

# STANDARDS METHODS FOR THE RECOVERY AND ENUMERATION OF HELMINTH OVA IN WASTEWATER, SLUDGE, COMPOST AND URINE-DIVERSION WASTE IN SOUTH AFRICA

Report to the  
Water Research Commission

by

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The publication of this report emanates from a project entitled: *Adopting internationally acceptable methods and building capacity to measure Helminth Ova in wastewater and sludge samples* (WRC Project No. K5/1662)

This report has a CD attached consisting of:

- 2 Posters: (1) The methods and (2) Photographs of helminth ova commonly seen,
- The approach to the development of the methods, as well as the procedure followed, supporting information and a list of the trainees,

#### **DISCLAIMER**

This report has been reviewed by the Water Research Commission (WRC) and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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## PREFACE

This manual has been developed to specify a standard analytical method for water and wastewater laboratories to recover and enumerate helminth ova in wastewater, sludge, compost and urine-diversion waste. The manual serves to guide microbiologists and laboratory technicians by means of step-by-step procedures to analyse various types of waste samples and to recognize different helminth species found in South Africa. It has been developed in a manner that will allow both the experienced practitioner and the trainee technician to understand, interpret and carry out the method. It also includes a collection of photographs to assist the laboratory practitioner with the microscopic analysis of the helminth ova that are recovered.

The adoption of this method as a standard by the various laboratories around South Africa will assist in creating the much needed capacity and expertise in the recovery, identification and enumeration of helminth ova. It will also enable the determination of a set of prevalence statistics on helminth ova, which is currently lacking for the country.

While this manual has been developed as a stand-alone document, supporting posters outlining the method and depicting the photographs of helminth ova commonly seen have also been developed. These are available on the CD attached to this document. In addition, the approach to the development of the method, as well as the procedure followed and supporting information were also documented as a separate document on the CD attached to this document.

The method documented in this manual is an attempt at documenting a standard, simple and cost effective analytical method for South Africa for the recovery and enumeration of helminth ova. It is thus considered to be a living publication, and will be reviewed periodically based on local and international scientific advancements and analytical developments that may take place. All users are urged to take a critical view regarding the manual in terms of usefulness, suitability and applicability.

## ACKNOWLEDGEMENTS

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Mr I Bailey	Water Environment
Mrs S Jackson	eThekweni Municipality: Water & Sanitation

### **Project team:**

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Mr D Hawksworth	University of KZN – Pollution Research Group (method development and training session)
Ms C Maya-Rendon	National Autonomous University of Mexico (method development)
Ms Lee-Ann Boyd	Golder Associates Africa (Pty) Ltd

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- The Water Research Commission for the funding of the project.
- The World Health Organisation for use of photographs.
- C.E. Archer & C.C. Appleton of the University of KwaZulu-Natal for use of photographs and the life cycle diagram of *Ascaris lumbricoides*.
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### CD CONTENTS

- Procedure and approach followed for methods development and information on training sessions
- 2 Posters



1 INTRODUCTION

The helminths are a very large and varied group of multicellular parasitic worms. Some infect humans, others animals or plants, while many may be free-living in the soil. Some produce male and female worms, others are hermaphrodites. All have differentiated organs, and their life cycles include the production of ova (eggs) or larvae as well as the complex alternation of generations which can include up to three different hosts. There are three major groups of helminths containing members that have man as their host viz. flukes (Trematoda), tapeworms (Cestoda), and roundworms (Nematoda). Helminth infections are spread through the ingestion or inhalation of their ova, some of which can survive outside the host for long periods of time, or via larvae/cercariae penetrating skin exposed to infected soil/water. Once inside the body, helminth ova hatch and many undergo maturation in the tissues before re-entering the gut and lodging in the intestines. Here they grow and undergo sexual reproduction, resulting in the production of eggs or larvae which are passed out via the faeces to the environment (Figure 1).

Helminths may damage tissues (e.g. visceral larva migrans caused by the dog and cat round worm, genus *Toxocara*), cause blood-loss (e.g. hookworm species) and result in more serious effects like epilepsy when man becomes an accidental intermediate host (e.g. the pork tapeworm, *Taenia solium*). Contamination of crops with helminths can take place through direct faecal contamination with both human and animal excreta, or through the use of contaminated sludge or wastewater for agriculture. While worldwide the prevalence of helminth ova and the incidence of infections are well documented, in southern Africa research on the prevalence of the different helminth species in wastewater and sludge samples is severely lacking.

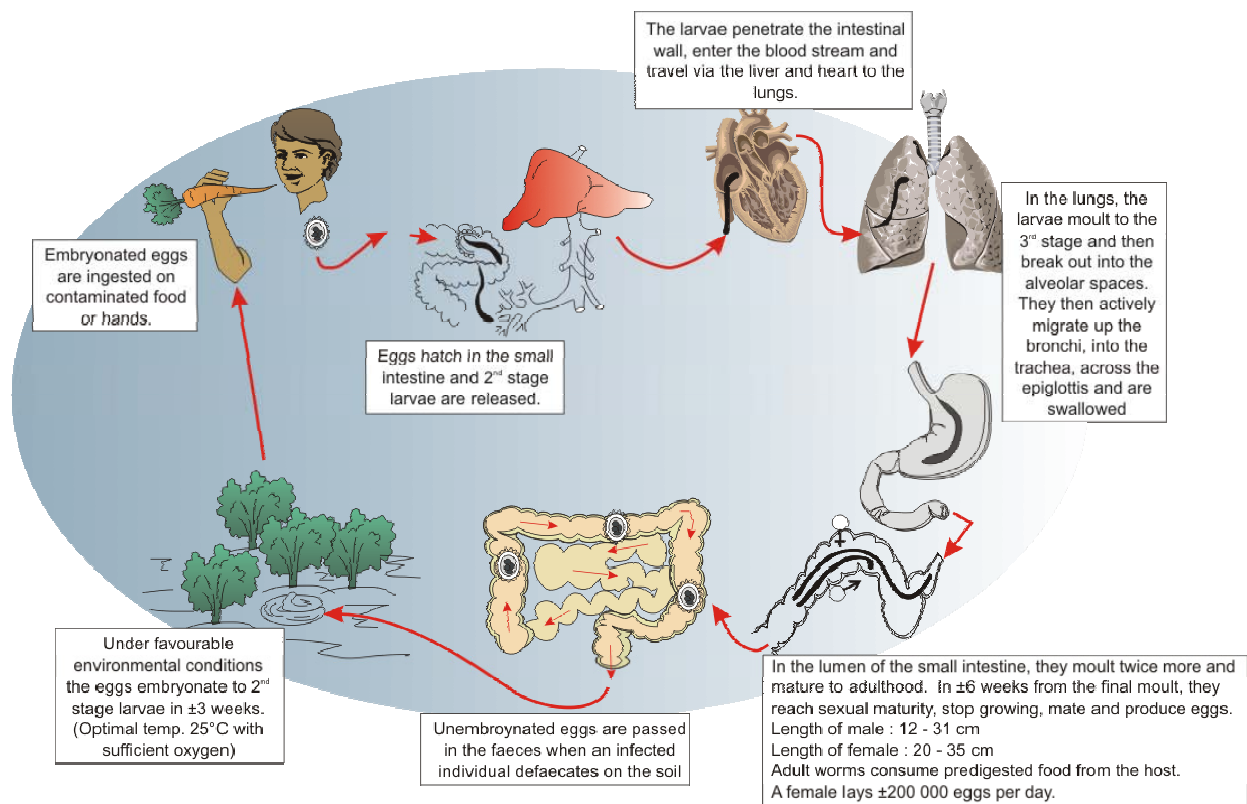


Figure 1: Diagram of the life cycle of *Ascaris lumbricoides*, the most common human nematode infection worldwide (Adapted from C.E. Archer & C.C. Appleton, Unpublished ©).



## 2 THE PROBLEM

To date in South Africa, no standard method exists for measuring helminth ova in wastewater, sludge or faecal samples mixed with soil. Helminth ova concentrations in wastewater and sludges have thus been largely underestimated or never reported, as the presence of *Ascaris lumbricoides* eggs is frequently the only standard that has been used. While reporting numbers of viable *Ascaris* ova in sludges is a requirement in terms of certain regulatory obligations, no analytical method has been specified. As a result, different laboratories in South Africa have adopted different methods. Several studies done internationally however, show evidence that *Ascaris* ova are often not the most prevalent of the helminths in wastewater samples. For example, while in the USA, the relative prevalence of different helminth species is *Ascaris* > *Toxocara* > *Toxascaris* > *Trichuris* > *Hymenolepis*; in France the relative prevalence of helminth species is *Taenia* > *Trichuris* > *Toxocara* > *Ascaris* (Cabaret *et al.*, 2002). Research on the prevalence of the different helminth species in South African wastewater and sludge samples could not be located. It should be mentioned, though, that there is a reasonable amount of data on the prevalence of these infections in humans, especially school children. According to Cabaret *et al.* (2002), the prevalence of human taeniasis (*Taenia spp.*) in Africa is 2-10% while Belgium, France, Germany, Hungary, Italy, Montenegro, Poland, Spain and the Netherlands show a prevalence of 0.01-2%. This demonstrates that the continued reliance on the *Ascaris* standard could seriously underestimate the total helminth ova concentrations in South Africa. Consequently due to the lack of expertise and of no standardised method being available to do total helminth ova counts, their true prevalence in human waste in South Africa remains unknown. With the revised sludge guidelines soon to be implemented, total helminth ova counts will be a requirement of the sludge classification system. The viable helminth ova count will not only give an indication of the effectiveness of the disinfection process of the wastewater treatment facility but will also generate data on the prevalence of the different species, as well as provide some indication of the incidence of infection in communities serviced by these treatment plants. A standard method will also allow helminth ova counts to be reliably compared between laboratories and to regulatory guidelines.

## 3 THE APPROACH FOLLOWED TO DEVELOP A STANDARD METHOD

A method similar to the one currently in use in a several laboratories in South Africa was compared to other internationally used methods. Recommendations made were that an adapted Environmental Protection Agency (EPA) method be adopted to measure helminth ova based on the fact that this technique demonstrated a greater recovery rate than the rest of the procedures. While the original intention was to adopt this adapted EPA method for South Africa, with some refinement to suit the local context, a local group in South Africa also recently reported the development of an improved recovery protocol, the Ammonium Bicarbonate/Zinc Sulphate (AmBic/ZnSO<sub>4</sub>) method for the detection of helminth ova (particularly *Ascaris*).

The method documented in this manual is based on an adapted EPA method which allows a better recovery of helminth ova using the relative specific gravities of the ova of different helminth species, and the AmBic/ZnSO<sub>4</sub> method for the detection of helminth ova in soil-based samples. The resulting method is based on the strengths of both methods and has been tested to determine its suitability and appropriateness for the South African context.

The method, which is based on a combination of washes, filtrations and flotations, has been adapted into three separate procedures to suit the main groups of waste products, *viz.*

- wastewater or effluent,
- wet sludge, and
- dry or composted sludge and UD waste.





#### 4 FIELD OF APPLICATION

The method describes the detection, identification and quantification of total and viable helminth ova in wastewater or effluent, sludge and dry or composted sludge and UD waste.

#### 5 PURPOSE OF THIS MANUAL

The purpose of this manual is:

- to promote the use of a standard method for the recovery and enumeration of total helminth ova in wastewater, sludge, compost and UD waste by all laboratories in South Africa;
- to encourage the analysis of waste and wastewater samples for helminth ova by providing a simple, cost-effective and accurate method suitable for the South African context;
- to create an understanding and provide guidance on performing the method for the recovery and enumeration of helminth ova;
- along with training workshops and quality control, to create much needed expertise and skills in the application of the methodology and in the recovery and enumeration of total helminth ova.

#### 6 WHO SHOULD USE THIS MANUAL?

This manual has been developed to provide a standard method to support various types of water and wastewater laboratories in South Africa undertake analysis of wastewater or effluent, sludge and dry or composted sludge and UD waste for the recovery and enumeration of total helminth ova. The manual is therefore targeted at:

- Water treatment service providers
- Wastewater treatment service providers
- Local authorities and town/city councils that own and operate their own water/wastewater laboratories
- Water/wastewater scientists
- Research organisations/institutions
- Regulators

#### 7 SUPPORTING INFORMATION

In addition to the step by step methodology that is provided, some photographs have been included in the manual to enable the untrained eye to examine samples and recognize different helminth species found in South Africa. Posters outlining the method and depicting these photographs have also been developed and are available from the WRC.



## 8 METHODS FOR THE DETERMINATION, IDENTIFICATION AND QUANTIFICATION OF HELMINTH OVA

The method has been adapted into three separate procedures to suit specific types of waste samples. Thus this manual includes a separate procedure for the analysis of each of the following:

- **wastewater or effluent,**
- **wet sludge,** and
- **dry or composted sludge and UD waste.**

Also included is an example of the **manipulation of the method** to accommodate **variations in sludge types.**

## 9 THE PRINCIPLE BEHIND THE METHOD

The method is based on three fundamental processes viz. (1) washing, (2) filtering one or more times, and then (3) floating and sedimenting of the retrieved parasites. A flotation step is used for the isolation of helminth ova using density gradient centrifugation and a chemical solution that is saturated at a specific gravity of 1.3 so that all helminth ova having relative densities that range from 1.13 (e.g. *Ascaris*) to 1.27 (e.g. *Taenia*) are able to float in that solution.

## 10 PERFORMING THE METHOD

The following components with regard to performing the method are described in detail in the sections that follow:

- **Equipment required**
- **Reagents required**
- **Solution preparation**
- **Step by step method for each waste type:**
  - *Analysis of wastewater or effluent*
  - *Analysis of wet sludge, and*
  - *Analysis of dry or composted sludge and UD waste*
  - *Analysis of sludges that may vary from the above waste types*



## 10.1 EQUIPMENT REQUIRED

- A centrifuge with a swing-out rotor and buckets that can take 15 mL and/or 50 mL plastic conical test tubes
- Vortex mixer
- Retort stand with at least 2 clamps on it
- Large plastic funnels to support the filters ( $\pm$  220 mm diameters)
- Filters / sieves: 1x 150  $\mu$ m; 1x 100  $\mu$ m; 1x 20  $\mu$ m mesh sizes
- Approximately 6 plastic beakers (500 mL volume)
- Plastic wash bottles
- At least 4 glass (Schott) bottles: 1 L, 2 L and 5 L sizes, for make-up and storage of the chemical solutions and de-ionized water
- Magnetic stirrer and stirring magnets
- 15 mL and 50 mL plastic conical test tubes
- 3 x small glass beakers (100 mL)
- Plastic Pasteur pipettes and plastic stirring rods
- Glass microscope slides (76 x 26 x 1.2 mm)
- Square and rectangular cover-slips (22 x 22 mm and 22 x 40 mm)
- A binocular compound microscope with 10x eyepieces, a 10x objective and a 40x objective



**WORKING OUT THE G-FORCE OF THE CENTRIFUGE:**

$$\text{G-force (or g)} = (1.118 \times 10^{-5}) r s^2 = 0.0001118 \times r \times s^2$$

*where:*

**s** = revolutions per minute (i.e. the speed you spin at)

**r** = the radius (the distance in centimetres from the centre of the rotor to the bottom of the bucket holding the tubes, when the bucket is in the swing-out position)

**Calibration and Standardization**

All equipment used **must be** calibrated with certified or adjusted standards according to the manufacturer's specifications.

**10.2 REAGENTS**

- **Zinc Sulphate (ZnSO<sub>4</sub>)**
- **Ammonium Bicarbonate (AmBic) or Tween80**

The make up of the above reagents is described in Section 10.3.



### 10.3 SOLUTION PREPARATION

#### Zinc Sulphate

1.  $\text{ZnSO}_4$  (heptahydrate) is made up by dissolving 500 g of the chemical in 880 mℓ de-ionised or distilled water.
2. A **hydrometer must be used** to adjust the specific gravity (SG) to 1.3, using more chemical if the SG is too low or more water if it is >1.3.

This high specific gravity facilitates the floating of heavier ova such as *Taenia* sp. (SG = 1.27). It is not critical if the SG of the  $\text{ZnSO}_4$  solution is just over 1.3 but it should **never** be below!

#### Ammonium Bicarbonate (AmBic)

The AmBic solution is essentially a saturated ammonium bicarbonate solution made up as follows:

1. Ammonium bicarbonate\* is made up by dissolving 119 g of the chemical in 1000 mℓ of de-ionised water.

*\*can be obtained from Sigma Aldrich*

#### 0.1% Tween80

1. 1 mℓ of Tween80 is measured out using a pipette and placed in 999 mℓ of de-ionized or distilled water to give a 0.1% wash solution.

**Note:** Tween80 is extremely viscous and it is necessary to wash all of it out into the water in which it is made up, by alternately sucking up water and blowing it out using the same pipette.



## 10.4 STEP BY STEP METHOD

**Note:** If more than one water, sludge, compost or UD sample is to be processed simultaneously, label the samples, any corresponding beakers, test tubes and microscope slides with an appropriate laboratory number so as not to mix up results.

### PROCEDURE FOR EFFLUENT OR WASTEWATER

1. An amount of 5 l of wastewater, or the total volume received from the Plant, can be used. Write down the volume used for testing.
2. Supporting the 150 µm and 20 µm filters in 2 funnels one beneath the other in a retort stand, filter the sample through the 150 µm filter onto the 20 µm filter, swirling the 20 µm filter to facilitate draining of the fluid. If preferred, a plastic stirring rod can be used instead, to stir the filtrate on the filter, which also helps the fluid to pass through.
3. Any matter held back by the 150 µm filter is discarded, whilst the solids collected on the 20 µm filter are kept and rinsed off into a plastic beaker. Make sure to rinse off everything on this filter into the plastic beaker so as not to lose any of the parasites.
4. Pour the contents of the beaker into as many test tubes as are needed to accommodate the whole filtrate, and centrifuge at 1389 g ( $\pm 3000$  rpm) for 3 min. Pour, or suction off, the supernatant fluid and discard it.
5. Combine the deposits into a suitable number of tubes so that there is not more than 1 ml in a 15 ml test tube or 5 ml in a 50 ml test tube.
6. Re-suspend each deposit in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.
7. Centrifuge the tubes at 617 g ( $\pm 2000$  rpm) for 3 min. Carefully remove the tubes from the centrifuge and, using a plastic Pasteur pipette, transfer the supernatant to 3 or 4 test tubes. Fill the tubes with distilled water to **reduce** the SG of the ZnSO<sub>4</sub> so as not to damage the eggs and also to allow them to **deposit** upon centrifugation.
8. Centrifuge the tubes at 964 g ( $\pm 2500$  rpm) for 3 min; remove & discard the supernatant fluid. Combine the deposits into one test tube, using water to recover all the eggs from the other tubes. Then centrifuge again at 964 g for 3 min to get one deposit.
9. The deposit is transferred to one or more microscope slides, each one is covered with a coverslip, and examined under the microscope to enumerate each species of helminth ova using the 10x objective and the 40x objective to confirm any uncertainties. A calculation is performed so that the ova count can be expressed as eggs per litre of effluent, for each parasite species. (E.g. If 215 *Ascaris* eggs were found in 5 l of effluent, then there are  $215/5 = 43$  *Ascaris* eggs/l).



**TO TEST FOR VIABILITY**

- Perform steps 1 to 8 of procedure above, then continue as follows
- 10.** Once there is a final deposit in the test tube and re-suspend it in 4 ml of 0.1 H<sub>2</sub>SO<sub>4</sub>. Before incubating mark the test tube with the level of liquid and incubate at a temperature of 26°C for three to four weeks. Check the level of liquid in each one of the test tubes and add the reagent every time that is necessary, compensating for any evaporation that may occur.
- 11.** Once the incubation time is over, homogenize the deposit and proceed to quantify the eggs. Remove all of the deposit using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a cover slip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses. Only those ova where the larva is observed are considered viable.

**Note:** Viability of *Ascaris* ova may be determined at step 9 when enumeration is being done. However this requires experience in microscopy and a trained eye. While the *Ascaris* ova are being examined and counted they may be categorized as follows:

- Undeveloped
- Containing a motile larva
- Containing an immotile larva (in good condition)
- Containing an immotile larva (necrotic i.e. dead)
- Egg broken, dead

Only those *Ascaris* ova with motile and immotile larva in good condition are considered viable.



## PROCEDURE FOR WET SLUDGE/SLUDGE TO LAND

**Note:** It is always preferable to work with small sub-samples as eggs may not be as easily released from a large sample to float out of the sludge when doing the ZnSO<sub>4</sub> flotation technique. Rather increase the number of sub-samples than overload each test-tube in order to keep the number of tubes down.

The number of sub-samples will also be dependent on the helminth ova load expected. This will require knowledge of the epidemiology of helminths in the particular area in South Africa. Consequently, more sub-samples must be done in an area of low endemicity and less in a highly endemic area.

1. Mix the sludge sample well by swirling and stirring with a plastic rod. From the total sample take 4 x 15 ml sub-samples and place them into 4 x 50 ml test tubes. (If the solid content is high this should be sufficient sample. If it is low, take more 15 ml sub-samples).
2. Add either a few millilitres of 0.1% Tween80 **or** AmBic solution to the samples, vortex and add more wash solution. Repeat this procedure until the tubes are filled to approximately a centimetre from the top.
3. Place the 150 µm sieve in a funnel in a retort stand with a plastic beaker underneath to catch the filtrate. Filter the well-mixed contents of the tubes one at a time, rinsing out each tube and washing this water through the sieve as well.
4. Pour the filtrate into test tubes and centrifuge at 1389 g (±3000 rpm) for 3 minutes. Suction off the supernatant fluids and discard. Combine the deposits into a suitable number of tubes so that there is not more than 1 ml in a 15 ml tube or 5 ml in a 50 ml tube
5. Re-suspend each of these deposits in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.
6. Centrifuge the tubes at 617g (±2000 rpm) for 3 minutes. Remove from the centrifuge and pour the supernatant fluids through the 20 µm filter, washing well with water.
7. Collect the matter retained on the sieve and wash it into test tubes.
8. Centrifuge the tubes at 964 g (±2500 rpm) for 3 minutes; remove & discard the supernatant fluid. Combine the deposits into one test tube, using water to recover all the eggs from the other tubes. Then centrifuge again at 964 g for 3 minutes to get one deposit.
9. Once there is one final deposit, remove all of it using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a coverslip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses.
10. Each species of helminth ova is enumerated separately and reported as eggs per gram of wet sludge.





## PART 2: THE METHOD

**Note:** If reporting needs to be specified in number of ova/dry weight the following is required:

- Take a sub-sample from the original sample prior to step 1.
- Weigh the sub-sample and dry in an oven. Weigh the sample again after drying. This will indicate the moisture content of the wet sludge sample.
- Analyse the sludge sample (wet sludge) as described above from steps 1 to 9.
- The result can then be calculated back to dry weight as follows: Viable helminth ova count in wet sample \* (100/moisture content) / wet weight

### TO TEST FOR VIABILITY

- Perform steps 1 to 8 of procedure above, then continue as follows:
  11. Once there is a final deposit in the test tube and re-suspend it in 4 ml of 0.1 H<sub>2</sub>SO<sub>4</sub>. Before incubating mark the test tube with the level of liquid and incubate at a temperature of 26°C for three to four weeks. Check the level of liquid in each one of the test tubes and add the reagent every time that is necessary, compensating for any evaporation that may occur.
  12. Once the incubation time is over, homogenize the deposit and proceed to quantify the eggs. Remove all of the deposit using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a cover slip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses. Only those ova where the larva is observed are considered viable.

**Note:** Viability of *Ascaris* ova may be determined at step 9 when enumeration is being done. However this requires experience in microscopy and a trained eye. While the *Ascaris* ova are being examined and counted they may be categorized as follows:

- Undeveloped
- Containing a motile larva
- Containing an immotile larva (in good condition)
- Containing an immotile larva (necrotic i.e. dead)
- Egg broken, dead

Only those *Ascaris* ova with motile and immotile larva in good condition are considered viable.

**Note:** Samples may be examined slightly differently from that described in step No. 9 above by doing the following:

The deposits are filtered through a 12 µm ISOPORE membrane, which is then rinsed with distilled water. The membrane is air-dried, cut in half and placed on a microscope slide. Immersion oil is used to clear the membrane before examining under the microscope.



## PROCEDURE FOR COMPOSTED SLUDGE OR URINE DIVERSION WASTE

1. Weigh out 2 or more 1 g samples into 15 ml test tubes. 50 ml test tubes may be used if a centrifuge that can take these large tubes is available. If using 50 ml tubes, weigh out 3 g maximum per tube. See note at beginning of WET SLUDGE method for reasons.
2. Add a few millilitres of AmBic **or** 0.1% Tween80 and vortex well. Add more solution to about 6 ml (in a 15 ml tube) or 20 ml (in a 50 ml tube) and vortex on and off, repeating the addition of solution and vortexing until the tubes are filled to 10 ml / 40 ml and have been vortexed over a period of about 30 minutes in total.
3. Centrifuge the tubes at 1389 g ( $\pm 3000$  rpm) for 3 min and discard the supernatant. Re-suspend in de-ionized water and vortex to wash off the AmBic or Tween80, and centrifuge again at 1389 g for 3 minutes. Discard the supernatant.
4. Re-suspend each deposit in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.
5. Centrifuge the tubes at 617 g ( $\pm 2000$  rpm) for 3 minutes. Carefully remove the tubes from the centrifuge and, using a plastic Pasteur pipette, transfer the supernatant to 3 or 4 test tubes. Fill these tubes with distilled water to **reduce** the SG of the ZnSO<sub>4</sub> so as not to damage the eggs and also to allow them to **deposit** upon centrifugation.
6. Centrifuge at 964 g ( $\pm 2500$  rpm) for 3 minutes. Remove tubes and discard the supernatant fluid. Combine the deposits into one test tube, using water to recover all the eggs from the other tubes. Centrifuge again at 964 g for 3 minutes to get one deposit.

**Note:** At this point if the sample contains a lot of large particles of light debris that floated with the eggs e.g. grass, filter the deposit through a 100  $\mu$ m filter, collect the filtrate in test tubes and centrifuge again to get a deposit for microscopy.

7. Once there is one final deposit, remove all of it using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a coverslip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses.
8. Each species of helminth ova is enumerated separately and reported as eggs per gram of compost or UD waste.

### TO TEST FOR VIABILITY

- Perform steps 1 to 6 of procedure above, then continue as follows:
9. Once there is a final deposit in the test tube and re-suspend it in 4 ml of 0.1 H<sub>2</sub>SO<sub>4</sub>. Before incubating mark the test tube with the level of liquid and incubate at a temperature of 26°C for three to four weeks. Check the level of liquid in each one of the test tubes and add the reagent every time that is necessary, compensating for any evaporation that may occur.



**10.** Once the incubation time is over, homogenize the deposit and proceed to quantify the eggs. Remove all of the deposit using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a cover slip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses. Only those ova where the larva is observed are considered viable.

**Note:** Viability of *Ascaris* ova may be determined at step 9 when enumeration is being done. However this requires experience in microscopy and a trained eye. While the *Ascaris* ova are being examined and counted they may be categorized as follows:

- Undeveloped
- Containing a motile larva
- Containing an immotile larva (in good condition)
- Containing an immotile larva (necrotic i.e. dead)
- Egg broken, dead

Only those *Ascaris* ova with motile and immotile larva in good condition are considered viable.



## PROCEDURE FOR SLUDGES THAT VARY IN CHARACTERISTICS

Due to variations in sludges from different wastewater treatment plants, one may be required to manipulate the method slightly to accommodate these differences in characteristics. However the three basic principles (washing, filtering, floating and sedimenting) are always maintained. Here is an example for Dry Sludge/"Biltong" Sludge.

### EXAMPLE: DRY COMPOST / "BILTONG" SLUDGE

1. Weigh out 10 g of the **very** dried out pieces of compost in a 500 ml plastic beaker.
2. Add a measured amount (probably 150 ml should be sufficient) of either 0.1% Tween80 or distilled or deionised water and allow to soak overnight.
3. The next morning, break up the softened sample and blend well with a plastic stirring rod.
4. Place the 150 µm sieve in a funnel in a retort stand with a plastic beaker beneath to catch the filtrate. Filter the well-mixed softened compost through this filter, stirring it and using distilled water to make sure all parasite eggs are sieved through the filter into the beaker.
5. Pour the filtrate into test tubes (15 ml or 50 ml tubes) and centrifuge at 1389 g ( $\pm 3000$  rpm) for 3 minutes. Suction off the supernatant fluids and discard. Combine the deposits into a suitable number of tubes so that there is approximately 1 ml in a 15 ml tube or 5 ml in a 50 ml tube.
6. Re-suspend each of these deposits in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.
7. Centrifuge the tubes at 617 g ( $\pm 2000$  rpm) for 3 minutes. Remove from the centrifuge and pour the supernatant fluids through the 20 µm filter, washing well with water.
8. Collect the matter retained on the sieve. Using a squeeze bottle of distilled water, rinse everything off the top of the filter into the test tubes. (Squirt the water from the underside of the filter as well to make sure that any eggs that may be a bit stuck on the filter will also wash off into the test tubes.)
9. Centrifuge the tubes at 964 g ( $\pm 2500$  rpm) for 3 minutes; remove & discard the supernatant fluid. The deposits can then be combined into one test tube, using water to rinse out all the eggs from the other tubes and then centrifuge again at 964 g for 3 minutes to get one deposit.
10. Once there is one final deposit, remove all of it using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a coverslip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses.
11. Each species of helminth ova is enumerated separately and reported as eggs per gram of dry sludge.



## 11 REFERENCE PHOTOGRAPHS

The photographs in this section have been included as a source of reference to aid in microscopic analysis for the identification of helminth ova species.

### THE MOST COMMON HELMINTH OVA



Normal fertile *Ascaris lumbricoides* ovum showing brownish coloured mamillated outer shell.

Size : 55 - 75 x 35 - 50  $\mu\text{m}$ .

(WHO, 1994)



Normal fertile *Ascaris* ovum but without the mamillated layer - referred to as decorticated.

Size : 55 - 75 x 35 - 50  $\mu\text{m}$ .

(WHO, 1994)



Infertile *Ascaris* ovum - It is longer and thinner than a fertile egg. The brownish coloured mamillated layer is irregular and the egg contents are granular and disorganized.

Size : 85 - 95 x 43 - 47  $\mu\text{m}$ .

(WHO, 1994)



PART 3: PHOTOGRAPHS OF HELMINTH OVA



Hookworm sp. ovum has a thin wall and is usually seen in the 4 - 8 cell stage in fresh faeces.  
Size: 60 - 75 x 36 - 40  $\mu\text{m}$ .

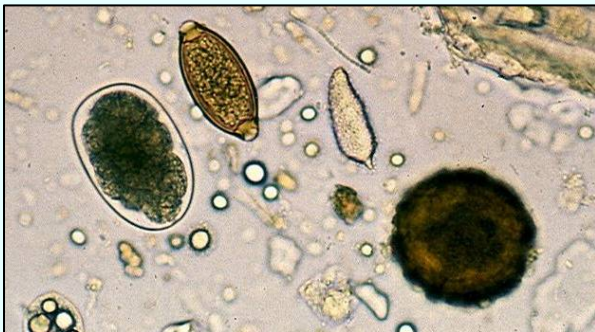
(WHO, 1994)



*Trichuris trichiura* ovum has a smooth brown coloured shell with bipolar "plugs".

Size: 50 - 55 x 22 - 24  $\mu\text{m}$

(WHO, 1994)

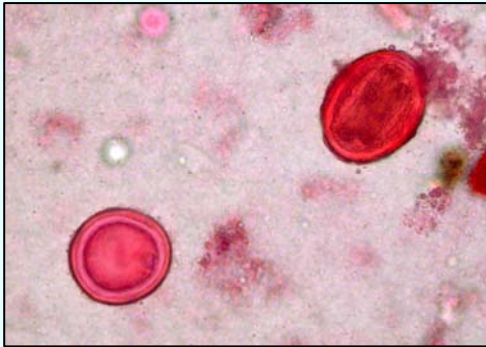


A combination photo to show the different egg sizes of *Ascaris* (right) *Trichuris* (middle) and Hookworm sp. (left).

(WHO, 1994)



PART 3: PHOTOGRAPHS OF HELMINTH OVA



(Top Right) : *Ascaris* ovum  
 (Bottom Left): Spore that can be confused with *Ascaris*  
 (Archer and Appleton, Unpublished)



*Taenia* sp. ova - the pork and beef tapeworm eggs are identical. Each has a thick striated egg wall containing a 6-hooked oncosphere.  
 Size : 31 - 43  $\mu$ m in diameter.  
 (WHO, 1994)



(Left) *Toxocara cati* ovum in 2-celled stage. Size: 65 x 75  $\mu$ m  
 (Right) *Toxocara canis* ovum, fully embryonated. Size: 85 x 75  $\mu$ m

(Archer and Appleton, Unpublished)



LESS COMMON HELMINTH OVA, LARVAE AND CILIATE CYSTS



*Schistosoma mansoni* ovum has a thin transparent shell with a lateral spine and a miracidium inside.

Size: 114 – 175 x 45 – 70  $\mu\text{m}$ .

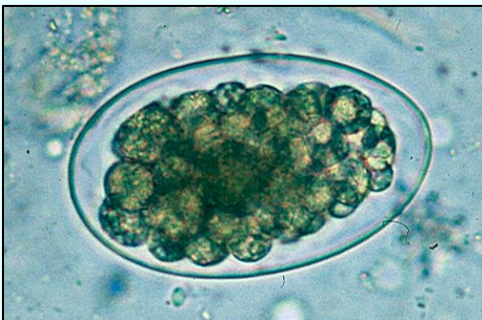
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*Schistosoma haematobium* ovum, normally found in urine, but also in faeces. It has a thin shell, a terminal spine and contains a miracidium.

Size: 112 - 170 X 50 - 70  $\mu\text{m}$ .

(WHO, 1994)



*Trichostrongylus* ovum resembles hookworm but is larger with one end more tapered than the other.

Size: 75 - 95 x 40 - 50  $\mu\text{m}$ .

(WHO, 1994)





PART 3: PHOTOGRAPHS OF HELMINTH OVA



*Fasciola hepatica* ovum - very large with an operculum at one end.  
Size: 130 - 150 x 63 - 90  $\mu\text{m}$ .  
(WHO, 1994)



*Hymenolepis diminuta* ovum is spherical, yellowish-brown in colour, with polar filaments that lie just inside the shell wall. In the middle of the egg is a 6-hooked oncosphere (embryo).  
Size: 70 - 85  $\mu\text{m}$  in diameter.  
(WHO, 1994)



*Hymenolepis nana* ovum is slightly more oval than spherical and has a thin clear shell. Polar filaments fill the area between the oncosphere and shell.  
Size: 30 - 47  $\mu\text{m}$  in diameter.  
(WHO, 1994)



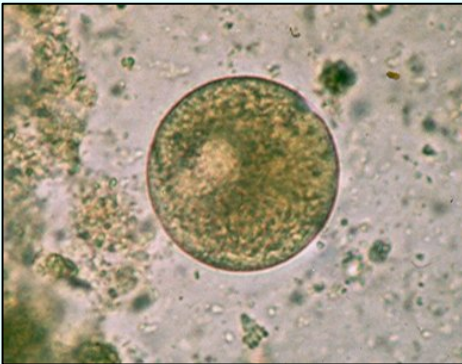
PART 3: PHOTOGRAPHS OF HELMINTH OVA



*Strongyloides stercoralis* rhabditiform larva. This is the first stage rhabditoid larva passed in faeces. However other stages of the free-living soil dwelling life-cycle may be seen in sewage and UD waste.

Size: 180 - 380 x 14 - 20  $\mu\text{m}$ .

(WHO, 1994)



*Balantidium coli* cyst - this is NOT a helminth egg, BUT a protozoan cyst.

Size: 45 - 75  $\mu\text{m}$  in diameter.

(WHO, 1994)



Three spirurid nematode ova: (left) *Streptopharagus* sp. found in rat faeces; (middle) *Physaloptera caucasia* from baboon faeces: 44 - 65 x 32 - 45  $\mu\text{m}$  and (right) *Streptopharagus* sp. From baboon faeces: 37,9 - 41,7 x 19,0 - 28,6  $\mu\text{m}$

(Archer and Appleton, Unpublished)



PART 3: PHOTOGRAPHS OF HELMINTH OVA



*Strongyloides* sp. ovum from  
rat faeces.  
Size: 50 - 58 x 30 - 34  $\mu$ m  
(Archer and Appleton, Unpublished)



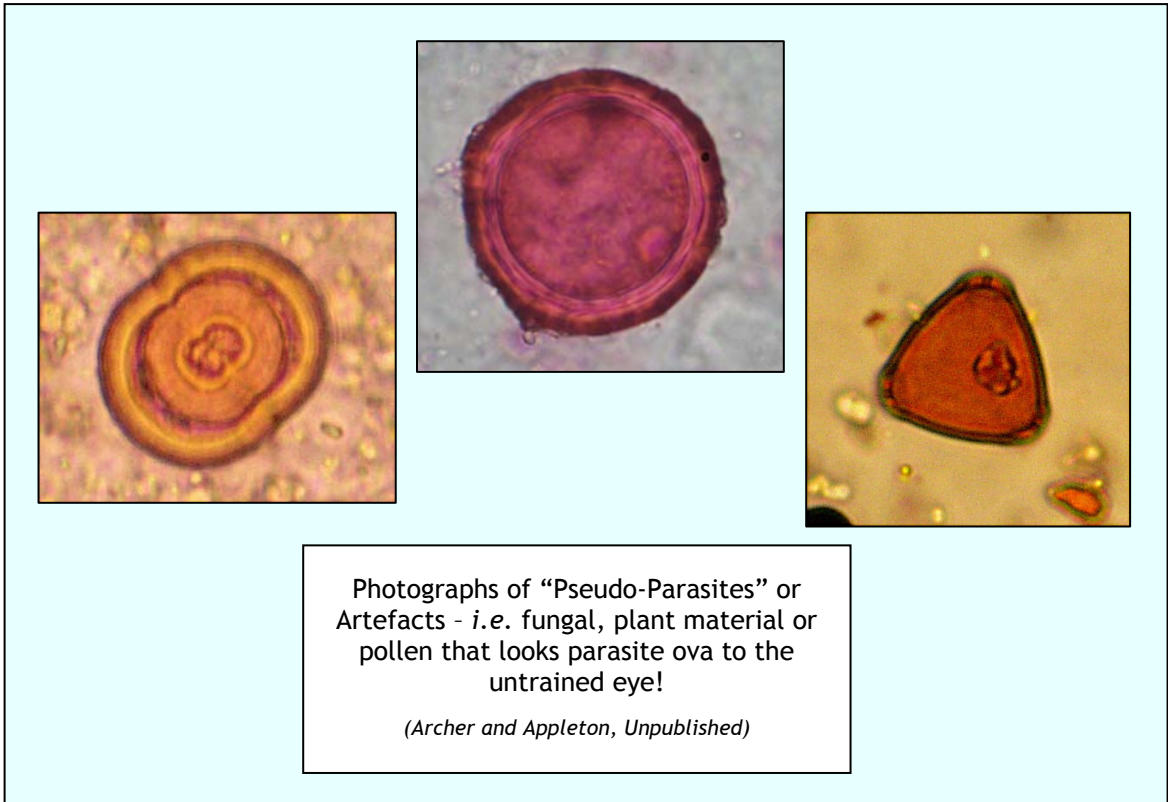
*Strongyloides stercoralis* -  
Free-living gravid female worm:  
 $\pm$ 1,2 mm x 65  $\mu$ m  
(Archer and Appleton, Unpublished)



*Strongyloides stercoralis* - Free-  
living male worm:  
 $\pm$ 0,7 mm x 40 - 50  $\mu$ m  
(Archer and Appleton, Unpublished)



PSEUDO-PARASITES



12 CONCLUSION

The method documented above is considered to be efficient and cost-effective for the measurement of helminth ova burdens in wastewater, sludge, compost and UD waste. It is proposed that it be adopted as the standard methodology for South Africa. However it is also known that sample qualities and characteristics vary throughout the country and thus it is up to the laboratory practitioner to include additional wash and filtration steps as required. Thus the laboratory practitioner should adapt the method accordingly to suit his or her sample qualities as well as the existing laboratory facilities/equipment at his/her disposal. It is important however to maintain the principle behind the method so that comparable results are obtained.

This manual presents an attempt to document a standard and simple analytical methodology for South Africa for the recovery and enumeration of helminth ova. It is thus considered to be a living publication, and will be reviewed periodically based on local and international scientific advancements.

A contact list is provided in Appendix 1, of the persons that have been involved in the development of the method. This serves as a support base to users of this manual, so that guidance and assistance can be elicited from them when the need arises. In addition, the report submitted to the WRC as part of the project deliverable (available on CD from the WRC) also includes the contact information of persons from laboratories throughout South Africa that have been trained on the method as part of the capacity building component of the project. These persons could offer assistance to users of the manual in resolving teething problems through information sharing and skills transfer between laboratories, as well as sharing experiences and daily realities.



### 13 REFERENCES

ARCHER C, MAYA-RENDON C, HAWKSWORTH D, LEIBACH L, RODDA N, SMITH MT, APPLETON CC, BUCKLEY C and MOODLEY P (2006). University of KwaZulu-Natal Pollution Research Group / Catalina Maya collaboration in association with Zitholele Consulting to find the simplest, most cost-effective methods for determining helminth ova burdens in wastewater, sludge, compost and urine-diversion waste in South Africa. Report to the Water Research Commission. Project Number K5/1662.

CABARET J, GEERTS S, MADELINE C and BARBIER D (2002). The use of urban sewage sludge on pastures: the Cysticercosis threat. *Vet. Res.* **33**: 575-597.

EPA Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge. (Revised October 1999). EPA/625/R-921013.

HAWKSWORTH D, ARCHER C, RODDA N, SMITH M, APPLETON C and BUCKLEY C (in preparation). Method for the recovery of *Ascaris* ova from solid waste from urine diversion toilets. University of KwaZulu-Natal. Pollution Research Group. Unpublished.

JIMÉNEZ B and MAYA C (2006). Techniques for determining Helminth ova in wastewater and sludge. Instituto de Ingeniería, UNAM, Mexico.

W.H.O. (1998). Training manual on diagnosis of intestinal parasites based on the WHO Bench Aids for the Diagnosis of Intestinal Parasites. World Health Organization, Geneva. WHO/CTD/SIP/98.2

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World Health Organization, Geneva, 1994: Bench Aids for the Diagnosis of Intestinal Parasites.

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2. PROJECT/METHOD RELATED INFORMATION

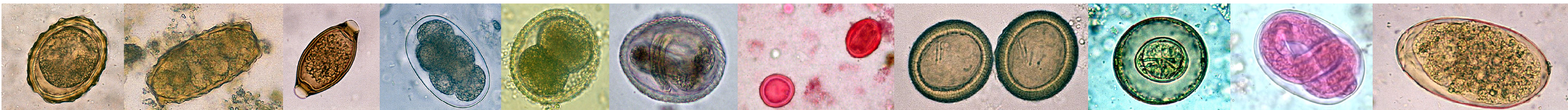
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# A STANDARD METHOD FOR THE RECOVERY AND ENUMERATION OF HELMINTH OVA IN WASTEWATER, SLUDGE, COMPOST AND URINE-DIVERSION (UD) WASTE IN SOUTH AFRICA

## Effluent or Waste Water

An amount of 5l of wastewater, or the total volume received from the Plant, can be used. Write down the volume used for testing.

Supporting the 150µm and 20µm filters in 2 funnels one beneath the other in a retort stand, filter the sample through the 150µm filter onto the 20µm filter, swirling the 20µm filter to facilitate draining of the fluid. If preferred, a plastic stirring rod can be used instead, to stir the filtrate on the filter, which also helps the fluid to pass through.

Any matter held back by the 150µm filter is discarded, whilst the solids collected on the 20µm filter are kept and rinsed off into a plastic beaker. Make sure to rinse off everything on this filter into the plastic beaker so as not to lose any of the parasites.

Pour the contents of the beaker into as many test tubes as are needed to accommodate the whole filtrate, and centrifuge at 1389g (±3000rpm) for 3 min. Pour, or suction off, the supernatant fluid and discard it.

Combine the deposits into a suitable number of tubes so that there is not more than 1ml in a 15ml test tube or 5ml in a 50ml test tube.

Re-suspend each deposit in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.

Centrifuge the tubes at 617g (±2000rpm) for 3 min. Carefully remove the tubes from the centrifuge and, using a plastic Pasteur pipette, transfer the supernatant to 3 or 4 test tubes. Fill the tubes with distilled water to reduce the SG of the ZnSO<sub>4</sub> so as not to damage the eggs and also to allow them to deposit upon centrifugation.

Centrifuge the tubes at 964g (±2500rpm) for 3 min; remove & discard the supernatant fluid. Combine the deposits into one test tube, using water to recover all the eggs from the other tubes. Then centrifuge again at 964g for 3 min to get one deposit.

The deposit is transferred to one or more microscope slides, each one is covered with a coverslip, and examined under the microscope to enumerate each species of helminth ova using the 10x objective and the 40x objective to confirm any uncertainties. A calculation is performed so that the ova count can be expressed as eggs per litre of effluent, for each parasite species.

## Composted Sludge or UD waste

Weigh out 2 or more 1g samples into 15ml test tubes. 50ml test tubes may be used if a centrifuge that can take these large tubes is available. If using 50ml tubes, weigh out 3g maximum per tube. See note at beginning of WET SLUDGE method for reasons.

Add a few millilitres of AmBic or 0.1% Tween80 and vortex well. Add more solution to about 6ml (in a 15ml tube) / 20ml (in a 50ml tube) and vortex on and off, repeating the addition of solution and vortexing until the tubes are filled to 10ml / 40ml and have been vortexed over a period of about 30 min in total.

Centrifuge the tubes at 1389g (±3000rpm) for 3 min and discard the supernatant fluids. Re-suspend in de-ionized water and vortex to wash off the AmBic or Tween80 and centrifuge again at 1389g for 3 min. Discard the supernatant fluids.

Re-suspend each deposit in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.

Centrifuge the tubes at 617g (±2000rpm) for 3 min. Carefully remove the tubes from the centrifuge and, using a plastic Pasteur pipette, transfer the supernatant to 3 or 4 test tubes. Fill these tubes with distilled water to reduce the SG of the ZnSO<sub>4</sub> so as not to damage the eggs and also to allow them to deposit upon centrifugation.

Centrifuge at 964g (±2500rpm) for 3 min. Remove tubes and discard the supernatant fluid. Combine the deposits into one test tube, using water to recover all the eggs from the other tubes and then centrifuge again at 964g for 3 min to get one deposit.

**Note:** At this point if the sample contains a lot of large particles of light debris that floated with the eggs e.g. grass, filter the deposit through a 100µm filter, collect the filtrate in test tubes and centrifuge again to get a deposit for microscopy.

Once there is one final deposit, remove all of it using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a coverslip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses.

Each species of helminth ova is enumerated separately and reported as eggs per gram of compost or UD waste.

## Wet Sludge/Sludge to Land

**Note:** It is always preferable to work with small sub-samples as eggs may not be as easily released from a large sample to float out of the sludge when doing the ZnSO<sub>4</sub> Flotation Technique. Rather increase the number of sub-samples than overload each test-tube in order to keep the number of tubes down.

The number of sub-samples will also be dependent on the helminth ova load expected. This will require knowledge of the epidemiology of helminths in your particular area in South Africa. Consequently, more sub-samples must be done in an area of low endemicity and less in a highly endemic area.

Mix the sludge sample well by swirling and stirring with a plastic rod. From the total sample take 4 x 15ml sub-samples and put them into 4 x 50ml test tubes. (If the solid content is high this should be sufficient sample. If it is low, take more 15ml sub-samples).

Add either a few millilitres of 0.1% Tween80 or AmBic solution to the samples, vortex and add more wash solution. Repeat this procedure until the tubes are filled to about a centimetre from the top.

Place the 150µm sieve in a funnel in a retort stand with a plastic beaker underneath to catch the filtrate. Filter the well-mixed contents of the tubes one at a time, rinsing out each tube and washing this water through the sieve as well.

Pour the filtrate into test tubes and centrifuge at 1389g (±3000rpm) for 3 min. Suction off the supernatant fluids and discard. Combine the deposits into a suitable number of tubes so that there is not more than 1ml in a 15ml test tube or 5ml in a 50ml test tube.

Re-suspend each of these deposits in a few millilitres of ZnSO<sub>4</sub> and vortex well to mix. Keep adding more ZnSO<sub>4</sub> and mixing until the tube is almost full.

Centrifuge the tubes at 617g (±2000rpm) for 3 min. Remove from the centrifuge and pour the supernatant fluids through the 20µm filter, washing well with water.

Collect the matter retained on the sieve and wash it into test tubes.

Centrifuge the tubes at 964g (±2500rpm) for 3 min; remove & discard the supernatant fluid. Combine the deposits into one test tube, using water to recover all the eggs from the other tubes. Then centrifuge again at 964g for 3 min to get one deposit.

Once there is one final deposit, remove all of it using a plastic Pasteur pipette and place it onto one or more microscope slides. Place a coverslip over each deposit and examine microscopically using the 10x objective and the 40x objective to confirm any unsure diagnoses.

Each species of helminth ova is enumerated separately and reported as eggs per gram of sludge.

## Solutions

### Zinc Sulphate (Flotation Solution)

- ZnSO<sub>4</sub> (heptahydrate) is made up by dissolving 500g of the chemical in 880ml de-ionised or distilled water.
- A hydrometer must be used to adjust the specific gravity (SG) to 1.3, using more chemical if the SG is too low or more water if it is > 1.3.

This high specific gravity facilitates the floating of heavier ova such as *Taenia sp.* (SG = 1.27). It is not critical if the SG of the ZnSO<sub>4</sub> solution is just over 1.3 but it should never be below!

### Ammonium Bicarbonate (Wash Solution)

The AmBic solution is essentially a saturated ammonium biocarbonate solution.

- Ammonium biocarbonate can be obtained from Sigma Aldrich and is made up by dissolving 119g of the chemical in 1000ml of de-ionised water.

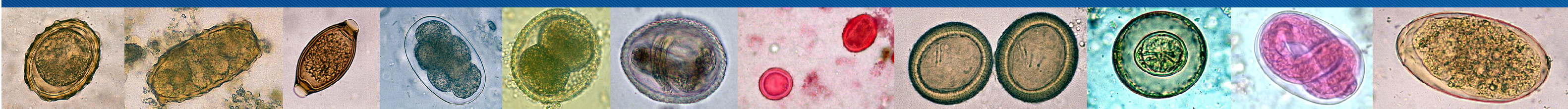
### 0.1% Tween80 (Wash Solution)

- 1ml of Tween80 is measured out using a pipette and placed in 999ml of de-ionised or distilled water to give a 0.1% wash solution.

**Note:** Tween80 is extremely viscous and it is necessary to wash all of it out into the water in which it is made up, by alternately sucking up water and blowing it out using the same pipette.

## REFERENCES

- Jiménez B and Maya C (June, 2006). Techniques for Determining Helminth Ova in Wastewater and Sludge. Instituto de Ingeniería, UNAM, Mexico.
- Hawksworth D, Archer C, Rodda N, Smith M, Appleton C, Buckley C (in preparation). Method for the Recovery of Ascaris Ova from Solid Waste from Urine Diversion Toilets.
- Archer C; Maya C; Hawksworth D; Leibach L; Rodda N; Smith M. T; Appleton C. C.; Buckley C; Moodley P. (2006). University of KwaZulu-Natal Pollution Research Group / Catalina Maya collaboration in association with Zitholele Consulting to find the simplest, most cost-effective methods for determining Helminth Ova burdens in wastewater, sludge, compost and urine diversion waste in South Africa. Report to the Water Research Commission. Project Number K5/1662.



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Size : 55 – 75 x 35 – 50 µm.



• Infertile *Ascaris* ovum – note it is longer and thinner than a fertile egg. The brownish coloured mottled layer is irregular and the egg contents are granular and disorganized.  
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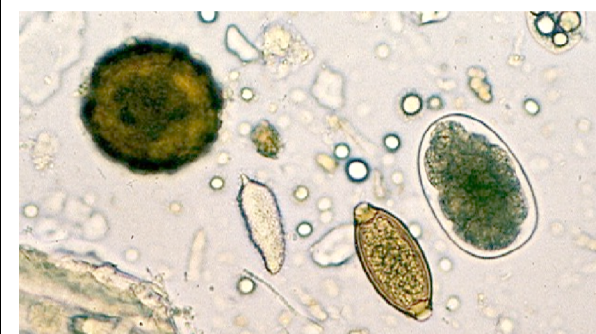
• *Taenia* sp. ova – the pork and beef tapeworm eggs are identical. Each has a thick striated egg wall containing a 6-hooked oncosphere.  
Size: 31-43 µm in diameter



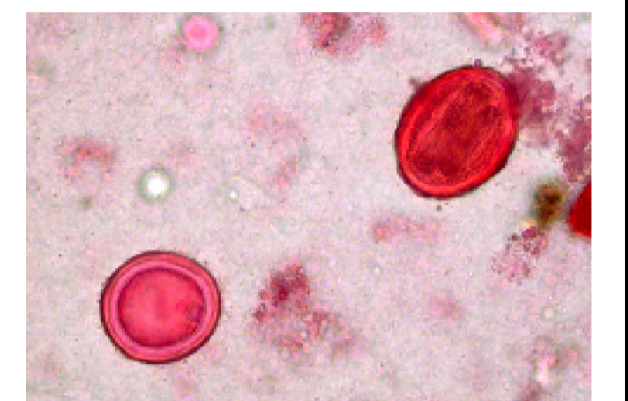
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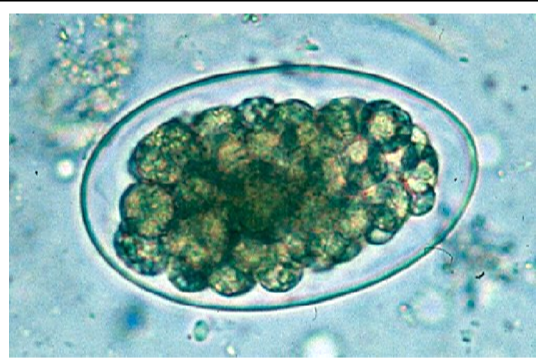


•• (Left) *Toxocara cati* ovum in 2-celled stage : 65 x 75 µm (Right) *Toxocara canis* ovum, fully embryonated : 85 x 75 µm

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• *Schistosoma mansoni* ovum has a thin transparent shell with a lateral spine and a miracidium inside.  
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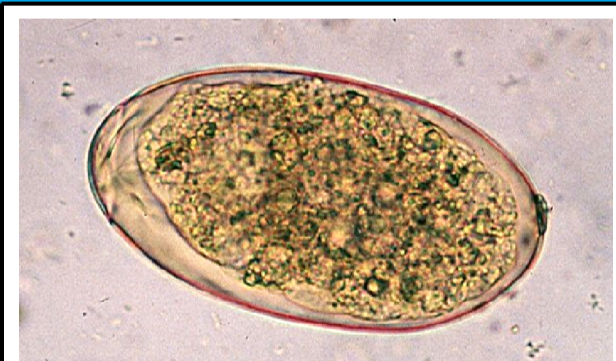
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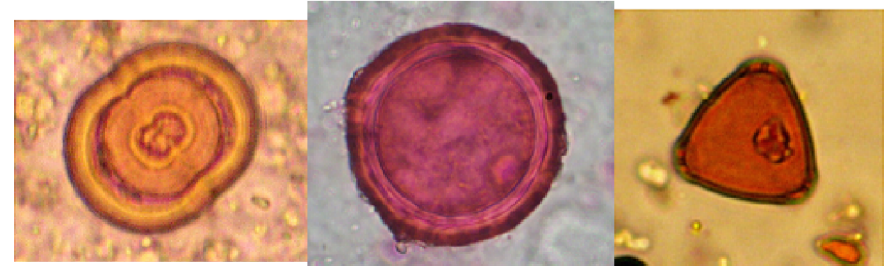


•• Three spirurid nematode ova : (left) *Streptopharagus* sp. found in rat faeces; (middle) *Streptopharagus* sp. from baboon faeces : 37,9 – 41,7 x 19,0 – 28,6 µm and (right) *Physaloptera caucasica* from baboon faeces : 44 – 65 x 32 – 45 µm



•• *Strongyloides* sp. ovum from rat faeces  
Size : 50 – 58 x 30 – 34 µm

# PSEUDO PARASITES



•• These 3 photos are of "Pseudo-Parasites" or Artefacts – i.e. Fungal or plant material, or pollen, that looks like it must be parasite ova to the untrained eye!

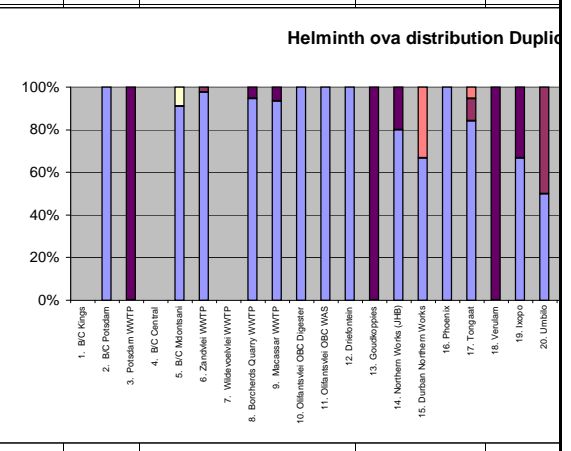
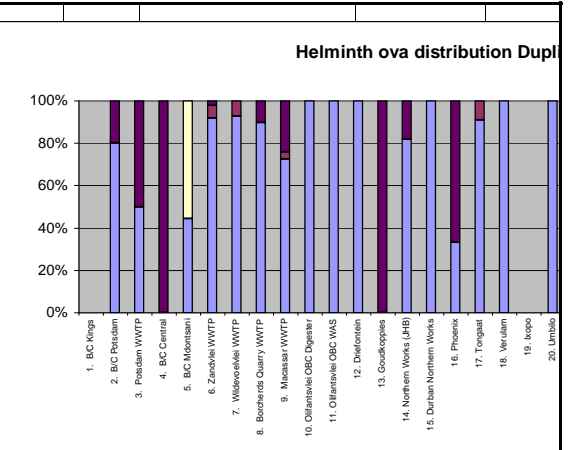
## ACKNOWLEDGEMENTS

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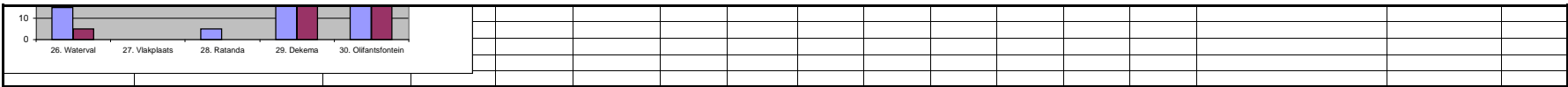
Duplicate 1									
Area (Town/City)	WWTP (Sample)	Ascaris	Trichuris	Taenia	Toxocara	Other	Unknown	Total/10g	
East London	1. B/C Kings							10	
East London	2. B/C Potsdam	4				1		55	
East London	3. Potsdam WWTP	1				1		10	
East London	4. B/C Central					1		15	
East London	5. B/C Mdontsani	4		5				45	
Cape Town	6. Zandvlei WWTP	45	3			1		245	
Cape Town	7. Wildevoelmei WWTP	26	2					140	
Cape Town	8. Borchers Quarry WWTP	35				4		200	
Cape Town	9. Macassar WWTP	21	1			7		145	
Johannesburg	10. Olifantsvlei OBC Digester	9						45	
Johannesburg	11. Olifantsvlei OBC WAS	6						30	
Johannesburg	12. Driefontein	1						5	
Johannesburg	13. Goudkoppies					1		5	
Johannesburg	14. Northern Works (JHB)	9				2		55	
Durban	15. Durban Northern Works	6						30	
Durban	16. Phoenix	1				2		15	
Durban	17. Tongaat	10	1					55	
Durban	18. Verulam	1						5	
Pietermaritzburg	19. Ixopo							0	
Durban	20. Umbilo	2						10	
Pietermaritzburg	21. Darville	3						15	
Tswane	22. Rietgat						1	5	
Tswane	23. Sunderland Ridge	2	1				1	20	
Tswane	24. Rooiwal	0						0	
Tswane	25. Daspoort							0	
Ekurhuleni	26. Waterval	3						15	
Ekurhuleni	27. Vlakplaats	0						0	
Ekurhuleni	28. Ratanda						1	5	
Ekurhuleni	29. Dekema	10						50	
Ekurhuleni	30. Olifantsfontein	10						50	
Duplicate 2									
Area (Town/City)	WWTP (Sample)	Ascaris	Trichuris	Taenia	Toxocara	Other	Unknown	Total/10g	
East London	1. B/C Kings							0	
East London	2. B/C Potsdam	6						50	
East London	3. Potsdam WWTP					2		10	
East London	4. B/C Central							0	
East London	5. B/C Mdontsani	10		1				55	
Cape Town	6. Zandvlei WWTP	41	1					210	
Cape Town	7. Wildevoelmei WWTP	0						0	
Cape Town	8. Borchers Quarry WWTP	18				1		95	
Cape Town	9. Macassar WWTP	14				1		75	
Johannesburg	10. Olifantsvlei OBC Digester	11						55	
Johannesburg	11. Olifantsvlei OBC WAS	7						35	
Johannesburg	12. Driefontein	4						20	
Johannesburg	13. Goudkoppies					1		5	
Johannesburg	14. Northern Works (JHB)	8				2		50	
Durban	15. Durban Northern Works	2					1	15	
Durban	16. Phoenix	1						5	
Durban	17. Tongaat	16	2				1	95	
Durban	18. Verulam							5	
Pietermaritzburg	19. Ixopo	4					2	30	
Durban	20. Umbilo	1	1					10	
Pietermaritzburg	21. Darville	1						5	
Tswane	22. Rietgat							0	





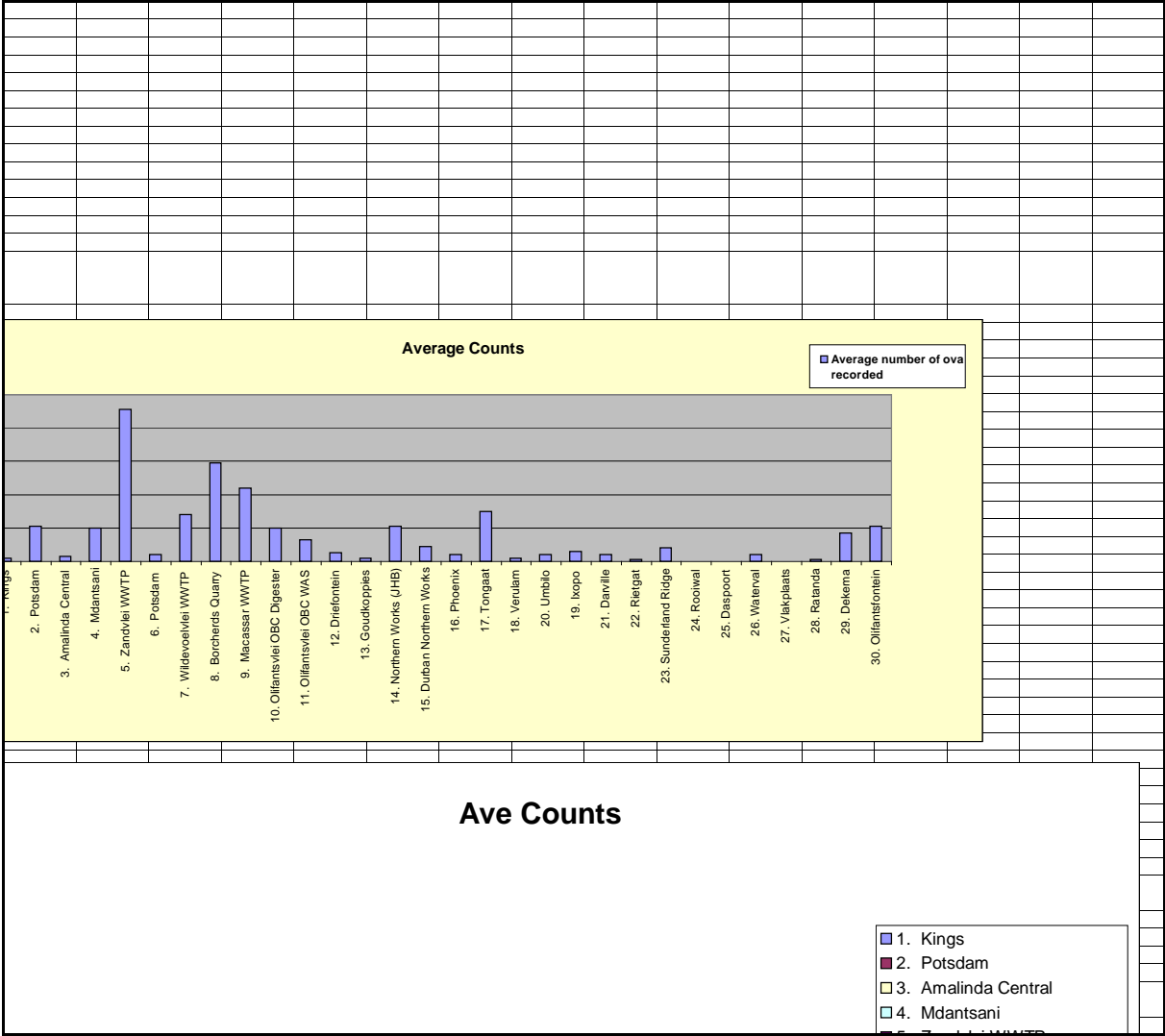


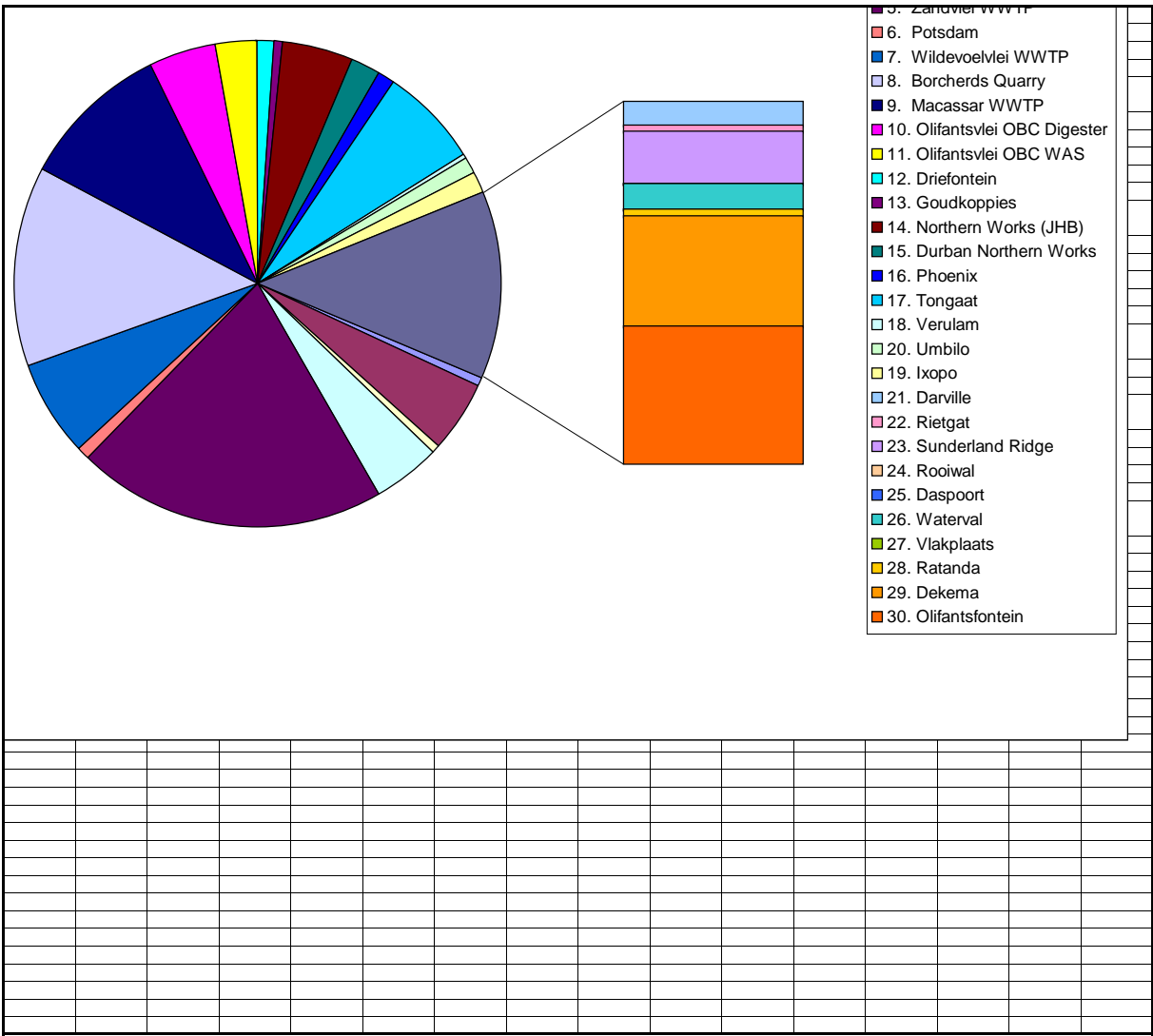












**This report documents the procedure and approach followed for the development of standard methods for the enumeration and recovery of helminth ova and the training sessions conducted.**

## EXECUTIVE SUMMARY

During 2006 the Water Research Commission (WRC), commissioned a project to adopt and document an internationally acceptable method to measure helminth ova in wastewater, sludge and soil samples, and subsequently build capacity in South African Water and Wastewater Laboratories on the method. The motivation for the project is that in South Africa, currently no standard method exists for measuring Helminth ova in wastewater, wastewater sludge or soil samples. Helminth ova concentrations in our wastewater and wastewater sludges are thus largely underestimated or never reported, as the presence of *Ascaris lumbricoides* eggs is frequently the only “standard” that is used. While reporting of viable *Ascaris* ova numbers in sludges is a requirement in terms of certain regulatory obligations, no analytical method has been specified. As a result, different laboratories in South Africa have adopted different methods.

The aim of the project was thus to develop a standard and cost effective method to measure Helminth ova burdens in wastewater, sludge, compost and urine – diversion (UD) waste, based on the work done by Professor B. Jiménez’s Research Group, at the Institute of Engineering, National Autonomous University of Mexico, and by a local group in South Africa – the Pollution Research Group (PRG) at the University of KwaZulu-Natal (UKZN), led by Dr Nicola Rodda. Professor B. Jiménez’s Research Group recommended an adapted EPA method which allowed a better recovery of Helminth ova based on the relative specific gravities of the ova of different helminth species. The PRG – UKZN group recently reported the development of an improved recovery protocol (the Ammonium Bicarbonate/Zinc Sulphate - AmBic/ZnSO<sub>4</sub> method) for the detection of helminth ova (particularly *Ascaris*) in soil-based samples. This project thus sought to evaluate the strengths of both methods and develop the most appropriate, efficient and cost – effective Helminth Ova method for South Africa.

The adapted EPA method (and rapid, shortened versions of it), the Visser® Filter method (a commonly used method) and the PRG’s AmBic/ZnSO<sub>4</sub> method, for the extraction of helminth ova from human waste materials, were tested in a collaboration between the Mexican group, the UKZN-PR Group and ERWAT. This work resulted in a simpler, cost-effective and accurate method for the South African context. The method, which is based on a combination of washes, filtrations and flotations, has been adapted into three “separate” methods to suit the main groups of waste products, viz. (1) wastewater or effluent, (2) wet sludge, and (3) dry or composted sludge and UD-waste.

Once the method was confirmed, a further objective of this project was to build some capacity in South African Water and Wastewater Laboratories on the use of the method. In this regard seven laboratories were trained on the method at two training sessions that were held during February 2007.

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## 1 INTRODUCTION

In South Africa, no standard method exists for measuring helminth ova in wastewater, wastewater sludge or soil samples. Helminth ova concentrations in our wastewater and wastewater sludges are thus largely underestimated or never reported, as the presence of *Ascaris lumbricoides* eggs is frequently the only “standard” that is used. While reporting of viable *Ascaris* ova numbers in sludges is a requirement in terms of certain regulatory obligations, no analytical method has been specified. As a result, different laboratories in South Africa have adopted different methods. Several studies done internationally however, show evidence that *Ascaris* ova are often not the most prevalent in wastewater samples. For example, while in the USA, the relative prevalence of different Helminth species is *Ascaris* > *Toxocara* > *Toxascaris* > *Trichuris* > *Hymenolepis*; in France the Helminth species relative prevalence is *Taenia* > *Trichuris* >> *Toxocara* > *Ascaris* (Cabaret *et al.*, 2002). Research on the prevalence of the different Helminth species in South African wastewater sludge and wastewater samples could not be located. However, according to Cabaret *et al.* (2002), the prevalence of human taeniasis (*Taenia saginata*) in Africa is 2-10% while Belgium, France, Germany, Hungary, Italy, Montenegro, Poland, Spain and the Netherlands show a prevalence of 0.01-2%. Thus the continued use of the *Ascaris* standard only, could seriously underestimate the helminth ova concentrations in South Africa. Consequently, due to the lack of expertise and no standardised method being available to do total helminth ova counts, the true prevalence of helminth ova in human waste in South Africa remains unknown. With the revised sludge guidelines soon to be implemented, total helminth ova counts will be a requirement of the sludge classification system. The viable helminth ova count will not only give an indication of the effectiveness of the disinfection process of the wastewater treatment facility but will also generate data on the prevalence of the different species, as well provide some indication of incidence of parasitic infections.

## 2 AIMS OF THE PROJECT

The primary aim of the project was two-fold:

- to develop a standard and cost effective method to measure helminth ova burdens in wastewater, sludge, compost and urine – diversion (UD) waste; and
- to demonstrate the newly adopted method to South African Water and Wastewater Laboratories and build some capacity in its application.

## 3 THE APPROACH

A method similar to the one currently in use in a few laboratories in South Africa was compared to other internationally used methods in 2005 (Professor B. Jiménez’s Research Group, at the Institute of Engineering, National Autonomous University of Mexico). Recommendations made were that an adapted EPA method be adopted to measure helminth ova based on the fact that this technique demonstrated a greater recovery rate than the rest of the procedures. Also, the method utilizes a specific gravity which allows better recovery of helminth ova by using their relative densities. While the original intention of this project was to adopt the adapted EPA method for South Africa, with some refinement, a local group in South Africa – the Pollution Research Group (PRG) at the

University of KwaZulu-Natal (UKZN), also recently reported the development of an improved recovery protocol (the Ammonium Bicarbonate/Zinc Sulphate - AmBic/ZnSO<sub>4</sub> method) for the detection of helminth ova (particularly *Ascaris*) in soil-based samples. This identified a need to evaluate the strengths and weaknesses of both methods and develop the most appropriate, efficient and cost-effective helminth ova method for South Africa.

Once determined, the identified method would then be tested and validated to determine its appropriateness and applicability. Based on this analysis the method would be further refined to suit local conditions in South Africa.

The method once validated and tested through practical application would then have to be demonstrated to various laboratories from around South Africa to build some capacity and expertise in its application.

#### **4 INTERNATIONAL COLLABORATION**

Prof Jiménez of Mexico had agreed to assist with the validation of the translation of the adapted EPA Method (NMX Anexo NOM 004 SEMARNAT 2000 - in Spanish) that is based on the EPA Method 625R92013AppI. Prof Jiménez had also kindly agreed to share the validated method with the South African Water Industry at no cost as long as due reference and acknowledgement was given. Through this project a member of her Mexican team, Ms Catalina Maya Rendon was kind enough to avail herself to demonstrate the method in South Africa. We were thus able to bring over Ms Catalina Maya to visit South Africa for a two week period to test and validate the method, and allow for much need knowledge sharing and transfer of expertise between the groups.

#### **5 THE PROCEDURE FOLLOWED**

The collaboration between the Mexican group, the UKZN-PR Group and ERWAT took place at the laboratories of the School of Biological and Conservation Sciences, UKZN, in August 2006, over a two-week period. The adapted EPA method (and rapid, shortened versions of it), the Visser® Filter method and the PRG's AmBic/ZnSO<sub>4</sub> method, for the extraction of helminth ova from human waste materials, were tested. This work resulted in a simpler, cost-effective and accurate method for the South African context. The method, which is based on a combination of washes, filtrations and flotations, has been adapted into three "separate" methods to suit the main groups of waste products, viz. (1) wastewater or effluent, (2) wet sludge, and (3) dry or composted sludge and UD-waste.

The method was then tested and validated by ERWAT who measured helminth ova in duplicate wastewater and wastewater sludge samples from 30 wastewater treatment plants throughout South Africa. This exercise, while confirming the suitability of the method and refining it to a further degree, also provided a first order baseline prevalence for helminth ova from these plants.

Together these groups formulated the method outlined below for extracting helminth ova from the various types of waste produced by treatment plants and UD toilets in South Africa.



## 6 THE PRINCIPLE BEHIND THE METHOD

The method is based on the difference in density between the helminth eggs and other solids present in wastewater. It includes three fundamental separation processes of sedimentation, flotation and decantation, as well as an extraction stage. A flotation step is used for the isolation of helminth ova using density gradient centrifugation by use of a wash solution. Helminth ova have specific gravities that range from 1.13 (e.g. *Ascaris*) to 1.27 (e.g. *Taenia*) when using a solution with a specific gravity of 1.3.

## 7 METHODS FOR MEASURING HELMINTH OVA

The helminth ova method has been adapted into three separate methods to suit the main groups of waste products, *viz.*

- wastewater or effluent,
- wet sludge, and
- dry or composted sludge and UD-waste.

Also included is an example of the manipulation of the method to accommodate variations in sludge types.

These methods are documented in TT report 322/08, together with the equipment needed and reagents required.

## 8 FIRST ORDER BASELINE PREVALENCE OF HELMINTH OVA IN WASTEWATER SLUDGE SAMPLES

Sludge samples from various wastewater treatment plants were tested for helminth ova as a means to validate the methods and to determine a baseline first order prevalence. The purpose of this exercise was merely to get some indication of the helminth ova species that do prevail and was thus not meant to be a statistical analysis. The plants varied in size, treatment processes and wastewater inflow characteristics, and thus it was not possible to determine any trends or specific correlations. What is presented here are merely the results as observed. The process however did assist in refining the method to suit the quality and variations of South African wastewater sludges.

Sludge samples were collected from 30 wastewater water treatment plants from around South Africa. The cities and the number of plants per city from which samples were collected are listed in **Table 8.1** below.

**Table 8.1: Areas in South Africa and number of plants from which sludge samples were collected**

AREA	NUMBER OF PLANTS
East London (Buffalo City)	4
Durban (Ethekeweni)	6
Pietermaritzburg and surrounding area (Umgeni Water plants)	3
Cape Town	5
Johannesburg (Johannesburg Water Plants)	3
Tshwane	4
Ekurhuleni (ERWAT plants)	5

The wastewater treatment plants sampled, their locations, treatment technology used and their approximate capacity are listed in **Table 8.2**.

Duplicate sludge samples from the wastewater treatment plants (60 samples in total) were analysed at ERWAT laboratories using earlier versions of the methods that are defined in section 10. Initially some teething problems were experienced in applying the method however these have been overcome, and have resulted in changes and refinement of the methods to the final versions included in this report.

To make the microscopic work easier the deposit was filtered through a 12 µm ISOPORE membrane. Immersion oil was added on the membrane for microscopic analysis. The slides were examined firstly using 10X magnification and the photographs were taken at 40X magnification.

The first order prevalence analysis on the 30 wastewater treatment tested indicated *Ascaris* spp. as the most abundant Helminth found in all areas. *Trichuris* sp was identified in some samples however the number of ova reported was low. In terms of occurrence within the different areas, the most abundant to the less abundant number of helminth ova recorded is as follows: Cape Town > Johannesburg > East London > Ekurhuleni > Durban > Pietermaritzburg > Tshwane.

**Table 8.2: Description of wastewater treatment plants (WWTP) sampled**

	<b>WWTP</b>	<b>Area (Town/City)</b>	<b>Main Treatment Processes</b>	<b>Size of Plant (Ml) Range</b>
1	Kings	East London /King Williams Town?	Biofilters; Anaerobic Digestion	1-5
2	Potsdam	East London	Biofilters; Anaerobic Digestion	5-20
3	Amalinda Central	East London	Biofilters; Anaerobic Digestion	1-5
4	Mdantsani	East London	Biofilters; Anaerobic Digestion	5-20
5	Potsdam	Cape Town	Biofilters; Anaerobic Digestion	20-100
6	Zandvlei	Cape Town	Activated Sludge	20-100
7	Wildvoëlsvlei	Cape Town	Activated Sludge	5-20
8	Borcherds Quarry	Cape Town	Activated Sludge	20-100
9	Macassar	Cape Town	Activated Sludge	20-100
10	Olifantsvlei OBC Digestor	Johannesburg	Activated Sludge; Anaerobic Digestion	>100
11	Olifantsvlei OBC WAS	Johannesburg	Activated Sludge; Anaerobic Digestion	>100
12	Driefontein	Johannesburg	Activated Sludge; Anaerobic Digestion	5-20
13	Goudkoppies	Johannesburg	Activated Sludge; Anaerobic Digestion	>100
14	Northern Works	Johannesburg	Activated Sludge	>100
15	Durban Northern Works	Durban	Activated Sludge; Anaerobic Digestion	20-100
16	Phoenix	Durban	Activated Sludge; Anaerobic Digestion	20-100
17	Tongaat	Durban	Biofilters; Activated Sludge; Anaerobic Digestion	5-20
18	Verulam	Durban	Biofilters; Activated Sludge; Anaerobic Digestion	5-20
19	Umbilo	Durban	Biofilters; Activated Sludge; Anaerobic Digestion	20-100
20	Darville	Pietermaritzburg	Biofilters; Activated Sludge; Anaerobic Digestion	2-100
21	Ixopo	Pietermaritzburg	Activated Sludge	<1
22	Rietgat	Tshwane	Activated Sludge; Anaerobic Digestion	20-100
23	Sunderland Ridge	Tshwane	Activated Sludge; Anaerobic Digestion	20-100
24	Rooiwal	Tshwane	Biofilters; Activated Sludge; Anaerobic Digestion	>100
25	Daspoort	Tshwane	Biofilters; Activated Sludge; Anaerobic Digestion	20-100
26	Waterval	Ekurhuleni	Activated Sludge; Anaerobic Digestion	>100
27	Vlakplaats	Ekurhuleni	Biofilters; Activated Sludge; Anaerobic Digestion	20 -100
28	Ratanda	Ekurhuleni		
29	Dekema	Ekurhuleni	Biofilters; Anaerobic Digestion	20-100
30	Olifantsfontein	Ekurhuleni	Activated Sludge	20-100

The % industrial effluent is < 10% for all the works sampled.

## 8.1 Results

The average counts of helminth ova recorded for each wastewater treatment plant is reflect in **Table 8.3** below and depicted in **Figure 8.1**.

**Table 8.3: Average number of helminth ova recorded per wastewater treatment plant**

WWTP (Sample)	Average number of ova recorded
1. Kings	5
2. Potsdam	53
3. Amalinda Central	8
4. Mdantsani	50
5. Zandvlei WWTP	228
6. Potsdam	10
7. Wildevoelvlei WWTP	70
8. Borchers Quarry	148
9. Macassar WWTP	110
10. Olifantsvlei OBC Digester	50
11. Olifantsvlei OBC WAS	33
12. Driefontein	13
13. Goudkoppies	5
14. Northern Works (JHB)	53
15. Durban Northern Works	23
16. Phoenix	10
17. Tongaat	75
18. Verulam	5
20. Umbilo	10
19. Ixopo	15
21. Darville	10
22. Rietgat	3
23. Sunderland Ridge	20
24. Rooiwal	0
25. Daspoort	0
26. Waterval	10
27. Vlakplaats	0
28. Ratanda	3
29. Dekema	43
30. Olifantsfontein	53

The occurrence of helminth ova (number) from an area perspective around South Africa is reflected in **Figure 8.2** below.

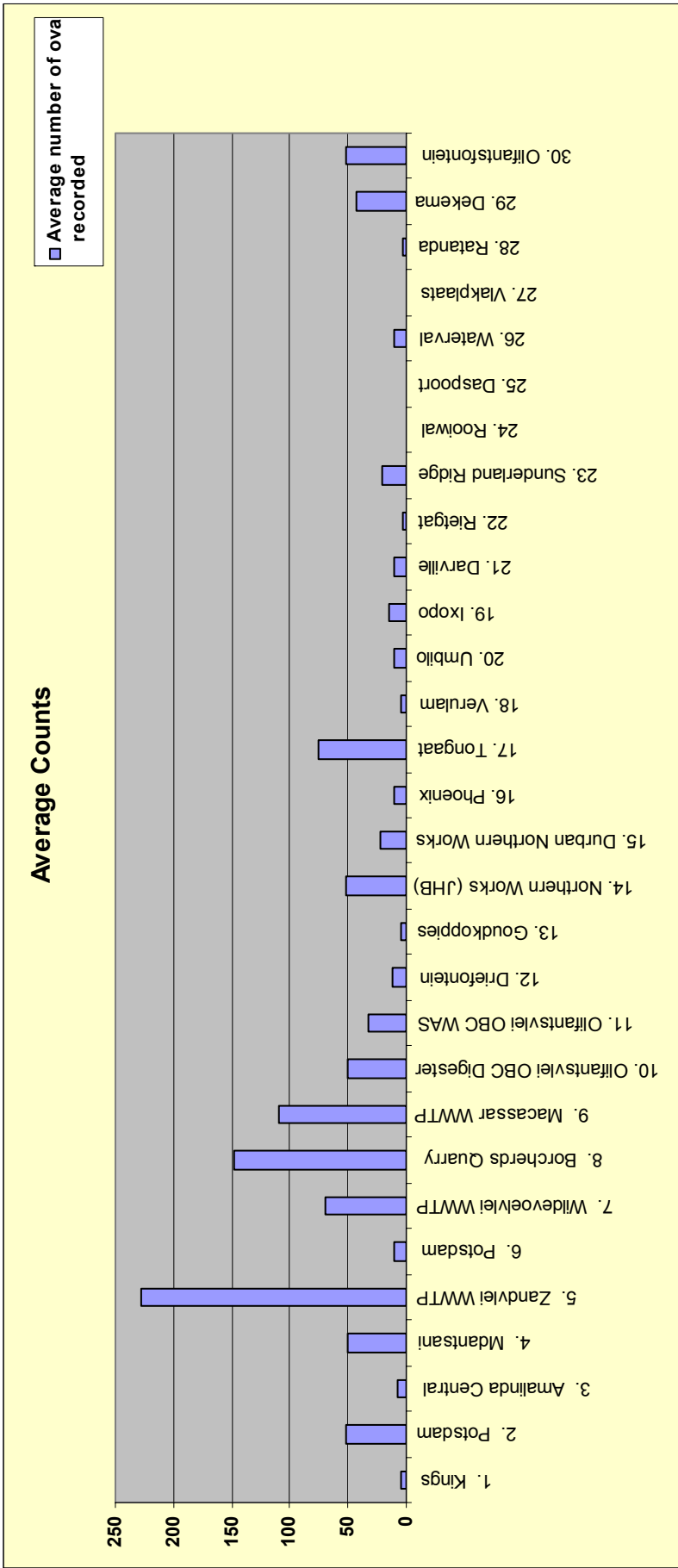
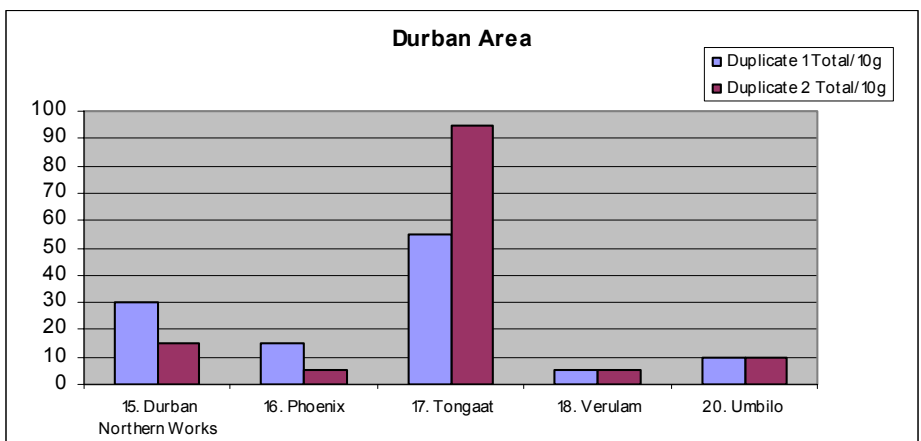
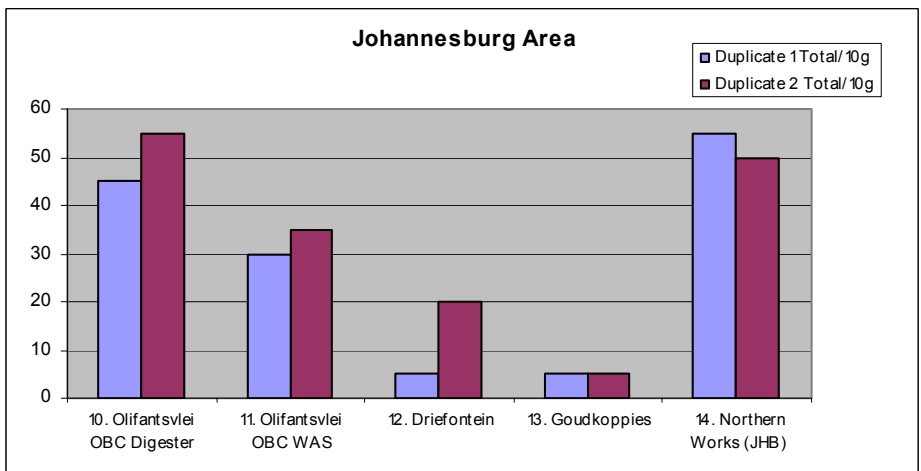
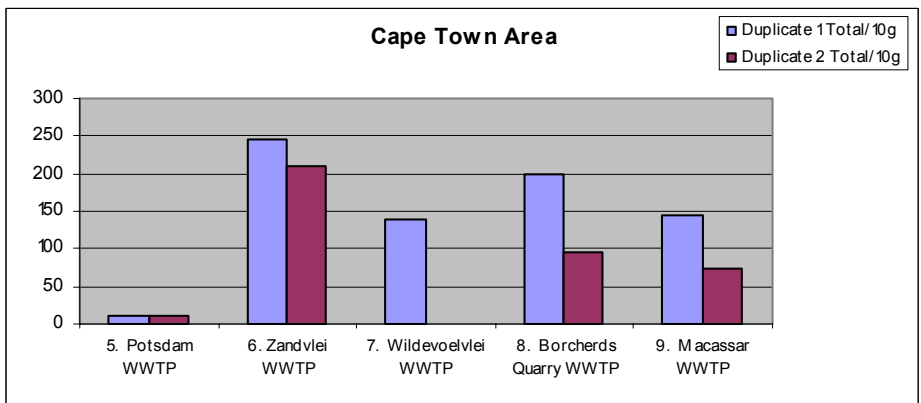
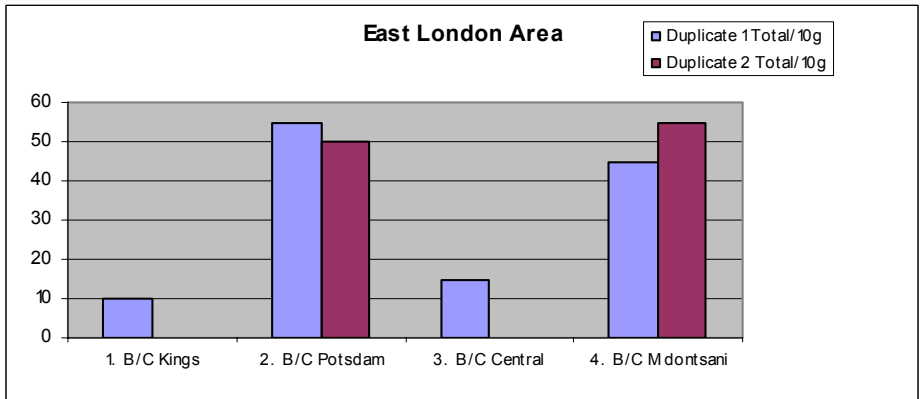
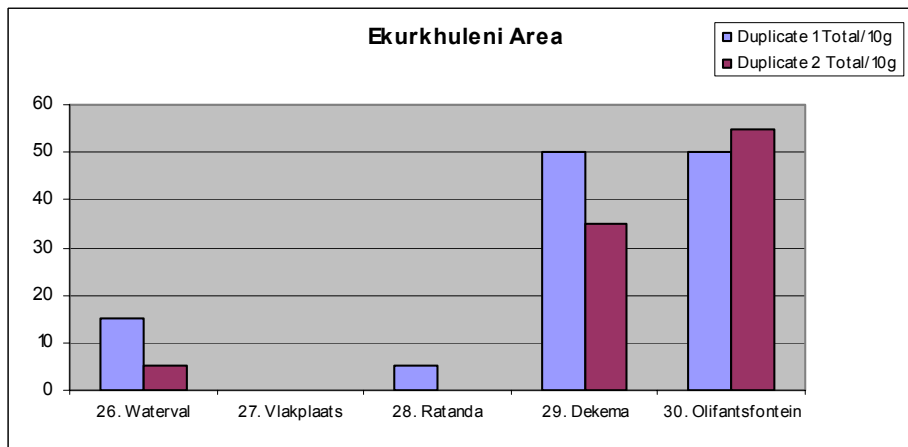
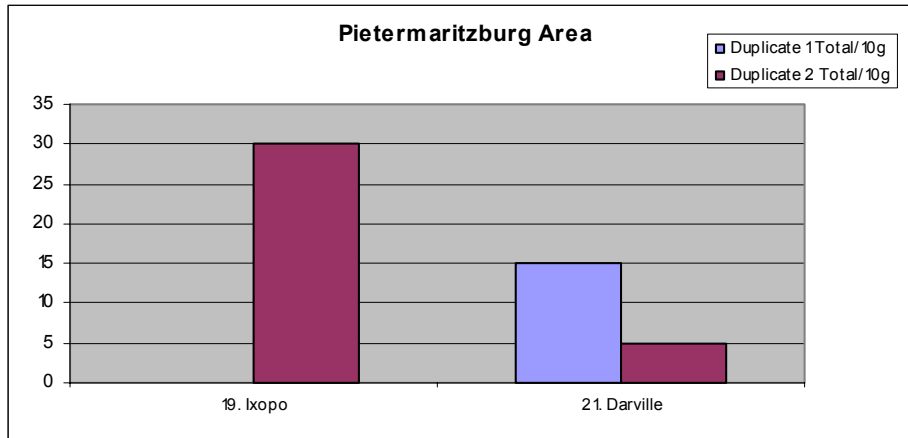


Figure 8.1: Average number of helminth ova recorded per wastewater treatment plant





**Figure 8.2: Graphical representation of the number of helminth ova (in duplicate) recorded per wastewater treatment within the various areas around South Africa**

The percentage occurrence of the different helminth ova species that were recorded per wastewater treatment from the duplicate samples are reflected in **Figure 8.3** below.

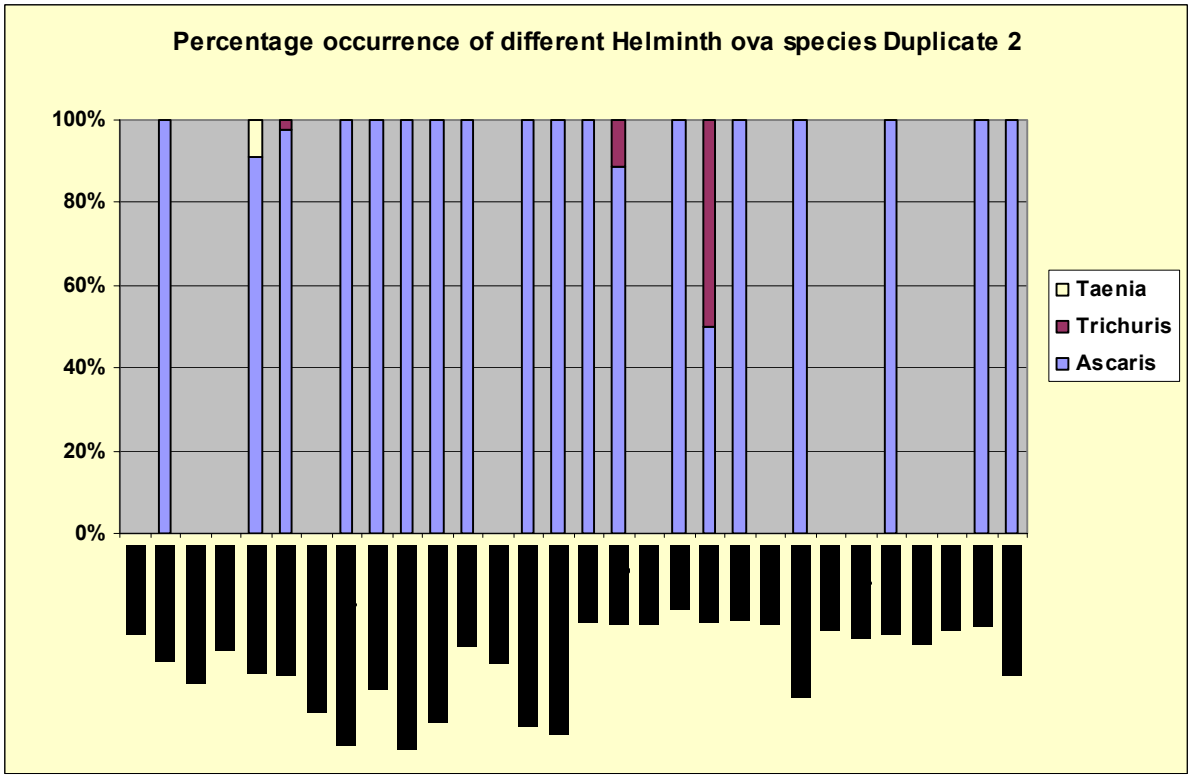
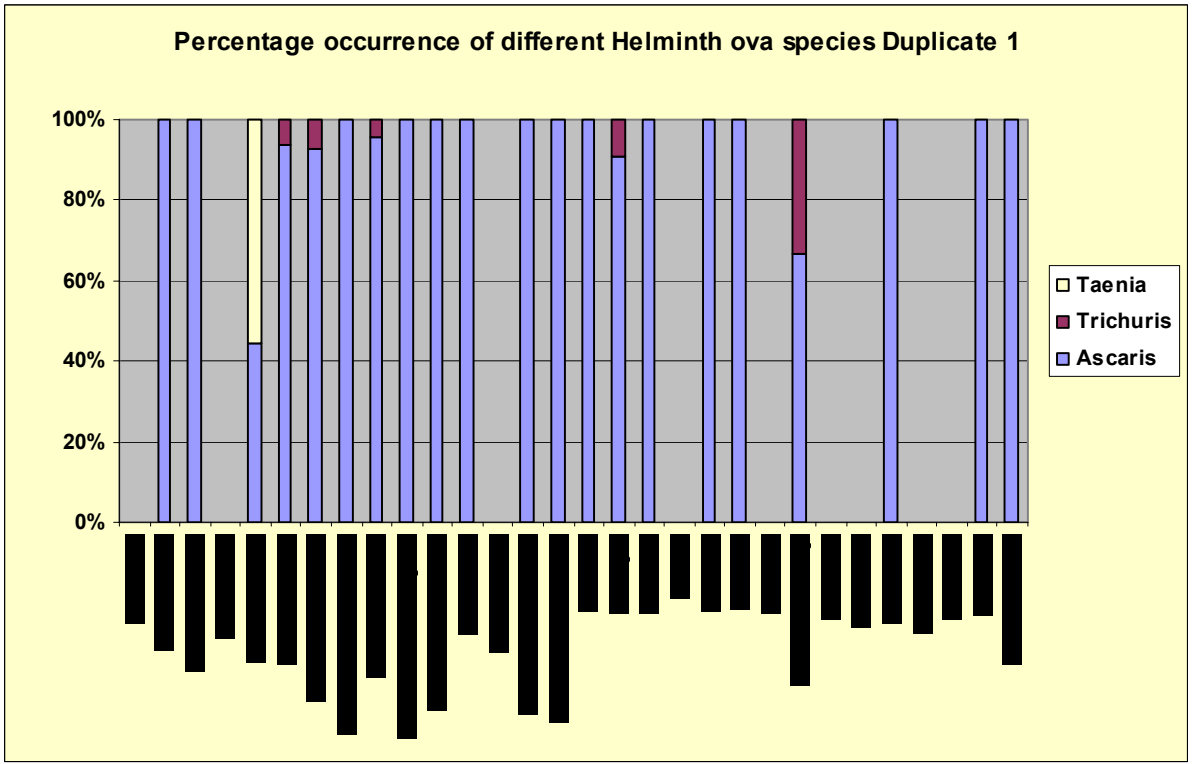
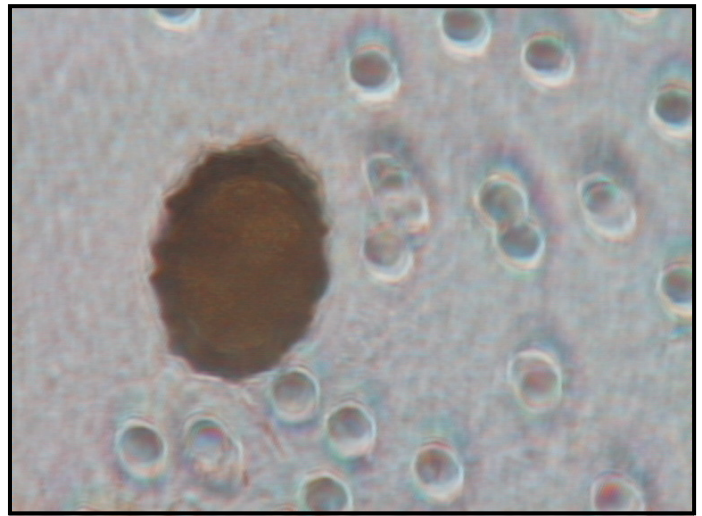
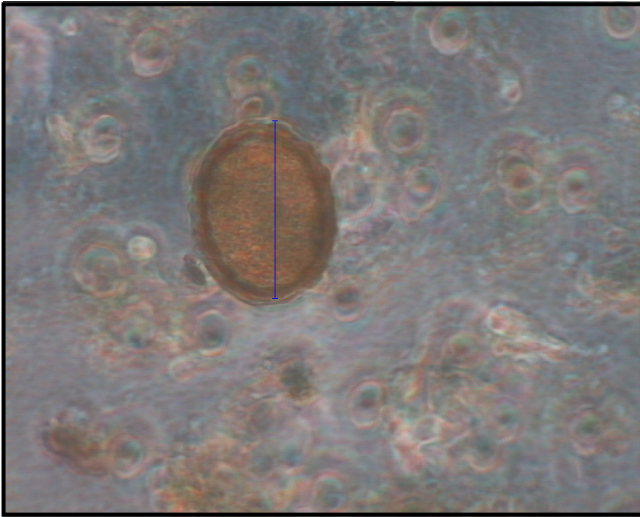


Figure 8.3: Percentage occurrence of the different helminth ova species recorded per duplicate sample for each of wastewater treatment plant

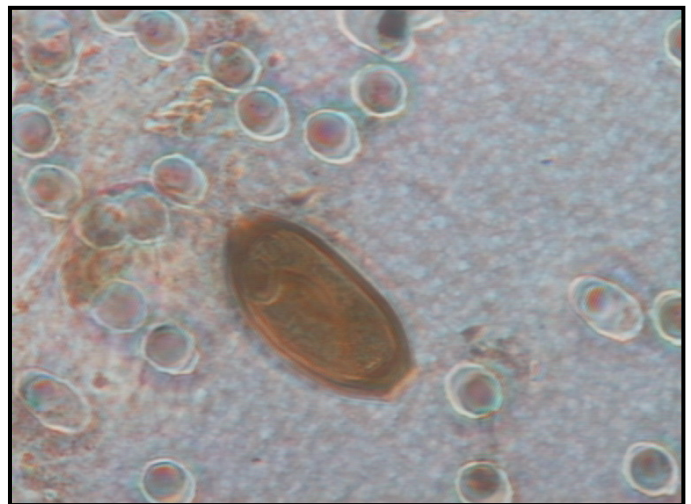
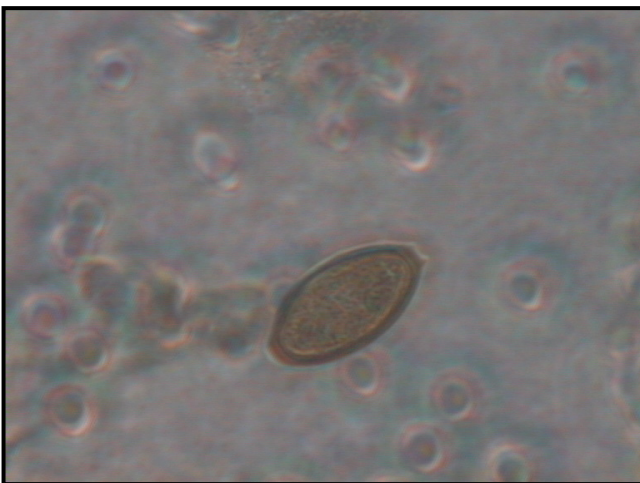


**Photographs of helminth ova observed:**

*Ascaris sp.* - 40X Magnification



*Trichuris sp.* - 40X Magnification



In conclusion - the first order prevalence analysis on the 30 wastewater treatment tested indicated *Ascaris* sp. as the most abundant Helminth found in all areas. From the results *Trichuris* sp. and *Taenia* sp. were identified in some samples however these are considered insignificant in number. The analysis did allow for vigorous testing of the method; however due the differences in wastewater sludge treatment processes, wastewater inflow characteristics, and plant capacities it was not possible to draw any real statistical analysis of the data obtained. A scientific study needs to be undertaken to provide validated data and statistics. The information obtained however does give some indication of helminth ova prevalence in South Africa. This task did achieve the objective of validating the method.

## 9 TRAINING SESSIONS TO BUILD CAPACITY ON HELMINTH OVA METHODS DEVELOPED

The second primary objective of this project was to transfer skills and develop capacity in the Helminth ova method that was adopted for South Africa. In this regard three training sessions of three days each were arranged. The 15 laboratories that were invited and only 7 laboratories were willing or able to send representatives. The laboratories that participated are listed below in **Table 9.1**.

**Table 9.1 Laboratories and attendees that participated in the training sessions**

	<b>Laboratory</b>	<b>Participant</b>	<b>Position</b>
1	Johannesburg Water	Mrs Jennifer Culpan	Scientist
2	Tshwane Municipality	Ms Ida Böhmer	Microbiologist
3	Ethekweni Municipality	Mrs Kimoni Dhunpath	Senior scientist
4	Nelson Mandela Metro	Mrs Phumza Matyolo	Laboratory technician
5	Midvaal Water	Mrs Flavia Letseka	Laboratory assistant
6	Umgeni Water	Mr Lloyd Donnelly	Laboratory technician
7	Magalies Water	Mr Chris Jansen van Vuuren	Snr laboratory technician

The training was held at ERWAT laboratories in Kempton Park. The training was conducted by Ms Colleen Archer from the PRG of UKZN who was assisted by David Hawksworth also from the PRG of UKZN and Lizette Leibach of ERWAT. The contact details of participants are included in **Appendix A**.

**Table 9.2: List of attendees**

<b>Laboratory</b>	<b>Participant</b>	<b>Position</b>
Johannesburg Water	Mrs Jennifer Culpan	Scientist
Tshwane Municipality	Ms Ida Böhmer	Microbiologist
Ethekweni Municipality	Mrs Kimoni Dhunpath	Senior scientist
Nelson Mandela Metro	Mrs Phumza Matyolo	
Midvaal Water	Mrs Flavia Letseka	Laboratory assistant
Umgeni Water	Mr Lloyd Donnelly	Laboratory technician
Magalies Water	Mr Chris Jansen van Vuuren	Snr laboratory technician

Each participant was given a training manual for use at the training session and for further reference in their own laboratories. The manual was compiled in such a manner that it could be used as a reference guide. The training manual has been revised to include the comments of the attendees and now forms TT report 322/08.

This skills transfer and capacity development that transpired during the training sessions was aimed at a stepping stone to building further capacity in the country as the training was based on the “train the trainer” model. These 7 individuals will have to now transfer the skills and train their staff and colleagues. By doing so South Africa will within the next few years, have some expertise available for measuring Helminth ova, as well as have some indication of prevalence by routine application of the method.

## **10 CONCLUSION**

The helminth ova method that was developed through this project has proven to be simple, cost-effective and appropriate for South Africa. The next step would now be to propose it as a standard method for South Africa to adopt. The poster/brochure documenting the method that will be finalised shortly through this project will also serve as a mechanism to create awareness of the existence of the method and its applicability for wastewater, wastewater sludge and soil samples.

A recommendation from the participants of the training session is that the training needs to happen again at more centres around the country and more frequently – not just as a once-off exercise as was the case in this instance. It was found to be very valuable and worthwhile. It is therefore recommended that the WRC consider running the training sessions throughout the major centres of South Africa to build much needed capacity and promote desperately needed skills transfer to more laboratory technologists.

A further recommendation would be that the WRC commission a project to determine the prevalence of helminth ova in South Africa. With a verified method now being available, the next logical step would be to try and generate some helminth ova prevalence statistics for South Africa. This will allow for determination of the most abundant helminth species as well provide an indication of the incidence of parasitic infections.

While having an easy and cost – effective method available has taken South Africa forward, an issue that has emerged through the training is the microscopy analysis and the ability to identify helminth ova and the species once the eggs have been extracted. Microscopy is a “dying” art in South Africa and the key to understanding prevalence and occurrence of helminth ova lies in the ability to identify them after isolation. Thus while we have now been able to adopt a standard method, a gap that still needs to be addressed is the building of capacity in microscopy analysis and in the helminth ova identification. The project team did attempt to assist in some regard with this by the inclusion of photographs of examples of helminth ova in the poster/brochure and the training manual to serve as a reference guide for inexperienced laboratory technologists – however this is still a small step in addressing the need.

## 11 REFERENCES

Archer C., Maya C., Hawksworth D., Leibach L., and Moodley P. 2007. Methods for Determining Helminth Ova Burdens in Wastewater, Sludge, Compost and Urine Diversion Waste in South Africa. Training manual prepared for training sessions conducted as part of project. WRC Project K5/1662.

Cabaret, J., Geerts, S., Madeline, C. and Barbier, D. 2002. The use of urban sewage sludge on pastures: the Cysticercosis threat. *Vet. Res.* 33 (2002) 575-597.

EPA Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge. EPA/625/R-92/1013, Revised October 1999.

Hawksworth D., Archer C., Rodda N., Smith M., Appleton C., Buckley C (not yet published). Method for the Recovery of Ascaris Ova from Solid Waste from Urine Diversion Toilets.

Jiménez B., Maya C. and Schwartzbord J. 2005. Comparison of Techniques for the Detection of Helminth Ova in Wastewater, *Journal of Water Environment Research*.

Jiménez B and Maya C .2006. Techniques for Determining Helminth Ova in Wastewater and Sludge. Instituto de Ingeniería, UNAM, Mexico.

Maya C; Jimenez B; Schwartzbrod J. 2006. Comparison of Techniques for the Detection of Helminth Ova in Drinking Water and Wastewater. *Water Environment Research*, 78 (2), 118 – 124.

## **APPENDIX A**

### **PARTICIPANT CONTACT LIST**

**METHODS FOR THE DETERMINATION OF HELMINTH OVA BURDENS IN WASTEWATER EFFLUENT, SLUDGE,  
COMPOST AND URINE-DIVERSION WASTE**

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