

Exploring methodologies from isolation to excystation for *Giardia lamblia* : a systematic review

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INTRODUCTION & AIM

Giardia lamblia is a flagellated protozoan and the etiological agent of giardiasis, a leading cause of epidemic and sporadic diarrhoea globally (Ahmed, 2023). The parasites life cycle consists in two main stages: a cyst stage and a trophozoite stage (Figure 1).

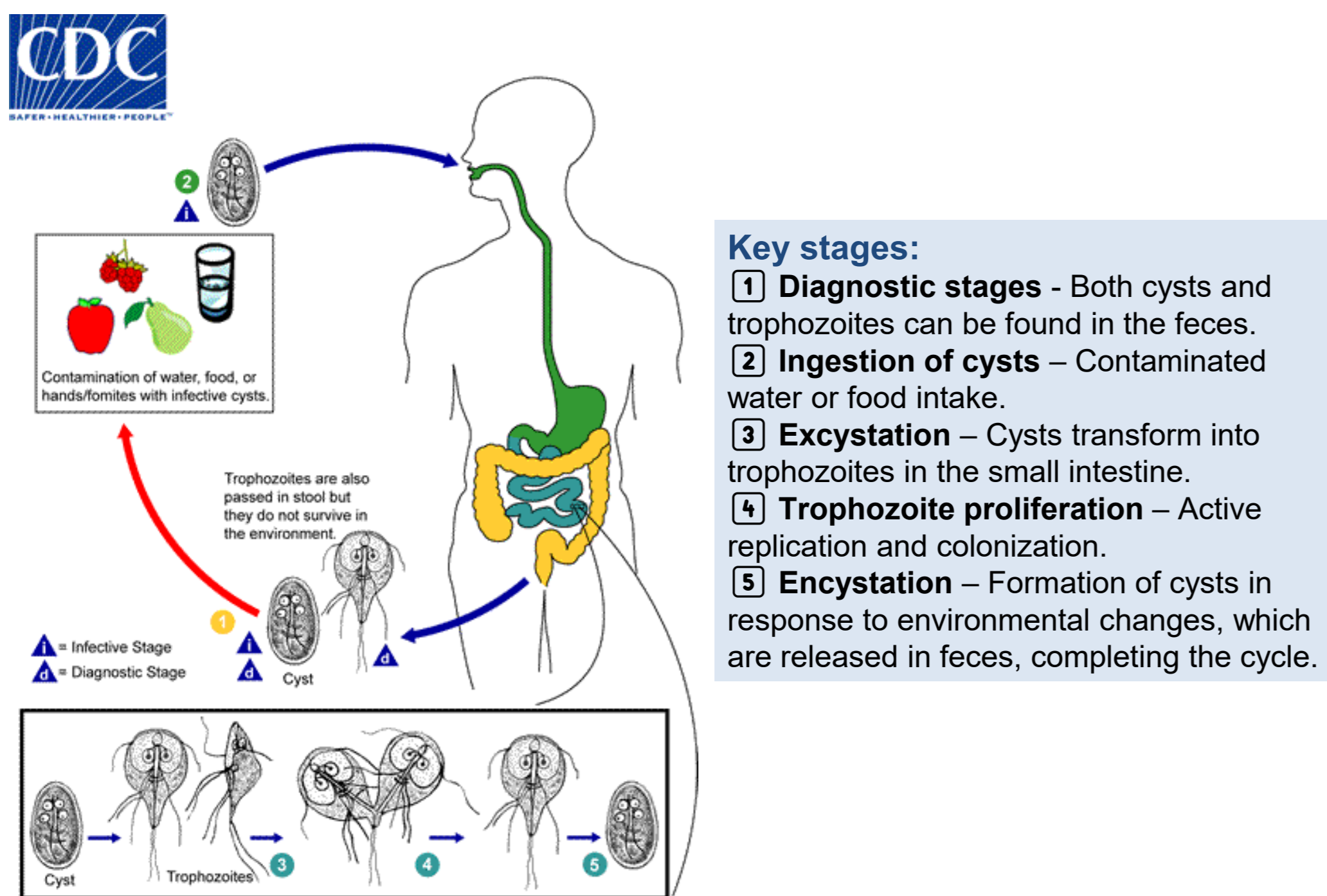


Figure 1: Life cycle of protozoan parasite *Giardia lamblia*. Adapted from Centers for Disease Control and Prevention, (2017).

Given the clinical significance and public health impact of giardiasis, studying the life cycle of *G. lamblia* requires robust methodologies for **isolation, purification, axenization, excystation, and encystation**.

This study systematically reviews the main methodologies described in the literature for studying the life cycle of *G. lamblia*.

METHODS

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020)

Data bases: MEDLINE, ScienceDirect, Web Of Science

Search equation: ("axenization" OR "isolation" OR "excystation" OR "encystation" OR "purification") AND ("method") AND ("giardia")

Inclusion criteria: Research articles published in English, Portuguese or Spanish, assessing methods to evaluate the processes of encystation, excystation, isolation, purification and axenization of *G. lamblia*.

Exclusion criteria: Studies lacking explicit method descriptions were excluded, as were reviews, systematic reviews, and meta-analyses.

CONCLUSION

- ✓ Methods for the isolation and purification of *G. lamblia* exhibit notable variability and lack uniformity compared with the more consistent methods that are used for other life cycle stages.
- ✓ These findings underscore the urgent need for the development of standardized methodologies to enhance the reproducibility and reliability of research outcomes in this field.

RESULTS & DISCUSSION

A total of 39 studies were included in the review (Figure 2).

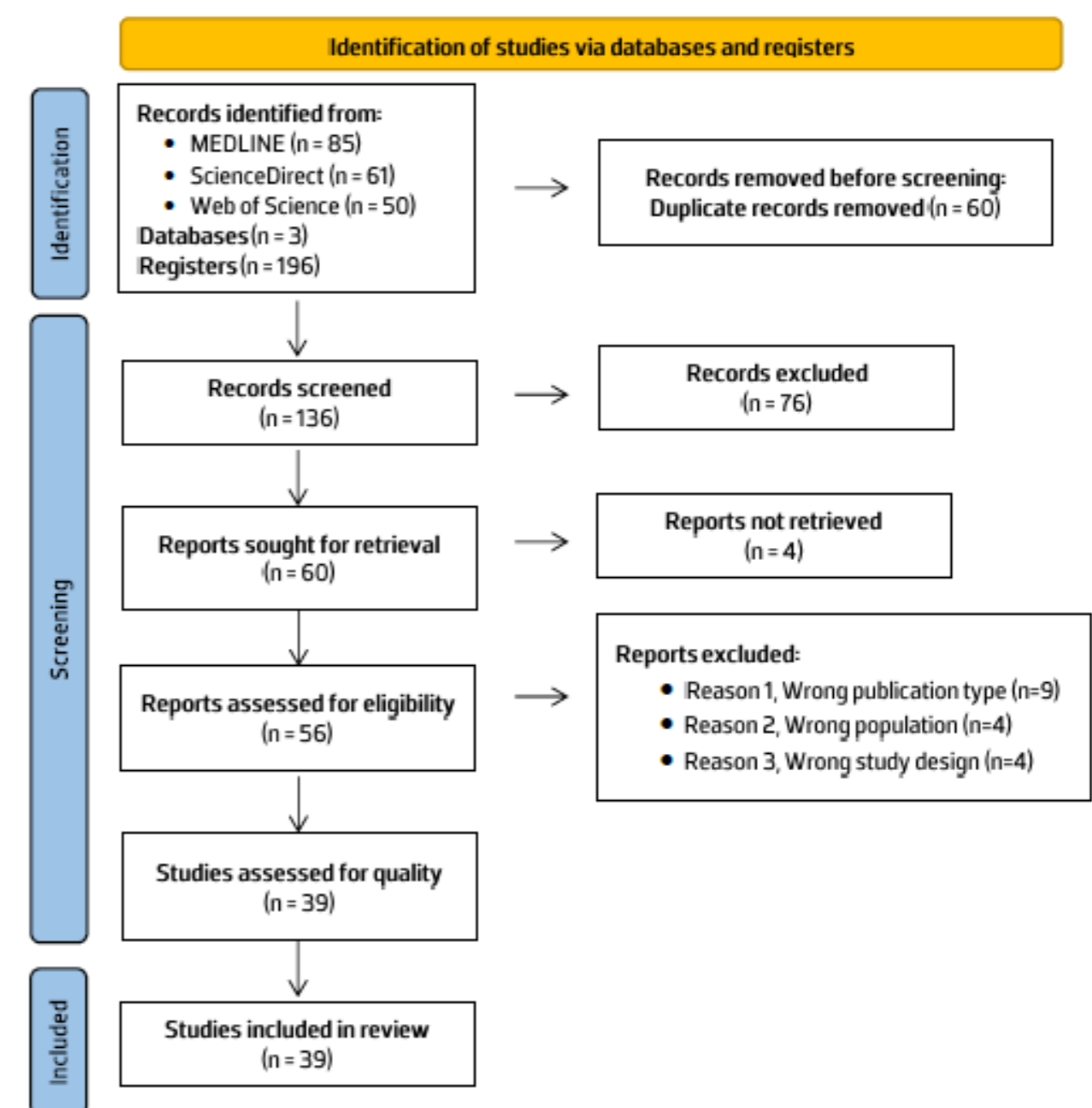


Figure 2: Flowchart of the selection procedure adapted from PRISMA 2020 statement (Page et al., 2021).

A total of 56 methods for isolation and purification, 7 methods for excystation, and 3 methods for axenization and encystation were found in the included studies (Table 1).

Table 1: Methods for *G. lamblia* through its life cycle.

Stage	Nº of methods	Most cited
Isolation and purification	56	<u>Initial separation:</u> combination of filtration, low-speed centrifugation and multiple washing steps. <u>Further purification:</u> density gradients and Immunomagnetic Separation (IMS).
Excystation	7	<u>In vitro:</u> performed in two phases: an initial low-pH induction phase using acid solutions (pH≤2,0) and a subsequent excystment phase. <u>In vivo:</u> inoculation of cysts into gerbils, followed by examination of the small intestinal contents.
Axenization	3	Incubation at 37°C with Keisters' modified TYI-S-33 medium, with an alkaline pH ranging from 7,0 to 7,2, using different concentrations and combinations of antimicrobials and supplemented with bile salts and bovine serum.
Encystation	3	Induction with filter-sterilized TYI-S-33 media supplemented with different concentrations of bovine bile, adjusted to pH 7,8.

- Isolation and purification methods exhibited significant variability, often involving two phases: an initial separation using simple techniques, followed by a purification phase using density gradient for faecal samples and IMS for water samples or nucleic acid extraction.
- Other stages showed more uniform methods, although it was notable that the effectiveness of the employed methods differed depending on the source and sample type.

REFERENCES

