



The Inactivation of *Ascaris suum* Eggs by Short Exposure to High Temperatures for the Purpose of Sanitizing VIP Latrine Sludge by Viscous Heating.

Danica Naidoo

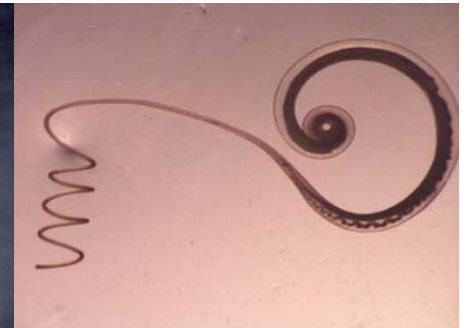
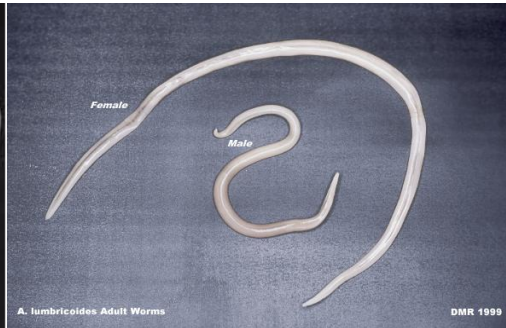
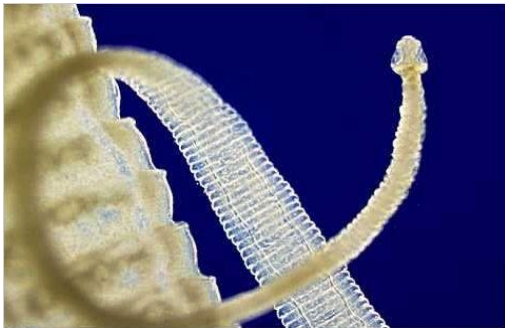
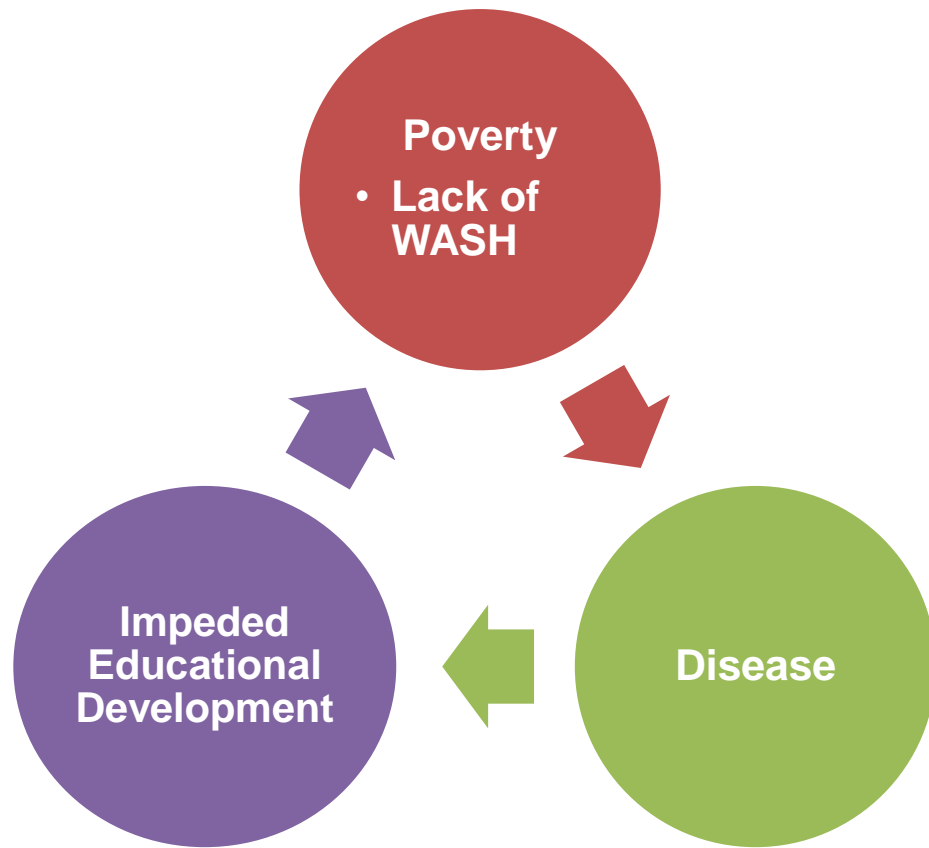
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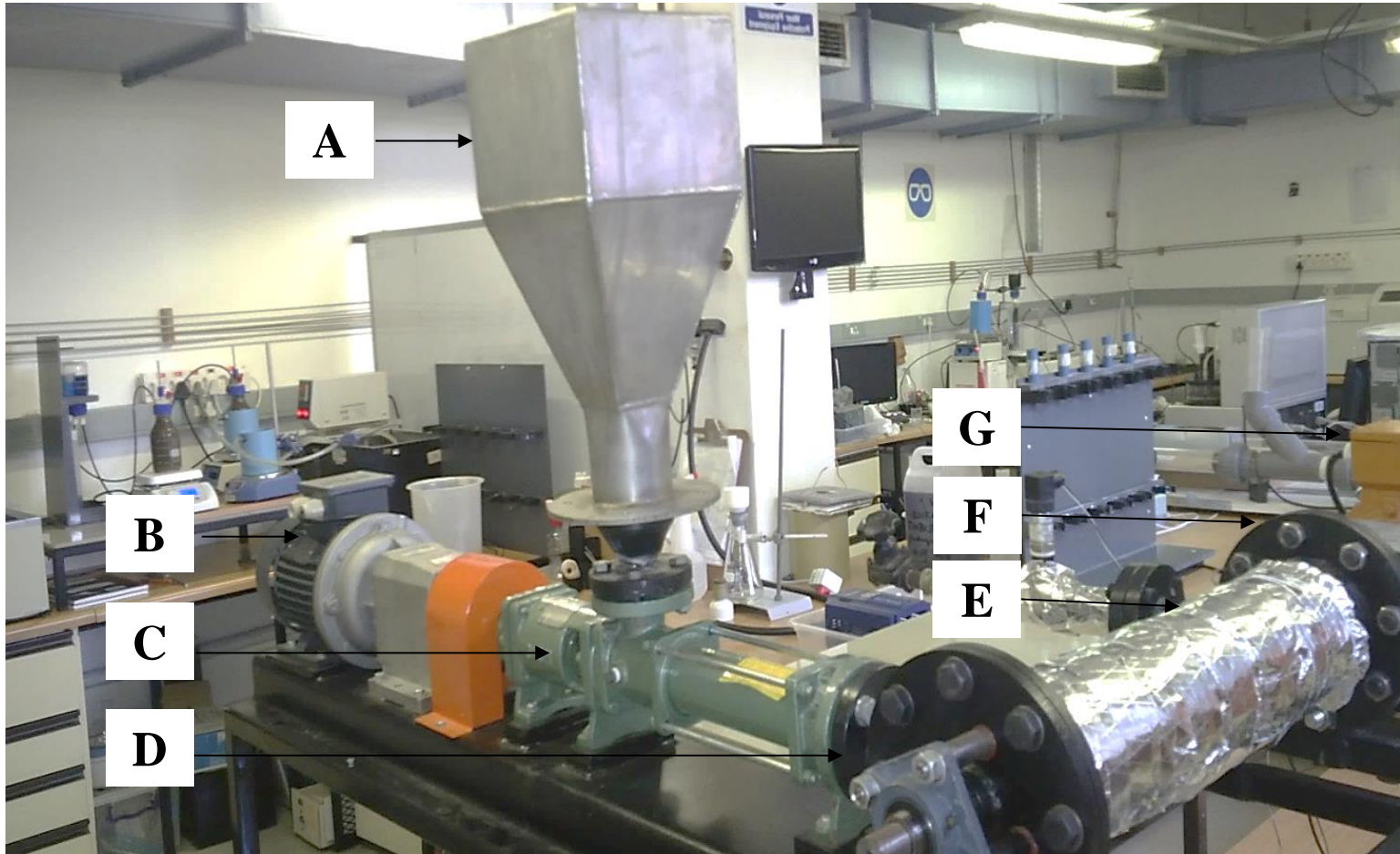


**BILL & MELINDA
GATES foundation**



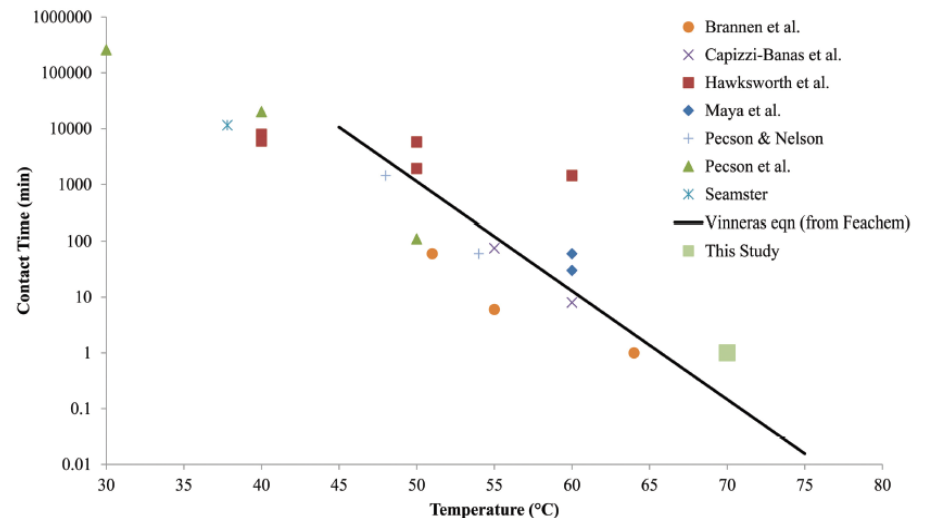


Viscous Heating Device

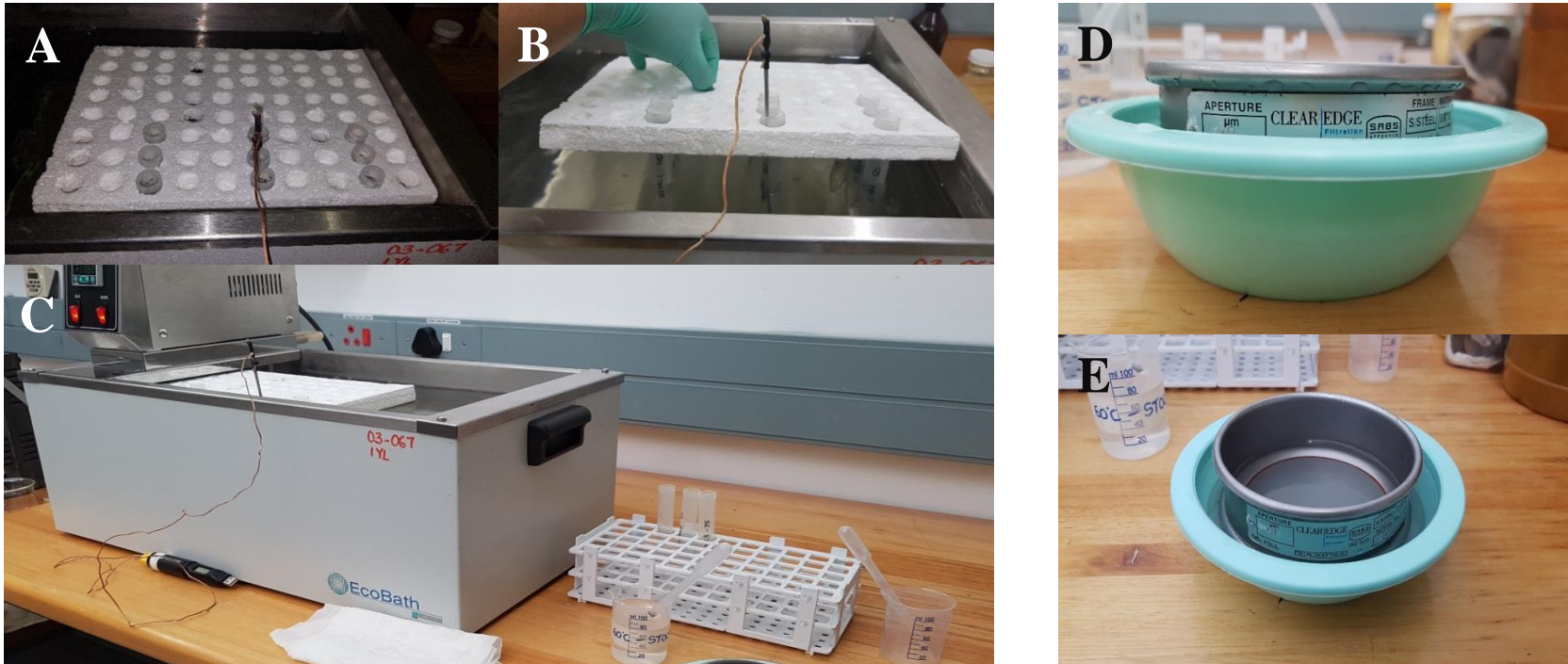


Rationale and Aims:

- Previous studies on viscous heating reported exposure times of 2-80 seconds sufficient for egg inactivation.
- Literature has focused on egg inactivation by heat treatment with exposure times of 5 minutes and above.
- Most studies also focused on the inactivation of eggs suspended in sludge.
- This study therefore aimed to investigate the effects of heat on isolated eggs when treated at short exposure times for the purpose of the viscous heating device:
- At what time*temperature combination do eggs die-off?

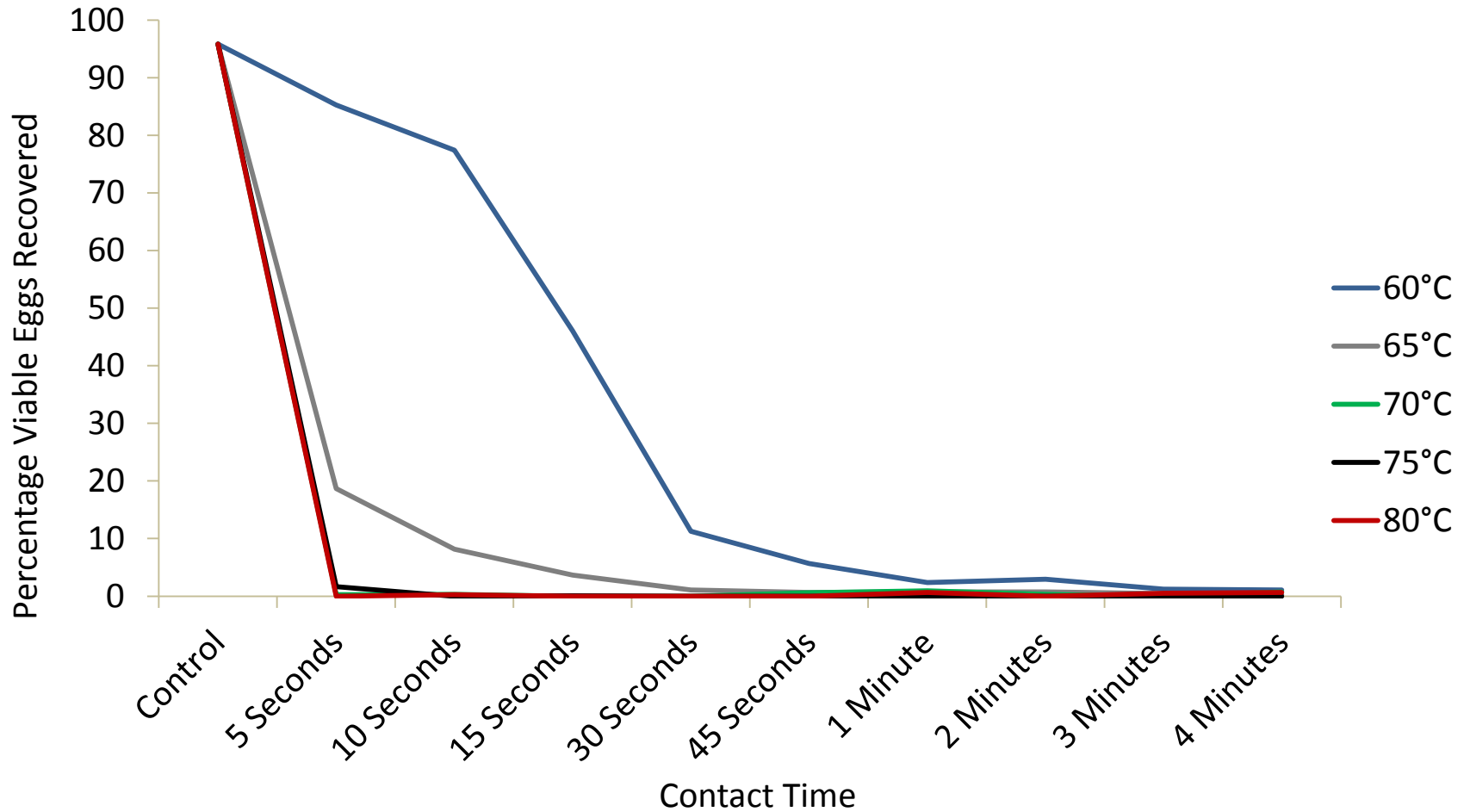


Methodology

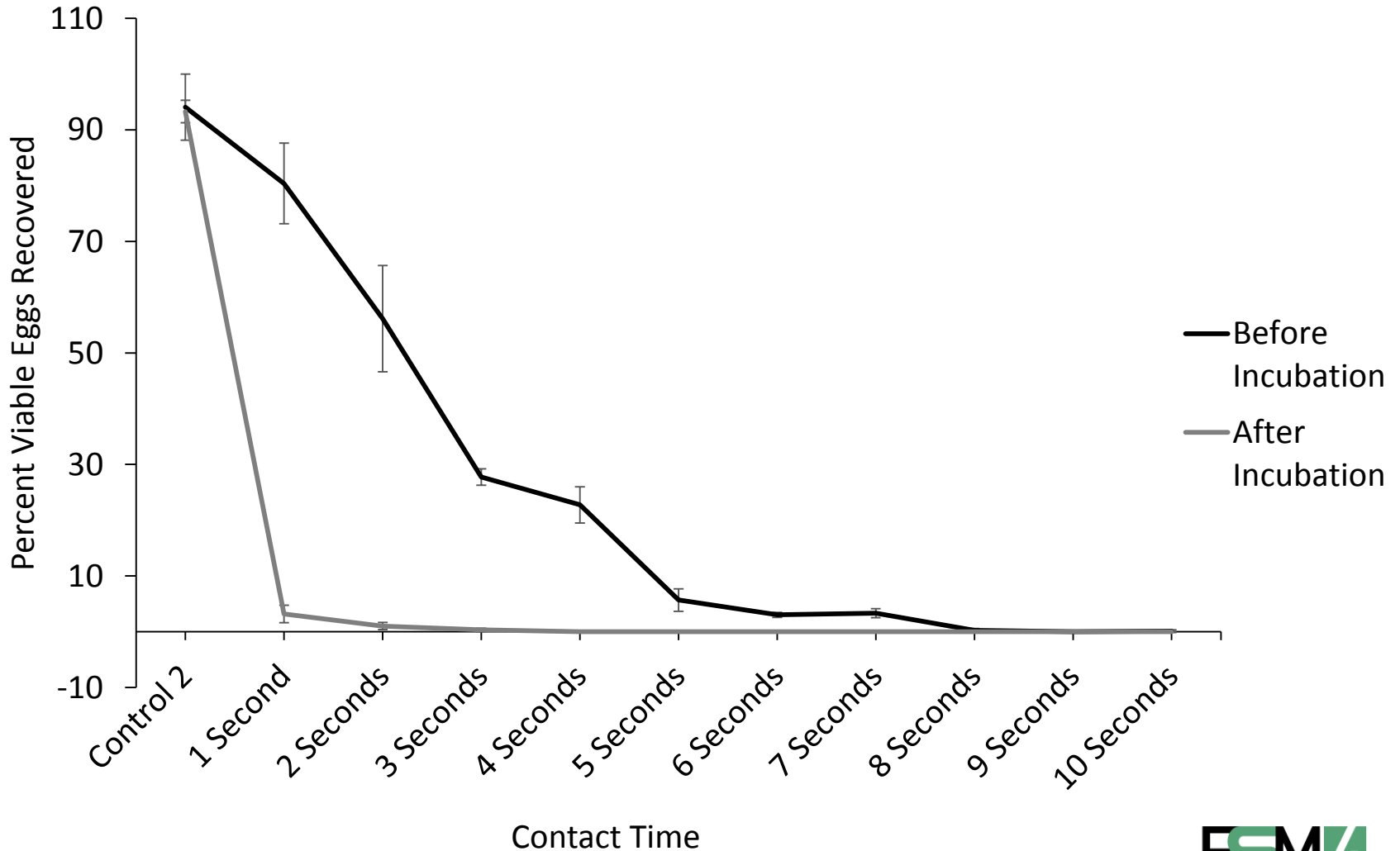


- Test temperatures: 60°C, 65°C, 70°C, 75°C and 80°C.
- Tested exposure times: 5, 10, 15, 30 and 45 seconds, and 1, 2, 3 and 4 minutes.
- Criterion for successful inactivation: <10% viable eggs recovered after treatment.

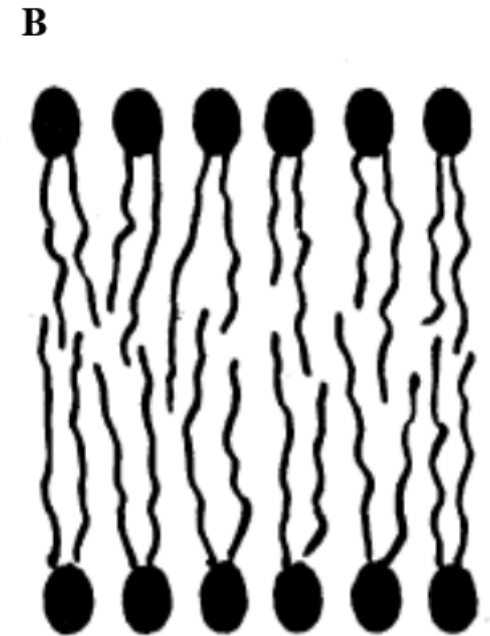
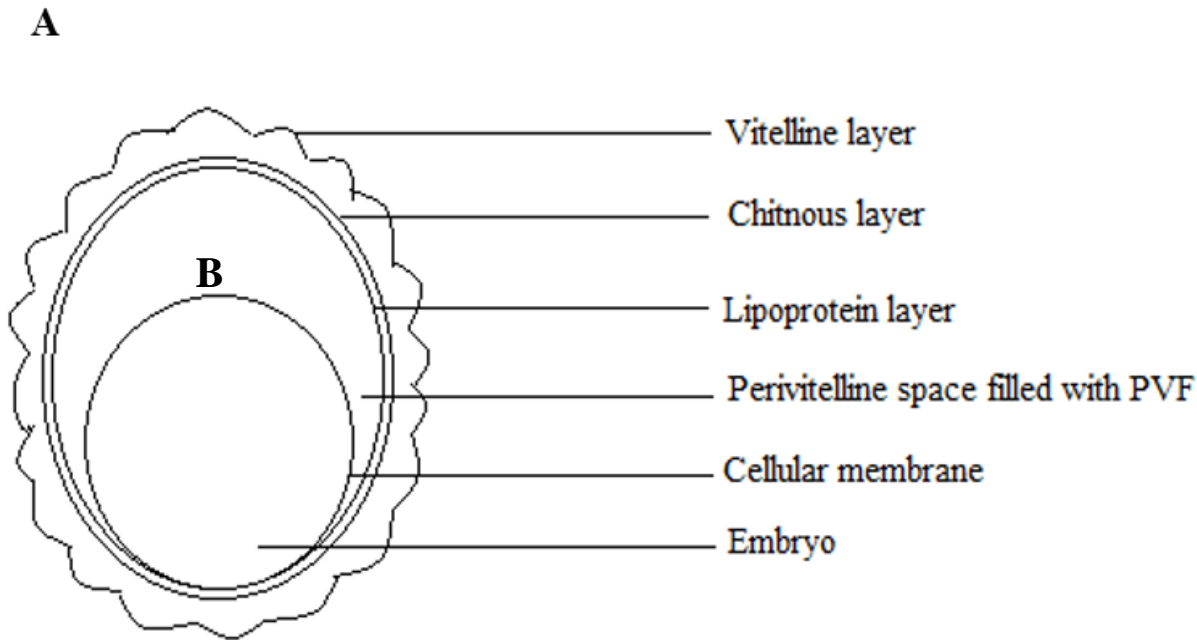
Overall Inactivation Data:



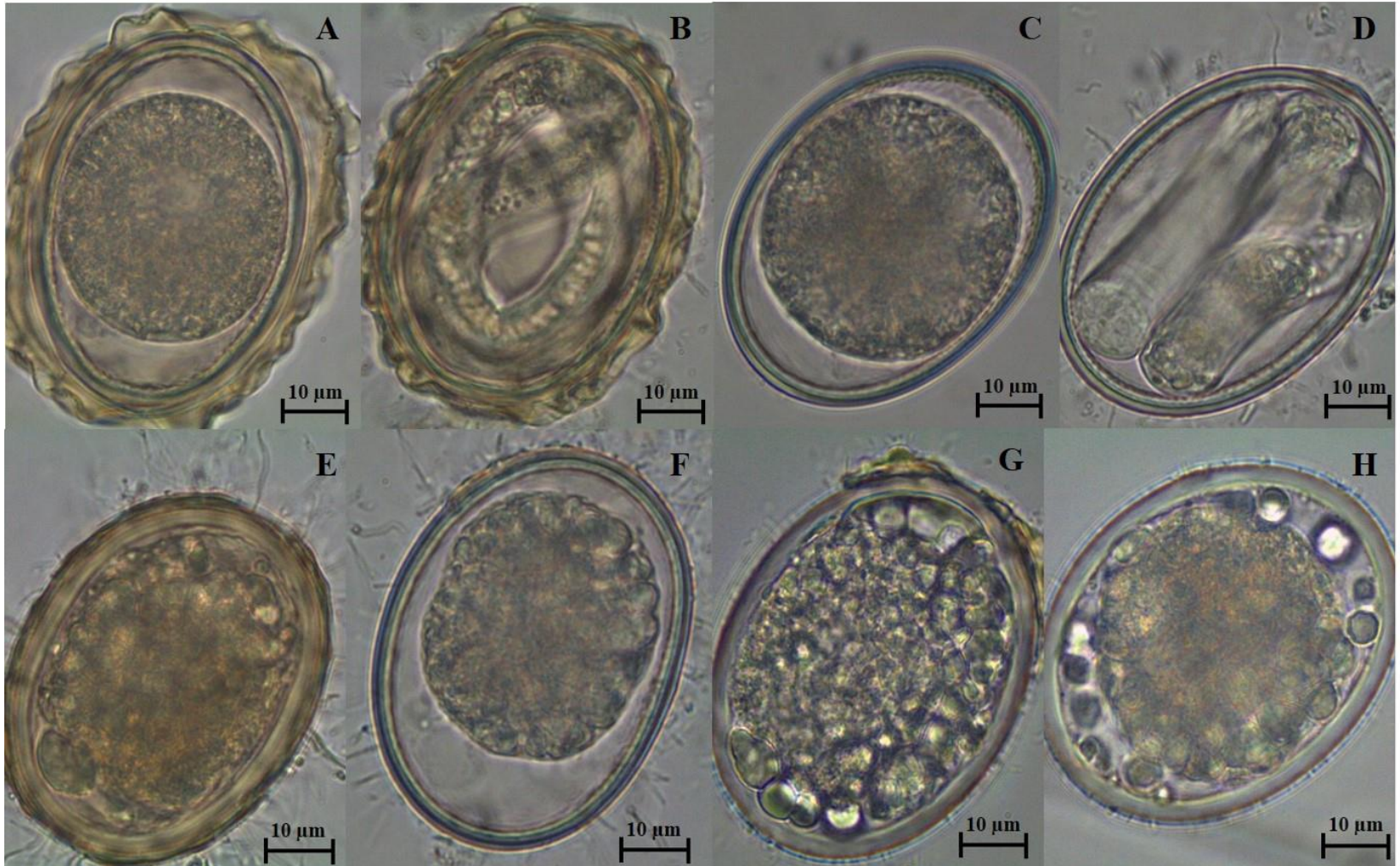
Inactivation at 80°C (1 – 10 seconds):



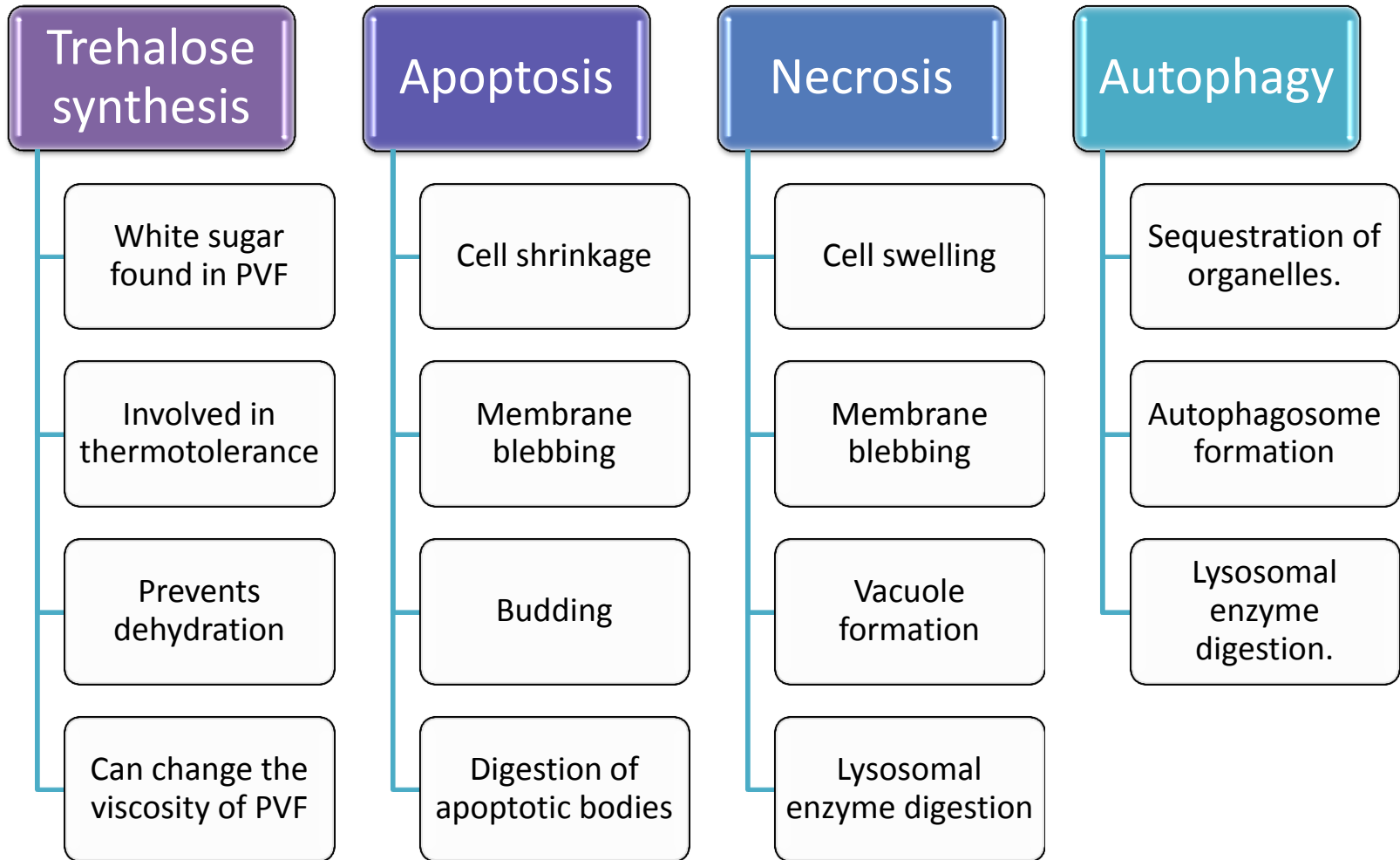
Morphology of *Ascaris* egg



Observed egg morphology:



Trehalose and Programmed Cell Death (PCD):



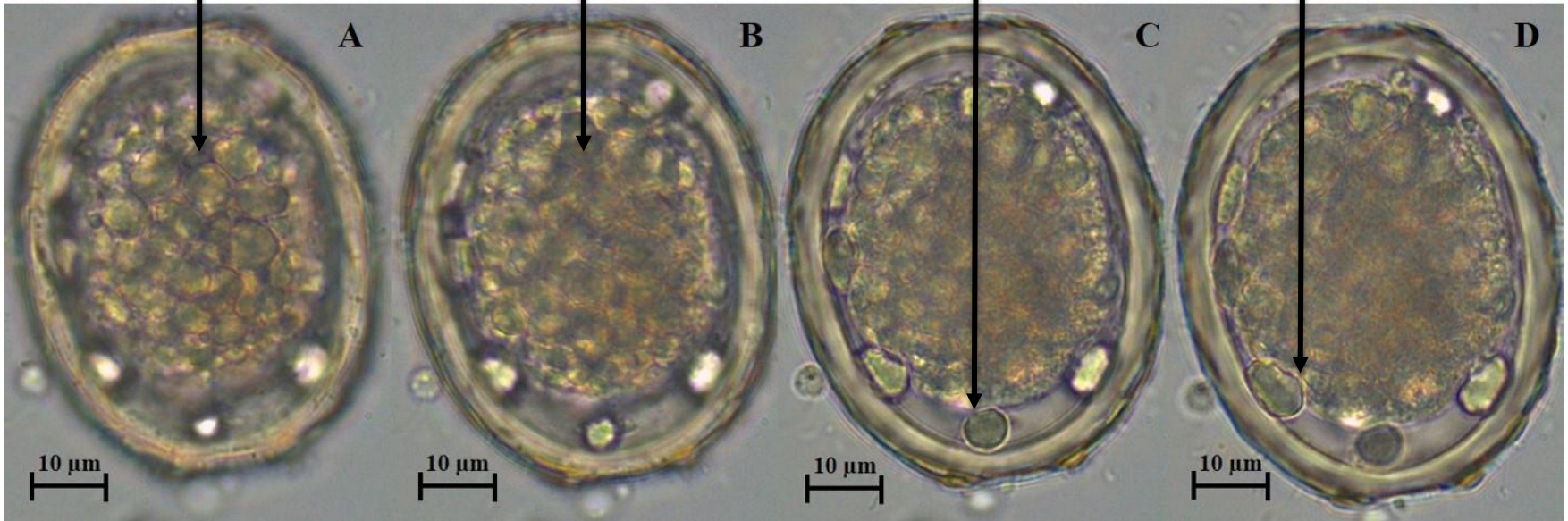
Treated egg showing globule formation:

Globule formation –
shift in
membrane
fluidity

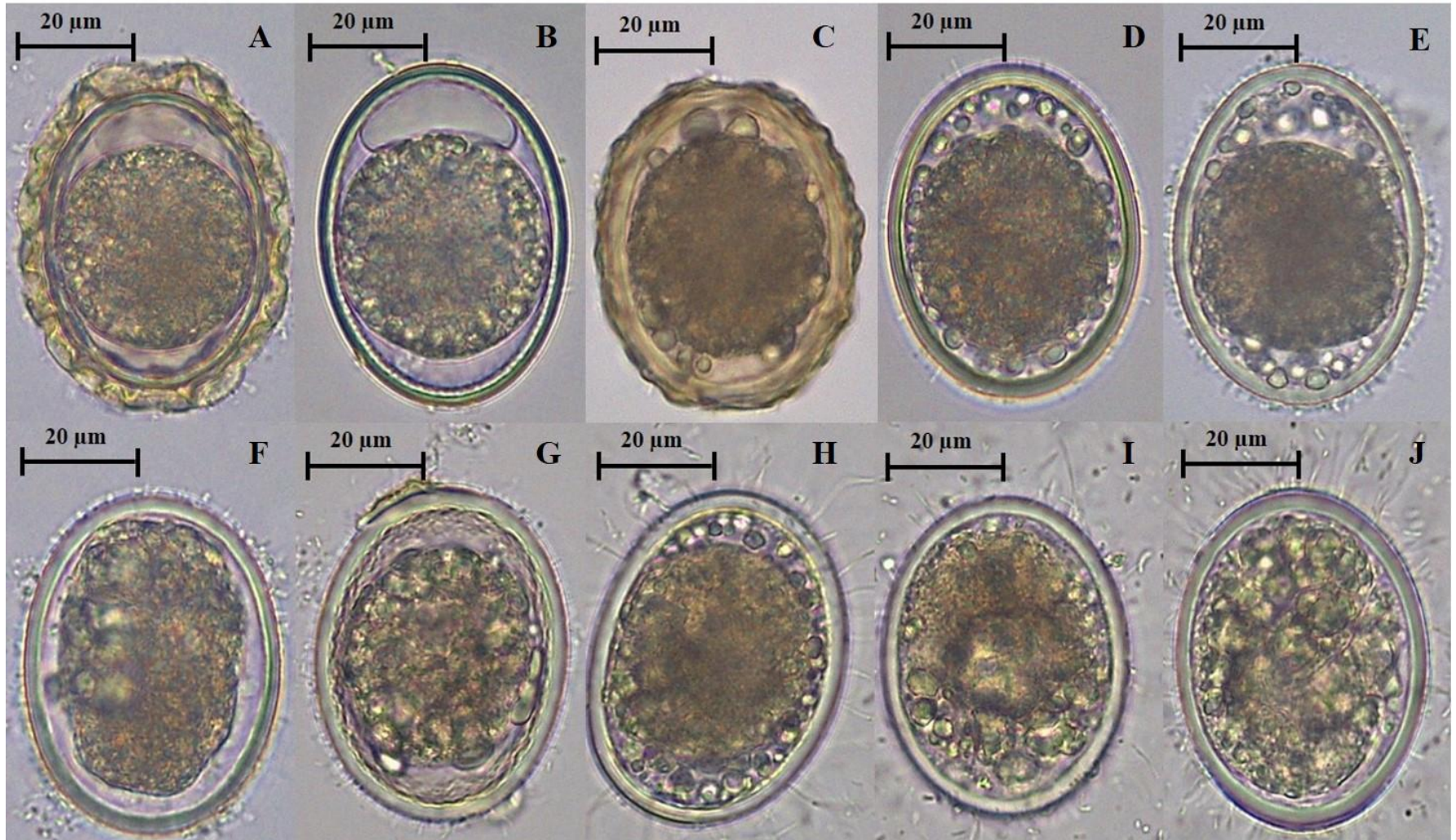
Possible
membrane
blebbing –
apoptosis or
necrosis

Possible
budding -
apoptosis

Possible
vacuole
formation -
autophagy



Egg damage at 80°C, after incubation:



Conclusions:

Table 1: Summary of the results for each test temperature of the current study.

Temperature (°C)	Damage Not Visible	Visible Damage	Complete Die-off
60°C	30 seconds	3 minutes	-
65°C	15 seconds	3 minutes	-
70°C	5 seconds	2 minutes	15 seconds
75°C	5 seconds	1 minute	10 seconds
80°C	-	5 seconds	5 seconds
80°C - *Short	1 second	4 seconds	4 seconds



Conclusions:

- 80°C may be recommended as the most effective temperature for application to the viscous heating device.
- 70°C and 75°C may also be recommended, however longer exposure times may be required to ensure successful inactivation.
- Further investigation is needed to determine the effects of heat on eggs in sludge: does sludge provide insulation to the eggs?
- Further investigation is also required to investigate the effects of viscous heating on eggs in sludge.
- Egg damage: molecular and cellular work is required to determine the mode-of-action of heat stress: apoptosis, necrosis or autophagy?

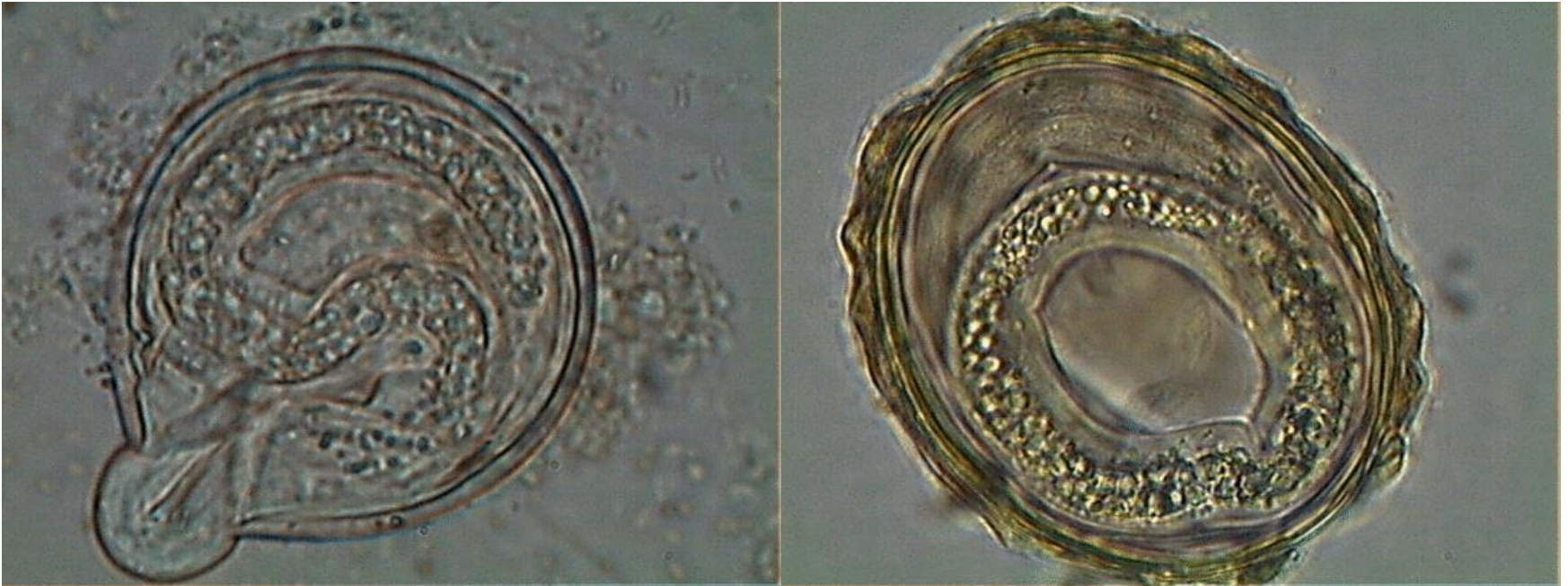


References:

- Ayçiçek, H., Yarsan, E., Sarimehmetoğlu, H.O., Tanyüksel, M., Girginkardeşler, N., Özyurt, M. (2001) Efficacy of some disinfectants on embryonated eggs of *Toxocara canis*. Turk. J. Med. Sci **31**, 35-39.
- Bardosh, K. (2015) Achieving “Total Sanitation” in rural African geographies: poverty, participation and pit latrines in Eastern Zambia. Geoforum **66**, 53-63.
- Behm, C. A. (1997). The role of trehalose in the physiology of nematodes. Int. J. Parasitol **27** (2), 215 – 229.
- Belcher, D., Foutch, G.L., Smay, J., Archer, C. and Buckley, C.A. (2015) Viscous heating effect on deactivation of helminth eggs in ventilated improved pit sludge. Wat. Sci. Tech. **72** (7), 1119-1126.
- Bhagwan, J.N., Still, D., Buckley, C. and Foxon, K. (2008) When last did we look down the pits. *WISA Paper*.
- Brownell, S.A. and Nelson, K.L. (2006) Inactivation of single-celled *Ascaris suum* eggs by low-pressure UV radiation. Appl. Environ. Microbiol. **72** (3), 2178-2184.
- Dunn Jr, W. A., 1990. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. J. Cell. Biol., **110** (6), 1923-1933.
- Elmore, S. (2007). Apoptosis: a review of programmed cell death. Tox. Path **35** (4), 495 - 516.
- Fewtrell, L., Kaufmann, R.B., Kay, D., Enanoria, W., Haller, L. and Colford, J.M. (2005) Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. Lancet. Infect. Dis. **5** (1), 42-52.
- Podichetty, J.T., Islam, M.W., Van, D., Foutch, G.L. and Johannes, A.H. (2014) Viscous heating analysis of simulant feces by computational fluid dynamics and experimentation. J. Water. Sanit. Hyg. Dev. **4** (1), 62-71.
- Voronina, E., Wessel, G. M. (2001). Apoptosis in sea urchin oocytes, eggs, and early embryos. Mol Reprod. Dev. **60** (4), 553 - 561.



Thank You!!



A special thank you to Dr. Gary Foutch, Professor Christopher Appleton, Colleen Archer and Professor Chris Buckley for continuous guidance and support. Thank you to the NRF and the Bill and Melinda Gates Foundation for funding this project. Much appreciation goes out to PRG and UKZN as well.