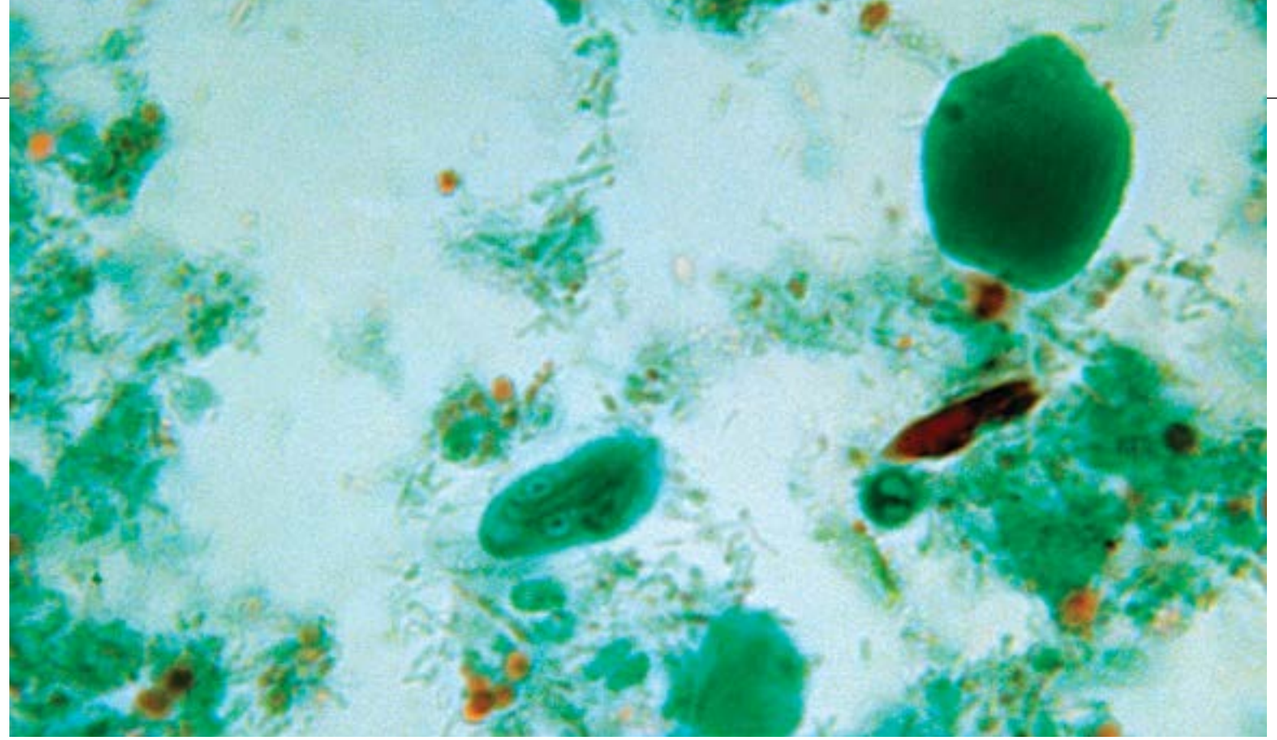
HOW TO...
USE TRICHROME STAINING FOR ENVIRONMENTAL SURVEILLANCE OF PROTOZOAN HUMAN PARASITES

Can trichrome staining be a cost-effective tool? Parasitology PhD student **Umar Anjum** and colleagues report on a pilot study.

Environmental contamination by protozoan intestinal human parasites has become a significant global threat due to increasing globalisation, climate change and population increase globally.

In this communication, we describe a simple laboratory procedure, trichrome staining, as a preliminary step for determining the environmental presence of *Giardia duodenalis*, for which, despite being a worldwide leading diarrhoeagenic human parasite, infection remains underdiagnosed in the UK. To highlight the necessity of the implementation of routine monitoring studies for determining biological contamination in highly populated areas to minimise infections, we describe a pilot study in which we detected the presence of *Giardia* in urban and recreational areas of Leicester. The trichrome staining technique proved to be a sensitive and cost-effective technique for the detection of this parasite in urban areas. The procedure could be easily adopted by relevant authorities with moderate training to perform surveillance studies as a preliminary screening to identify potential risks and perform further thorough studies that are more expensive.



Photomicrograph of a *Giardia duodenalis* cyst seen using a trichrome stain

IMAGE: GETTY

Introduction

Giardia duodenalis (syn. *G. lamblia* and *G. intestinalis*) is an intestinal protozoan capable of infecting a range of different animal species, including humans, which can be found in soil, water and food contaminated with infected animal faeces. Identification of intestinal parasites traditionally depends upon demonstration of their infective forms (e.g. cysts, oocysts, ova or trophozoites) in wet faecal mounts in saline and/or iodine. However, these methods require relatively skilled personnel who can recognise the morphological structures of the infective forms in the very heterogeneous environment found in a stool smear. Normal trichrome stain could be a more sensitive staining technique for detecting *Giardia* and other emerging human protozoan intestinal parasites such as *Entamoeba*.

Trichrome stain

Small portions of the internal part of fresh animal droppings are sampled from urban surfaces, preferably in dry weeks to avoid weather influence. Thin and uniform smears are prepared within a biological safety cabinet by using a small amount of homogenised faecal sample. Smears are then fixed in methanol for 10 minutes and air-dried. The staining process consists of immersion of fixed slides in Coplin jars containing 70% v/v ethanol for five minutes and then trichrome stain for 10 minutes. Smears are then immersed in acidified alcohol solution (ethanol 90% v/v and acetic acid 1% v/v) for 5-10 seconds, for colour differentiation. Slides are then immersed twice in 96% v/v ethanol to stop the action of the acidified alcohol solution.

The final step consists of the following dehydration phases: a) 96% v/v ethanol for 5 minutes; b) repeating the same step in a separate 96% v/v ethanol container; c) 3 minutes in 100% ethanol; d) 10 more minutes in xylene and finally e) mounted with DPX to avoid re-hydration. Slides are then ready to be examined under a light microscope.

Environmental pilot study

A total of 21 fresh animal faecal samples were collected from Humberstone Park in Leicester in August 2017. A veterinarian identified the animal species as: seven avian and 14 dog. Smears were stained with trichrome as, described. *Giardia* spp. cysts were observed in three dog faecal samples (21.4%). The cytoplasm of cysts appear dark blue-green being clearly differentiated from other potential artefacts and background.

This pilot study confirmed a preliminary study in which we specifically detected the presence of *G. duodenalis* in one dog faecal sample from 18 animal stool samples collected in Castle Park (central Leicester) in winter 2016, using a highly specific immunoassay technique. Our results would indicate the presence of *Giardia* spp. in Leicester, in which dogs might play a potential role in transmission.

> *Normal trichrome stain could be a sensitive and cost-effective technique for survey studies*

Applicability of trichrome stain

Trichrome stain could be a sensitive and cost-effective technique for the preliminary detection of protozoan parasites in the environment, as compared to conventional wet-mount methods and prior to performing other, more species-specific, techniques. Wet preparations may not be adequate for performing environmental surveillance studies to protect human health, as protozoan parasites could be difficult to detect due to their smaller size and resemblance with artefacts such as food particles, air bubbles and vegetable or fat cells. The chromotrope 2R, a specific component of trichrome stain, has a strong affinity to chromatin, making the protozoan parasites more prominent and easily identifiable.

Conclusions

Trichrome stain could be a sensitive and cost-effective method for the establishment of routine environmental survey studies in highly populated cities for identifying potential hot-spots and risks due to the presence of protozoan human parasites in urban areas, prior to performing more sophisticated species-specific tests.

The staining test could be easily adopted by the relevant authorities with moderate training and/or resources for quick identification of these risks. BMS

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