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Nationwide autumn-winter survey of *Giardia duodenalis* in Polish dogs – diagnostic comparison and PCR-RFLP characterization

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Abstract

Introduction and Objective. *Giardia duodenalis* is an important intestinal protozoan of dogs and a potential environmental and zoonotic concern. The aim of the study is to determine the occurrence of *G. duodenalis* in Polish dogs, assess selected host factors associated with infection, compare microscopy and rapid immunochromatographic assay, and characterise positive Giardia isolates by PCR-RFLP.

Materials and Method. Between October – December 2025, 2,251 dogs in Poland were examined using 3-day faecal sample sets. Microscopy was performed by zinc sulfate flotation. A subset of 818 sample sets was additionally tested with a rapid immunochromatographic coproantigen assay. Microscopy-positive samples were subjected to PCR-RFLP targetting the gdh and beta-giardin loci.

Results. Microscopy-based prevalence was 13.06% (294/2,251; 95% CI: 11.73–14.52). Infection was strongly associated with age, with the highest prevalence in dogs aged <1 year (26.86%). The immunochromatographic assay showed 98.18% sensitivity and 96.78% specificity relative to microscopy. At least one molecular marker was detected in 241/294 microscopy-positive samples (81.97%). Assemblages D and C predominated, accounting for 48.96% and 48.13% of genotyped samples, respectively, whereas assemblages B (2.07%) and A (0.83%) were rare.

Conclusions. *Giardia duodenalis* is common in dogs in Poland, especially in young animals. Canine-adapted assemblages C and D were the most prevalent, while potentially zoonotic assemblages A and B were only occasionally detected. Combined use of microscopy and immunochromatographic testing may improve routine diagnosis.

Key words

prevalence, PCR-RFLP, Giardia duodenalis, glutamate dehydrogenase, assemblages, canine giardiasis, zinc sulfate flotation, rapid immunochromatographic assay, beta-giardin

INTRODUCTION

Giardia duodenalis is one of the most prevalent intestinal protozoa in dogs remains of significant importance in veterinary, environmental, and public health contexts in addition to veterinary medicine. Infected dogs, including asymptomatic carriers, may shed cysts into the environment and contribute to the contamination of households, public parks and kennels [1, 2]. *Giardia duodenalis* is a genetically diverse species complex involving several assemblages that differ in host range and epidemiological relevance. Assemblages A and B are most commonly associated with giardiasis in humans, assemblages C and D are typically canine-adapted, assemblage E is mainly found in livestock, and assemblage F is typically feline-adapted [1–3].

Previous studies conducted in Poland have shown that *Giardia* infection occurs in dogs with variable frequency depending on the examined population, geographic area, and diagnostic method. Available molecular data indicate that canine *Giardia* isolates are most often assigned to host-adapted assemblages C and D, although assemblages with potential zoonotic relevance were also reported [3–5]. However, there is still a lack of comprehensive nationwide data that combines epidemiological analysis with practical diagnostic comparison and molecular characterization in dogs across different regions of Poland [4–6].

Due to the parasite burden, intermittent cyst shedding, and quality of the faecal sample, the routine microscopy-based diagnosis of canine giardiasis may be challenging. Rapid immunochromatographic (IC) assays may improve detection, but agreement between conventional microscopy and antigen-based assays is not always complete [2, 7]. Therefore, combining parasitological and molecular methods may provide a more accurate assessment of infection prevalence and its epidemiological significance.

Molecular identification of *G. duodenalis* assemblages is of particular importance from the perspective of public and environmental health, as dogs may harbour both host-adapted and potentially zoonotic genotypes [1, 5, 8].

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Additionally, characterization of canine *Giardia* isolates may improve our understanding of the role of dogs in circulation of *Giardia* between humans, animals, and the environment.

The aim of this study is to determine the occurrence of *G. duodenalis* in dogs in Poland, assess selected epidemiological factors associated with infection, compare microscopy and rapid IC assay, and characterize microscopy-positive *Giardia* isolates using PCR-RFLP analysis of the glutamate dehydrogenase (*gdh*) and beta-giardin (*bg*) markers to evaluate their potential zoonotic significance.

MATERIAL SAND METHOD

Study population and sample processing. Between October – December 2025, a total of 2,251 canine faecal sample sets from Poland were examined. All samples were submitted for diagnostic testing to a commercial veterinary diagnostic laboratory in Warsaw, Poland. For each dog, three faecal samples collected over three consecutive days were used for analysis. Because this was a laboratory-based retrospective study, detailed information on previous antiparasitic treatment or prophylaxis was not consistently available for all submissions. Exclusion of follow-up cases was based on diagnostic request forms and laboratory records. However, prior undocumented treatment could not be entirely ruled out and is acknowledged as a limitation.

Microscopic examination and rapid immunochromatographic assay. The three faecal samples collected from each dog were examined as a pooled three-day sample set. Approximately 1 g of faeces from each daily sample were combined before flotation. All pooled faecal sets were examined for the presence of *G. duodenalis* cysts using a zinc sulfate solution with a specific gravity of 1.31 g/cm^3, which differs from the classical Faust technique. The higher specific gravity reflects the routine diagnostic protocol used in the laboratory and was applied to improve recovery of *Giardia* cysts from canine faecal sample sets. However, this modification may affect recovery efficiency and morphology and should be considered when comparing results with studies using standard flotation protocols. Additionally, 818 pooled faecal sample sets were tested for *G. duodenalis* coproantigen using a rapid immunochromatographic (IC) assay, Giardia Stick (Operon, S.A., Zaragoza, Spain), according to the manufacturer's instructions. Selection for coproantigen testing was based on the diagnostic request of the referring veterinarian.

Assessment of cyst shedding intensity. For flotation-positive samples, cyst shedding intensity was measured microscopically in 10 fields of view at 400× magnification. Cyst counts were classified as follows: single, 1–2 cysts in 10 fields of view; moderately numerous, 3–9 cysts in 10 fields of view; and numerous, ≥10 cysts in 10 fields of view. This semi-quantitative classification was based on routine parasitological diagnostic recommendations described by Myjak et al., and standard veterinary parasitology practice [9].

Recovery of cysts and DNA extraction. Cysts of *G. duodenalis* detected by flotation were recovered from the cover glass by rinsing with distilled water. The obtained suspension was centrifuged, the supernatant was discarded, and the remaining pellet frozen in –20 °C until DNA extraction. DNA was extracted using the TANBead Maelstrom 4800 Nucleic Acid Extraction System (Taiwan Advanced Nanotech Inc., Taoyuan City, Taiwan). The extracted DNA was stored at –20 °C until further analyses.

PCR-RFLP analysis of the gdh marker. Semi-nested PCR-RFLP targeting the *gdh* locus was performed according to the protocol of Read et al. [10]. In the primary reaction, primers GDHeF (5′-TCAACGTYAAYCGYGGYTTCCGT-3′) and GDHiR (5′-GTTRTCCTTGCACATCTCC-3′) were used, whereas the secondary reaction was performed with primers GDHiF (5′-CAGTACAACTCYGCTCTCGG-3′) and GDHiR (5′-GTTRTCCTTGCACATCTCC-3′). The expected size of the secondary PCR product was 432 bp. Thermal cycling conditions for both rounds consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. When both duplicate reactions were negative, nested PCR was repeated using a modified primary-round annealing temperature of 50 °C instead of 55 °C. Secondary *gdh* amplicons were digested with NlaIV (NIPPON Genetics EUROPE, Düren, Germany), and restriction fragments were separated on 3% agarose gel and interpreted according to published reference patterns for the *gdh* locus [10].

PCR-RFLP analysis of the beta-giardin marker. Nested PCR-RFLP targeting the *bg* locus was carried out using a modified protocol based on Lalle et al. [11]. The primary PCR used primers G7 (5′-AAGCCCGACGACCTCACCCGCAGTGC-3′) and G759 (5′-GAGGCCGCCCTGGATCTTCGAGACGAC-3′), whereas the secondary PCR used primers G99 (5′-GAACGAGATCGAGGTCCG-3′) and G609 (5′-CTCGACGAGCTTCGTGTT-3′). The expected sizes of the primary and secondary amplicons were 753 bp and 511 bp, respectively. The primary-round cycling profile consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. For the secondary PCR, the same profile was used except that the annealing temperature was 55 °C. When both duplicate reactions were negative, nested PCR was repeated with the primary-round annealing temperature lowered from 65 °C to 55 °C. Secondary PCR products were digested with HaeIII (EURX, Gdańsk, Poland), and the restriction fragments were separated on 3% agarose gel; restriction patterns were interpreted according to published reference profiles for assemblage differentiation at the *bg* locus [11].

For both loci, PCR was performed in a 25 µL reaction mixture containing 12.5 µL of StartWarm HS-PCR Mix (A&A Biotechnology, Gdańsk, Poland), 1 µL of each primer (10 µM), 2 µL of template DNA in the primary reaction, and ultrapure water (A&A Biotechnology, Gdańsk, Poland) added to a final volume of 25 µL. All PCR and nested-PCR reactions were performed in a MultiGene optiMAX thermocycler (Labnet International, USA). Each sample was analysed in duplicate. First-round PCR products were diluted 20-fold, and 1 µL of the diluted product was used as template for the secondary reaction. Selected PCR products representing different RFLP profiles were subjected to Sanger sequencing to confirm assemblage assignment. Sequence identity was verified by comparison with reference sequences available in GenBank. To compare nucleotide sequences with the

NCBI GenBank database, the BLAST platform was used. The *Giardia* assemblages were identified by comparing the consensus sequences using MEGA v. 6 software [12]. Phylogenetic analysis was performed using the Neighbour-Joining method based on nucleotide sequences of *gdh* and *bg* loci and the Kimura two-parameter model. Bootstrap analysis was performed with 1,000 replicates.

Statistical analysis. Statistical analysis was performed to assess the occurrence of *G. duodenalis*, its association with selected host factors, the diagnostic performance of the rapid IC assay, and the relationship between cyst burden and molecular detection. Prevalence was calculated as the proportion of microscopy-positive samples with 95% confidence intervals (95% CI). Associations with gender, age category, and province were evaluated using the chi-square test or Fisher's exact test, while age as a continuous variable was analysed with the Mann-Whitney U test. The effects of age and gender on microscopy-positive *Giardia* detection were further assessed by binary logistic regression and expressed as odds ratios (ORs) with 95% CIs. Using microscopy as the operational reference method, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the IC assay were calculated. It is important to note that microscopy does not represent a true gold standard due to intermittent cyst shedding and limited sensitivity. The association between cyst burden and PCR-RFLP detection at the *gdh* and *bg* loci, including repeat amplification after lowering the primary-round annealing temperature, was analysed using the chi-square test or Fisher's exact test. Assemblage distribution was presented descriptively as numbers and percentages overall and by province. A p-value of <0.05 was considered significant. All analyses were performed in Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS

A total of 2,251 canine faecal sample sets were analyzed. *Giardia duodenalis* cysts were detected microscopically in 294 samples, reaching an overall prevalence of 13.06% (95% CI: 11.73–14.52). No significant association was found between microscopy-positive results and dog gender. *Giardia* was detected in 117/937 females (12.49%) and 177/1,314 males (13.47%) (p = 0.536). In contrast, infection was strongly associated with age. The highest prevalence was observed in dogs younger than one year (209/778; 26.86%), whereas significantly lower values were found in dogs aged one to <3 years (42/701; 5.99%) and ≥3 years (43/772; 5.57%) ($p = 4.68 \times 10^{-44}$). In logistic regression, increasing age significantly reduced the odds of microscopy-positive *Giardia* detection (OR 0.69; 95% CI: 0.63–0.76; $p = 5.86 \times 10^{-16}$), while gender was not significant (OR 1.15; 95% CI: 0.89–1.48; p = 0.298) (Tab. 1). Microscopy-based prevalence differed numerically between provinces, ranging from 9.0% – 15.8%, but these differences were not statistically significant (p = 0.759) (Fig. 1).

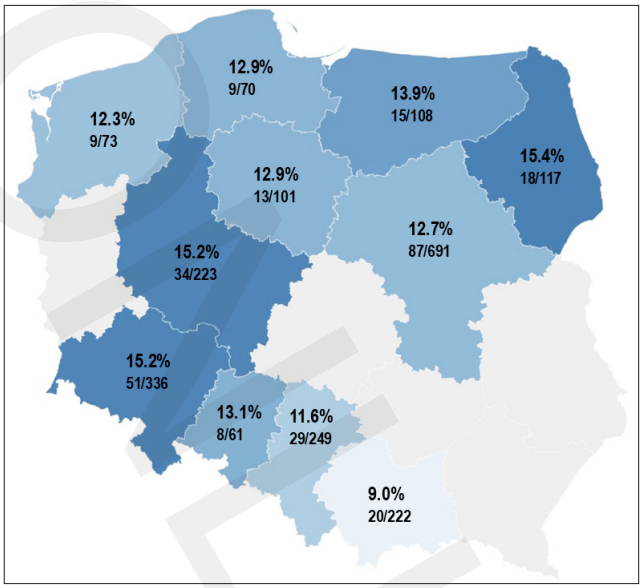

Figure 1. Microscopy-based prevalence of Giardia duodenalis in dogs by voivodeship in Poland. Values shown on the map indicate the percentage of microscopy-positive fecal sample sets in each voivodeship, with the corresponding number of positive cases and the total number of dogs examined in that voivodeship given in parentheses (n/N, where n = number of positive dogs and N = total number of dogs examined). Grey areas indicate voivodeships not represented in the study

Both microscopy and rapid IC assay results were available for 818 samples. Using microscopy as the operational reference method, the rapid test showed a sensitivity of 98.18% (95% CI: 94.79–99.38) and a specificity of 96.78% (95% CI: 95.13–97.89). Positive and negative predictive values were 88.52% and 99.53%, respectively, with an overall diagnostic accuracy of 97.07% (Tab. 2).

Table 2. Agreement between microscopy and immunochromatographic (IC) assay for detection of Giardia duodenalis in canine faecal samples

	Microscopy positive	Microscopy negative	Total
IC positive	108	23	131
IC negative	2	685	687
Total	110	708	818

Table 1. Microscopy-based occurrence of G. duodenalis according to selected host factors, with logistic regression analysis

Variable	Category	No. tested	No. positive	Prevalence (%)	Univariable p-value	OR (95% CI)	Multivariable p-value
Gender	Female	937	117	12.49	0.536	Reference	—
	Male	1,314	177	13.47		1.15 (0.89–1.48)	0.298
Age category	<1 year	778	209	26.86	4.68×10^{-44}	—	—
	1 to <3 years	701	42	5.99		—	—
	≥3 years	772	43	5.57		—	—
Age	Per 1-year increase	2,251	—	—	—	0.69 (0.63–0.76)	5.86×10^{-16}

Age categories were used for descriptive and univariable analyses, whereas age as a continuous variable was included in the logistic regression model

At the standard annealing temperatures, the *gdh* marker was detected in 128/294 (43.54%) microscopy-positive samples and the *bg* marker in 155/294 (52.72%). Re-amplification of initially negative samples using a lower primary-round annealing temperature increased the number of PCR-positive results by detecting 40 additional *gdh*-positive and 68 additional *bg*-positive samples, increasing the final detection rates to 168/294 (57.14%) and 223/294 (75.85%), respectively. However, because this modified protocol was not independently validated, results obtained after lowering the annealing temperature were interpreted with caution. Selected amplicons were subjected to sequencing to confirm assemblage assignment, and only confirmed results were considered in the final interpretation. Overall, amplification of at least one molecular marker was obtained in 241/294 (81.97%) samples, and 150/294 (51.02%) samples were positive by both markers, whereas 18/294 (6.12%) only for *gdh*, and 73/294 (24.83%) only for *bg*.

Final PCR detectability increased significantly with increasing cyst burden. For the *gdh* marker, positivity rose from 26.57% in samples with single cysts to 66.67% in those with moderately numerous cysts, and 98.90% in those with numerous cysts ($p = 6.25 \times 10^{-28}$). A similar pattern was observed for the beta-giardin marker, with corresponding values of 51.05%, 98.33%, and 100.00%, respectively ($p = 1.19 \times 10^{-20}$) (Tab. 3). Lowering the annealing temperature significantly improved molecular detection for both loci. For *gdh*, additional positives obtained after lowering the primary-round annealing temperature accounted for 11/143 (7.69%) in samples with single cysts, 15/60 (25.00%) in samples with moderately numerous cysts, and 14/91 (15.38%) in samples with numerous cysts ($p = 0.0038$). When only primarily PCR-negative samples were considered, the proportion of newly detected *gdh*-positive cases increased from 9.48% in samples containing single cysts to 42.86% in those with moderately numerous cysts and 93.33% in those with numerous cysts ($p = 1.15 \times 10^{-13}$). For *bg*, additional positives after lowering the annealing temperature were found in 37/143 (25.87%) samples with single cysts and 31/60 (51.67%) samples with moderately numerous cysts, whereas no further gain was observed in samples with numerous cysts because all of them had already been positive before protocol modification ($p = 9.02 \times 10^{-13}$) (Tab. 4).

Among the 241 samples positive for at least one molecular marker, assemblages D and C predominated, representing 118/241 (48.96%) and 116/241 (48.13%) samples, respectively. Assemblage B was detected in 5/241 (2.07%) samples and assemblage A in 2/241 (0.83%). Assemblages C and D were identified in all provinces represented among molecularly positive samples, whereas assemblage A was found only in the Mazovian and Silesian provinces, and assemblage B only in the provinces of Mazovia, Małopolska and Silesia. No mixed-assemblage infections were documented in this study (Fig. 2 and 3).

Table 3. Molecular detection of G. duodenalis, assemblage distribution, and association between cyst burden and PCR positivity in microscopy-positive canine faecal samples

Variable	Category	N	n	%	*p*-value
Molecular detection	*gdh* positive (in total)	294	168	57.14	—
	bg positive (in total)	294	223	75.85	—
	positive by at least one marker	294	241	81.97	—
	positive by both markers	294	150	51.02	—
	gdh positive only	294	18	6.12	—
	bg positive only	294	73	24.83	—
	negative by both markers	294	53	18.03	—
Assemblage distribution	D	241	118	48.96	—
	C	241	116	48.13	—
	B	241	5	2.07	—
	A	241	2	0.83	—
PCR positivity by cyst burden – *gdh*	single cysts	143	38	26.57	6.25×10^{-28}
	moderately numerous cysts	60	40	66.67	
	numerous cysts	91	90	98.90	
PCR positivity by cyst burden – *bg*	single cysts	143	73	51.05	1.19×10^{-20}
	moderately numerous cysts	60	59	98.33	
	numerous cysts	91	91	100.00	

Assemblage distribution was calculated among samples positive by at least one molecular marker.

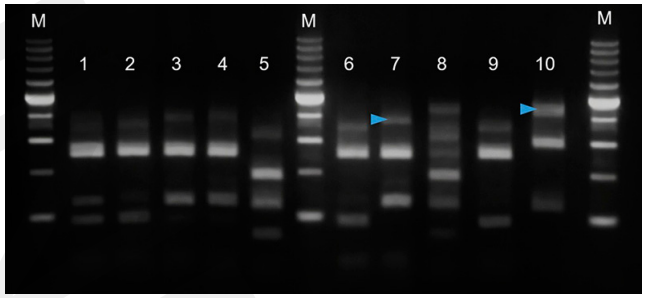

Figure 2. Representative electrophoretic profiles of gdh PCR-RFLP amplicons following digestion with NlaIV and separation by agarose gel electrophoresis. Lanes 1 (D039), 2 (D111), 3 (D114), 4 (D225), 6 (D466), 7 (D469), and 9 (D513) show restriction patterns consistent with assemblage D. Lanes 5 (D006) and 8 (D044) correspond to assemblage C, and lane 10 (D301) to assemblage B. Blue arrowheads indicate incompletely digested amplicons, which were excluded from pattern interpretation. M, 100-bp DNA ladder (A&A Biotechnology, Gdańsk, Poland).

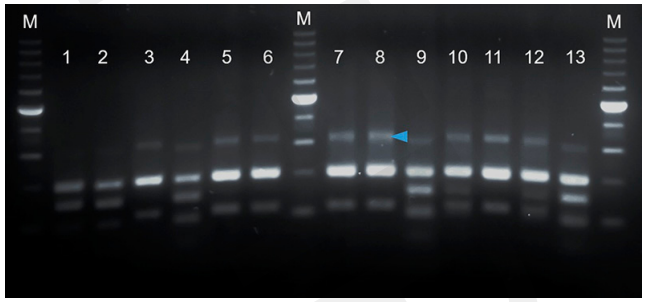

Figure 3. Representative electrophoretic profiles of beta-giardin PCR-RFLP amplicons following digestion with HaeIII and separation by agarose gel electrophoresis. Lanes 1 (D006), 2 (D044), 4 (D100_50), 9 (D187_50), and 13 (D199_50) show restriction patterns consistent with assemblage C. Lanes 3 (D039), 5 (D111), 6 (D114), 7 (D225), 8 (D055_50), and 11 (D371_50) correspond to assemblage D, whereas lanes 10 (D201) and 12 (D205) represent assemblage A. Sample IDs marked with the suffix "_50" indicate amplicons obtained after lowering the primary-round annealing temperature from 55 °C to 50 °C. The blue arrowhead indicates an incompletely digested amplicon, which was excluded from pattern interpretation. M, 100-bp DNA ladder (A&A Biotechnology, Gdańsk, Poland).

Overall, the molecular results indicated a clear predominance of canine-adapted assemblages C and D, with only sporadic detection of potentially zoonotic genotypes. Representative isolates were additionally analysed by sequencing and phylogenetic analysis, which confirmed the assemblage assignment obtained by PCR-RFLP (Fig. 4).

Table 4. Effect of lowering the annealing temperature on PCR detectability of the gdh and bg markers according to cyst burden

Marker	Cyst burden	Positive before lowering annealing temperature, n/N (%)	Additional positives after lowering annealing temperature, n/N (%)	Total positives after protocol modification, n/N (%)	Overall p-value across cyst-burden categories
gdh	single cysts	27/143 (18.88)	11/143 (7.69)	38/143 (26.57)	0.0038
	moderately numerous cysts	25/60 (41.67)	15/60 (25.00)	40/60 (66.67)	
	numerous cysts	76/91 (83.52)	14/91 (15.38)	90/91 (98.90)	
bg	single cysts	36/143 (25.17)	37/143 (25.87)	73/143 (51.05)	9.02×10^{-13}
	moderately numerous cysts	28/60 (46.67)	31/60 (51.67)	59/60 (98.33)	
	numerous cysts	91/91 (100.00)	0/91 (0.00)	91/91 (100.00)	

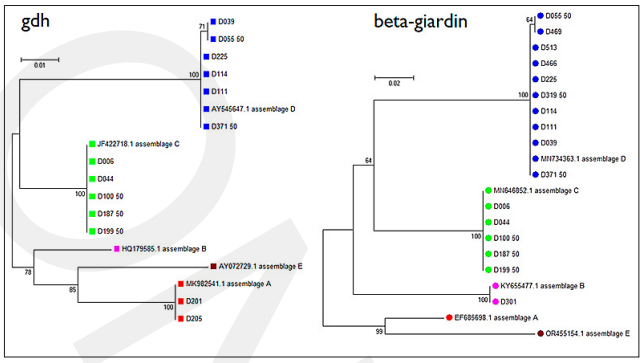

Figure 4. Phylogenetic analysis of Giardia duodenalis isolates from dogs based on partial gdh and beta-giardin gene sequences. The Neighbor-Joining tree was constructed using the Kimura 2-parameter model in MEGA v. 6. Sequences obtained in this study are shown in bold and labeled with sample IDs corresponding to those presented in Figures 2 and 3. Reference sequences were retrieved from GenBank. Bootstrap values (1,000 replicates) are indicated at nodes. The tree confirms clustering of isolates within assemblages consistent with PCR-RFLP results

DISCUSSION

This study provides nationwide data on the occurrence, diagnostic detection, and molecular epidemiology of *G. duodenal*is in dogs in Poland. The overall microscopy-based prevalence of 13.06% confirms that canine giardiasis remains common in routine veterinary diagnostics. This value is consistent with previous Polish studies reporting that the frequency of *Giardia* infection in dogs may vary considerably depending on the examined population, geographical area, and diagnostic method [3–6]. The results obtained in the current study support the view that canine giardiasis should still be regarded as an important parasitological and environmental issue in Poland.

A major epidemiological finding was the strong association between age and infection. Dogs younger than one year had a significantly higher prevalence than older animals, and increasing age significantly reduced the odds of microscopy-positive infection. This finding is in agreement with previous Polish studies and with more recent reports from other dog populations, in which young animals were consistently identified as the main reservoir of *Giardia* infection [4, 5, 13–17]. In contrast, no significant association with gender was observed, which is also consistent with the available literature [14, 15, 17].

The rapid IC assay showed very high sensitivity and specificity when compared with microscopy, indicating its practical usefulness as a screening tool in veterinary diagnostics. However, several samples were antigen-positive despite the absence of microscopically detectable cysts or trophozoites. This discrepancy can be explained by the intermittent shedding of *Giardia* cysts, low parasite burden, uneven distribution of parasitic stages in faeces, and the inherently imperfect sensitivity of flotation-based microscopy, particularly when a single sample is examined [2, 7, 18–20]. In addition, immunochromatographic assays detect soluble parasite antigens rather than intact cysts, and may therefore remain positive even when dispersive stages are not visible in the examined faeces [18, 20, 21]. These findings support the complementary use of microscopy and rapid IC testing in routine practice.

Molecular detection was strongly influenced by cyst burden. Both *gdh* and *bg* loci were detected much more frequently in samples with moderate or high cyst counts than in samples containing only single cysts. This result is biologically expected and indicates that the amount of parasitic material recovered from flotation preparations has a major impact on PCR success. Importantly, repeat nested PCR with a lower primary-round annealing temperature was associated with increased PCR positivity.

Although lowering the annealing temperature increased PCR positivity, this approach may also increase the risk of non-specific amplification. Therefore, results obtained under modified conditions should be interpreted with caution and require independent validation [16, 22].

With regard to assemblage distribution, the present study demonstrated a clear predominance of canine-adapted assemblages C and D, which together accounted for more than 97% of all genotyped samples. This pattern is highly consistent with current European data. A systematic review of dog studies conducted in European countries revealed that assemblages C and D are the predominant genotypes in dogs across the continent, while zoonotic assemblages A and B are less common [23]. More recent European studies have confirmed this pattern, including reports from The Netherlands, Italy, Croatia, and Germany, in which canine assemblages C and D predominated despite occasional detection of zoonotic variants [13, 14, 16, 17]. The predominance of C and D in the material in the current study is also in line with previous findings from Poland [3–5].

The zoonotic importance of canine giardiasis should therefore be interpreted in a balanced manner. On the one hand, the overwhelming predominance of assemblages C and D suggests that most infections in the studied dogs belonged to a host-adapted canine transmission cycle. On the other hand, the sporadic detection of assemblages A and B indicates that the zoonotic dimension cannot be completely excluded [1, 8, 22]. Importantly, although human giardiasis is still predominantly associated with assemblages A and B, sporadic reports suggest that infections with canine-adapted assemblages C and D may also occur in humans [22, 24–27]. From a One Health perspective, even a low

frequency of potentially zoonotic genotypes may be relevant in situations of close human-dog contact and environmental faecal contamination.

Strengths and limitations of the study. The main strengths of the study include the large number of faecal sample sets, the use of both routine parasitological and antigen-based diagnostic methods, and molecular characterization based on two loci. At the same time, several limitations should be acknowledged. Molecular analyses were performed only on microscopy-positive samples, which precluded genotyping of microscopy-negative but antigen-positive cases. Furthermore, microscopy was used as the reference method for calculating rapid IC assay performance, although microscopy itself may underestimate infection because of intermittent shedding and limited analytical sensitivity [18–21].

Despite these limitations, the collected data offer a comprehensive understanding of the epidemiology of canine Giardia in Poland and its potential implications for public health.

Additional limitations include the lack of full treatment history for all dogs, the use of microscopy as an operational rather than definitive reference method, and the absence of quantitative cyst counts (CPG). Furthermore, although selected samples were confirmed by sequencing, not all PCR-RFLP results were validated at the sequence level.

CONCLUSIONS

Giardia duodenalis remains a common intestinal parasite in dogs in Poland, particularly in animals younger than one year. The high diagnostic performance of the IC assay and the occurrence of antigen-positive but microscopy-negative samples support the complementary use of both methods in routine veterinary diagnostics. Molecular analysis showed a clear predominance of canine-adapted assemblages C and D, whereas assemblages A and B were detected only sporadically, indicating a limited but non-negligible zoonotic potential.

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