

USE OF A FAECAL PARASITE CONCENTRATOR 'MINI PARASEP®' FOR DETECTING MICROSPORIDIA IN URBAN AREAS.

Haafizah Hoosen¹, Fernando Izquierdo², Carmen del Aguila², Soledad Fenoy², Angela Magnet², Umar Anjum¹ Antonio Peña-Fernández¹

^{1.} Faculty of Health & Life Sciences, De Montfort University, Leicester, UK

^{2.} Facultad de Farmacia, Universidad San Pablo CEU, Madrid, Spain

Haafizah.hoosen@dmu.ac.uk; Antonio.pena-fernandez@dmu.ac.uk

INTRODUCTION

Recent outbreaks of infection affecting large groups of population in Europe has been related with the emerging group of pathogens microsporidia (Decraene et al., 2012).

MATERIALS & METHODS

40 faecal samples were collected from two locations on 28/06/2016, monitored areas and sources shown in Figs. 1 &2 using Parasep® tube. The samples were treated according to manufacturer's instructions, the solution smeared onto a microscope slide and stained using a modified trichrome stain (Galván-Díaz et al., 2014; Fig. 3).

OBJECTIVES

The aim of this study was twofold: **a)** to determine the possible presence of microsporidia in recreational environments where there is a risk of exposure to the public; and **b)** to determine if the novel device Parasep® tube (Apacor, UK) with Alcorfix® (alcohol-based fixative) can be used to collect safely animal faecal samples for parasitic diagnoses. This was tested with or without Triton-X.





Figure 1: Bradgate Park & Watermead Country Park, Leicester.

Chart 1: Animal source of faecal samples.

RESULTS

11 of the 40 faecal samples collected were found to have

1) Sample Preparation

2) Emulsification

Introduce a pea sized faecal samples to the fixative using the spoon on the end of the Mini Parasep [®] filter. Mix in thoroughly with the Mini Parasep [®] spoon.

Seal the Mini Parasep[®] by screwing in the

Vortex or shake to with the sedimentation

filter/sedimentation cone unit.

cone pointing upwards

3) Centrifugation

Invert Mini Parasep [®] and centrifuge at 1200g for three minutes. Mini Parasep [®] fits all 15ml centrifuge buckets. NO TE: TO CALCULATE THE REQUIRED RPH FOR ANY CENTRIFUGE. FIPM = √ <u>9</u> x 1000 1.12r RPM - retor speed in ress/min. g - centrifugal force (max.1000g) r - radius, herizortal distance between sementation cone tip and spindle centre measured in mm. DEBRIS FATTY PLUG SUPERNATANT

4) Examination

Direct – Unscrew and discard the filter and mixing tube. Pour off all the liquid above the sediment. Pipette one drop of sediment onto slide, then proceed as appropriate for staining technique.

Figure 2: Process of using Mini Parasep® faecal concentrators. Instructions and illustrations taken from Apacor Instruction manual.



either compatible or positive structures with microsporidia (Table 1; Chart 2; Figs. 4 & 5).

Table 1. Samples found to have positive or compatible structuresfor microsporidia.

Park/Area	RESULT	SAMPLE SOURCE	DIFFERENCE WHEN TREATED WITH Triton – X?
Bradgate	Compatible with Enterocytozoon bieneusi	Dog	No
Bradgate	Encephalitozoon	Unidentified	No
Bradgate	Encephalitozoon	Deer	No
Bradgate	Encephalitozoon	Deer	No
Watermead	<u>Compatible with</u> Enterocytozoon bieneusi	Waterfowl	No
Watermead	Encephalitozoon	Unidentified	No
Watermead	Encephalitozoon	Unidentified	No
Watermead	Encephalitozoon	Dog	No
Watermead	Compatible with Enterocytozoon bieneusi	Fox	No





Watermead	Compatible with Enterocytozoon bieneusi	Dog	No
Watermead	Encephalitozoon	Unidentified	No

Figure 4: Modified trichrome staining displaying <u>compatible</u> structure for *E. bieneusi* at x100 magnification.

Figure 5: Modified trichrome stain displaying a microsporidia spore at x100 magnification.

REFERENCES

Decraene V., Lebbad M., et al. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. Epidemiol Infect. 2012 Mar;140(3):519-27. **Garcia,L (2002)**, Laboratory identification of the Microsporidia. Journal of Clinical Microbiology; 40(6):1892-1901

Galván-Díaz AL., Magnet A., Fenoy S., et al. Microsporidia detection and genotyping study of human pathogenic E. bieneusi in animals from Spain. PLoS One. 2014 Mar 20;9(3):e92289.

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CONCLUSIONS

- The detection of human pathogenic microsporidia (*Enterocytozoon bieneusi* & *Encephalitozoon*) may be public health risk.
- Using Parasep® does not affect the detection of microsporidia (with or without Triton-X) through microscopy and therefore may be an appropriate way to collect hazardous samples safely.