scil v-Giardia

Giardia detection made easy

Challenges and Benefits of Giardia Diagnostics

Abstract:

This study intensively discusses Giardia detection out of fecal samples. During routine daily work, Giardia diagnostic is an important tool and it is facilitated by the aid of rapid tests. Difficulties of direct antigen detection are, however, not always known and not taken into account when a diagnosis is made. Irregular distribution of cysts in fecal samples and relatively long shedding periods can thereby lead to problems and insecurities. This paper will evaluate these points in detail and will provide guidance for a correct and optimal Giardia diagnostic. We are also giving an introduction to Giardia infection in dogs and cats. Goal of the present study is providing reference points for an optimal giardia diagnostic of high quality.

First, presence and distribution of cysts in fecal samples were evaluated. Thereby it became obvious that extremely irregular distribution of cysts can occur in feces. Different fecal samples were evaluated using an ELISA and results are displayed and discussed. Different diagnostic tools exist to detect Giardia cysts in the clinic. In the second part of the study these tools were compared and advantages and disadvantages outlined. Finally, Giardia detection using the lateral flow assay scil v-Giardia was compared to a previously validated ELISA using 84 canine and feline fecal samples. In this final evaluation a high agreement could be detected between both assays. In negative (Optical density, OD ≤0.5) and highly positive (OD >1.5) samples agreement was about 99%. Samples with a small number of cysts (OD between >0.5 and ≤1.5) ELISA is more sensitive than scil v-Giardia. In summary, overall agreement is 95.24%, which makes the scil v-Giardia an accurate diagnostic tool to detect Giardia cysts directly in the clinic. It is also a beneficial tool for follow-up examinations.

Introduction

Giardia organisms are flagellated protozoan parasites, which occur in the intestines of many mammals. Giardiasis is a zoonotic disease for which dogs and cats are important vectors. In small animals six *Giardia species* can be differentiated based on their trophozoite-morphology. Of these six species, three are described as being zoonotic.

The following species can be differentiated:

Species	Reservoir
Giardia duodenalis	Man Small animals Non-domestic mammals
Giardia muris	Infected rodents
Giardia psittaci	Birds
Giardia ardeae	Birds
Giardia agilis	amphibians

Life-cycle of Giardia can be described easily. Cysts are excreted via the feces and are the resistant stage of the parasite. Entering the environment, cysts are immediately infectious. As they are very robust, cysts remain vital, and thereby infectious, over month if present in cold water and humid surroundings. Dry weather and high temperatures lead on the other hand to a rapid death of the cysts. A minimal dosage of 10 to 100 cysts can already lead to a profound giardiasis.

Infection occurs via orally, via smear infections, or during ingestion of contaminated food or water. After ingestion, oocysts persist in the small intestines and change into trophozoites. These adhere to the upper layer of the intestinal mucosa and multiply there. Continuous mucosal damage occurs with subsequent loosening of the epithelium. The clinical picture often shows chronic intermittent mucoid diarrhea, with pale and soft feces which are rarely bloody. Especially in kittens and puppies Giardia may lead to profound and recurrent diarrheic episodes with slimy diarrhea.

In addition to the visibly affected animals, Giardia infection may also occur silently. Many men and animals serve as asymptomatic carrier while excreting the infectious organism. *Giardia spp*. occurs in 20% of the adult dog and cat population in Germany. Puppies and young animals are more often affected and prevalence in this population can rise up to 70% in dogs and up to 75% in cats. In infected animals, in addition to the veterinary therapeutic care, special hygienic procedures need to be taken care of due to the high risk of reinfections in Giardiasis. This is especially important in animal shelters or households with multiple animals as there an increased infectious pressure is present.

scil v-Giardia

Giardia detection made easy

Established available detection methods

Different detection methods are available for Giardia analysis but these vary in their sensitivities.

1) Fecal smear

A small amount of feces is placed on a glass slide, mixed with one drop of physiologic saline solution and covered with a cover slip. Using the 20x or 40x objective, trophozoites or oocysts can be detected.

2) Concentration techniques

To find parasites under the microscope an additional enrichment procedure is recommended. This method is quite sensitive and can already detect small numbers of parasites. An example is the i.e. flotation method using a saturated salt or sugar solution. Disadvantage of this method is that flotation results need to be evaluated immediately under the microscope. Otherwise oocysts will dry-out and disrupt in the saturated flotation solution.

3) Immunological methods

Very sensitive techniques are immunological methods. In these procedures proteins of the cystic walls are usually detected. In case of infection with *Giardia duodenalis* cystic wall proteins are released and excreted with the feces. These proteins can then be detected using Enzyme Immunoassays (ELISA) or Lateral Flow Assays (LFA).

Enzyme Immunoassays (ELISA)

A specific monoclonal antibody against *Giardia duodenalis* cyst and trophozoite cellular wall proteins is bound to the surface of microtiter plates. In these wells diluted fecal samples or control material are inserted. A second monoclonal antibody against *Giardia duodenalis,* conjugated with horseradish peroxidase, is added in the next step. After addition of substrates/ chromogens and the stop solution, automatic photometric detection takes place at 450 nm wavelength.

Lateral Flow Immunoassays (LFA)

scil v-Giardia is a LFA and can be described in the following manner: On a test strip a specific monoclonal antibody against *Giardia duodenalis* cyst and throphozoite cellular wall proteins is bound. In a separate sample tube, the fecal sample gets diluted and is subsequently administered to the sample well on the test cassette. A second monoclonal antibody against *Giardia duodenalis*, which is conjugated to a Latex particle, gets in contact with the fecal sample and runs through the test strip. If Giardia organisms are present in the sample, these will be catched by one of the monoclonal antibodies and a test line will become visible. Evaluation of the result can easily be done visually.

4) PCR

PCR is the newest detection method. Via PCR it is possible to differentiate different genotypes of the agents. This procedure is rarely used in clinical practice. In different studies, i.e. from year 2013, PCR showed a lower sensitivity than ELISA methods. Using PCR as the gold standard for Giardia detection therefore needs to be judged critically.

In clinical practice, immunological methods are mostly used. Therefore, in the following study, we will concentrate on these detection methods. Different ELISA will be compared with the scil v-Giardia (LFA).

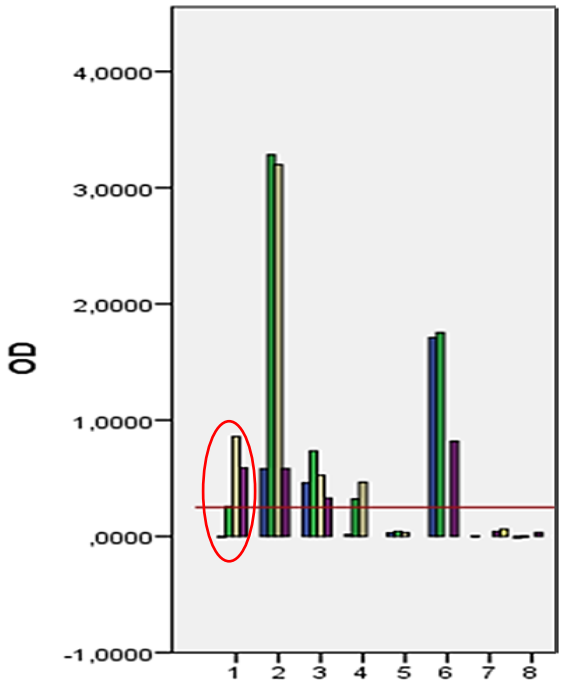
Giardia detection in fecal samples

As described above, evaluation of fecal samples can aid in the diagnosis of a Giardia infection in our patient. A definitive diagnosis should base on an analysis of fecal samples collected over multiple days, as Giardia organism are shedded intermittently. In addition, observations have shown that Giardia organism are irregularly dispersed in the fecal samples. If you are dividing a fecal sample containing irregularly dispersed Giardia organism in 10 equal tubes, not every tube will give a positive Giardia test result. This makes the analysis more complicated.

In our first small study, 20 fecal samples (negative and positive ones) were evaluated using an ELISA. From each sample, different sections were evaluated. Results showed that clearly negative samples also revealed negative results using the ELISA method. Positive samples, however, showed remarkable differences concerning the Giardia concentration in the different subsamples of the positive samples. This could be shown based on different optical density (OD) results of the subsamples. The higher the OD value, the higher the respective Giardia concentration in the evaluated sample.

scil v-Giardia

Giardia detection made easy



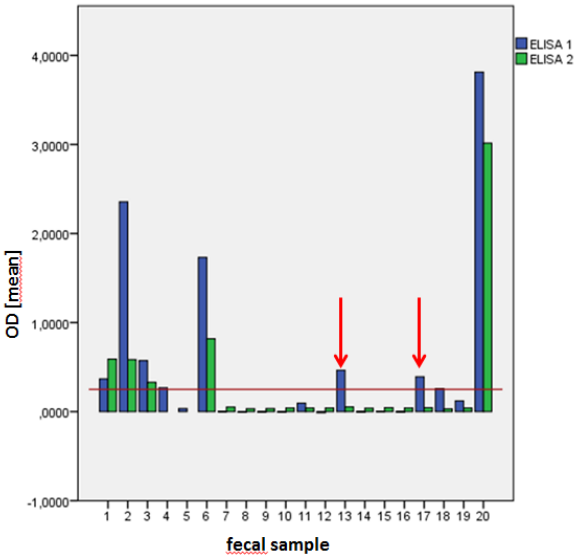
In the first seven fecal samples, a difference was obvious. Four subsamples had been evaluated from the first fecal sample (see circle). Two of these evaluations showed a positive test result (result above the horizontal red line). The first two evaluations did, however, provide a negative test result. From these seven first samples, only samples number five and seven showed clearly negative test results using the ELISA method. This example shows, how variable cysts can be dispersed in fecal samples.

Due to the described irregular shedding of organism as well as the variable dispersion of organism in one fecal sample it is generally recommended to work with the highest care in the diagnostic of Giardia infection.

ELISA comparison

The second study also revealed that different results can occur in samples with low numbers of Giardia organism. In this study, two different ELISA were compared using 20 samples.

In samples with a high Giardia burden and samples without Giardia organism (negative results), results of both ELISA were identical. Differing results were, however, observed in samples containing low numbers of Giardia organism. This is also visible in the next graph. The OD results have been normalized to gain a uniform basis for the direct comparison of the ELISA results. Differences concerning the quantitative results ranged up to 10%. This is exemplified in the graph in samples 13 and 17 (see red arrows). In both cases, ELISA results were positive for one ELISA and negative for the second ELISA. Sample number 18 could also not be identified as definitively negative by one ELISA. Therefore, three of twenty samples showed a deviating result with the two different ELISAs. This reveals a concordance of 85%.

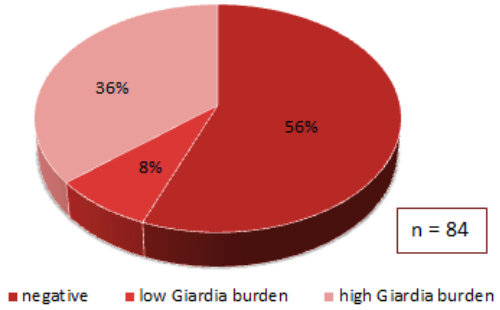


scil v-Giardia Test Performance

In the third evaluation, 84 fecal samples were tested using one ELISA and the lateral flow assay scil v-Giardia. The following table shows the height of the Giardia burden of the 85 samples evaluated in this study based on their ELISA OD results. The following thresholds were defined for samples with a high, or low Giardia burden as well as for negative samples:

Giardia burden	Threshold (OD result)
negative	≤ 0.5
low	> 0.5 to ≤ 1.5
high	> 1.5

Of the evaluated samples (n=84), 56% of the samples were free for Giardia cysts, while 8% showed a low and 36% a high Giardia burden.



scil v-Giardia LFA showed the results after 10 minutes and was remarkably easy and hygienic to use. The fecal sample had been taken up with a swab, homogenized in a sample tube pre-filled with a buffer solution, and then administered to the sample well of the test cassette. The administered fluid run undisturbed through the test strip in all 84 fecal samples and control lines were clearly visible in all samples evaluated. Please note that control line intensity did not serve as a reference line. This is further strengthened in the package insert. A test line of higher intensity than the control line does also provide an accurate result and has to be judged as positive.
The ELISA was performed simultaneously to the scil v-Giardia assay. On the surface of the microtiter plate wells, Giardia specific antibodies against specific *Giardia duodenalis* cyst and trophozoite antigens are bound. A suspension of the fecal sample or of controls were pipetted into the sample wells and incubated at room temperature together with biotinylated anti-Giardia antibodies (conjugate 1). After the washing step streptavidin-poly-peroxidase conjugate (conjugate 2) was added and everything was incubated at room temperature for a second time. After adding the substrate, the bound enzyme converts the clear solution in the sample wells into a blue solution in case of a positive result. Addition of the stop-solution leads to another color change from blue to yellow. Extinction is proportional to the concentration of the *Giardia duodenalis* antigens in the sample.

Results were compared and are summarized in the following table:

Test performance	
Sensitivity	91.89%
Specificity	97.87%
TTP (Total Test Performance)	95.24%

For samples being highly positive, or in the definitively negative fecal samples, comparison of results showed a nearly perfect agreement between scil v-Giardia and ELISA. Only samples with a low Giardia burden were detected with higher sensitivity using the ELISA method. In summary, scil v-Giardia showed a Total Test Performance of 95.24% with a sensitivity of 91.89%, and a specificity of 97.87%.

Summary of Results

Results of the first study showed clearly how heterogeneous *Giardia duodenalis* cysts are dispersed in fecal samples. Out of seven samples, which were tested by four ELISA, only two samples showed concordant ELISA results. This discrepancy is i.e. exemplified by the results of the first sample, in which two ELISA yielded a negative, and two ELISA a positive result for Giardia.

The second study focused on samples with a low Giardia burden. Heterogeneity of Giardia cyst distribution could again be proven in this study. A set of samples was tested with two ELISA. Results of one ELISA were again positive, and for the second ELISA negative, however. Therefore, three of twenty samples showed finally differing result leading to an agreement in 85% of the cases.

Finally, in the last, and third study, results of the ELISA were compared to results of scil v-Giardia assay. A very high agreement was present between the two assays, leading to a scil v-Giardia test performance of 95.24% with a sensitivity of 91.89% and a specificity of 97.87%.

Conclusion

We can finally conclude that diagnosing a low burden of Giardia cysts remains challenging for all test systems. This makes Giardia diagnostics and result interpretation especially complex. We therefore recommend collecting multiple fecal samples, pool them, and evaluating a carefully mixed final pooled fecal sample. Such a procedure overcomes the heterogeneous distribution of Giardia cysts in the feces.

Our study clearly proved that scil v-Giardia rapid test is a highly accurate diagnostic tool for diagnosis of Giardia cysts in the general clinical setting and for follow-up examination

References

Heusinger T., Galian M., Müller E. (2010): Article in German, Giardia sp. In dogs and cats in Germany. Comparison of results of microscopic evaluation and antigen detection using ELISA *(orig.: Giardia sp. bei Hunden und Katzen in Deutschland. Vergleich der Ergebnisse von der mikroskopischen Untersuchung und einem Antigen Nachweis mittels ELISA.)* Proceedings DVG-Congress of the Parasitology and Parasitic Disease subgroup, Munich, Germany.

Jelinek T., Neifer S., (2013): Detection of *Giardia lamblia* stool samples: a comparison of two enzyme-linked immunosorbent assays. F1000Research 2013, 2:39

Luján H.D., Svärd S. (2011): Giardia - A Model Organism, Springer-Verlag Wien 2011

Rishniw M., Liotta J., Bellosa M., Bowman D., Simpson KW. (2010): Comparison of 4 Giardia Diagnostic Tests in Diagnosis of Naturally Acquired Canine Chronic Subclinical Giardiasis. J. Vet. Intern Med.