

Epidemiology of and Risk Factors for Amebic Meningoencephalitis, Kerala, India, 2025

Appendix

Laboratory Diagnosis

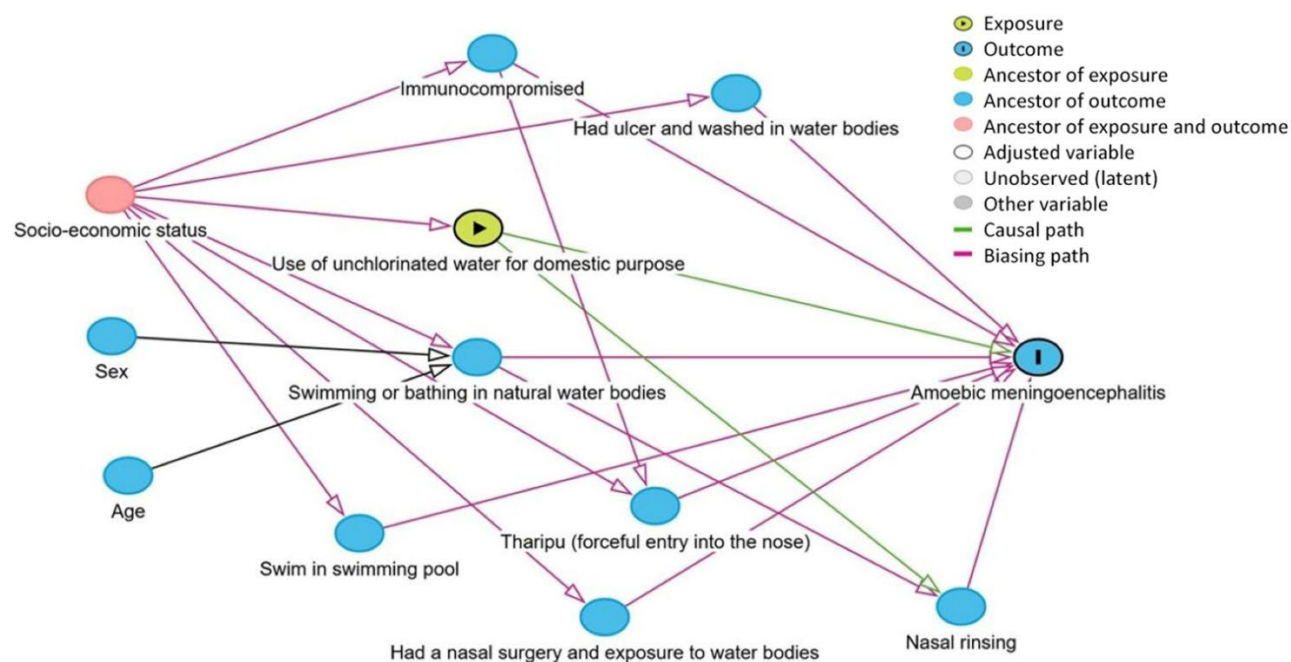
Molecular assays included conventional pan-genus FLA and genus-specific PCR for *Acanthamoeba*, *Naegleria*, and *Balamuthia* species and real-time PCR assays, the *Acanthamoeba*-specific primer pair used was the forward primer (5'-GGCCCAGATCGTTTACCGTGAA-3') and the reverse primer (5'TCTCACAAGCTGCTAGGGAGTCA-3') (1). The conventional PCR amplified products were sequenced for confirmation. The *Naegleria*-specific primer pair used was the forward primer NaeglF192 (3'-GTG CTG AAA CCT AGC TAT TGT AAC TCA GT-5'), and the reverse primer (5'-CAC TAG AAA AAG CAA ACC TGA AAG G-3') (2).

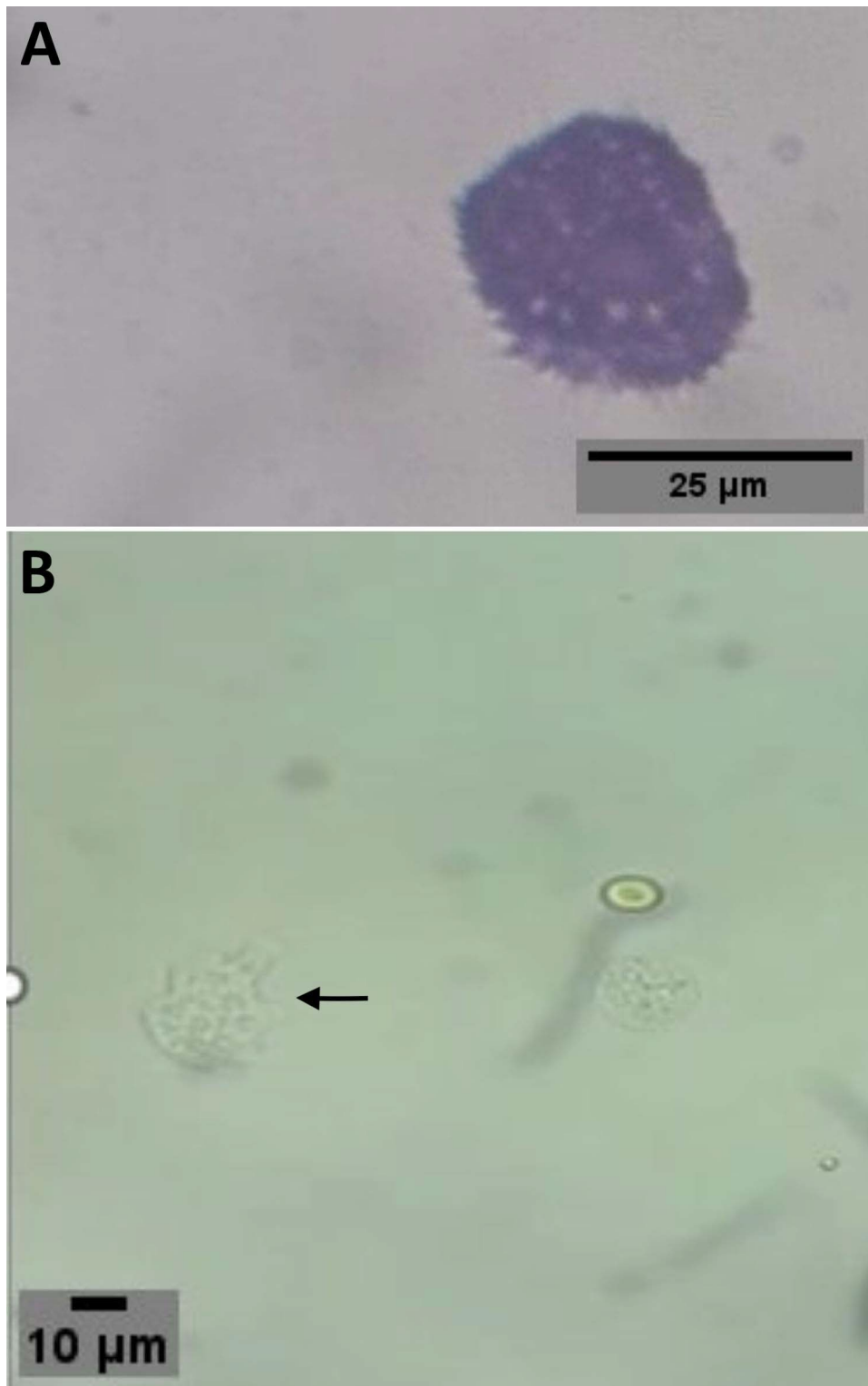
References

1. Sankar P, Moorkoth AP, Khurana S, Mohan A, Vadekkandyil S, M SA, et al. Microbiological profile of meningoencephalitis due to pathogenic free-living amoebae: a case series from Kerala, India. *Cureus*. 2026;18:e103922. [PubMed https://doi.org/10.7759/cureus.103922](https://doi.org/10.7759/cureus.103922)
2. Qvarnstrom Y, Visvesvara GS, Sriram R, da Silva AJ. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J Clin Microbiol*. 2006;44:3589–95. [PubMed https://doi.org/10.1128/JCM.00875-06](https://doi.org/10.1128/JCM.00875-06)

Appendix Table. CSF profile of AME cases

Parameter (n = 128)	Category / Summary	Value
Total cell count (/μL)	Median (IQR)	12 (7–50)
	Range	0–12,900
	Missing, n	2
Protein (mg/dL)	Median (IQR)	67.6 (43–94)
	Range	0.5–5763
	Missing, n	1
Glucose (mg/dL)*	Median (IQR)	69.5 (59–91)
	Range	2–209
	Missing, n	2
Blood sugar/RBS (mg/dL)	Median (IQR)	132.5 (107–182)
	Range	71–850
	Missing, n	36
CSF: Blood glucose ratio	Median (IQR)	0.56 (0.40–0.68)
	Missing, n	37
Pleocytosis (n = 126)	Normal (≤ 5 cells/ μ L)	23 (18.3%)
	Mild (6–50 cells/ μ L)	73 (57.9%)
	Moderate (51–500 cells/ μ L)	16 (12.7%)
	Marked (>500 cells/ μ L)	14 (11.15%)
Predominant cell type (n = 128)	Lymphocytic	90 (70.3%)
	Neutrophilic/Polymorph predominant	3 (2.3%)
	Mixed	3 (2.3%)
	Non-lymphocytic/Other	12 (9.4%)
	Unspecified/Unknown	20 (15.6%)
CSF: Blood glucose ratio (n = 91)	<0.5	34 (37.4%)

**Appendix Figure 1.** Directed acyclic graph of risk factors associated with AME, Kerala, 2025.



Appendix Figure 2. Microscopic images of free-living amoeba. A) *Acanthamoeba* trophozoite: Giemsa stain showing Acanthopodia, nucleus, and contractile vacuoles (under oil immersion, original magnification $\times 100$). B) *Naegleria* trophozoite in CSF wet mount.