A modified assay for the enumeration of ascaris eggs in fresh raw sewage

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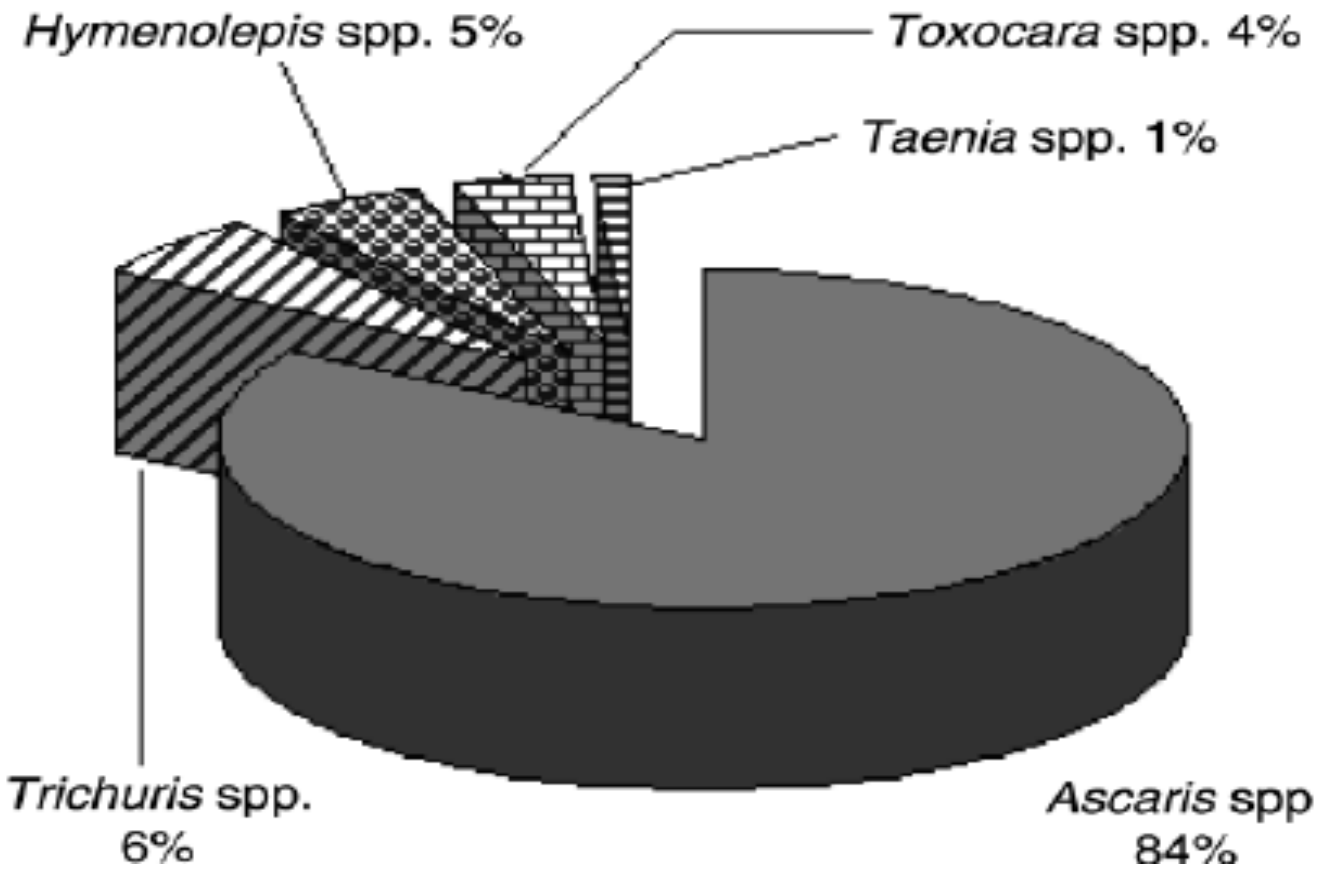
PATHOGENS in sewage

Organism	Numbers in Raw Sewage per Litre
Bacteria	
Escherichia coli (indicator)	10^5 -10^{10}
E. coli (pathogenic)	Low
Enterococci (indicator)	10^6 -10^7
Shigella	10^1 -10^4
Salmonella	10^3 -10^5
Clostridium perfringens (indicator)	10^5 -10^6
Viruses	
Enteroviruses	10^2 -10^6
Adenoviruses	10^1 -10^4
Noroviruses	10^1 -10^4
Rotaviruses	10^2 -10^5
Somatic coliphages (indicator)	10^6 -10^9
F-RNA coliphages (indicator)	10^5 -10^7
Protozoa	
Cryptosporidium sp.	0 -10^4
Entamoeba histolytica	4.5 x 10^4
Giardia sp.	10^2 -10^5
Helminth ova	
Ascaris lumbricoides	10^2
Hookworm	10 - 10^2

Distribution of helminth ova genera in wastewater



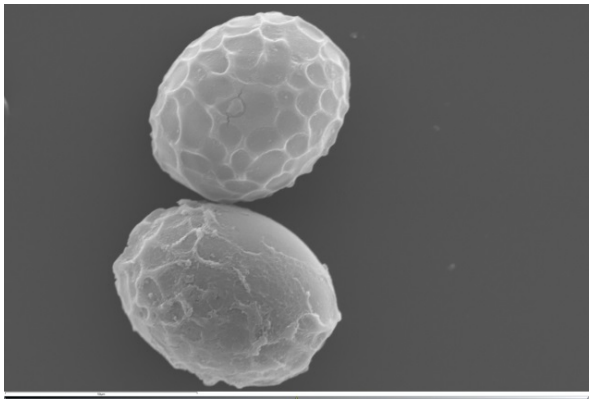
Source: Jiminez *et al*., 2007

Ascaris – The neglected parasite

- 1.5 billion people infected worldwide
- Enormous egg production (240,000 eggs/ day/ female)
- Eggs are highly resistant and may remain viable for several years

Fertlized egg

Unfertlized egg



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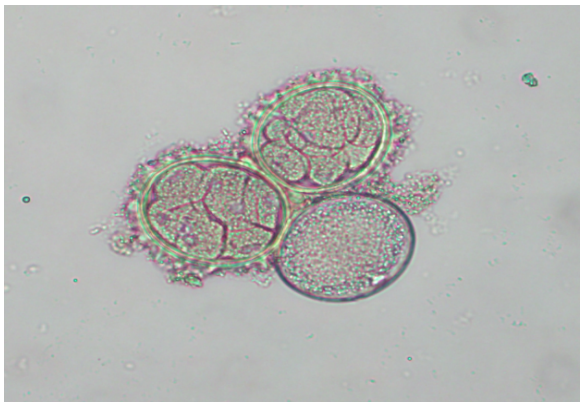
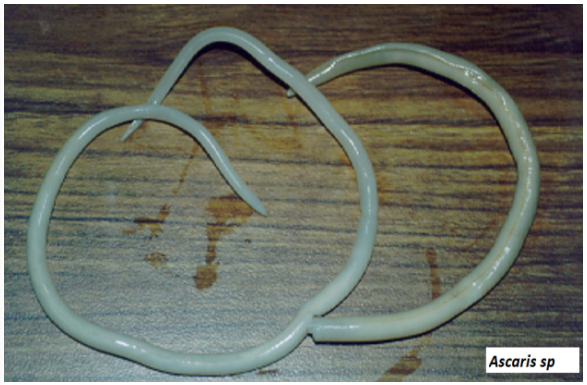


A.Suum 7 days embryonation

larvae emerging from the egg



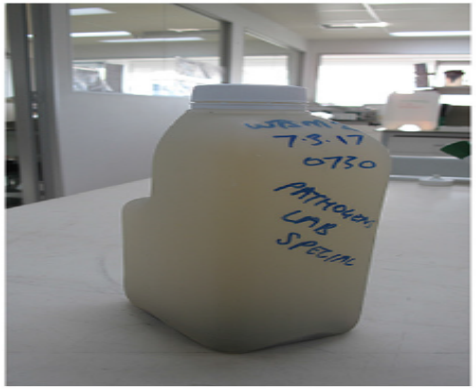
Adult worms

AIM & OBJECTIVES

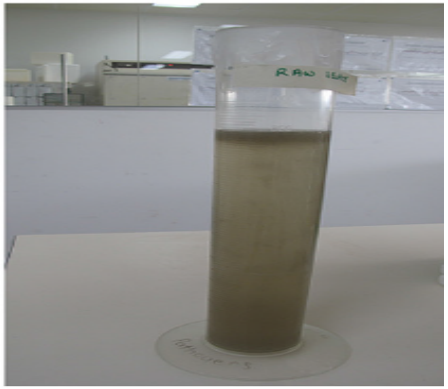
- Optimize the concentration and enumeration of helminth eggs

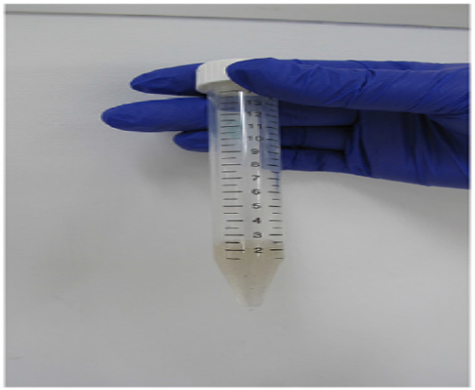
- Develop a reproducible and cost effective protocol for yielding better recovery rates of eggs from sewage

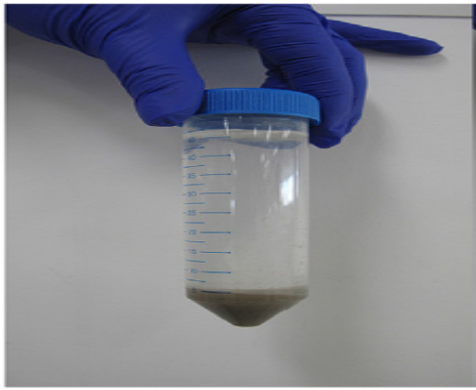
MODIFIED BOWMAN METHOD

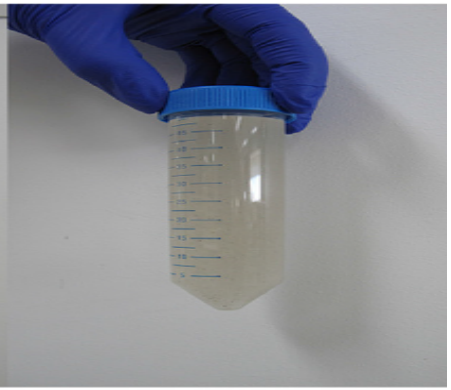

Raw sewage sample after overnight sedimentation


Blending of sample


Sedimentation of blended sample in graduated cylinder


Final sample concentrate

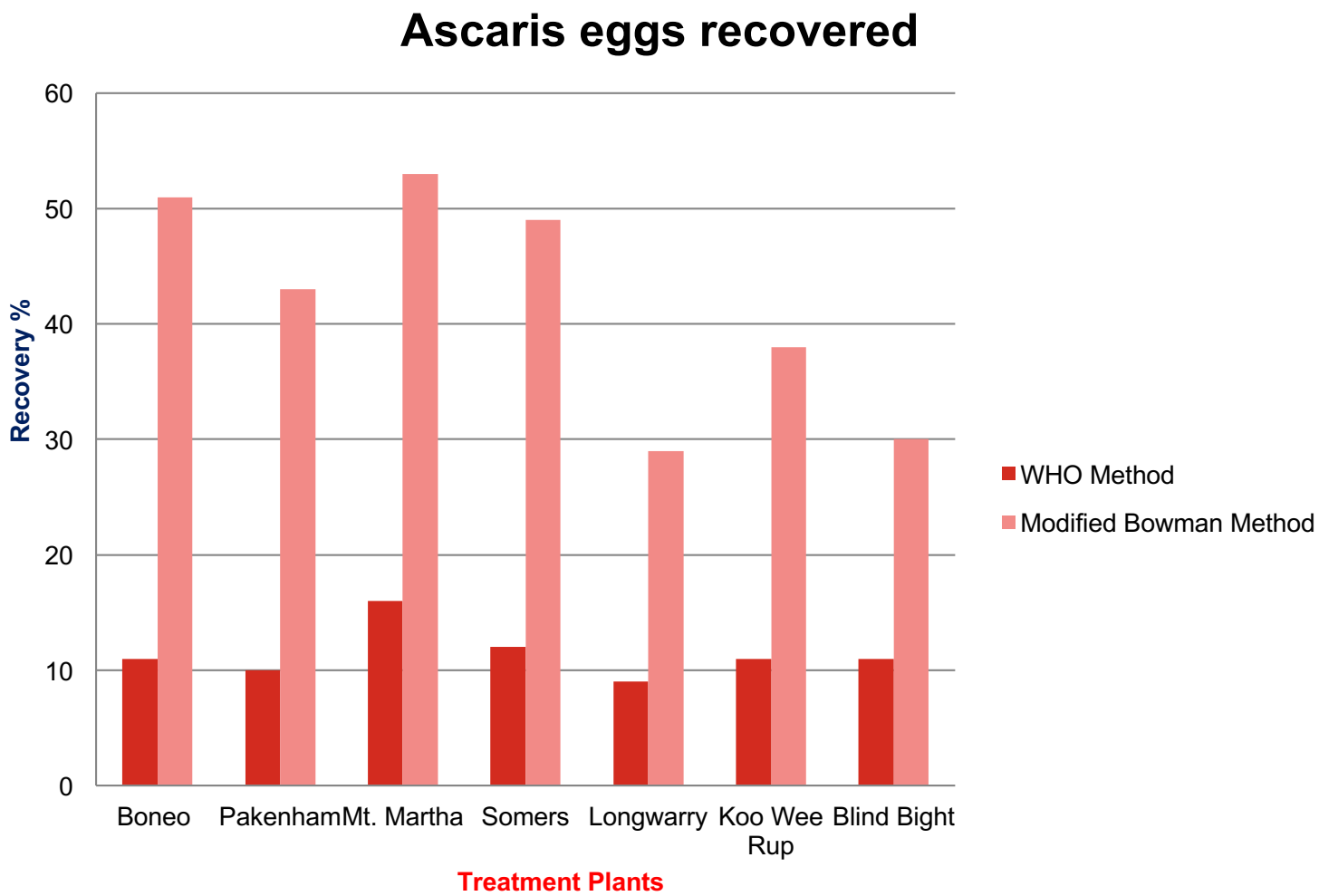

Sample concentrate after flotation step


Sample concentrate in magnesium sulphate flotation solution


Final sample concentrate in Whitlock counting chamber


Microscopic examination of sample for helminth eggs

Results


Ascaris eggs recovered
Recovery %
60
50
40
30
20
10
0
Boneo
Pakenham
Mt. Martha
Somers
Longwarry
Koo Wee Rup
Blind Bight
Treatment Plants
WHO Method
Modified Bowman Method

Conclusion

- Better recovery of 42% with modified Bowman method compared to the standard Bowman method
- Modified Bowman method is a simple, low cost method for routine use by commercial laboratories and researchers
- Need to improve identification by developing and comparing methods of detection
- Ongoing identification methods in my project:
  - Propidium Monoazide-qPCR to detect viable Ascaris ova from wastewater
  - RPA-SERS/Lateral strips for point-of-care diagnosis
  - Enzyme based -Colorimetric analysis
  - Nanozyme activity (*in-situ* synthesized metal nanoparticles)

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- ✓ Dr. Nicholas Crosbie, Melbourne Water, Australia

Methods X :
http://www.sciencedirect.com/science/article/pii/S2215016117300158

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