ACCURATE TESTING AND DIAGNOSIS OF *GIARDIA*

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ACCURATE TESTING AND DIAGNOSIS OF *GIARDIA*

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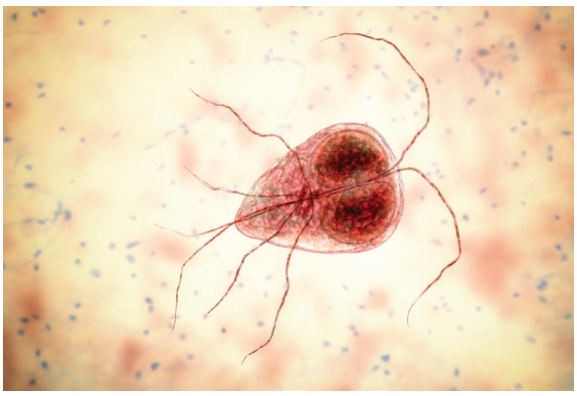
INTRODUCTION

Giardia duodenalis (syn *G. lamblia*, *G. intestinalis*) is a gastrointestinal parasite of mammals found worldwide.[1] Even though *G. duodenalis* infections are common in dogs and cats, it can be challenging to diagnose.[2,3] The prevalence of *G. duodenalis* infection varies depending on the age, clinical status, housing and geographic region of the animal and is influenced by the detection method employed.[4,5]

Country	Dogs	Cats
US	6.82%[6]	4.03%[26]
Canada	7.2%-12.62%[7,8]	2.4%-4.94%[27,28]
Ireland	4.2%-6.02%[9,10]	1.84%-4.2%[9,10], 3%-7%[12]
UK	8.4%[11], 3%-7%[12]	3%-7%[12]
Italy	1.9%-25.58%[13-15]	1%-36.84%[13,14]
Spain	1%-37.4%[16,17]	34.2%[29]
Germany	0.9%-30.6%[18-20]	0.7%-17.9%[18-20]
AUSTRALIA	**9.3%–22.1%[21,22]**	**2%[21]**
New Zealand	7.7%–24.7%[23]	2.8%–6.7%[23]
China	8.61%–12.8%[24,25]	1.9%–3.6%[30,31]

3D ILLUSTRATION OF TROPHOZOITE



Giardia exists in 2 stages:

TROPHOZOITE
Motile stage in small intestine

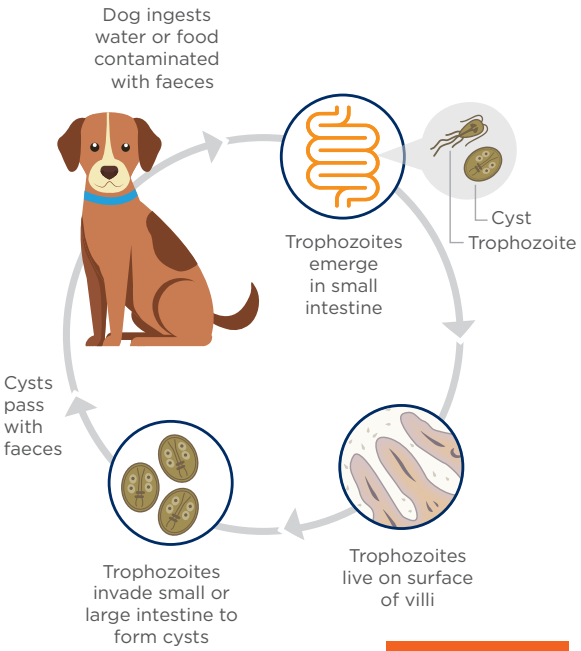
Trophozoites are usually 12 to 17 µm by 7 to 10 µm in size.[32] They are motile, flagellated organisms that originate from cysts and appear teardrop or pear-shaped.[33] Trophozoites are bilaterally symmetrical and have two nuclei, each with a large endosome. They also have a pair of transverse, dark-staining median bodies. Trophozoites inhabit the mucosal surfaces of the small intestine where they attach to the brush border, absorb nutrients and multiply by binary fission. They usually live in the proximal portion of the small intestine.[4,34]

CYST
Infective stage responsible for environmental contamination

Trophozoites invade the small or large intestine to form cysts. Cysts are ellipsoidal, nonmotile and contain 2 to 4 nuclei with long- and short-curved rods. They are 9 to 13 µm by 7 to 9 µm in size and possess a thick refractile wall. Newly formed cysts pass in the faeces. Cyst shedding may be continual over several days and weeks but is often intermittent, especially in the chronic phase of infection. Cysts can survive for several weeks to months in the environment, whereas trophozoites cannot.[2,32,34]

Upon infection, dogs and cats may be asymptomatic or present with symptoms such as weight loss and diarrhoea, which can be continual or intermittent, particularly in puppies and kittens. When symptomatic, faeces are usually soft, poorly formed, pale, malodorous, contain mucus and appear fatty. Watery diarrhoea is unusual in uncomplicated cases, and blood is usually not present in faeces. Occasionally, vomiting occurs. Minimum database clinical laboratory findings are usually normal.[2]

LIFE CYCLE OF *GIARDIA*



TESTING FOR *GIARDIA* PRESENTS CHALLENGES

It is very challenging to correctly diagnose a *Giardia* infection, and in fact, *Giardia* is one of the most commonly over and underdiagnosed parasites. It can easily be mistaken for many other objects (pseudoparasites) within the faeces, such as yeast, that can lead to potential overdiagnosis. *Giardia* can also be underdiagnosed, as cysts may be shed intermittently and can deteriorate in faecal solutions very quickly, making detection and identification more difficult. Given these complexities, there is no easy, quick test with 100% accuracy when it comes to diagnosing a *Giardia* infection, but there are many tests that can be used in conjunction to detect its presence.[3,34]

WHEN IS TESTING FOR *GIARDIA* APPROPRIATE?

According to expert organisations, veterinarians should screen cats or dogs for *Giardia* when they show symptoms such as chronic or intermittent diarrhoea.[34]

Additionally, patients at higher risk for *Giardia* infection, such as young animals or those at higher risk of contracting giardiasis due to lifestyle, may benefit from *Giardia* screening at routine wellness examinations.[3,34,35]

HOW TO TEST FOR *GIARDIA*

GIARDIA DIAGNOSIS

Expert organisations recommend testing symptomatic animals for *Giardia* with a combination of direct smear (detection of trophozoites), faecal flotation with centrifugation (detection of cysts), and faecal antigen test optimised for use in companion animals to detect *Giardia* antigen.[12,34,35] Multiple tests performed over several (usually alternating) days may be necessary to identify infection because of the possibility of intermittent shedding of cysts.[3]

TESTING ALGORITHM

Giardia suspected; Patients with symptoms →

- ✓ Saline direct smear
- ✓ Sugar + $ZnSO_4$ flotations
- ✓ *Giardia* antigen test

Testing for a broader range of parasites by faecal flotation with centrifugation using both 33% Zinc Sulfate solution and sugar solutions may also be warranted at wellness visits in patients who are asymptomatic but have high-risk factors for giardiasis. In asymptomatic patients with fewer risk factors, faecal flotation with centrifugation using only a sugar solution is recommended.[36]

SOLUTION ALGORITHM[36]

Wellness

Patients with high-risk factors:

- Clients who want a broader range of parasitic detection performed on their cat or dog
- Younger (<2 years old)
- Mostly outdoor
- Active (eg, swimming, hunting, visiting dog parks)
- Live with other animals
- Never tested for intestinal parasites

→ ✓ Sugar + 33% $ZnSO_4$ flotations

Patients with fewer risk factors:

- Older
- Mostly indoor
- Less active
- Only animal in household
- Have regular faecal examinations

→ ✓ Sugar flotation

The 3 tests recommended to aid in the diagnosis of a *Giardia* infection differ in several ways, including methodology, what is being detected (cyst vs trophozoite), and level of expertise and resources needed to conduct the test.[34]

DIRECT SMEAR

Direct smear is used primarily for detection of trophozoites in diarrhoeic stools. It is recommended to use a small sample of fresh, unrefrigerated faeces (preferably less than 30 minutes old). The sample should be mixed into 2 to 3 drops of saline (not water) on a glass slide to make a fine suspension. Then a coverslip is added, and the sample is viewed under a microscope. A Lugol iodine stain may be added to aid in identification.[34]

ANTIGEN TESTING

Giardia antigen testing can be a helpful test to use in conjunction with faecal flotation.[37] Diagnosis of *Giardia* infection by visual microscopy can be very difficult, especially when there are only a few *Giardia* cysts/trophozoites on a slide, and that can lead to a false-negative result.[3,37] The WITNESS® *Giardia* test is a Rapid Immuno-Migration (RIM) assay for detection of *Giardia intestinalis* antigen in faeces.[38]

FAECAL FLOTATION WITH CENTRIFUGATION

Faecal flotation with centrifugation is used primarily for detection of cysts in solid or semisolid stools. Faecal flotation requires the utilisation of flotation solutions to concentrate and separate intestinal parasite eggs and cysts from faecal debris. When choosing which solution to use, one should consider the specific gravity of the solution, the viscosity of the solution and the rate of water loss of the parasite egg or cysts caused by the hypertonicity of the solution.[32]

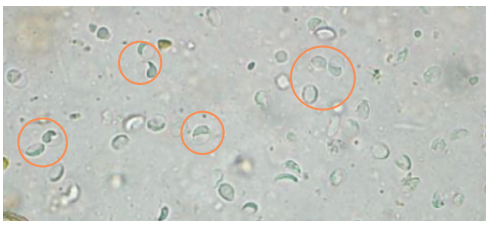
Many solutions can distort or damage *Giardia* cysts, further complicating cyst identification. A 33% $ZnSO_4$ solution is the preferred solution for *Giardia* cyst identification but is less effective for flotation testing of other common intestinal parasite eggs.[32]

ADVANTAGES AND DISADVANTAGES OF DIFFERENT SOLUTIONS[32]

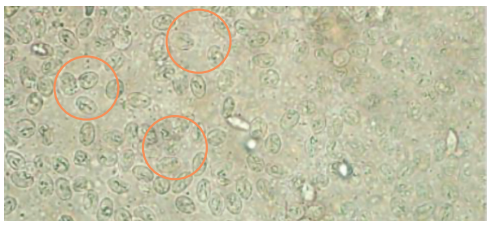
FLOTATION SOLUTION	SPECIFIC GRAVITY	ADVANTAGES	DISADVANTAGES
33% Zinc sulfate ($ZnSO_4$)	1.18	Floats common helminth and protozoa eggs and cysts; preferred for *Giardia*	Less effective for flotation of common tapeworm eggs than others; does not float some fluke and some unusual tapeworm and nematode eggs
Sheather's sugar solution	1.25	Floats common helminth and protozoa eggs and cysts; causes less damage to parasite eggs and cysts than salt solutions	Less sensitive than 33% $ZnSO_4$ for *Giardia*; creates sticky surfaces

THE IMPORTANCE OF 33% $ZnSO_4$ SOLUTION ON *GIARDIA* SAMPLES

Improved *Giardia* detection with $ZnSO_4$ vs sugar



Giardia cysts in sugar solution



Giardia cysts in 33% $ZnSO_4$ solution

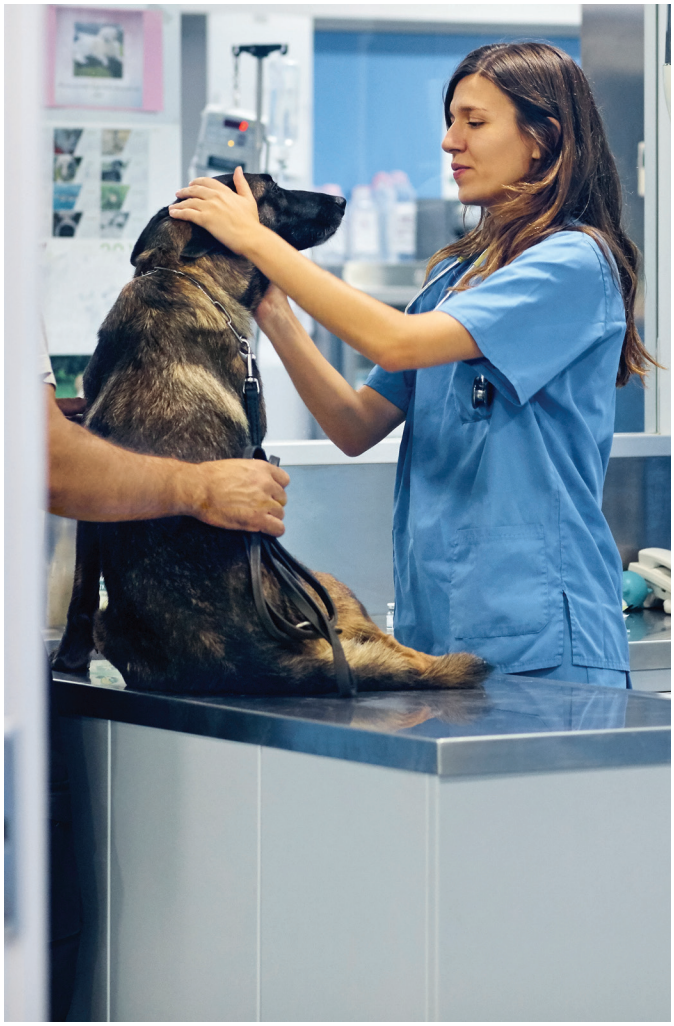
Images courtesy of Dr. Yoko Nagamori.

FAECAL FLOTATION USING vetscan IMAGYST™

Even when best practices for faecal testing are followed, *Giardia* cysts may still be difficult to correctly identify, especially for less experienced or suboptimally trained staff. The VETSCAN IMAGYST™ was developed to provide a simple, easy and structured faecal examination that is less influenced by different faecal preparation methods or level of experience of an examiner. The VETSCAN IMAGYST utilises an automated scanner and a cloud-based, deep-learning algorithm to locate, classify and identify parasite eggs and cysts found on faecal microscope slides to help overcome the challenges in manual scanning and identification.[37]

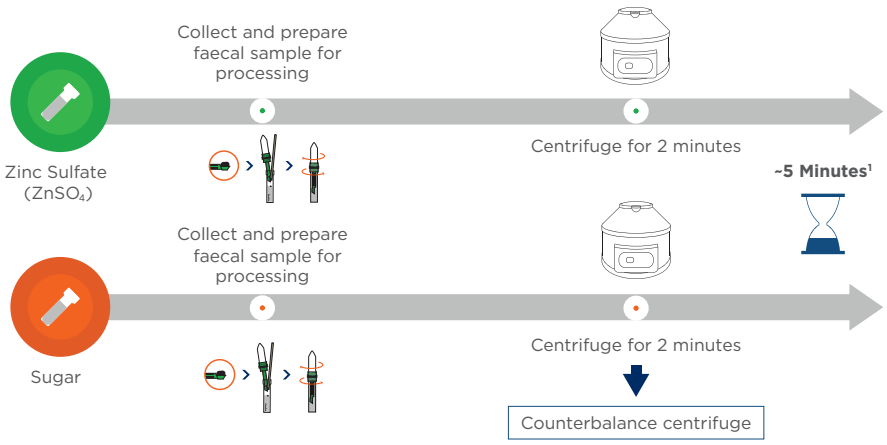
The VETSCAN IMAGYST system consists of 3 components: sample preparation, scanning of the sample by whole slide imaging (WSI) and analysis performed by a cloud-based, deep-learning algorithm. The VETSCAN IMAGYST sample preparation device is used for a centrifugal flotation technique with a transfer loop for easy transfer of the sample to a microscope slide. The VETSCAN IMAGYST applies a deep-learning, object-detection algorithm that automatically learns the most differentiating features of interest, allowing the algorithm to perceive and distinguish the morphology of parasite eggs and cysts from other objects on faecal flotation slides.[37,39]

In a performance study, the VETSCAN IMAGYST sample preparation method was shown to be comparable to the performance of conventional centrifugal flotation for *Giardia* as read by an expert (clinical parasitologist) with manual microscopy. The VETSCAN IMAGYST algorithm showed good performance (sensitivity and specificity) across the different parasites tested when compared to the clinical parasitologist. In addition, using the VETSCAN IMAGYST, the availability of both sugar and 33% $ZnSO_4$ solutions can provide a more complete faecal diagnostic evaluation.[37]

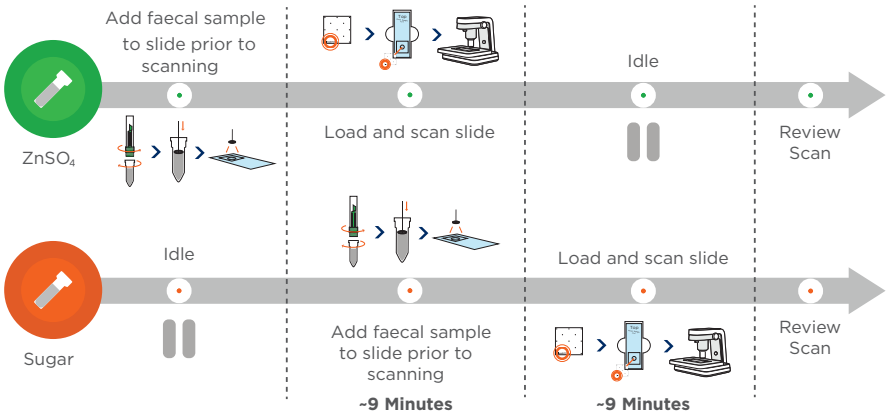


TESTING FOR *GIARDIA* WITH THE VETSCAN IMAGYST USING 33% $ZnSO_4$ AND SUGAR SOLUTIONS[40,41]

SAMPLE PREPARATION
(Done in parallel)



SAMPLE PROCESSING AND ANALYSIS



RETESTING MY PATIENT
WHEN AND HOW?

After a giardiasis diagnosis and treatment initiation, follow-up tests are very important, especially if symptoms have not been resolved after the conclusion of therapy. Follow-up testing may be conducted 24 to 48 hours after the completion of therapy.[34] This can be accomplished with centrifugal faecal flotation with zinc sulfate; and if this centrifugation was negative and the animal seems to be healthy, no further testing is needed.[3] It is important to remember that the antigen tests can provide a false-positive result in an animal that is no longer infected with *Giardia*, because *Giardia* antigen excretion can persist for several weeks after treatment.[37]

RETESTING FOR *GIARDIA*: ALGORITHM USING 33% $ZnSO_4$ SOLUTION[3,34]:

Negative centrifugation results with no remaining symptoms	Negative centrifugation results but animal continues showing symptoms after treatment
↓	↓
No further testing is needed	Further diagnostic workup is warranted

CONCLUSION

Testing for *Giardia* is complicated and requires the use of several types of diagnostic tools. In addition to best practices described in this paper, there are innovative platforms that can also be used to aid in the identification of *Giardia* cysts, such as the VETSCAN IMAGYST.

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