

Seeing what floats: Comparing fecal diagnostic techniques for the detection of zoonotic cestode eggs.



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INTRODUCTION

- *Echinococcus* species are parasitic cestodes (tapeworms) that infect humans, wildlife, livestock, and domesticated dogs and cats¹.
- Definitive hosts are primarily canids, especially coyotes and foxes.¹
- Reports of infection in non-endemic regions are increasing.²
- As urbanization increases worldwide, humans and domesticated animals are at greater risk of being infected through more frequent interactions with wild canid hosts.³
- Given the increased risk of transmission, there is a need to identify the most effective and sensitive fecal flotation methods to detect *Echinococcus* eggs.

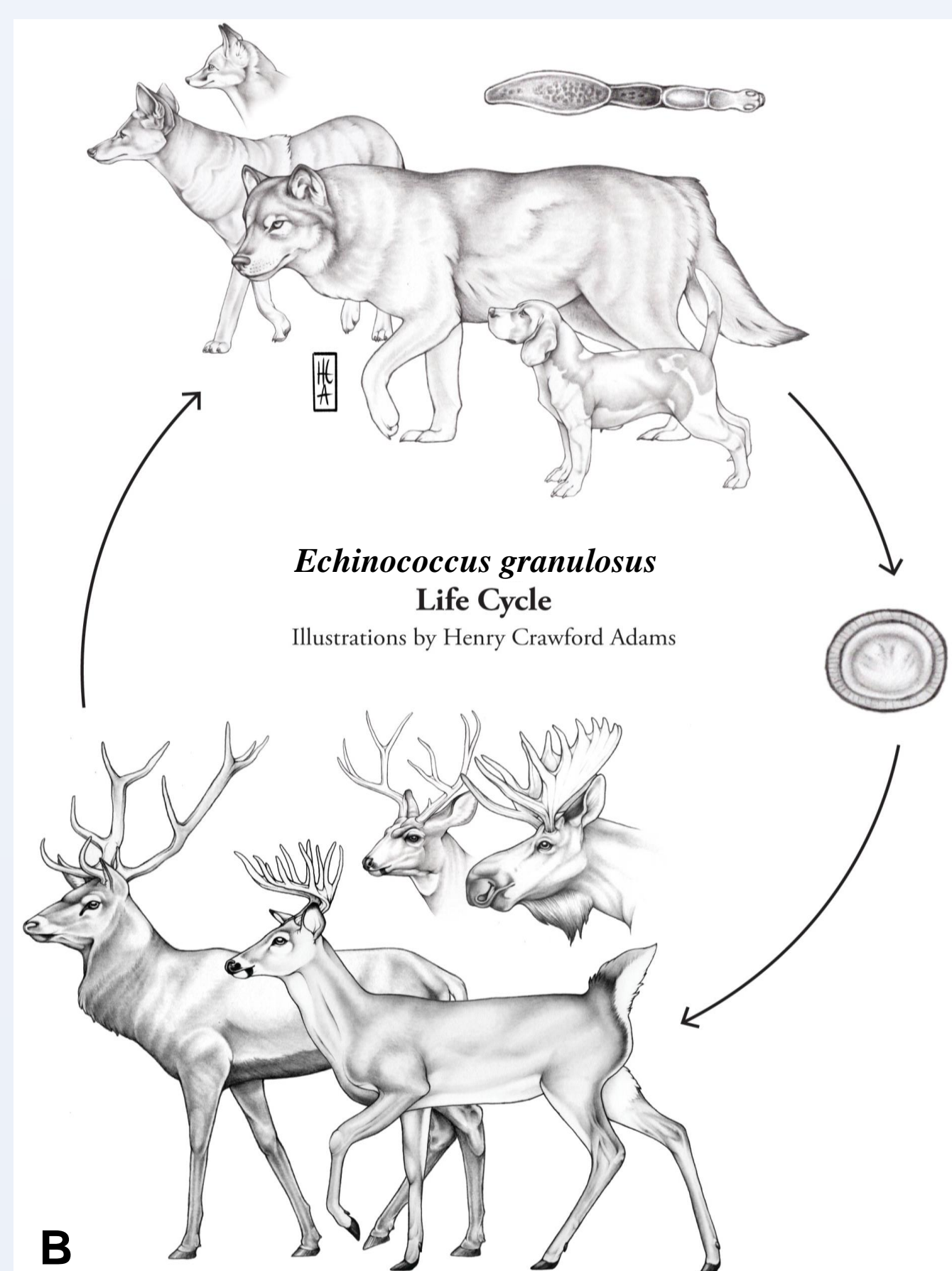
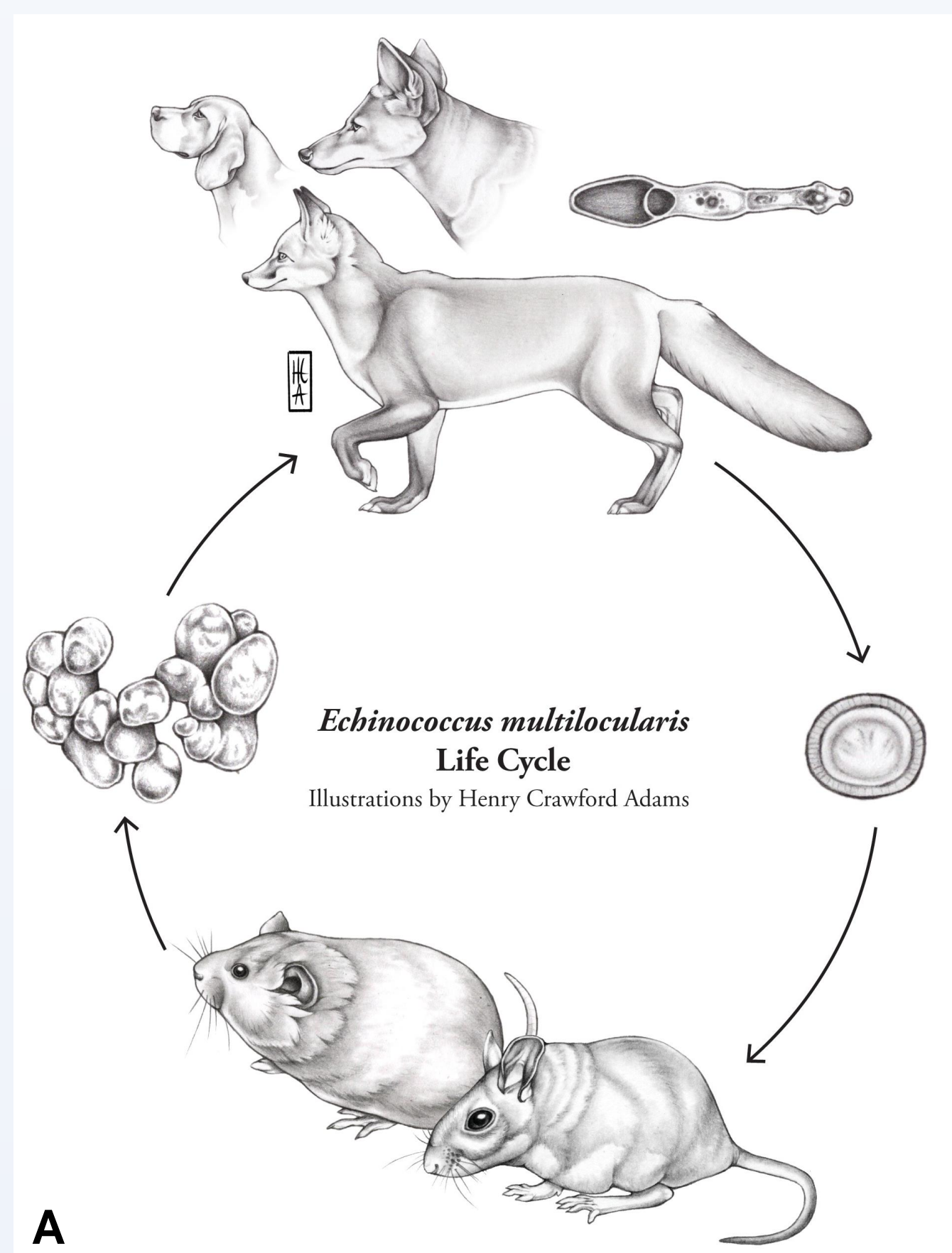


Figure 1. Lifecycles of (A) *E. multilocularis* and (B) *E. granulosus*. Diagrams courtesy of Henry Adams.

RESEARCH OBJECTIVE & HYPOTHESIS

- Different fecal flotation methods are employed by researchers and veterinary clinics, yet a *gold standard* test has not yet been widely adopted.
- The objective of this project was to compare the sensitivity & detection limit of three methods:
 - 1) Centrifugal
 - 2) Passive
 - 3) Mini-FLOTAC
- **Hypothesis:** Of the three methods, the centrifugal fecal float will be most sensitive for the detection of *Echinococcus* spp. eggs.

MATERIALS & METHODS

- Isolated eggs from *Echinococcus* spp. proglottids
- Spiked negative fecal samples with known concentrations of *Echinococcus* spp. eggs: 25, 40, and 60 eggs per gram of feces.
- Performed 6 replicates of each method^{7,8,9} using zinc sulfate flotation solution (1.18 SG):

Centrifugal fecal float

- Homogenized 10mL flotation solution with 1g feces.
- Centrifuged for 10 minutes at 500 rpm then read.

Passive fecal float

- Homogenized 1g of feces with 10mL flotation solution.
- Let sit in conical tube for 20 minutes then read.

Mini-FLOTAC

- Homogenized 18mL flotation solution with 2g feces.
- Filled chambers, let sit 10 minutes, then read.



Figure 2. (A) Centrifuging fecal samples. (B) *Echinococcus* spp. egg.

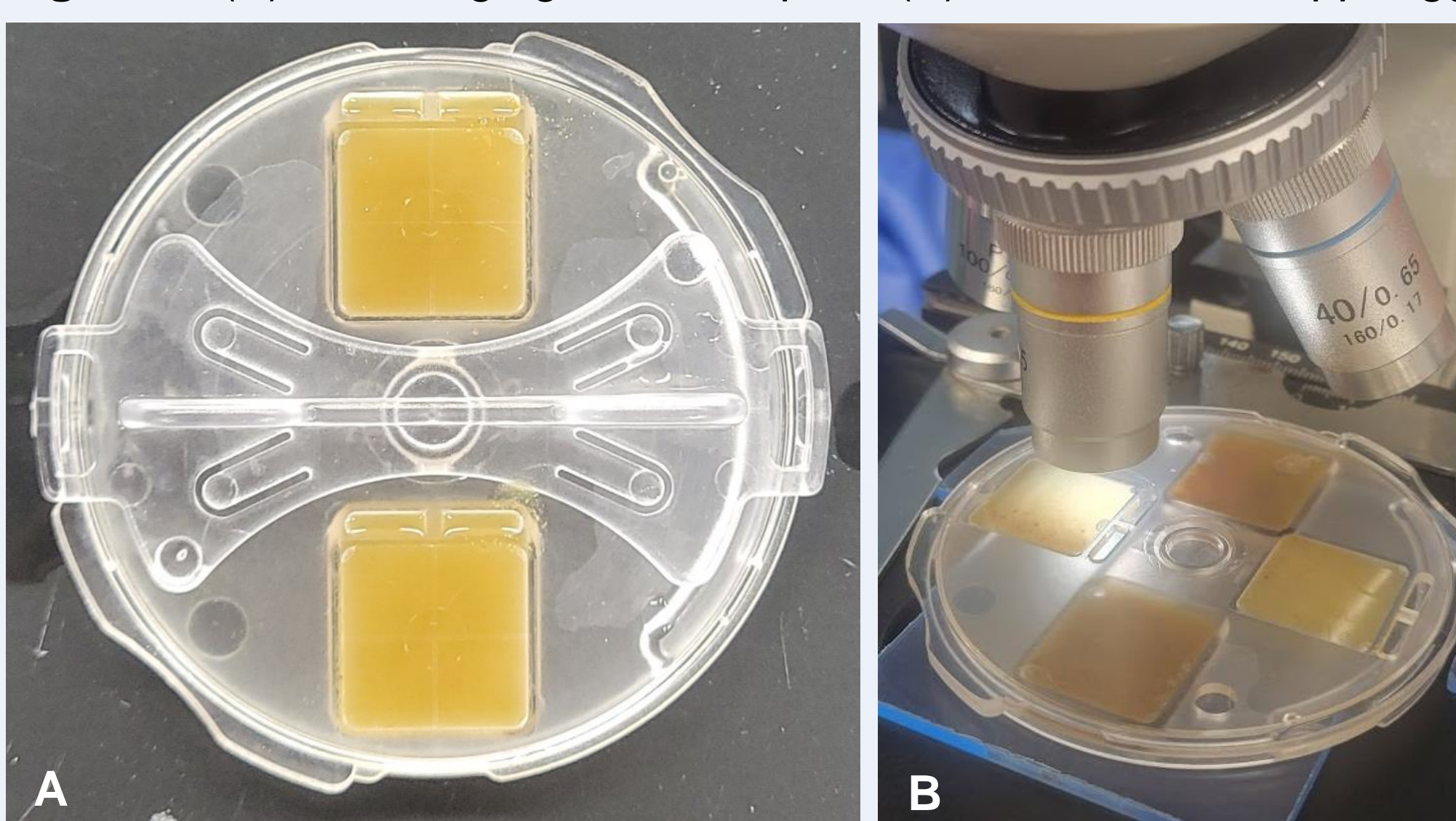


Figure 3. Mini-FLOTAC apparatus. (A) Filled chambers. (B) Mini-FLOTAC apparatus under microscope for reading.

RESULTS

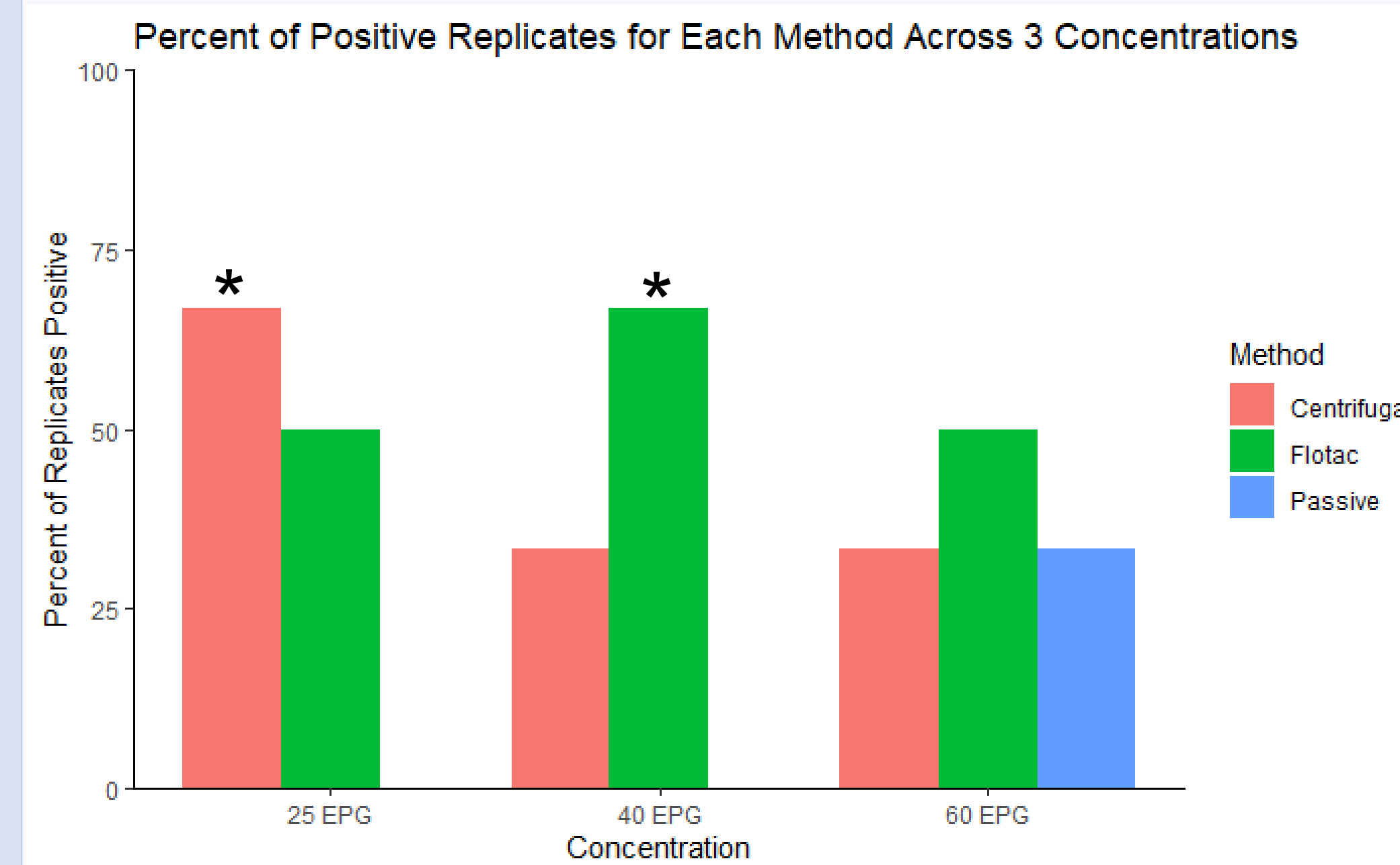


Figure 4. Percentage of replicates that were positive across three methods and concentrations. Asterisks denote statistically significant differences at a significance level of 0.05. EPG = eggs per gram.

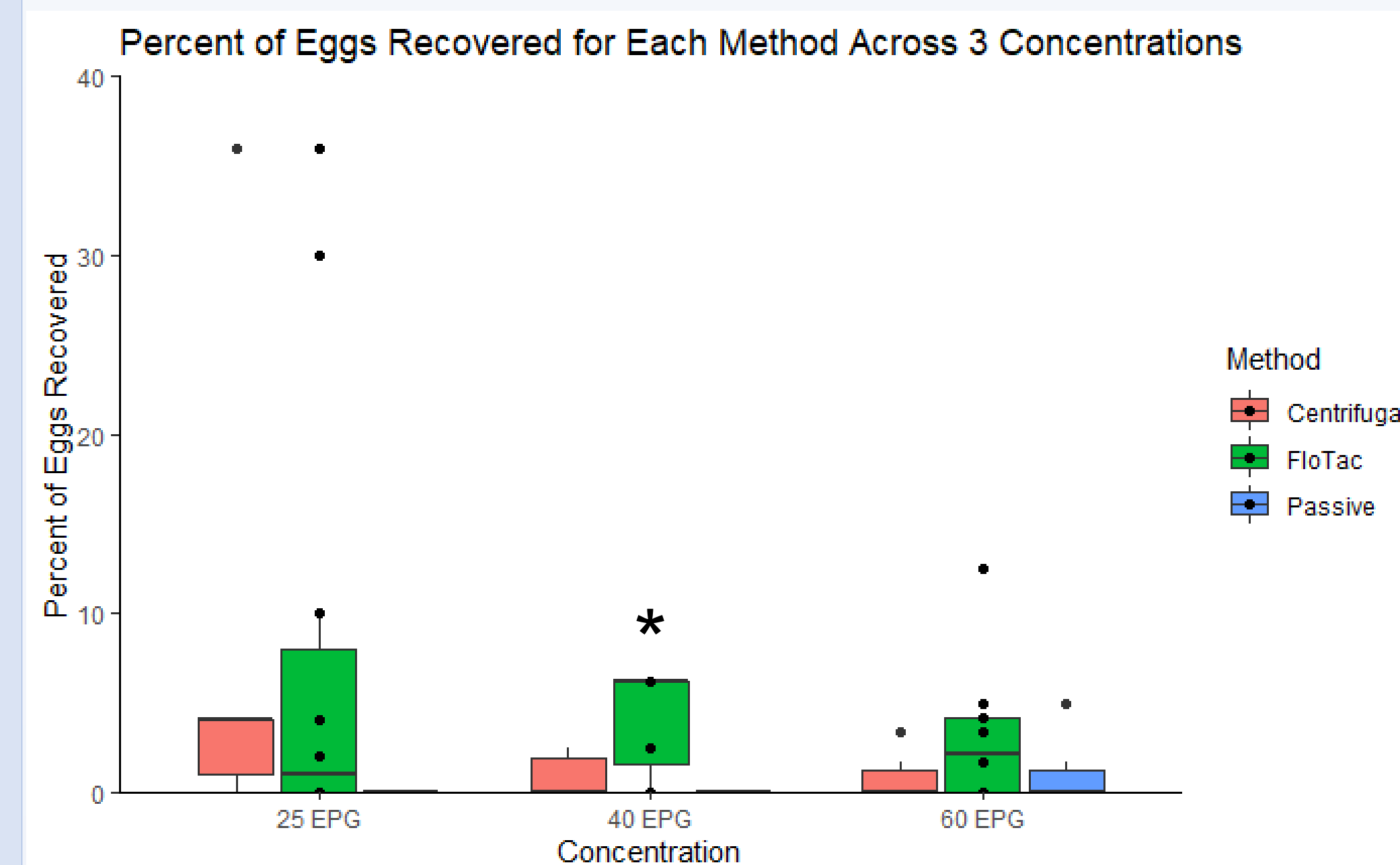


Figure 5. Percent recovery of eggs across three different methods at differing concentrations. Asterisk denotes a statistically significant difference at a significance level of 0.05. EPG = eggs per gram.

LIMITATIONS

- Eggs adhering to tools during sample preparation could result in lower concentrations.⁴
- Human error in collecting and counting eggs.
- Centrifugal fecal floats are prone to loss of coverslips during centrifugation.

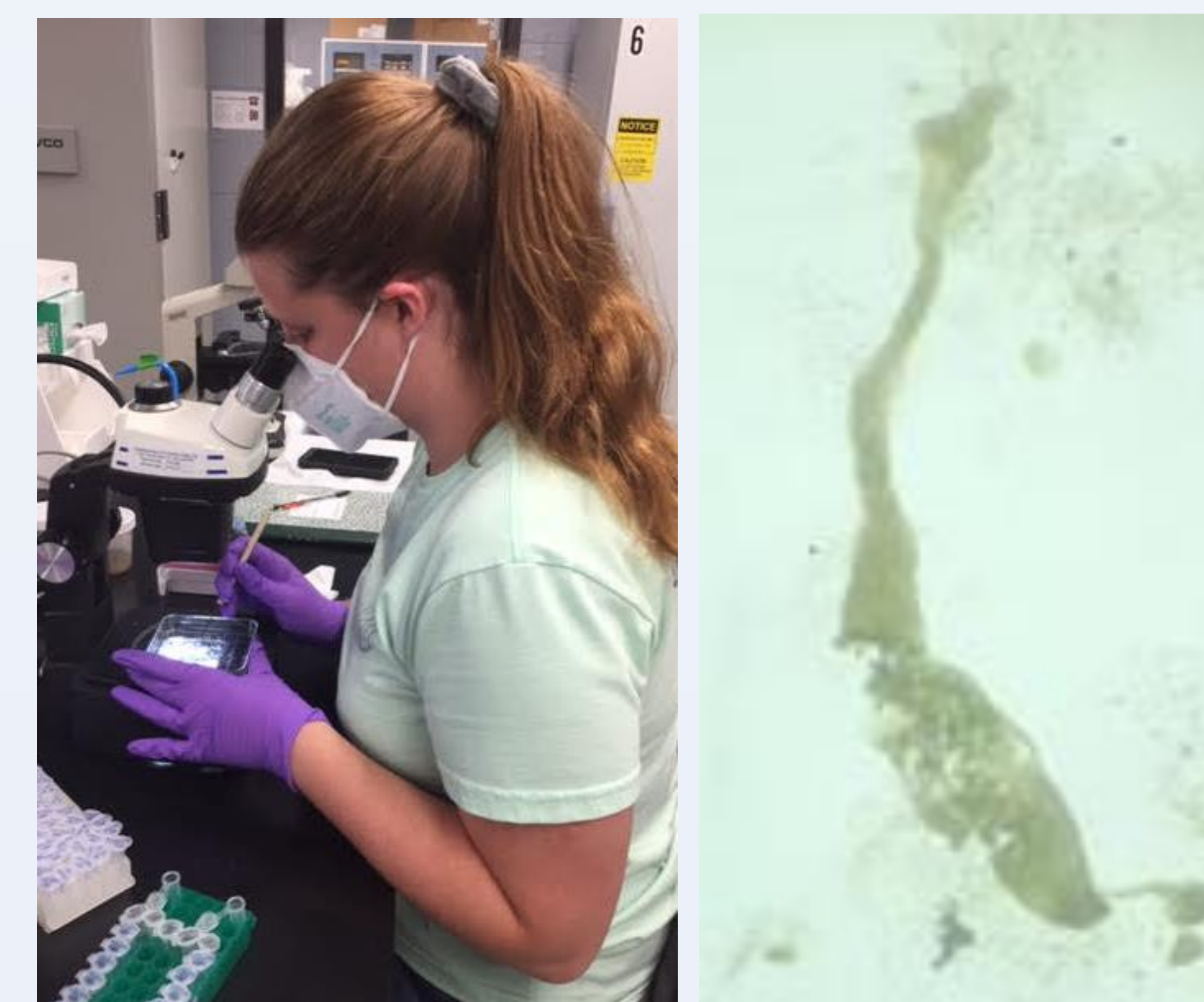


Figure 6. Isolating *Echinococcus* sp. eggs.

CONCLUSIONS & SIGNIFICANCE

- Centrifugal flotation and the Mini-FLOTAC had more positive results than passive flotation.
- The Mini-FLOTAC tended to detect more *Echinococcus* spp. eggs than the other methods.
- FLOTAC appears to be the best fecal flotation method to detect *Echinococcus* spp. eggs.
- Veterinarians in previously unaffected regions may not screen for *Echinococcus* spp.
- Public & veterinary health concern:
 - Alveolar echinococcosis (AE) is particularly harmful to humans. Causative agent: *E. multilocularis*.⁵
 - Cystic hydatid disease (CE) is less pathogenic. Causative agent: *E. granulosus*.⁵
- *Echinococcus* spp. currently infect 2-3 million people worldwide, costing \$200-800 million annually.⁶
- Infected livestock produce less meat, milk, and wool, resulting in an estimated \$1.5-\$2 billion USD loss annually.⁶
- Reliable detection methods are needed to address increasing *Echinococcus* spp. infections.

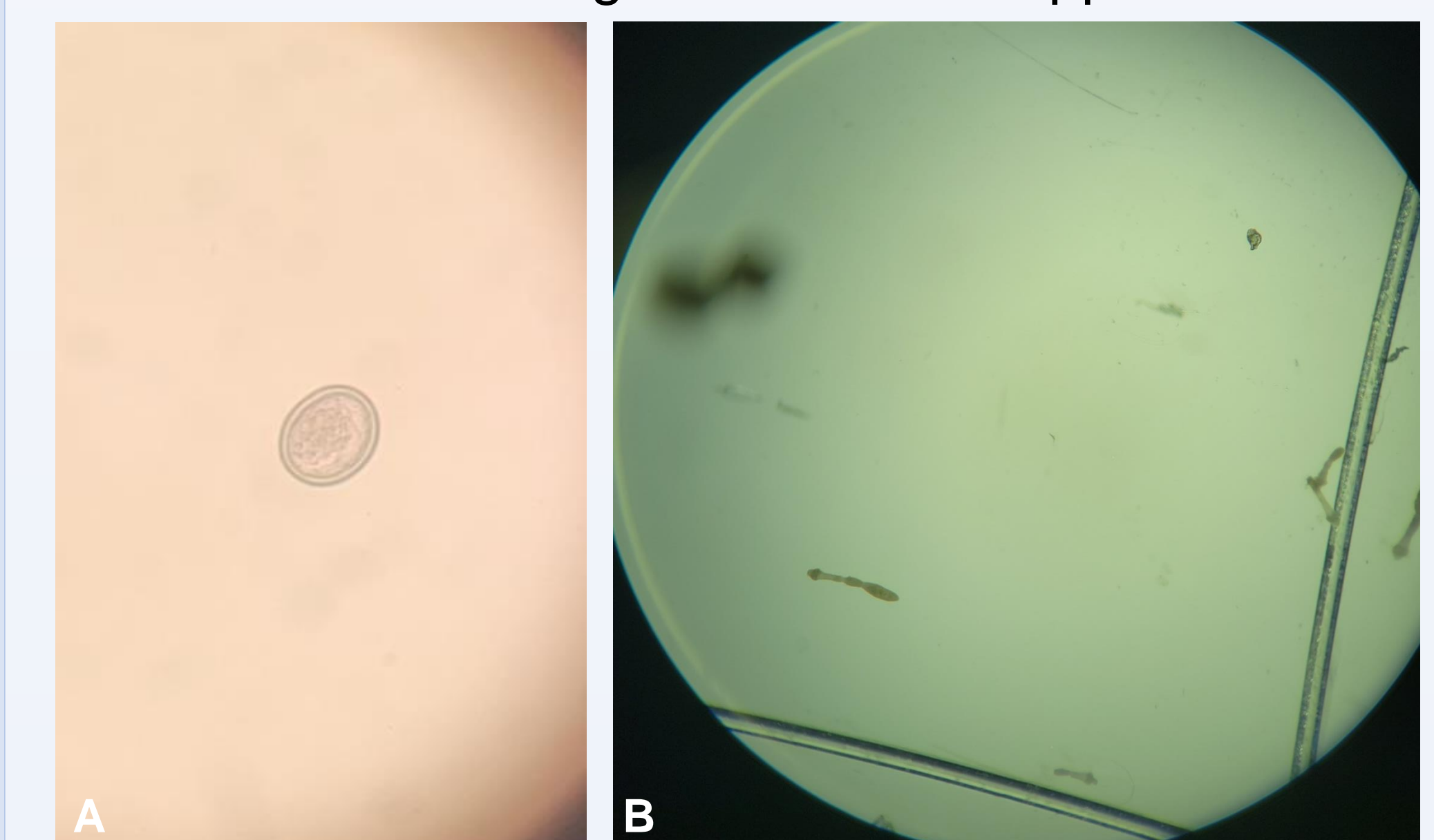


Figure 7. (A) *Echinococcus* spp. egg with distinct membrane. (B) *Echinococcus* spp. present in small intestinal sieve.

FUTURE DIRECTIONS

- Continue data collection using larger sample sizes and different egg concentrations
- Co-author a manuscript detailing fecal flotation methods best suited for detection of *Echinococcus* spp. eggs.

LITERATURE CITED

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