Monitoring of parasitic protozoans in effluent of wastewater treatment works

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INTRODUCTION

☑ Availability safe drinking water quality is important

☑ Deterioration of water quality dams, lakes and rivers

☑ Water Pollution – industries, urbanisation and human

☑ Wastewater treatment plants - major polluter of water sources
  - i.e. 1st barrier in multi-barrier system of ensuring good quality drinking water
THE ROLE AND IMPACT OF WWTP ON WATER QUALITY

✓ WWTP - preventing pollution of water sources
  o Inactivate & remove pollutants in wastewater
    □ Plant nutrients; Pharmaceutical; Bacteria; Viruses & Protozoan parasites

✓ WWTP – ineffective pollution prevention
  o Urbanization and associated activities – increase in wastewater – pressure in WWTP
  o Poor management of WWTP

✓ Discharging effluent not complying to regulations
  o Chemical & microbiological
POSSIBLE POLLUTANTS IN WASTEWATER

✓ Plant nutrients
  - Phosphate,
  - Nitrates & Nitrites

✓ Emerging organics contaminants
  - Pharmaceuticals

✓ Bacteria
  - *E. coli*, *Salmonella* & *Vibrio cholera*

✓ Viruses
  - Rotavirus

✓ Protozoan parasite
  - *Entamoeba histolytica*, *Cryptosporidium* & *Giardia*
PROTOZOAN PARASITES

✓ *Cryptosporidium* and *Giardia* are enteric protozoans parasites:
  o Self-limiting illness in immuno-competent
  o Serious to fatal in immuno-compromised
  o Infect wide range of animals & also man
  o Transmitted through
    q Human to human
      v Unhygienic actions
    q Water & food
    q Recreational
    q Feecal-oral route
LIFE CYCLE OF CRYPTOSPORIDIUM

1. Thick-walled oocyst (sporulated) exits host
2. Contamination of water and food with oocysts.
3. Thick-walled oocyst ingested by host
   - Recreational water
   - Drinking water
CRYPTOSPORIDIOUIM AND GIARDIA

Importance to water industry:

- Caused a number of outbreaks - deaths
- Can escape coagulation/filtration processes and remain viable due to their small size
- Resistance to conventional chlorine based disinfectants
- Highly resistant to environmental conditions and can survive in water for longer periods
- Prominent protozoan parasites in wastewater
Methodology for the detection of *Cryptosporidium* and *Giardia* (oo)cysts in water

In 2001, USEPA 1623 & 1622: Universally approved methods

- In 2005, 1622 adapted for wastewater (1693)
- In 2012, 1623.1 new revision with operational changes
  - Concentration – Filtration
  - Separation – Immuno-magnetic separation
  - Enumeration – fluorescein stains
CHALLENGES IN DETECTION METHODS

Wastewater samples

Method limitation

- Immuno-magnetic separation kit inhibition
- Turbidity
- pH sensitive
- Fats
- Unknown substances
- Identification challenges
- False positive (experience)
AIM AND OBJECTIVES

• Asses the method (USEPA 1623.1) performance/recovery in wastewater effluent

• Monitor physical and microbiological quality of wastewater effluent from the two WWTP

• Determine the removal efficiency of Cryptosporidium and Giardia by two WWTP
WASTEWATER QUALITY MONITORING

- **Why**: The reason for monitoring wastewater quality.
- **What**: The aspects of wastewater that are monitored.
- **When**: The timing of the monitoring.
- **Where**: The location of the monitoring.
- **How**: The methods used for monitoring.
WASTEWATER QUALITY MONITORING

**What**

- Microbiological determinants
  - Cryptosporidium oocysts
  - Giardia cysts
  - Escherichia coli

- Physical determinants
  - Turbidity
WASTEWATER TREATMENT PLANT 1

- Capacity 36 ML/day (40 ML/day)
- Activated sludge system
- Treats wastewater from Vereeniging, Sharpville, Kwaggastroom
- Final effluent discharged to Vaal River
- Sample 2 x month (6 months)
WASTEWATER TREATMENT PLANT 2

✓ Carrying capacity of 2.1 ML/day
✓ Trickling filters
✓ Treats water from Deneysville and Refengkgotso township
✓ Final effluent discharged to Vaal Dam
✓ Sample 2x a month (6 months)
METHODOLOGY

Sample collection
Turbidity measurement
*Escherichia coli* detection and enumeration
USEPA 1623.1 method
- Sample filtration & elution
- Sample concentration & separation
- *(Oo)*cyst detection & enumeration
SAMPLE COLLECTION

- Rod sampling method
- Sampling using a beaker
- Sampling using an attached beaker
- On-site measurements using YSI
USEPA 1623.1 METHOD: SAMPLE CONCENTRATION

Matrix

Filtration

Elution

Elution

Elution
USEPA 1623.1 METHOD: SAMPLE CONCENTRATION

CENTRIFUGATION → SUPERNATANT ASPIRATION → PELLET
USEPA 1623.1 METHOD: SAMPLE SEPARATION

Giardia

Cryptosporidium

CAPTURE

Giardia antibodies paramagnetic beads

Cryptosporidium antibodies paramagnetic beads

ASSOCIATION

Magnet
USEPA 1623.1 METHOD: SAMPLE SEPARATION

DISSOCIATION: HCL
USEPA 1623.1 METHOD:
DETECTION AND ENUMERATION

*Cryptosporidium* and *Giardia* FITC-mAb stain

❖ Florescence
❖ Size
USEPA 1623.1 METHOD: DETECTION AND ENUMERATION

_Cryptosporidium_ and _Giardia_ DAPI stain

- Confirmatory stain – identification of nuclei (viability)
# METHOD CHALLENGES EXPERIENCED IN THE PROJECT

<table>
<thead>
<tr>
<th>Problem</th>
<th>Solution</th>
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<tr>
<td>• pH very high especially in water samples with algae</td>
<td>• pH measured before processing and lowered if necessary</td>
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<td>• IMS beads not attaching to the magnet. Especially in wastewater with high scum and fat content.</td>
<td>• Use kaolin which helps in remove particles bound to cell epitopes.</td>
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<td>• High turbidity in the samples that clogs the filters</td>
<td>• Use not filter samples with turbidity more than 500 NTU</td>
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RESULTS

Mean recovery = G (40) C (35)

*Cryptosporidium* and *Giardia* counts in WWTP 2 effluent

Counts/10L

Week 1  Week 2  Week 3  Week 4  Week 5  Week 6  Week 7  Week 8  Week 9  Week...

Effluent-Cryptosporidium  Effluent-Giardia
RESULTS

Cryptosporidium and Giardia counts in WWTP 1 effluent

Mean recovery = G (35)  
C (30)
Results

*E. coli* counts in WWTP 1 & 2 WWTP effluent

General limits = 1000/100ml
Specific limit = 0/100ml

- E. coli- Deneysville WWTP effluent
- E. coli- Leeuwkuil WWTP effluent
CONCLUSION

✓ Cryptosporidium and Giardia need to be monitored in WWTP treated effluent
  - Prevalence in the population
  - WWTP can be potential source of contamination of water sources

✓ Monitoring/ analysis of effluent and estimation of removal efficiency
  - Useful in providing information on potential contamination sources
  - Cryptosporidium and Giardia should be included in permit requirements