Phase I Grand Challenges Explorations Financial Report

Organization Name: Universidad Nacional Autónoma de México
Project Title: Software to identify and quantify pathogenic helminth eggs
Report Type: (Interim 02)
Reporting Period *From* **(06/01/2012)**
To **(09/30/2012)**

Total Expenses by Type	PHASE I PROJECT: ACTUAL EXPENSES (in US dollars)
Personnel	$6,142.31 (two bourses)
Equipment	$284.11
Travel	$0.00
Consultants	$0.00
Supplies	$3,612.22
Subcontracts	$8,177.25
Project Expenses Subtotal	$18,215.89
Other sources of project support (see Section 1, number 3 above)	$4,000 (lab work)
Total Project Cost	$48,007.22

Introduction

Helminth egg content in wastewater, sludge and faecal sludge, is a critical environmental parameter in ensuring proper sanitation, which, today, is a major problem faced by most low income regions worldwide. Most developing countries are located in arid and semi-arid areas, where water demand for irrigation is as high as 81% (45% in developed countries) due to dependence on agriculture for food and produce for export (Blumenthal *et al.*, 2001). These countries also cover three quarters of the world's irrigated area, so wastewater reuse for irrigation is a common practice that encompasses two challenges: (a) the need to increase sanitation coverage with reliable treatment processes and (b) the need to manage wastewater not only as an ecological problem but also as a source of both water and nutrients. This opens up an opportunity to couple sanitation with reuse, allowing the development of new technologies.

Wastewater reuse in agriculture is a longstanding and beneficial practice. It saves significant volumes of fresh water and reduces reliance on artificial fertilizers or simply renders them accessible to poor farmers. It also increases incomes by allowing crops to be sown all year round. However, wastewater reuse also brings with it health risks, mainly as a result of its pathogen content. Helminth (parasitic worm) eggs are at the origin of at least part of these risks and are considered highly dangerous due to their low infectious dose and high persistence in the environment. Helminthioses are endemic in Africa, Latin America and East Asia, affecting more than 1,200 million people, who commonly suffer from diarrhea, undernourishment and anemia (Jiménez, 2007). Of these sufferers, 300 million are infected with serious clinical illnesses, and 155,000 die each year. Children, especially between 5 and 15 years of age, are most vulnerable because at this age undernourishment is a source of reduced physical, immune and mental development. For these reasons, since 1989, the WHO proposed a control limit of <1 helminth egg per liter (HE/ L) for the wastewater used for irrigation and, later, in 1992 USEPA established a limit of 0.25 HE/ g of sludge. In 2006, the WHO confirmed the importance of regulating helminth eggs in the reuse water for agriculture and aquaculture as well as the sludge.

However, there are two major barriers to the application of these controlling helminth eggs criteria:

(a) The lack of affordable and efficient treatment methods for the extremely high pathogen concentrations in wastewater, sludge and faecal sludge produced in developing countries.

(b) Scarce worldwide trained and available personnel to identify the eggs in influents, effluents and treated sludge in order to define reliable treatment processes.

The analytical technique used to identify and quantify helminth eggs basically consists of a series of steps in which these organisms are separated from particles and detritus contained in wastewater or sludge. Once helminth eggs are concentrated in a relatively clean sample, a laboratory technician needs to identify and quantity them using an optical microscope. This last step is critical and microbiologists are not capable of performing it without a long training program. In addition, this step is the major source of uncertainty in quantifying results. This proposal is focused on the development of automatic image analysis software that performs the identification and quantification steps, enabling in this way the development of effective, low cost, systems which could be implemented in several environmental monitoring facilities and laboratories throughout the world.

Objective

The development of image analysis software that will automatically perform helminth egg identification and quantification, reducing the consumption of materials and expenditure on highly trained personnel while obtaining reliable results.

System development

Primarily, a compilation and review of promising image recognition techniques was performed to select the most suitable to develop a specific recognition protocol for the helminth eggs identification.

At the same time, a microscopic image processing workstation was installed to create a digital database of up to 720 helminth eggs images. An optical microscope Carl Zeiss Axio Lab A1 and two digital photomicrography cameras were utilized to reach this objective. From this compilation, five species were selected, based on their medical importance and worldwide ubiquity, to be tested in a first version of the system: *Ascaris lumbricoides* as fertile and unfertile, *Trichuris trichiura, Toxocara canis, Taenia saginata* and *Schistosoma mansoni.*

A study of the particular characteristics of each species (size, shape, texture, internal structure, etc.) was carried out. Detailed and clear images were used for this purpose, but also pictures with visual artifacts, reproducing the most common difficulty levels on the examination of samples (Figure 1).

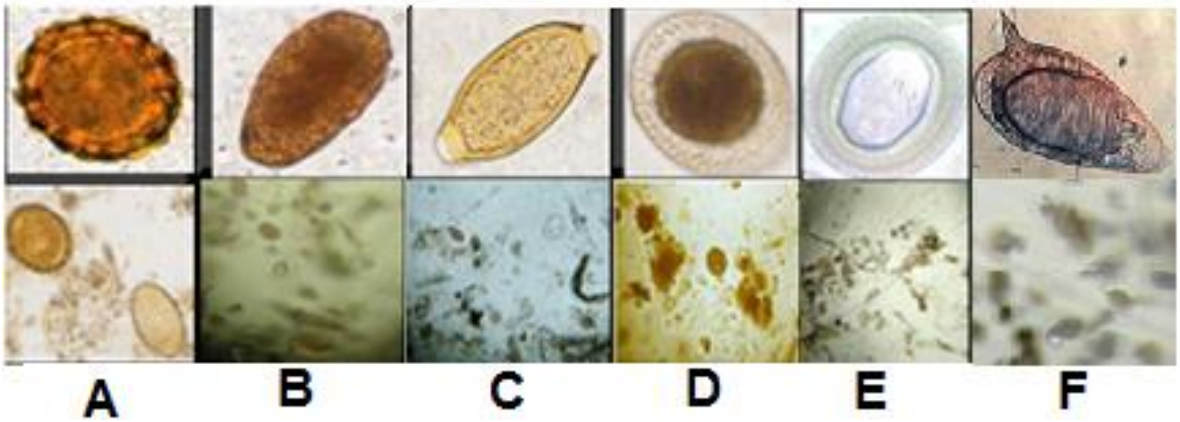


Figure 1. Clean and dirty images of some selected species: (A) *Ascaris lumbricoides* fertile, (B) *Ascaris lumbricoides* unfertile, (C) *Trichuris trichiura*, (D) *Toxocara canis*, (E) *Taenia saginata* and (F) *Shistosoma mansoni.*

Afterwards, a specific image processing algorithm for the helminth eggs was developed. This algorithm has two different stages: the first one consists in detecting and labeling all of the objects in an image using a median filter and applying algorithms to carry out adaptive histogram equalization, edge detection and watershed treatment. An example of these steps is presented in Figure 2.

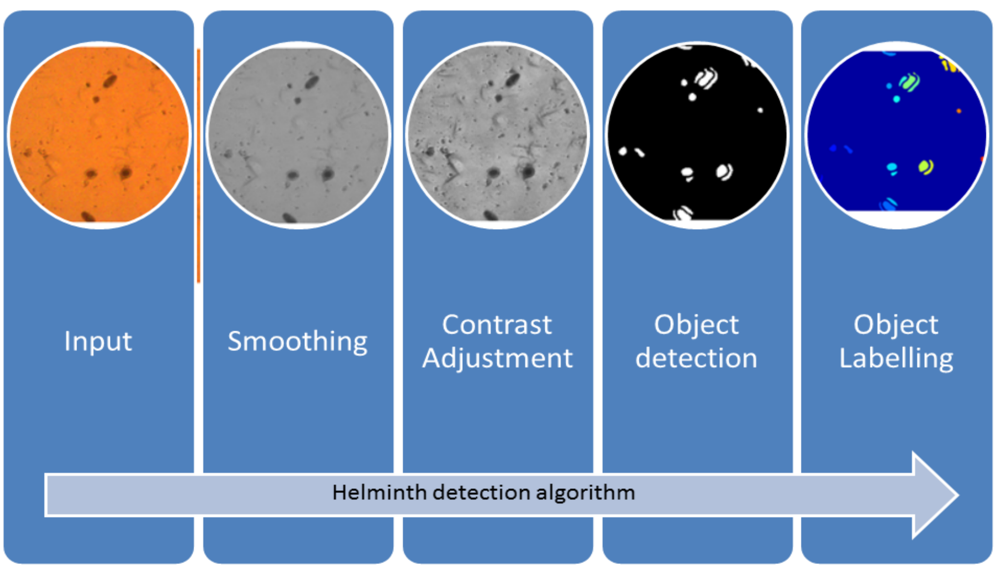


Figure 2. Flow diagram of the helminth detection algorithm.

The second stage of the algorithm performs the classification of each one of the detected objects. This classification is the resultant of measuring shape properties (area, perimeter and eccentricity) and texture properties (energy, mean gray level, contrast, correlation and homogeneity) for each object. 360 images regarding the selected species were used as training pictures to establish their range of values for each classification property. Therefore, by using such training data for the system, it is possible to identify a new object using a nearest neighbor classifier based on Mahalanobis distance (Figure 3).

$$D_M(x) = \sqrt{(\vec{x}-\vec{y})^T S^{-1}(\vec{x}-\vec{y})}$$

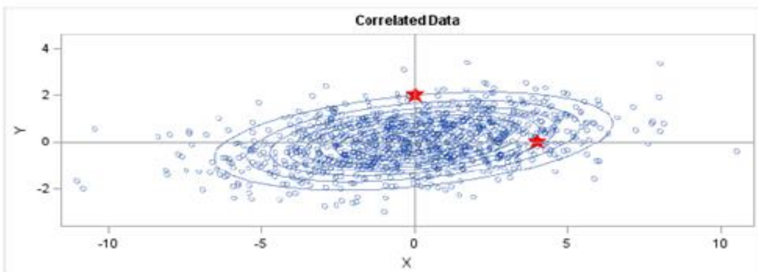
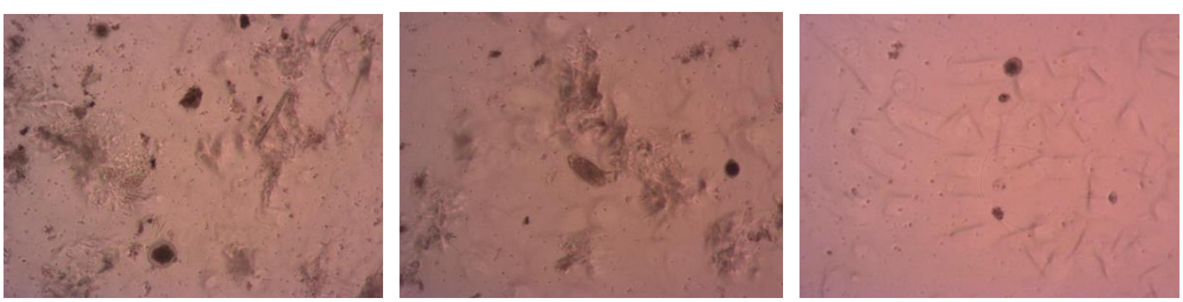


Figure 3. Mahalanobis distance between the properties *X* and *Y* compared to a group of previously classified cases.

The main characteristics of each species have been identified using images of filtered and unfiltered wastewater samples, as shown in figure 4.



(a) **(b)** **(c)**

Figure 4. Typical image of helminth eggs (a) raw wastewater, (b) treated wastewater, and (c) filtered wastewater.

So far, half of the 720 helminth images have been used for validation. The algorithm allows at this point an average true positive fraction (TPF) of 85% among the four genera tested, and a

TPF of 86% on identifying an unfertile *Ascaris*, as shown in table 1. Improvements are still needed, especially on the fertile *Ascaris* identification.

Table 1. Detection, identification and reliability results for helminth eggs ID system. Primary tests with base samples of 15 specimens of four genera.

Genus	Fertile *Ascaris*	Unfertile *Ascaris*	*Toxocara*	*Taenia*	*Trichuris*	Other Objects
Real number of eggs per sample	15	15	15	15	15	623
Correct detection as Helminth	15	13	14	14	13	72
Correct identification of genera	10	13	12	13	12	NA
Percentage of Reliability	66%	86%	86%	86%	80%	88%

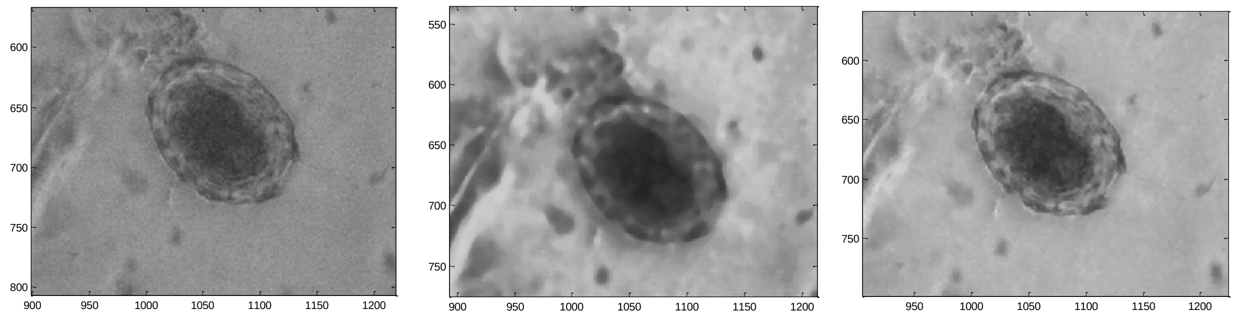
Although the algorithm is yet to be optimized, this system is capable at this time of distinguishing between four of the most common helminth eggs genera and also from other objects in water samples like detritus, air bubbles, pollen particles, etc. However, regarding the figure 1, it is clear that the algorithm so far (figure 2) does not take advantage of other differences among the genera. Thus, an improved version is expected to be developed and an increase on the number of involved species (*Hymenolepis nana, Hymenolepis diminuta* and *Schistosoma mansoni*). The additional genera were selected, in the case of *Hymenolepis*, because of the difficulty level of identification and, in the case of *Schistosoma*, because it is a widespread genus especially in Africa and South America.

Additionally new morphological descriptors such as perimeter roughness and internal structures will be included in the classifier in order to optimize the egg recognition results, as described below.

The next steps

Our objective is the development of the detection system with the maximum reliability. So now the team is focused on improving the identification algorithm to reach percentages of correct identification over 95% between fertile and unfertile *Ascaris* and among genera, and seeking a 99% of correct detection of helminth eggs among other objects.

To accomplish the goal, an extension of the software development is taking place, including new morphological descriptors. The first change will be a new filter algorithm. The median filter that has been used in the previous results has problems preserving the borders of the eggs as shown in figure 5. A better option would be an anisotropic diffusion filter because it has the capability to preserve the borders and edges while smoothing the rest of the image.



(a) **(b)** **(c)**

Figure 5. Filter comparison (a) Original Image, (b) Median filtered image, and (c) Anisotropic diffusion filtered image.

A further change will be the use of a bayesian classifier for improving the detection algorithm; it will be trained with new morphological characteristics of each egg genus, such as compactness index and form. In table 2 the next stages of the project are presented in chronological order. Once the improvements had been implemented in the system, a comparison of its performance will be made against the results obtained by the traditional laboratory technique to identify the helminth eggs. This shall lead us to new specific final recommendations about the system to be implemented before the final report of the project. At the end of this process it is expected to obtain software ready to be trial distributed and patentable.

A trial with selected local laboratories will be performed seeking a first external validation to the system as well as recommendations from field experts. Afterwards, the final report will be delivered to the Foundation Committee, and afterwards, it is intended to begin an international trial distribution to selected laboratories to evaluate the acceptance of the product. We hope this development shall be of great impact in many countries, but especially it will be a useful tool in those areas where water quality measurements are still a challenge.

Table 2. Following steps to take and project calendar in the development of the Helminth eggs identification system.

Product	Oct-Nov 2012	Dec-Jan 2012	Feb 2013	Mar-Apr 2013	May 2013	Jun 2013
Development of the second version of the system	XXXX					
Validation against traditional technique	XX	XXXX				
Second report and recommendations		XX	XXXX			
Development of the third version of the system			XX	XXXX		
Final report (first year)						XXXX

References

Blumenthal U., Cifuentes E., Bennett S., Quigley M., Ruiz-Palacios G. (2001). *The risk of enteric infections associated with wastewater reuse: the effect of season and degree of storage of wastewater*. *Trans Roy Soc Trop Med Hyg*. **95**: 131-137.

Jiménez B. (2007). Helminth ova removal from wastewater for agriculture and aquaculture reuse. Water Sci. Technol. ***55****(1-2):485-493.*

USEPA, 1992. Guidelines for water reuse. Report US EPA/625/R-92/004. U.S. Agency for Environmental Protection Agency, Washington, DC.

WHO (1989). Health Guidelines for the use of Wastewater in Agriculture and Aquaculture. Technical Report Series No 778, World Health Organization, Geneva Suiza.

WHO (2006). Guidelines for the safe use of wastewater, excreta and greywater. Vol. 1-2: Wastewater use in agriculture. Geneva.