Modification of the Alere GIARDIA Ag TEST immunochromatography KIT methodology for its use in frozen fecal sediment of dogs and cats

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ABSTRACT

Giardia duodenalis is a worldwide intestinal parasite and is one of the most frequent protozoa species infecting dogs and cats. This study aimed to modify the methodology of Alere GIARDIA Ag TEST KIT for its use in frozen fecal sediments with different storage times in a freezer (-20°C), thus expanding the range of use of this methodology. One hundred fecal sediments from dogs (n=50) and cats (n=50) previously examined by optical microscopy for Giardia cysts were selected for this study. The agreement between the modified immunochromatography and microscopy results was calculated by Kappa coefficient. To evaluate the performance of the modified immunochromatography assay on samples with different storage time, the fecal sediments were divided into three groups according to the time of storage in a freezer: (a) ≤ 1 year (n=37); (b) > 1 year and ≤ 3 years (n=39); (c) > 10 years (max. 13 years) (n=24). The results obtained by the modified immunochromatography assay demonstrates a higher sensitivity of this technique when compared with microscopy, regardless of the frozen storage time. These results allow for the use of this methodology in a greater scope of analysis, especially in frozen fecal sediment triage in sample collections, enabling epidemiological and comparative analysis along different decades.

Keywords: cats, diagnosis, dogs, Giardia duodenalis, immunochromatography.

INTRODUCTION

Giardia duodenalis is a worldwide intestinal protozoa that can infect a wide variety of mammal hosts, including humans, and is one of the most frequent protozoa species infecting dogs and cats (Thompson et al. 2000, Ballweber et al. 2010).

Giardia infection rates are variable among those hosts, and are associated with a number of factors, such as, study design, geographical location, climate and season, diagnostic methodology, studied population, health status of the host and animal management conditions (Dixon et al. 1997). Microscopy is the gold standard methodology for Giardia duodenalis diagnosis (Payne and Artzer 2009). However, this method has a high...
percentage of false-negative results because of
the intermittence, low number and size of the
cysts shed in fecal samples. Thus, a combination
of diagnostic methods is frequently indicated to
overcome such limitations (Tangtrongsup and
Scorza 2010). Several complementary diagnostic
methods have emerged in the past years, especially
immunological methods, as tools to improve
*Giardia* diagnosis.

The immunochromatography kit ALERE
GIARDIA Ag TEST KIT® is a method developed
for rapid qualitative diagnosis of *Giardia
duodenalis* cyst antigens in fecal samples from
dogs and cats by using a swab for sample collection.
However, Current and Garcia (1991) found that
parasites have a heterogeneous distribution in a
fecal sample, so the use of a swab for collection
of a fragment of solid or semi-solid feces could
result in false-negative tests, especially in low
parasite burdens. Therefore, the present study
aimed to modify the immunochromatography
test to allow its use in fecal sediments of dogs
and cats. Moreover, the performance of this
modified technique was evaluated on samples
from different storage freezing time in order
to expand the use of this test to samples (fecal
sediment) belonging to research laboratory
collections, allowing epidemiological analysis
and comparisons between different years.

**MATERIALS AND METHODS**

One hundred frozen fecal sediments from dogs
(n=50) and cats (n=50) belonging to two collection
of samples from research laboratories of the
Universidade Federal Fluminense were selected.
Fecal sediments were previously obtained using
concentration techniques usually employed in
parasitological exams (centrifuge-sedimentation
or spontaneous sedimentation). All samples were
previously examined by optical microscopy for
*Giardia duodenalis* cysts. Among those, 18 samples
(6 from dogs and 12 from cats) were positive for
*Giardia duodenalis* cysts by microscopy exam.

To evaluate the performance of the modified
immunochromatography on samples with different
storage time in a freezer (-20°C), fecal sediments
were divided in three groups according to the time
of storage by freezing: (a) ≤ 1 year (n=37); (b) >
1 year and ≤ 3 years (n=39); (c) > 10 years (max.
13 years) (n=24). The freezing times of the chosen
samples were based on the availability of samples
in the laboratory collection.

Because the immunochromatographic test was
developed for use in fresh feces by collecting a fecal
fragment with a swab, the modification to allow its
use in fecal sediments consisted in not using the
swab for sample collection, but homogenizing 50µl
of fecal sediment with 50µl of the kit dilution buffer
for approximately 10 seconds. The following steps
were performed according to the manufacturers’
recommendations, which consisted of removing a
sample of the supernatant using the pipette provided
by the kit manufacturer followed by the instillation
of 4 drops of the sample in the kit cassette well.
Two readings of test results were recorded 5 and
10 minutes after the procedure. Each sample was
tested in duplicate.

The agreement between the modified
immunochromatography and microscopy results
was calculated by Kappa coefficient (Smith 1995).
The evaluation of the influence of storage time on
the performance of the modified method was done
by Mann-Whitney test at a significance level of 5%.

**RESULTS**

The immunochromatography test was positive for
*Giardia duodenalis* antigens in 29 samples (29%),
12 from dogs (24%; 12/50) and 17 from cats (34%;
17/50). A comparison between microscopy and
immunochromatography results is shown on table I.

The frequency of agreement was 74% between
microscopy and immunochromatography. The
The results obtained by the modified immunochromatography demonstrate a higher sensitivity of this technique when compared with microscopy, regardless of the frozen storage time. Moreover, 18/29 samples were positive only by immunochromatography, contributing to a low level of concordance between the techniques evaluated.

**DISCUSSION**

A higher sensitivity of immunological methods when compared to conventional microscopy have
been demonstrated by several studies in a variety of hosts (Vidal and Catapani 2005, Garcia and Garcia 2006, Geurden et al. 2008, Ignatius et al. 2014). Such superiority could be explained by several factors, mainly by the capacity of detection of minimal quantities of antigens, even when the parasite burden is low, which would require several fecal samples for its detection by conventional microscopy methods, especially on chronic infections where the cyst shedding intermittency is frequently observed (Mohammed Mahdy et al. 2008). Moreover, the freezing and thawing of fecal samples may result in damage to cysts, thus, impairing the usefulness of microscopy with frozen samples.

It is worth noting that the usage of fecal sediment rather than the fecal sample swab could have favoured the diagnosis of samples with low cyst quantities, because of the concentration necessary for obtaining the fecal sediment. Moreover, the coproantigen detection can be performed even if the parasite is not intact in the sample, which could not be done by traditional microscopy.

However, it was not possible to detect antigens in seven samples that had cysts of *Giardia* sp. by microscopy exam, which also contributed to the low concordance of methods. Garcia and Garcia (2006) and Weitzel et al. (2006) also reported these discordant results. One possible explanation is the genetic heterogeneity of *Giardia* sp. isolates from different hosts (Mekaru et al. 2007). Additionally, possible reading errors during microscopy exam cannot be discarded because of the human error inherent to these techniques. Dixon et al. (1997) and Vidal and Catapani (2005) stated that the experience and the fatigue level of microscopists and the presence of a great amount of debris on the slides could influence the results of a microscopy analysis.

Although the immunochromatographic test allows a faster diagnosis (about 20 minutes) when compared with microscopy (about 1 to 2 hours, depending on the technique used) and does not require special equipment for diagnosis, it still has higher cost (about 5 times more).

The discordance between microscopy and immunochromatography results were mostly observed on samples with a lesser freezing time. However, 77% of these discordant samples were negative in microscopy and positive in immunochromatography. Such a result can be explained by a greater sensitivity of the immunochromatographic method compared to microscopy in samples with a low number of cysts, and thus there is no correlation with the freezing time.

Therefore, the modification of the ALERE GIARDIA Ag immunochromatography TEST KIT® showed good performance regardless of frozen storage time. These results allow for the use of this methodology in a greater scope of analysis, especially in frozen fecal sediment triage in sample collections, enabling epidemiological and comparative analysis along different decades.

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