

Use of immunochromatographic (IC) assays for the detection of *Giardia* antigen in rodent feces

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Received 06.01.2026

Accepted 02.02.2026

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Summary

The high overall prevalence of giardiasis (45.0%) observed in the present study confirms the important role of pet rodents as reservoirs of *Giardia* infection. Given that pet rodents are frequently purchased for and kept in close contact with young children, sensitive and regular screening for giardiasis is essential for limiting potential rodent-to-human transmission, particularly as infections in small mammals are often asymptomatic. The aim of this study was to evaluate whether commercially available immunochromatographic tests detecting *Giardia* antigen registered for dogs and cats can be successfully applied for the examination of rodent faecal samples. Immunochromatographic (IC) antigen assays based on the detection of the *Giardia*-specific antigen GSA-65 demonstrated high diagnostic utility. In comparison with conventional microscopic methods, IC testing showed an approximately 27% higher diagnostic sensitivity, substantially increasing the detectability of *Giardia* infection in faecal samples. Owing to the presence of GSA-65 across different species and assemblages of the genus *Giardia*, commercially available IC tests registered for dogs and cats can be effectively applied off-label in the diagnosis of giardiasis in rodents. Molecular analysis performed on samples positive by microscopy and/or IC testing confirmed the presence of parasites belonging to the *Giardia intestinalis* species complex, with predominance of the rodent-adapted assemblage G and sporadic detection of zoonotic assemblages A and B. These findings emphasize the added value of combining immunological, parasitological and molecular methods for accurate diagnosis and epidemiological assessment. Overall, the results of this study highlight the relevance of giardiasis in pet rodents and stress the importance of sensitive diagnostic approaches in veterinary practice, with clear implications for public health and the prevention of giardiasis.

Keywords: *Giardia*, rodents, diagnostics, antigen, immunochromatographic assay

Giardiasis is a parasitic disease of the gastrointestinal tract and represents one of the most important protozoan infections affecting humans as well as a wide range of livestock, wildlife and companion animals (3-5, 12, 14). It is currently considered the most common parasitic infection in dogs and cats. Meta-analyses of faecal surveys have reported a global prevalence of 15.2% in dogs and 12.0% in cats, while European data indicate mean prevalence rates of 19.8% in dogs and 15.9% in cats (2).

Among the numerous host species, rodents are regarded as an important factor in the environmental dissemination of giardiasis, contributing to contamination of water, food and soil (11, 13, 21). *Giardia* infections have been reported in both domestic and free-living rodent populations. Based on molecular evidence, four of the nine recognized *Giardia* spe-

cies have been identified in rodents: *Giardia muris*, *Giardia microti*, *Giardia cricetarum* and *Giardia intestinalis* (11, 13, 14, 21). To date, seven assemblages of *G. intestinalis* (A, B, C, D, E, F and G) have been reported in rodents (4, 10, 14). The rodent-adapted species *G. cricetarum*, *G. muris* and *G. microti* show marked host preferences. *G. microti* primarily infects sylvatic rodents of the family *Cricetidae*, such as voles and muskrats, whereas *G. muris* is mainly associated with synanthropic rodents of the family *Muridae*, including house mice and domestic rats. In addition, the zoonotic assemblages A and B of *G. intestinalis* have been repeatedly detected in rodents, underlining their potential epidemiological relevance.

In veterinary practice, giardiasis is most commonly associated with the intestinal form of infection. During invasion, trophozoites adhere to and damage the

mucosa of the duodenum, leading to clinical manifestations such as persistent or intermittent diarrhoea, malabsorption and weight loss. Although asymptomatic infections are frequent, clinical disease is more likely to occur in young animals, individuals in poor body condition, immunocompromised hosts or animals affected by concurrent infections. Sporadic reports have also documented extra intestinal localisation of *Giardia*, including the biliary tract, where infection may contribute to chronic cholecystitis, gallstone formation and impaired fat absorption.

A variety of diagnostic methods are currently available for the detection of giardiasis, including conventional coproscopical techniques, molecular assays and immunological tests aimed at detecting coproantigens in faecal or antibodies in serum. In routine veterinary practice, the most commonly applied methods include direct faecal smears, Lugol's iodine-stained smears, permanently stained smears (e.g. trichrome staining), centrifuged flotation using saturated zinc sulfate solution, and immunochromatographic antigen assays. However, commercially available veterinary immunochromatographic tests are currently recommended by manufacturers primarily for the diagnosis of giardiasis in dogs and cats (6, 19).

Therefore, the aim of the present study was to evaluate whether commercial veterinary immunochromatographic assays developed for the detection of *Giardia* antigens in dogs and cats can be effectively applied in the routine diagnosis of giardiasis in pet rodents.

Material and methods

The study was conducted in the Veterinary Parasitology Laboratory of the University of Life Sciences in Lublin in cooperation with the privately owned veterinary diagnostic laboratory Vet Diagnostyka. Fresh faecal material (approximately 1 g) was collected by owners of pet rodents and rabbits for routine parasitological examination.

In total, 180 faecal samples were examined (30 each from chinchillas, mice, rats, hamsters, guinea pigs and rabbits). Samples were initially screened for *Giardia* infection using (i) a direct smear, (ii) a centrifuged flotation technique with zinc sulfate solution (specific gravity SG 1.18) and (iii) an immunochromatographic (IC) antigen assay (Vet Expert Rapid Test Giardia Ag, VET PLANET, Poland).

Molecular detection and genotyping. Molecular analysis was restricted to samples that tested positive in at least one of the applied screening methods (microscopy and/or IC antigen assay). DNA was extracted from approximately 200 mg of faecal material using the Genomic Mini AX Stool kit, according to the manufacturer's instructions (A&A Biotechnology, Gdańsk, Poland). The extracted DNA was eluted in 200 µL of elution buffer (Tris buffer, 10 mM, pH 8.5) and stored frozen until further analysis. Because PCR was used only for multi locus molecular characterization of samples previously confirmed as positive, external positive and negative controls were not routinely included.

Molecular detection of *Giardia* spp. was performed by PCR targeting a fragment of the small subunit ribosomal

RNA (SSU rRNA) gene, according to a previously described protocol (16). All PCR-positive samples were subsequently subjected to multilocus genotyping of *Giardia intestinalis* using nested PCR assays targeting the β -giardin (*bg*), glutamate dehydrogenase (*gdh*), and triosephosphate isomerase (*tpi*) genes, which are commonly used for assemblage and sub-assemblage discrimination. Primer sets previously reported in the literature were used for the *bg*, *gdh*, and *tpi* loci, respectively (7, 9, 17, 18). A detailed summary of primers and PCR conditions is provided in Table 2.

PCR products were visualized by agarose gel electrophoresis, purified and sequenced bidirectionally. The obtained sequences were assembled into consensus sequences and compared with reference sequences deposited in GenBank using BLAST. Assemblages and sub-assemblages were assigned based on sequence similarity and phylogenetic analysis. When sequence data from two or more loci were available for a given isolate, multilocus genotypes (MLGs) were defined.

Statistical analysis. Statistical analyses were performed using Statistica v.13.1 (StatSoft, medical application). The prevalence of *Giardia* infection was calculated as the proportion of positive samples among the total number of faecal samples examined and expressed as a percentage. The presence of *Giardia* was treated as a binary outcome (positive/negative). Prevalence estimates are presented with 95% confidence intervals (CI) calculated using the Wilson score method. Differences in prevalence between animal species were assessed using contingency table methods (Pearson's chi-square test or Fisher's exact test when expected cell counts were low). A p-value < 0.05 was considered statistically significant.

Results and discussion

Overall, 81 of 180 samples were positive, resulting in an overall prevalence of 45.0% (81/180; 95% CI 37.9-52.3). Positive results were obtained in 26 chinchillas, 21 mice, 18 rats and 16 hamsters. All samples from guinea pigs (0/30) and rabbits (0/30) were negative.

Microscopic examination (direct smear and/or flotation) detected *Giardia* cysts in 66/180 animals, corresponding to 36.7% (66/180; 95% CI 30.0-43.9). The IC antigen assay was positive in 81/180 samples, i.e. 45.0% (81/180; 95% CI 37.9-52.3).

All samples positive by microscopy were also positive in the IC antigen test. In addition, 15 samples were positive by IC antigen testing despite no cysts or trophozoites being observed on smear or flotation. The complete results of the parasitological examination are presented in Table 1.

PCR amplification of the SSU rRNA gene confirmed the presence of *Giardia* DNA, identifying the parasite as belonging to the *Giardia intestinalis* species complex.

Multilocus genotyping at the *bg*, *gdh* and *tpi* loci enabled assemblage assignment in a subset of PCR-positive samples with sufficient DNA quality. The obtained genotyping profiles were consistent with those

most frequently reported in pet rodents, with predominance of the rodent-adapted assemblage G of *Giardia intestinalis*. In addition, sporadic detection of the zoonotic assemblages A and B was observed, as previously documented in companion and farmed rodent populations (4, 10). Due to incomplete amplification at all loci in some samples, full multilocus genotypes could not be obtained for all isolates. A summary of the molecular characterization of *Giardia intestinalis* isolates is presented in Table 3.

Tab. 1. Prevalence of *Giardia* infection in examined pet rodents and rabbits

Animal species	No. examined	No. positive	Prevalence (%)	95% CI (Wilson)
Chinchillas	30	26	86.7	70.3-94.7
Mice	30	21	70.0	52.1-83.3
Rats	30	18	60.0	42.3-75.4
Hamsters	30	16	53.3	36.1-69.8
Guineapigs	30	0	0.0	0.0-11.4
Rabbits	30	0	0.0	0.0-11.4
Total	180	81	45.0	37.9-52.3

Explanations: Prevalence is expressed as the proportion of positive samples among the total number of faecal samples examined. Confidence intervals (CI) were calculated using the Wilson score method.

Tab. 2. Primers and PCR conditions used for amplification of *Giardia intestinalis* DNA fragments

DNA fragment	Primer sequences (5'-3')	PCR conditions	Reference
ssurRNA (~130 bp)	<i>Outer:</i> RH11: CATCGGTCGATCCTGCC <i>Outer:</i> RH4: AGTCGAACCCTGATTCTCCGCCAGG <i>Inner:</i> GiarF: GACGCTCTCCCAAGGAC <i>Inner:</i> GiarR: CTGCGTCACGCTGCTCG	Nested PCR; 2 µL template; 95°C 3 min; 35 cycles of 95°C 30 s, 55°C 30 s, 72°C 60 s; final extension 72°C 7 min (both rounds)	(7)
gdh (~432 bp)	<i>Outer:</i> GDHeF: TCAACGYAAYCGYGGYTTCCGT <i>Outer/Inner:</i> GDHIR: GTTRTCTTGACACATCTCC <i>Inner:</i> GDHIF: CAGTACAACCTCYGCTCTCGG	Semi-nested PCR; 5 µL template; 95°C 3 min; 35 cycles of 95°C 30 s, 55°C 30 s, 72°C 60 s; final extension 72°C 7 min (both rounds)	(17)
bg (~511 bp)	<i>Outer:</i> G7: AAGCCCGACGACCTCACCCGAGTGC <i>Outer:</i> G759: GAGGCCGCCCTGGATCTTCGAGACGAC <i>Inner:</i> BG-F: GAACGAACGAGATCGAGGTCGG <i>Inner:</i> BG-R: CTCGACGAGCTTCGTGT	Nested PCR; 3 µL template; Primary: 96°C 5 min; 40 cycles of 95°C 30 s, 50°C 30 s, 72°C 60 s; final 72°C 7 min. Secondary: 96°C 5 min; 35 cycles of 96°C 45 s, 55°C 30 s, 72°C 45 s; final 72°C 7 min	(9)
tpi (~530 bp)	<i>Outer:</i> AL3543: AAATIATGCCTGCTCGTCC <i>Outer:</i> AL3546: CAAACCTTITCCGCAAACC <i>Inner:</i> AL3544: CCCTTCATCGGIGGTAACCT <i>Inner:</i> AL3545: GTGGCCACCACICCCGTGCC	Nested PCR; Primary: 94°C 5 min; 35 cycles of 94°C 45 s, 50°C 45 s, 72°C 60 s; final 72°C 10 min. Secondary: same as primary	(18)

Tab. 3. Molecular characterisation of *G. intestinalis* detected in positive faecal samples by host species

Animal species	No. PCR-tested*	Species identified	Predominant assemblage	Other assemblages detected	Notes
Chinchillas	26	<i>G. intestinalis</i>	Assemblage G	Assemblage A, B (sporadic)	Partial multilocus profiles obtained
Mice	21	<i>G. intestinalis</i>	Assemblage G	Assemblage B (sporadic)	Genotyping successful at ≥ 1 locus
Rats	18	<i>G. intestinalis</i>	Assemblage G	Not detected	Limited amplification at some loci
Hamsters	16	<i>G. intestinalis</i>	Assemblage G	Not detected	Incomplete multilocus data
Guinea pigs	0	–	–	–	All samples negative
Rabbits	0	–	–	–	All samples negative
Total	81	<i>G. intestinalis</i>	Assemblage G	Assemblage A, B	Full MLGs not obtained for all isolates

Explanations: *PCR-tested samples include only faecal samples that were positive by microscopy and/or IC antigen assay. Assemblage assignment was based on sequence similarity at one or more loci (*bg*, *gdh*, *tpi*). Due to incomplete amplification at all loci, full multilocus genotypes could not be obtained for every isolate, and mixed infections could not be excluded.

The overall prevalence of giardiasis observed in the present study (45.0%) confirms that *Giardia* infections are common among pet rodents (10, 12, 14, 21). This finding is consistent with previous reports indicating a high occurrence of *Giardia* spp. in rodent populations, although prevalence estimates vary widely depending on host species, husbandry conditions and the diagnostic methods applied. Rodents are therefore considered an important reservoir of *Giardia* infections. It is noteworthy that in our study we did not detect giardiasis in any of the fecal samples obtained from guinea pigs or rabbits, despite the fact that other studies have confirmed the presence of *Giardia* in these species as well (14). In a study conducted in Brazil on pet rabbits, the prevalence of *Giardia intestinalis* was 40% (1).

The negative results of faecal examinations in rabbits and guinea pigs are consistent with long-term observations from routine coproscopic studies in these species. Although the literature reports the occurrence of infection in rabbits, these studies were primarily conducted in slaughter rabbits, whereas the present study included exclusively pet animals kept individually. This difference in husbandry conditions likely explains the results obtained.

The highest prevalence rates reported in the literature are typically observed in laboratory rodents, where

infection levels in rats and mice may reach 96-100% (11, 12). Such high prevalence is likely influenced by high animal density, shared cages and the frequent occurrence of coprophagy, which facilitates parasite transmission. In contrast, prevalence in free-living rodent populations is generally lower. For example, studies conducted in Germany reported *Giardia* prevalence of 16% in *Apodemus* spp., 31% in *Microtus* spp. and 52% in *Myodes* spp. (8). Wild rodent studies from Poland have also demonstrated high infection rates in some species, with *Giardia* detected in all examined striped field mouse (*Apodemus agrarius*), yellow-necked mouse (*A. flavicollis*) and bank vole (*Myodes glareolus*) (15).

In comparison, data on giardiasis in pet rodents remains scarce. This gap is noteworthy given the close contact between pet rodents and humans, particularly children, who represent a recognized risk group for giardiasis. In the present study, the prevalence detected in pet hamsters (53.3%) was considerably higher than that reported in China, where infection rates of 22.2% were observed in pet hamsters of several species (13). Particularly high prevalence was recorded in chinchillas (86.7%), which is in agreement with previous studies indicating that this species is highly susceptible to *Giardia* infection under captive conditions (10, 14, 21).

The choice of diagnostic method represents a major factor influencing prevalence estimates in epidemiological studies. In the present investigation, all applied techniques were based on direct detection of *Giardia* but differed in their diagnostic targets. Direct smear and centrifuged flotation techniques rely on the visual detection of cysts or trophozoites, whereas immunochromatographic (IC) antigen assays detect the soluble *Giardia*-specific antigen GSA-65. Due to irregular and intermittent cyst excretion, reliance solely on microscopic methods may result in a substantial number of false-negative results. It has been estimated that examination of a single stool sample detects approximately 60-80% of infections, two samples increase detection to 80-90%, and three samples examined using centrifuged zinc sulfate flotation raise sensitivity to over 90% (6, 19).

The sensitivity of microscopic methods is also influenced by the experience of the diagnostician, as well as by sample storage and preservation conditions. These limitations affect IC antigen assays to a much lesser extent. In the present study, IC testing detected *Giardia* infection in 81 samples, whereas microscopic examination identified cysts in 64 samples, indicating an approximately 27% higher diagnostic sensitivity of faecal examination when IC tests were applied compared with microscopy alone. Despite the relatively frequent shedding of *Giardia* cysts in rodents, these findings clearly demonstrate that the use of IC assays significantly increases the probability of detection and thus markedly improves the overall diagnostic yield of giardiasis in this host group.

Rapid IC tests based on GSA-65 are particularly useful due to their simplicity, short turnaround time and high analytical sensitivity. GSA-65 is a thermostable, water-soluble glycoprotein present in both cysts and trophozoites, which appears in faecal prior to cyst shedding and disappears shortly after parasite elimination. This explains the superior performance of IC assays, especially in samples with low cyst counts. Depending on the manufacturer, reported sensitivities range from 83.3% to 97.5%, with specificities of 96.0-99.3%. An additional advantage of these assays is their suitability for frozen samples and faecal preserved in formalin.

Despite their high sensitivity, IC antigen tests detect *Giardia* at the genus level only and do not allow differentiation between species or assemblages. Consequently, a positive IC test result does not provide information on host specificity or zoonotic potential. This is particularly relevant for rodents, which may harbour rodent-adapted species such as *G. muris*, *G. microtia*, *G. cricetarum*, as well as *G. intestinalis* assemblage G. Molecular characterization performed in the present study confirmed that the detected isolates belonged to the *G. intestinalis* species complex, with predominance of the rodent-adapted assemblage G, consistent with previous reports in pet rodents. Importantly, sporadic detection of the zoonotic assemblages A and B was also observed (9-11, 13, 20, 21). Although the presence of these assemblages does not directly demonstrate transmission to humans, their detection in pet rodents kept in close contact with people indicates a potential zoonotic risk, particularly in households with children or immunocompromised individuals.

Overall, the present findings highlight the high prevalence of *Giardia* infection in pet rodents and emphasise the importance of combining immunochromatographic antigen assays with conventional parasitological and molecular methods. Such an integrated diagnostic approach substantially improves the detectability of giardiasis in rodents and allows for a more accurate assessment of their epidemiological and zoonotic significance.

Because pet rodents are kept in close contact with humans, particularly children, the sensitive detection of zoonotic protozoa, including *Giardia*, is of particular importance. The present findings indicate that, similarly to companion animals, rapid and sensitive immunological methods should be routinely applied in veterinary practice for the diagnosis of giardiasis in pet rodents.

Immunochromatographic antigen assays based on the detection of the *Giardia*-specific antigen GSA-65 offer substantial diagnostic advantages. This antigen is conserved across members of the genus *Giardia*, enabling reliable genus-level detection. Commercially available tests registered for dogs and cats can be successfully applied off-label for the diagnosis of giardiasis in rodents, demonstrating higher sensitivity than

conventional parasitological methods, such as direct smear and flotation.

Overall, the implementation of immunochromatographic antigen testing in routine diagnostics significantly improves the detectability of giardiasis in pet rodents and contributes to a more effective assessment of their potential epidemiological and zoonotic relevance.

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