Experimental Parasitology 147 (2014) 67–71

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Mini-FLOTAC for counting *Toxoplasma gondii* oocysts from cat feces – Comparison with cell counting plates

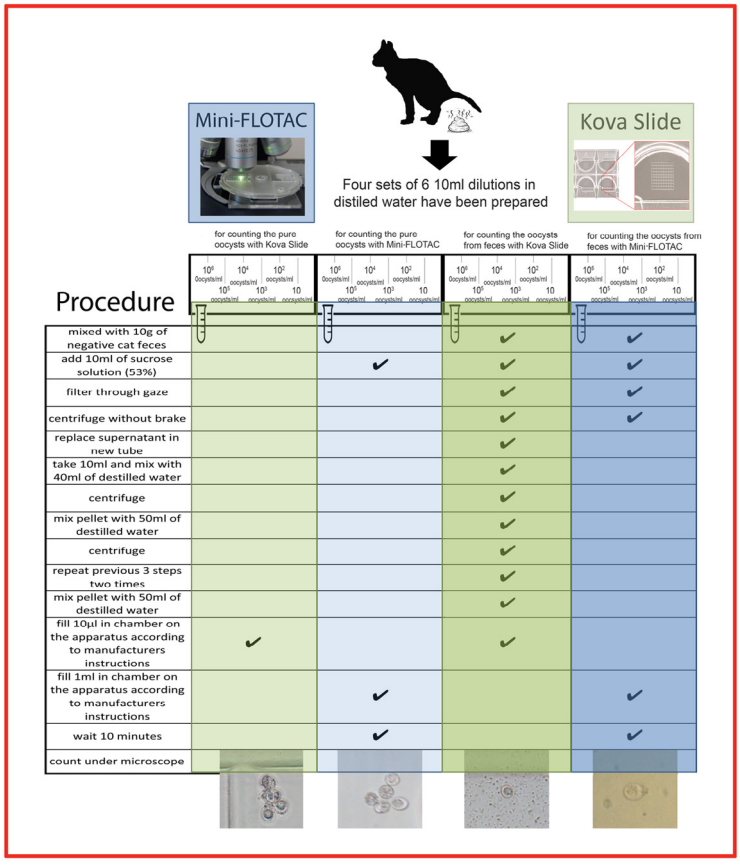
Vitomir Djokic [a,b,*], Radu Blaga [c], Laura Rinaldi [d], Delphine Le Roux [c], Tamara Ducry [a], Maria Paola Maurelli [d], Catherine Perret [a], Olgica Djurkovic Djakovic [b], Giuseppe Cringoli [d], Pascal Boireau [a]

[a] *ANSES, Laboratoire de santé animale de Maisons-Alfort, UMR BIPAR, Université Paris-Est, Maisons-Alfort, France*
[b] *National Reference Laboratory for Toxoplasmosis, Institute for Medical Research, University of Belgrade, Dr. Subotića 4, P.O. Box 102, Belgrade 11129, Serbia*
[c] *Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Université Paris-Est, Maisons-Alfort, France*
[d] *Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy*

HIGHLIGHTS

- The study compares Mini-FLOTAC with Kova Slide for detection of *T. gondii* oocysts.
- Thousand times higher sensitivity of Mini-FLOTAC has been shown.
- Mini-FLOTAC is more sensitive with more accurate quantification of oocysts.
- We therefore recommend its use for regular screening.

GRAPHICAL ABSTRACT



* Corresponding author. Fax: +33 149771316.
E-mail address: Vitomir.djokic@anses.fr; Vitomir.djokic@imi.bg.ac.rs (V. Djokic).

http://dx.doi.org/10.1016/j.exppara.2014.10.009
0014-4894/© 2014 Elsevier Inc. All rights reserved.

ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 6 October 2014
Accepted 16 October 2014
Available online 18 October 2014

Keywords:
Toxoplasma gondii
Oocysts
Detection
Mini-FLOTAC
Kova slide

ABSTRACT

Oocysts of *Toxoplasma gondii* represent one of the most common environmental contaminants causing the zoonotic infection toxoplasmosis. The aim of the present study was to compare the Mini-FLOTAC device with traditional cell counting plates (Kova Slide) for the detection of *T. gondii* oocysts from feline feces. Two types of experiments were performed: (i) purified oocysts were counted in different dilutions and (ii) specific pathogen free *T. gondii*-negative cat feces was inoculated with numbers of purified oocysts and counting was performed directly from feces. Our analysis showed a thousand times higher sensitivity of Mini-FLOTAC (5×10^2 oocysts) compared to Kova Slide (5×10^5 oocysts). Also, when compared by McNemar's test, counting of the purified oocysts showed a higher sensitivity of Mini-FLOTAC compared to Kova Slide, for a dilution of 10^3 oocysts/ml ($chi^2 = 6.1$; $P < 0.05$). A better sensitivity was also found with Mini-FLOTAC in dilutions of 10^5 and 10^4 oocysts/ml, when counted from feces ($chi^2 = 4.2$ and 8.1, respectively, $P < 0.05$). Our results show that Mini-FLOTAC is more sensitive than traditional methods of *T. gondii* oocysts detection and quantification is more accurate. Furthermore, Mini-FLOTAC simplicity and cost effectiveness allow it to be used with light microscopes in any laboratory or field conditions. We therefore recommend its use for regular screening. Further studies are needed to validate Mini-FLOTAC for the detection of oocysts in soil and water samples in field conditions.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

The cosmopolitan protozoan *Toxoplasma gondii*, able to infect humans and all warm-blooded animals, is estimated to be present in one third of the global human population, rendering it the most successful parasite on Earth (Dubey, 1996; Hill et al., 2011). The parasite's life cycle includes a sexual phase completed in the intestines of members of the Felidae family (definitive hosts) resulting in the production of oocysts, and an asexual one in which encysted parasites circulate between prey and predators (intermediate hosts). *T. gondii* achieves persistence in the host by conversion from the proliferative tachyzoite stage into quiescent encysted bradyzoites, a mechanism controlled by the host immune response (Sullivan and Jeffers, 2012). Thus, although generally mild and self-limiting in immunocompetent individuals, *T. gondii* infection may cause life-threatening disease in the fetus and in the immunocompromised host.

Humans get infected by ingestion or inhalation of oocysts from the environment (especially water, fruits and vegetables) contaminated by cat feces, and intake of tissue cysts present in the muscles of infected animals (Torrey and Yolken, 2013). Consumption of unwashed raw vegetables, poor hand hygiene and contaminated water sources were linked to cases of toxoplasmosis in humans (Jones and Dubey, 2010; Skinner et al., 1990). Considering the number of felids that can contaminate human environment and the number of oocysts shed, domestic cats are the species mostly associated with a risk of human infection (Hill and Dubey, 2002). Cats can excrete millions of oocysts after ingestion of a single bradyzoite. The importance of oocysts in the transmission of *T. gondii* is highlighted by the fact that, despite the world-wide distribution of the parasite, infection is virtually absent on small islands and atolls which have never been inhabited by cats (Yu et al., 2013).

Although consumption of undercooked meat has been identified as a major risk factor for *T. gondii* infection in humans, this finding does not explain outbreaks and high seropositivity in populations of vegetarians (Buffolano et al., 1996; Cook et al., 2000; Paul, 1998).

Many methods have been used to detect *T. gondii* oocysts in feces, environmental samples (soil, water), and fruits and vegetables (Aramini et al., 1999; Dumetre and Darde, 2003; Herrmann et al., 2011; Lalonde and Gajadhar, 2011; Meireles et al., 2008; Salant et al., 2010; Sullivan and Jeffers, 2012; Villena et al., 2004). Recent studies use PCR for the detection of oocysts (Lalonde and Gajadhar, 2011), while others use cell counter plates such as Kova Slide (Lelu et al., 2011) or Kato Katz (Meireles et al., 2008). Although these techniques are reliable, they either lack sensitivity/accuracy/precision or their cost effectiveness is not justified, and if not disposable, materials cannot be washed because of the water contamination. Different uses of multivalent techniques belonging to the "FLOTAC family" are described for the copro-microscopic detection of parasitic elements in human and animal feces (Cringoli, 2006; Cringoli et al., 2010).

Mini-FLOTAC is an easy-to-use apparatus from the FLOTAC family which is considered promising for detecting and counting helminth eggs and protozoa (oo)cysts in animals and humans (Cringoli et al., 2013).

In the present study, Mini-FLOTAC and Kova Slide were compared (in terms of sensitivity and accuracy) for the detection of *T. gondii* oocysts from cat feces in a controlled trial. An efficient and accurate method for the detection of *T. gondii* oocysts in cat feces would facilitate early identification of environmental contaminants and improve prevention of human infection.

2. Materials

2.1. Preparation of reference material

One four-month old specific pathogen free (SPF) cat was orally inoculated with a mouse brain containing approximately 100 cysts of *T. gondii* strain type II (76K) (Ethics committee approval number: 21/01/13-2). Prior to infection feces were examined by Kova Slide as described below for the presence of *T. gondii* oocysts. After infection (day 0), fecal samples were collected daily in plastic collection cups with screw lids, according to the manual of OIE for fecal samples collection (OIE Terrestrial Manual 2013). Each sample was stored at +4 °C, until analysis. The whole quantity of feces was homogenized and 10 grams were put in a 50 ml tube and mixed with a sucrose solution (52%, specific gravity = 1.2) as described by Lelu et al. (2011). Ten µl were examined under Kova Slide. Light microscope (Leica DMI 4000B) with ×200 magnification and blue ultra fluorescent light was used for oocysts detection taking advantage of auto fluorescence of *T. gondii* oocysts. For our study fecal sample from day six after inoculation was chosen as representative (approximately 10×10^6 oocysts/g, counted on Kova Slide).

2.2. Preparation of pure oocysts and fecal samples for counting

From a suspension of purified oocysts six-fold dilutions were made; from 1×10^6 to 10 oocysts/ml with 40 tubes for each dilution (in total 240 tubes). Feces for inoculation with oocysts (originating from another SPF cat – negative control), was divided in 120 tubes (10 g/tube). From each oocyst dilution 20 tubes were mixed with 20 tubes of feces. Out of 40 tubes for each dilution, 20 were used per method; 10 for counting the purified oocysts and 10

Download English Version:

https://daneshyari.com/en/article/4371052

Download Persian Version:

https://daneshyari.com/article/4371052

Daneshyari.com