

# Detection, Isolation, and *In Vitro* Cultivation of *Balamuthia mandrillaris*: A Systematic Review of Current Laboratory Approaches and Evidence

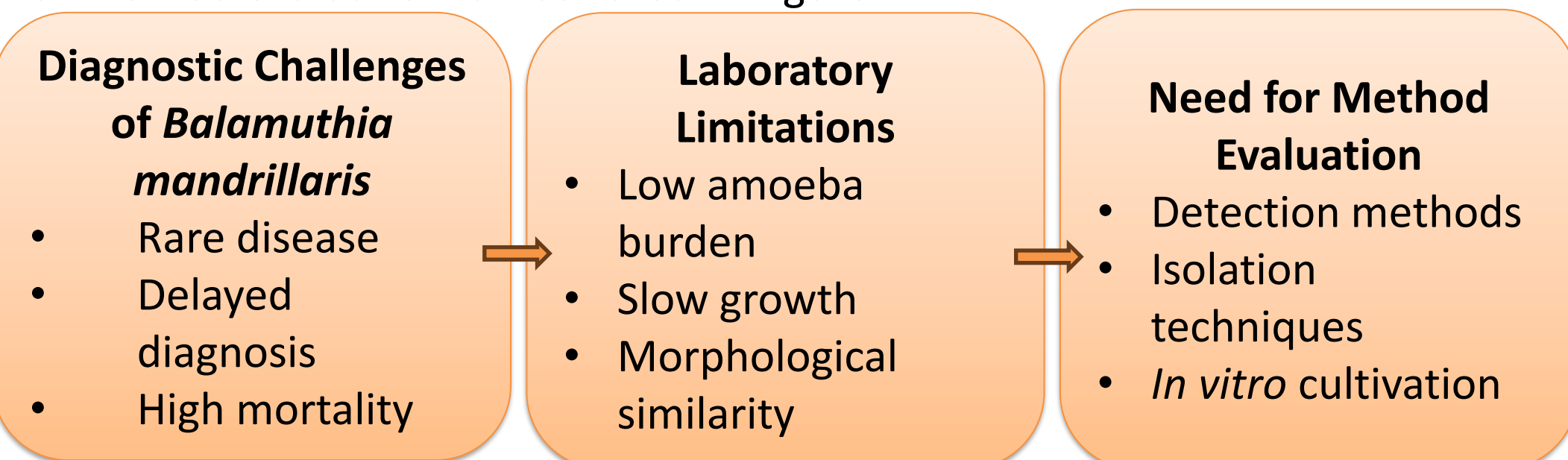
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## INTRODUCTION & AIM

*Balamuthia mandrillaris* is a pathogenic free-living amoeba responsible for granulomatous amoebic encephalitis, a rare but frequently fatal disease [1,2]. Laboratory diagnosis remains difficult due to low organism burden, slow growth, and morphological similarities with other free-living amoebae [3,4]. Multiple approaches have been developed for detection, isolation, and *in vitro* cultivation, though their performance and feasibility vary across studies [1]. The relationship between the current diagnostic challenges, laboratory limitations, and the need for method evaluation is illustrated in Figure 1.



**Figure 1.** Schematic representation of the relationship between the diagnostic challenges, laboratory limitations, and the need for method evaluation.

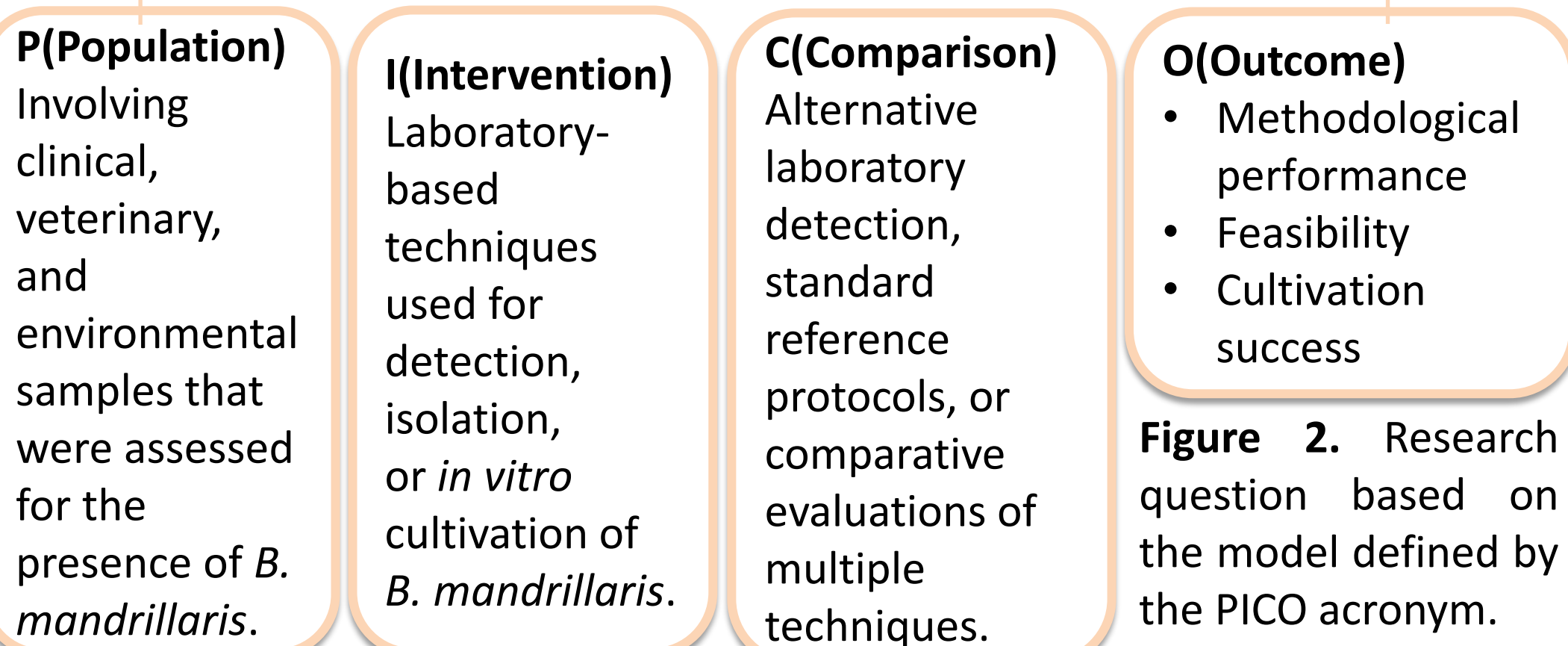
**Objective:** To summarize and compare the currently available laboratory methods.

## METHOD

Following PRISMA 2020 guidelines [5], a systematic search was conducted in Medline, Web of Science, ScienceDirect, and ProQuest using “*Balamuthia mandrillaris*” combined with “Detection”, “Isolation”, “Culture Techniques”, and “*In Vitro* Techniques” on 24<sup>th</sup> September 2025. Eligible studies included experimental, observational, and laboratory-based investigations assessing methods for detecting, isolating, or cultivating *B. mandrillaris* from clinical, veterinary, or environmental samples. Outcomes assessed included methodological performance metrics, feasibility indicators (e.g., turnaround time, technical complexity), and cultivation success. Research question based on the model defined by the PICO acronym is shown in Figure 2.

### Research Question:

What laboratory-based techniques have been developed for the detection, isolation, or *in vitro* cultivation of *B. mandrillaris*?



**Figure 2.** Research question based on the model defined by the PICO acronym.

### Inclusion Criteria

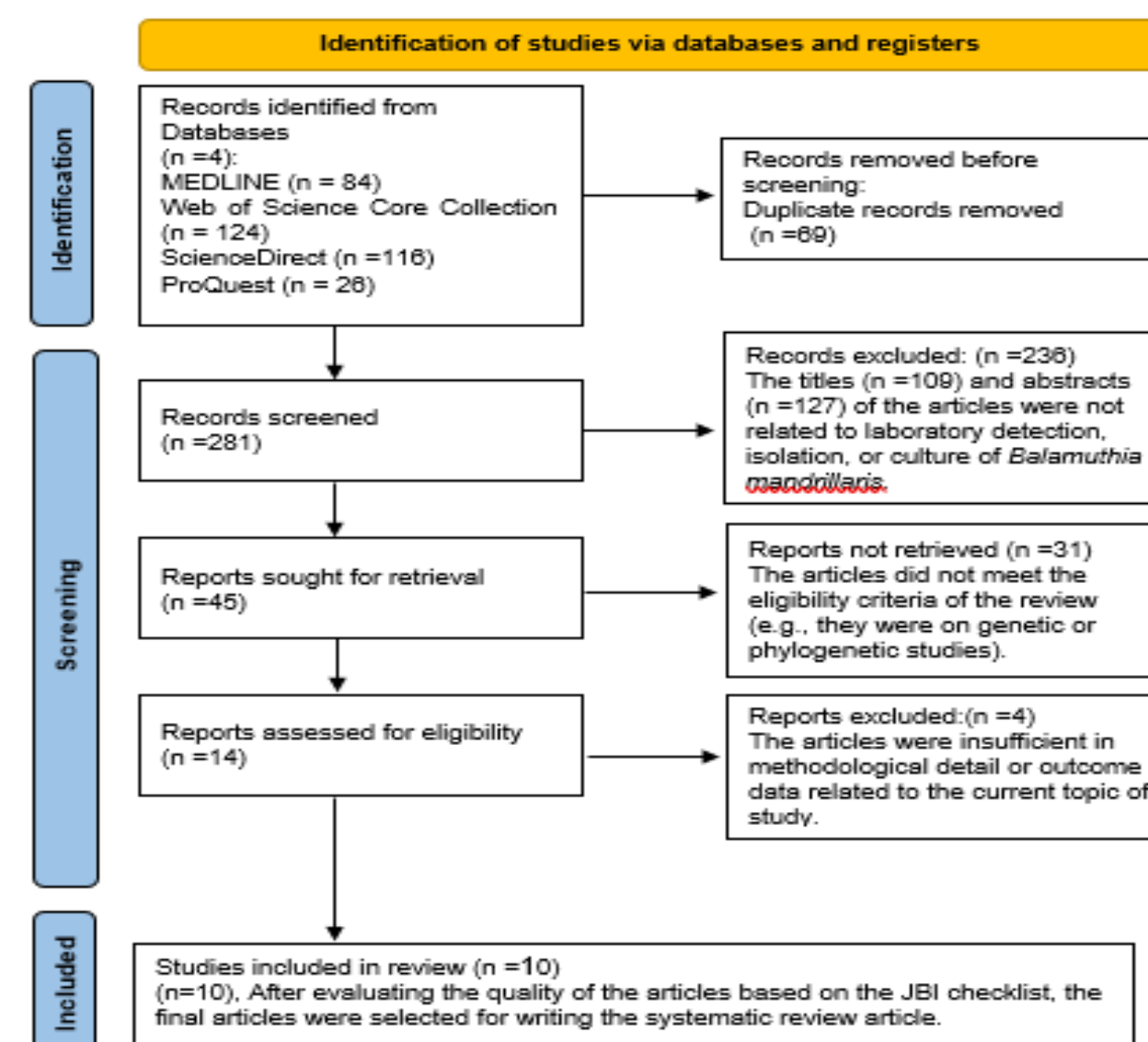
- Experimental, observational, or comparative laboratory studies
- Full-text original articles (English)
- Clinical, veterinary, and environmental samples
- Evaluation of detection, isolation, or cultivation methods
- No publication year restriction

### Exclusion Criteria

- Non-*Balamuthia* studies
- Reviews and conference abstracts
- Pure case reports or prevalence studies
- Genomic/taxonomic-only investigations
- Drug susceptibility studies only
- Insufficient methodological data
- Inaccessible full text

## RESULTS & DISCUSSION

Of 350 articles screened, 10 studies met the inclusion criteria. Laboratory approaches varied by sample type and methodology. Molecular methods, particularly PCR, demonstrated the highest analytical performance, with sensitivities up to 84% in formalin-fixed tissues and limits of detection as low as approximately one amoeba per sample [4,6]. In environmental studies, PCR showed higher detection rates than culture (e.g., 47.4% vs 18.4% for free-living amoebae) [6]. Culture-based methods enabled isolation of *B. mandrillaris* but required prolonged incubation periods (up to 60 days) and generally showed low recovery rates (e.g., 4.5% in water samples) [7,8]. Multi-step protocols combining environmental culture, mammalian cell co-culture, and axenization improved isolation efficiency but remained technically demanding [2,9,10]. *In vitro* cultivation advances demonstrated sustained growth in human fibroblast and endothelial cell lines and axenic media, achieving yields up to  $1.3 \times 10^6$  amoebae/mL with long-term viability [11]. Immunological assays were useful for exposure assessment but were insufficient as standalone diagnostic tools [1,3]. Figure 3 illustrates the PRISMA 2020 flow diagram.



**Figure 3.** PRISMA 2020 flow diagram.

- PCR showed highest performance: sensitivity up to 84% (FFPE tissues); LOD ~1 amoeba/sample.
- PCR outperformed culture in environmental detection (47.4% vs 18.4%).
- Culture-based methods: low recovery (4.5%) with prolonged incubation (≤60 days).
- Multi-step protocols (environmental culture → co-culture → axenization) improved isolation but remained technically demanding.
- Axenic cultivation achieved yields up to  $1.3 \times 10^6$  amoebae/mL with long-term viability.
- Immunological assays: useful for exposure assessment; insufficient as standalone diagnostics.

## CONCLUSION

Molecular methods provide the most sensitive and rapid detection of *B. mandrillaris*. While culture-based approaches, despite low recovery rates and prolonged incubation requirements, remain essential for isolation and biological characterization of the organism.

## FUTURE WORK / REFERENCES

A combined methodological strategy is recommended for both clinical diagnosis and environmental surveillance.

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