definition of outcome How outcome was assessed Method of measurement Number participants (exposed/non-exposed, missing/excluded) (if applicable)	
Statistics	Statistical methods used Details on statistical analysis (if any) Relative risk/odds ratio, confidence interval?	
Author's conclusion	Interpretation of results Assessment of uncertainty (if any)	
Reviewer comments	Results included/excluded in review (if applicable) Notes on study quality e.g. gaps, methods	

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