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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF GIARDIA SPP. IN CATTLE ON FARMS IN HUNEDOARA COUNTY, ROMANIA

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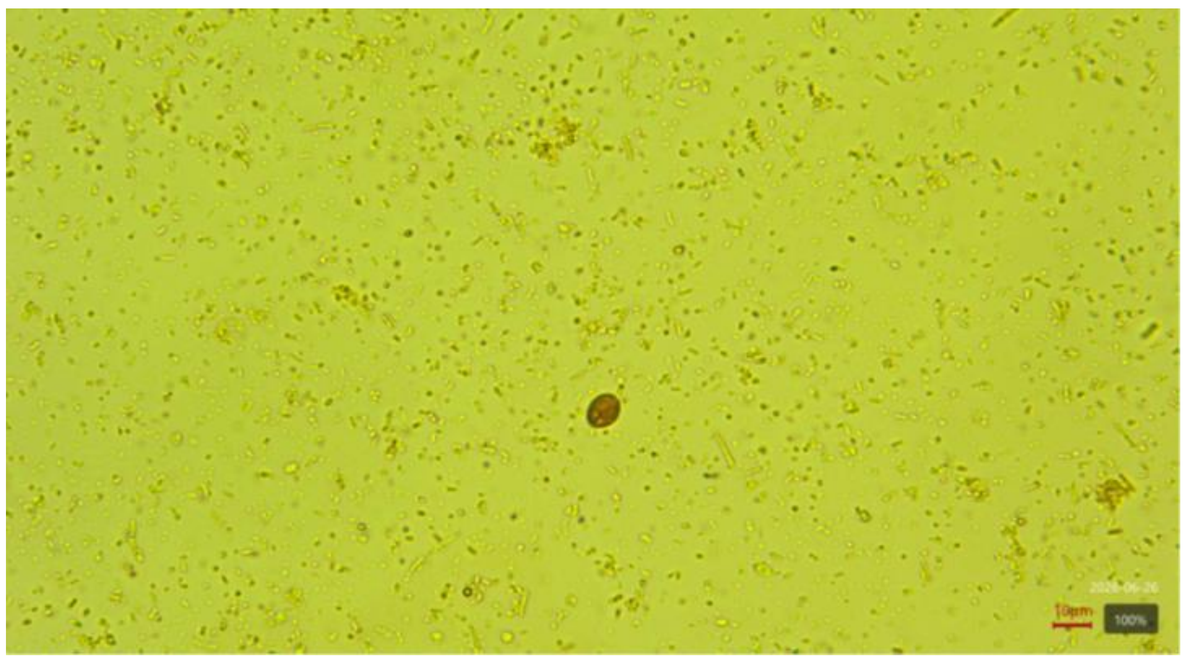
Abstract: *Giardia duodenalis is an intestinal protozoan of veterinary importance, and its detection and molecular characterization contribute to understanding infection in young cattle. This study aimed to investigate the occurrence of Giardia spp. in calves from Hunedoara County, western Romania, using morphological and molecular methods. Between October 2025 and March 2026, individual fecal samples were collected from 10 calves aged 1–5 months from farms in Bobaia, Boșorod, and Cioclovina. Both apparently healthy calves and animals exhibiting gastrointestinal signs were included. The diagnostic protocol comprised Willis flotation, direct microscopy with Lugol’s iodine, rapid coproantigen immunochromatographic testing, and PCR amplification targeting the glutamate dehydrogenase (gdh) gene. Molecular characterization included bidirectional Sanger sequencing and comparison with GenBank reference sequences using BLAST. Willis flotation detected six positive samples (60%), Lugol microscopy detected seven (70%), and PCR detected eight (80%). The proportions of Giardia-positive samples were 5/5 in Bobaia, 2/3 in Boșorod, and 1/2 in Cioclovina. These findings document Giardia infection in calves from the investigated farms and demonstrate that PCR detected more positive samples than either microscopic method. The results support the complementary use of microscopic and molecular approaches for investigating Giardia infection in calves and provide preliminary data for further epidemiological studies in the region.*
Keywords: *Giardia spp.; calves; microscopy; molecular detection; gdh gene; Romania.*

• Introduction

Giardia duodenalis is a globally distributed intestinal protozoan infecting humans and domestic and wild mammals. In calves, infection may be asymptomatic or associated with diarrhea, impaired nutrient absorption, and reduced growth. Transmission occurs through ingestion of cysts from contaminated water, feed, or the environment. Assemblage E is commonly associated with cattle, whereas assemblages A and B have broader host ranges, including humans. Molecular characterization complements microscopy and antigen detection by providing information on parasite genetic identity. Previous Romanian studies have identified distinct genetic groups in domestic and wild animals and assemblage E in buffalo calves. This study investigated *Giardia* spp. in calves from farms in Bobaia, Boșorod, and Cioclovina, Hunedoara County, western Romania. It aimed to compare the proportions of positive samples obtained by Willis flotation, Lugol-stained direct microscopy, and PCR targeting the gdh gene, and to compare available sequencing findings with GenBank reference sequences, providing preliminary observations for further regional epidemiological research.

• Material and method

Ten calves aged 1–5 months (eight females and two males) from farms in Bobaia (n = 5), Boșorod (n = 3), and Cioclovina (n = 2), Hunedoara County, western Romania, were investigated between October 2025 and March 2026. Both apparently healthy calves and animals with gastrointestinal signs were included. Individual fecal samples were collected directly from the rectum, transported under refrigerated conditions (4°C), and processed for DNA extraction within approximately 2–3 days.Parasitological examination included Willis gravitational flotation with saturated sodium chloride solution and Lugol-stained direct microscopy. Findings were recorded as positive or negative based on cyst morphology. Additional screening used a commercial coproantigen assay (B.GIA Ag Rapid Test Kit).Genomic DNA was extracted using the E.Z.N.A.® Stool DNA Kit. Nested PCR targeted an approximately 432 bp fragment of the glutamate dehydrogenase (gdh) gene, following Read et al. [2004] with minor modifications. The primary reaction used GDHEF (5′-TCAACGTYAAYCGYGGYTTCCGT-3′) and GDHIR (5′-GTTRTCCTTGCACATCTCC-3′), while the secondary reaction used GDHIF (5′-CAGTACAACTCYGCTCTCGG-3′) and the same reverse primer, GDHIR. Positive and negative controls were included in each run. PCR products were separated by electrophoresis in 1.5% agarose gels and visualized under UV light. Selected amplicons were purified and subjected to bidirectional Sanger sequencing by Macrogen Europe B.V. Sequences were edited for quality and compared with GenBank entries using BLAST to confirm pathogen identity.



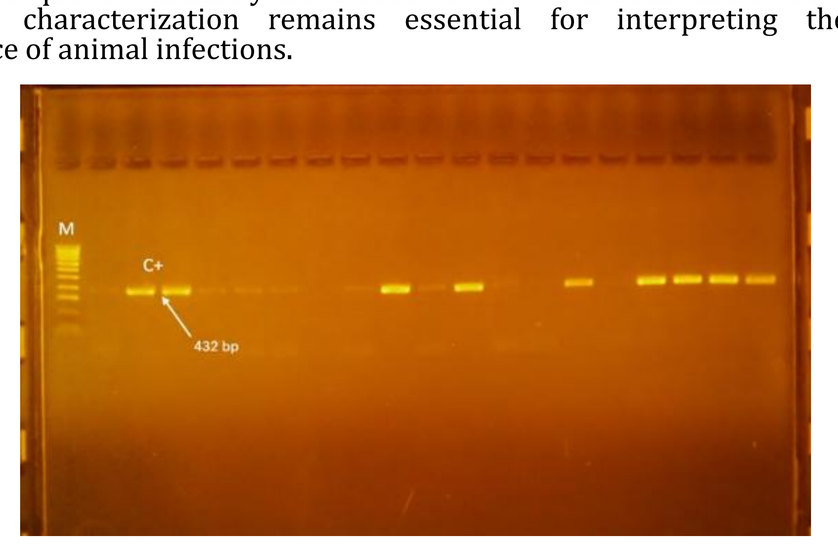
Microscopic identification of Giardia spp. cysts in fecal samples collected from cattle in Hunedoara County.

• Results and discussions

Giardia was detected in 8/10 samples (80%) by rapid antigen testing, 6/10 (60%) by Willis flotation, and 7/10 (70%) by Lugol-stained direct microscopy. Observed cysts exhibited morphological features consistent with Giardia spp. PCR targeting the gdh gene detected Giardia DNA in 8/10 samples (80%), producing amplicons of approximately 432 bp.Positive calves were identified in all three localities: Bobaia (5/5; 100%), Boșorod (2/3; 66.7%), and Cioclovina (1/2; 50%). Positivity was 66.7% in both the 1- and 2-month age groups (2/3 each); all calves aged 3–5 months were positive. Both males (2/2; 100%) and six females (6/8; 75%) tested positive.Bidirectional Sanger sequencing yielded partial gdh sequences matching *G. duodenalis* references in GenBank, including entries under the synonymous name G. intestinalis. Sample 3 showed one of its closest matches to assemblage E isolate H.QLD56 (KY655480.1), documenting sequence similarity to an assemblage E reference at the investigated locus.

Sample ID	Age (months)	Locality	Sex	Willis flotation	Lugol microscopy	Rapid antigen test	PCR
1	1	Bobaia	F	+	+	+	+
2	2	Cioclovina	F	−	−	−	−
3	2	Boșorod	F	+	+	+	+
4	2	Bobaia	F	+	+	+	+
5	1	Boșorod	F	−	−	−	−
6	4	Bobaia	F	+	+	+	+
7	1	Cioclovina	M	+	+	+	+
8	3	Bobaia	F	−	−	+	+
9	5	Bobaia	F	−	+	+	+
10	3	Boșorod	M	+	+	+	+
Positive, n (%)				6 (60%)	7 (70%)	8 (80%)	8 (80%)

The detection of *Giardia* in eight of ten calves by antigen testing and PCR, with positive animals across all examined age categories, supports investigation during the first months of life. Differences between microscopy, antigen testing, and PCR highlight their complementary diagnostic value; antigen testing and PCR agreed across all ten samples. Less favorable hygiene conditions recorded on some Bobaia farms suggest environmental exposure as a plausible contributor to the observed distribution. Cyst survival under cool conditions may facilitate infection persistence in contaminated farm environments. The partial gdh sequence from sample 3 showed similarity to assemblage E reference KY655480.1, consistent with the association of this assemblage with cattle and other hoofed livestock. This finding aligns with previous bovine investigations and complements Romanian molecular studies in other animal populations. However, the comparison concerns a single locus, and sequence similarity alone does not establish transmission between hosts. Molecular characterization remains essential for interpreting the zoonotic significance of animal infections.



• Conclusions

- *Giardia* infection was confirmed in calves from Hunedoara County: antigen testing and PCR detected 8/10 positive samples, Willis flotation 6/10, and Lugol microscopy 7/10.
- Complementary diagnostic methods provided evidence of infection; the *gdh* sequence from sample 3 showed similarity to a *G. duodenalis* assemblage E reference.
- Findings support *Giardia* testing in young calves and provide preliminary data for further regional epidemiological studies.