



SHORT COMMUNICATION

One-Pot RPA-CRISPR/Cas12a assay for Rapid Visual Detection of *Giardia duodenalis* in Cattle and Goats

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ABSTRACT

Giardia duodenalis is a widespread zoonotic protozoan causing giardiasis in humans and livestock. A one-pot RPA-CRISPR/Cas12a assay was developed for rapid, visual detection of *G. duodenalis* for the detection of a specific genomic segment (GenBank: CP110919.1). For this crRNA and RPA primers were designed and optimized. The assay completed the detection within 30min at 37°C in a single tube, which demonstrated high specificity with no cross-reactivity against six non-target pathogens. The limit of detection was 1 copy/μL for recombinant plasmids and 1 cyst per 10mL for cysts, surpassing nested PCR (10³ cysts/10mL). In 30 clinical fecal samples, the assay showed 100% concordance with nested PCR. This one-pot system eliminates aerosol contamination risk and provides intuitive visual readouts, offering a field-applicable tool for giardiasis screening in cattle and goats.

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INTRODUCTION

Giardia duodenalis is a zoonotic protozoan causing giardiasis in humans and animals, with over 280 million human cases annually (Cai *et al.*, 2021). In livestock, prevalence ranges from 1.09 to 74.2% in cattle (Taghipour *et al.*, 2022) and 0.7 to 33.8% in small ruminants (Geng *et al.*, 2023), causing substantial economic losses (Ryan *et al.*, 2019). Assemblages A and E predominate in ruminants, while assemblage B is rarely detected in livestock (Sprong *et al.*, 2009; Santin, 2020). Microscopy and ELISA are limited by low sensitivity and time-consuming procedures, potentially leading to false positives, whereas PCR requires expensive instrumentation (Dashti *et al.*, 2021).

RPA-CRISPR/Cas12a detection has emerged as a rapid and visual alternative (Chen *et al.*, 2018; Li *et al.*, 2018). The one-pot RPA-CRISPR/Cas12a platform integrates isothermal amplification and Cas12a-mediated cleavage within a single closed tube, effectively minimizing contamination risk while streamlining the detection workflow (Gao *et al.*, 2025). However, no one-pot RPA-CRISPR/Cas12a assay for *G. duodenalis* has

been reported. Consequently, a rapid, contamination-free, and field-applicable method is urgently needed. This study developed a one-pot RPA-CRISPR/Cas12a system completing detection within 30min at 37°C, providing intuitive visual readouts for *G. duodenalis* screening.

MATERIALS AND METHODS

Parasites and clinical samples: *G. duodenalis*, *Blastocystis hominis*, *Cryptosporidium parvum*, *Enterocytozoon bieneusi*, *Moniezia expansa*, *Fasciola hepatica* and *Escherichia coli* preserved by our laboratory were used for specificity testing. Thirty fecal samples (15 from dairy cattle and 15 from Leizhou black goats in Guangdong Province, China) were collected. Genomic DNA was extracted from approximately 200 mg of each fecal specimen using the E.Z.N.A.® Stool DNA Kit (Omega Bio-tek Inc., Norcross, GA, USA) in accordance with the manufacturer's protocol.

Primer and crRNA design: Based on a *G. duodenalis*-specific genomic segment encoding a dynein heavy chain

protein (GenBank: CP110919.1) (Yu *et al.*, 2024), three forward and four reverse RPA primers were designed using SnapGene software, and two crRNAs targeting PAM sequences were synthesized (Table 1). The pMD-19T-535 recombinant plasmid containing the target fragment was constructed as a standard template.

Table 1: Sequences of RPA primers and crRNAs used in the one-pot RPA-CRISPR/Cas12a assay

Assay	Name	Sequence (5'→3')
crRNA	crRNA1	ATCAATAGGGTACGCGATAACTT
crRNA	crRNA2	GCACAATCCACTGTTTAGAATG
RPA primer	RPA-F1	AGTTCAGGGACGATCTAAAGACCTTATTTCAA
RPA primer	RPA-F2	AACAAAAATATCGTGTTCATGCTGGACGATA
RPA primer	RPA-F3	AGTCACAAC TAGAAGATATCAACAACATTCTAA
RPA primer	RPA-R1	AATAAGTTTGGCACAATCCACTGTTTAGAA
RPA primer	RPA-R2	ATAATCTTGTCCAGCTCATCCTGCTCGAATAA
RPA primer	RPA-R3	AGTTATCGCGTACCCTATTGATGAAAAAGCGAA
RPA primer	RPA-R4	AACCAGTCTATGGTGAGATTGTGACAAGAGAA

One-pot RPA-CRISPR/Cas12a assay optimization: RPA amplification and CRISPR/Cas12a trans-cleavage were integrated in a single 30µL reaction tube at 37°C for 30min. The F1/R1 primer pair and crRNA1 combination were selected as optimal based on fluorescence curve analysis. The RPA reaction volume was reduced from 50 to 25µL for the standalone step to minimize reagent consumption.

Specificity, sensitivity, and reproducibility: Specificity was evaluated using DNA from *G. duodenalis* and six non-target pathogens. Sensitivity was determined using tenfold serial dilutions of the recombinant plasmid (10^4 to

10^{-1} copies/µL) and gradient-diluted cyst genomic DNA. Reproducibility was assessed through three independent assays. Conventional nested PCR targeting the β-giardin gene was used as a reference method (Yu *et al.*, 2023). The negative control (NC) consisted of a no-template reaction in which an equal volume of sterile water was substituted for template DNA and was included in every run to monitor background signal and contamination.

Clinical sample detection: Thirty fecal samples were tested using the one-pot assay, and results were compared with nested PCR. Positive samples were confirmed by Sanger sequencing.

RESULTS AND DISCUSSION

The one-pot RPA-CRISPR/Cas12a assay was optimized with the F1/R1 primer pair and crRNA1 combination, which exhibited the earliest fluorescence onset and highest reaction efficiency. The assay demonstrated high specificity, producing strong fluorescence signals only for *G. duodenalis* DNA, with no cross-reactivity against *B. hominis*, *C. parvum*, *E. bienewsi*, *M. expansa*, *F. hepatica*, or *E. coli*, visible under both blue light and UV illumination (Fig. 1). The limit of detection (LOD) was 1 copy/µL for the recombinant plasmid and 1 cyst per 10mL for *G. duodenalis* cysts, substantially surpassing nested PCR which detected only 10^3 cysts/10mL (Fig. 2). Three independent sensitivity assays demonstrated good reproducibility.

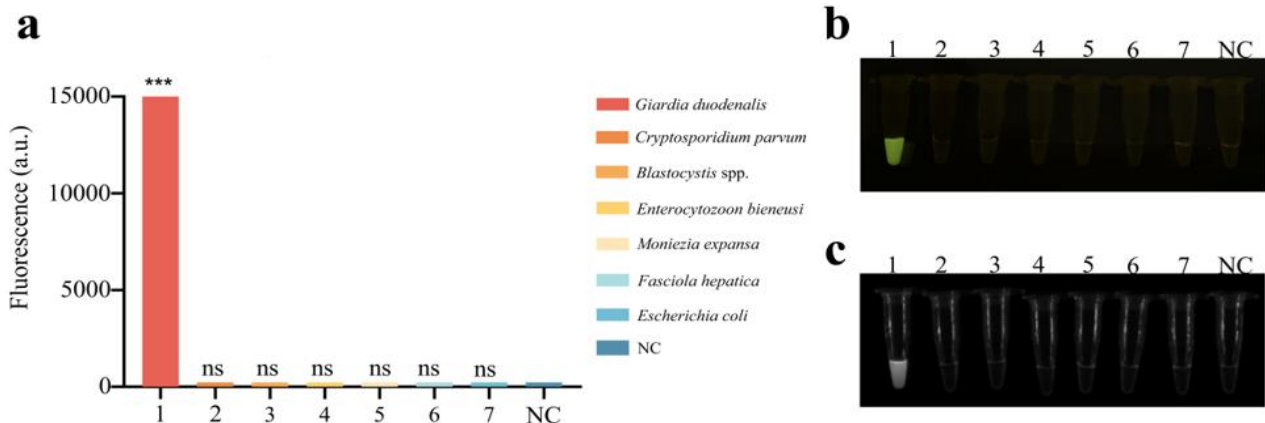


Fig. 1: Specificity of the one-pot RPA-CRISPR/Cas12a. (a) Specificity of the one-pot RPA-CRISPR/Cas12a system for *G. duodenalis* detection with the fluorometric assay, (b-c) Specificity of the one-pot RPA-CRISPR/Cas12a system for *G. duodenalis* detection by the naked eye under blue light and UV light. 1. *G. duodenalis*, 2. *C. parvum*, 3. *B. hominis*, 4. *E. bienewsi*, 5. *M. expansa*, 6. *F. hepatica* and 7. *Escherichia coli*, NC stands for negative control.

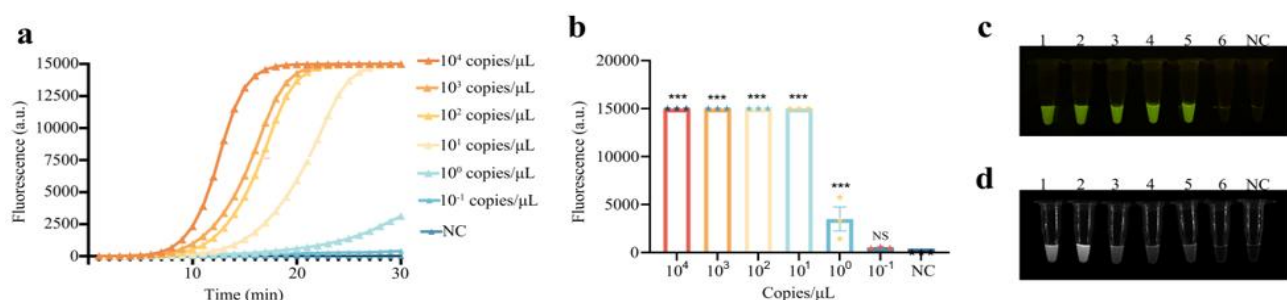


Fig. 2: Analysis of sensitivity of the one-pot RPA-CRISPR/Cas12a assay. (a-b) Sensitivity of the one-pot RPA-CRISPR/Cas12a platform for recombinant plasmids DNA detection with the fluorometric assay, NC stands for the negative control. Error bars represent the standard deviation (SD) of the mean, where $n = 3$ replicates. *** $P \leq 0.0001$, ** $P \leq 0.001$. (c-d) Sensitivity of the one-pot RPA-CRISPR/Cas12a system for *Giardia duodenalis* detection by the naked eye under blue light and UV light. Numbers 1-6 correspond to 10^4 copies/µL, 10^3 copies/µL, 10^2 copies/µL, 10^1 copies/µL, 10^0 copy/µL and 10^{-1} copies/µL, respectively; NC: negative control.

In 30 clinical fecal samples, the assay detected *G. duodenalis* in 3/15 cattle (20.0%) and 5/15 black goats (33.3%), with 100% concordance with nested PCR results. The higher prevalence in goats aligns with previous findings in southern China (Wang *et al.*, 2023; Yu *et al.*, 2023).

Giardiasis remains a significant burden in livestock farming (Ryan *et al.*, 2019). Unlike the previously reported two-step RPA-CRISPR/Cas12a method requiring 1.5h with tube opening (Zhao *et al.*, 2024), our one-pot system eliminates aerosol contamination risk and shortens detection to 30min at a constant 37°C without intermediate tube opening or product transfer. This integrated design enhances operational safety and field applicability, as the reaction can be performed in a water bath or even held in the palm of the hand without specialized equipment. Moreover, the selected target gene (GenBank: CP110919.1), identified through preliminary screening, achieves a detection limit of 10⁰ copies/μL for the standard plasmid and a sensitivity equivalent to 1 cyst per 10mL—1000-fold more sensitive than conventional nested PCR (1000 cysts/10mL). Regarding assemblage coverage, although the Cas12a recognition elements (PAM and seed region) are completely conserved among assemblages A, B and E in ruminant livestock, RPA primer binding regions exhibit approximately 82% identity for assemblage B with 3'-terminal mismatches that may compromise amplification efficiency. This detection range is consistent with the established epidemiological distribution of *G. duodenalis* assemblages, wherein assemblage B is primarily associated with human hosts and infrequently identified in ruminant livestock (Sprong *et al.*, 2009; Santin, 2020), whereas assemblages A and E predominate in ruminant livestock populations and are reliably detected by the current primer set (Ryan *et al.*, 2019). Future efforts will focus on designing degenerate RPA primers or assemblage-specific primer cocktails to expand pan-assemblage coverage. Notably, one-pot integration entails a well-documented trade-off, wherein analytical sensitivity is typically reduced relative to two-step workflows, since Cas12a trans- and cis-cleavage competes with RPA amplification and degrades primers/templates before sufficient amplicon can accumulate (Huang *et al.*, 2025). Our plasmid LOD of 1 copy/μL, although approximately 10-fold higher than the two-step RPA-CRISPR/Cas12a assay of Zhao *et al.* (2024) (0.1 copy/μL), is consistent with this one-pot penalty and reflects the operational advantage of tube-opening-free detection that prevents aerosol contamination.

In conclusion, we developed a rapid, sensitive and low-equipment-dependency one-pot RPA-CRISPR/Cas12a assay for visual detection of *G. duodenalis* in ruminant livestock, suitable for field applications in resource-limited settings.

Conflict of interest: The authors declare no conflict of interest.

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Institutional Review Board Statement: This study was approved by the Ethics Committee of Foshan University (FS2023109) and conducted in accordance with Chinese animal research ethics guidelines.

Authors contributions: XY, XM, QL, SW performed the experiments and drafted the manuscript; GL, XP, XM contributed to the experiments; XY, YH designed the study and revised the manuscript; DD, YL, XM, NL collected fecal samples and analyzed data; XY edited the manuscript; All authors have read and approved the final version.

REFERENCES

- Cai W, Ryan U, Xiao L, *et al.*, 2021. Zoonotic giardiasis: an update. *Parasitology Research* 120:4199-4218.
- Chen JS, Ma E, Harrington LB, *et al.*, 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* 360:436-439.
- Dashti A, Koster PC, Carmena D, 2021. *Giardia duodenalis*: Detection by quantitative Real-Time PCR and molecular diversity. *Methods in Molecular Biology* 2369:83-97.
- Gao X, Dong X, Song H, *et al.*, 2025. A one-pot CRISPR-Cas12a-based assay for rapid, on-site detection of African swine fever virus. *International Journal of Biology of Macromolecules* 321:146109.
- Geng H, Yan W, Wang J, *et al.*, 2023. Meta-analysis of the prevalence of *Giardia duodenalis* in sheep and goats in China. *Microbial Pathogenesis* 179:106097.
- Huang D, He Y, Xu C, *et al.*, 2025. Recent advances and applications in integrating nucleic acid amplification technologies and CRISPR systems by spatial/temporal separation strategies for one-pot diagnosis. *TrAC Trends in Analytical Chemistry* 193:118456.
- Li SY, Cheng QX, Liu JK, *et al.*, 2018. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Research* 28:491-493.
- Ryan U, Hijawi N, Feng Y, *et al.*, 2019. *Giardia*: an under-reported foodborne parasite. *International Journal of Parasitology* 49:1-11.
- Santin M, 2020. *Cryptosporidium* and *Giardia* in ruminants. *Veterinary Clinics of North America: Food Animal Practice* 36:223-238.
- Sprong H, Caccio SM and van der Giessen JVB, 2009. Identification of zoonotic genotypes of *Giardia duodenalis*. *PLoS Neglected Tropical Diseases* 3:e558.
- Taghipour A, Sharbatkhori M, Tohidi F, *et al.*, 2022. Global prevalence of *Giardia duodenalis* in cattle: a systematic review and meta-analysis. *Preventive Veterinary Medicine* 203:105632.
- Wang H, Mu X, Zhong M, *et al.*, 2023. Prevalence and molecular characterization of *Giardia duodenalis* in dairy cattle in parts of Guangdong province. *Progress in Veterinary Medicine* 44:16-22.
- Yu X, Wang H, Li Y, *et al.*, 2023. Occurrence and genotypic identification of *Blastocystis* spp., *Enterocytozoon bienersi* and *Giardia duodenalis* in Leizhou Black Goats in Zhanjiang City, Guangdong Province, China. *Animals-Basel* 13:2777.
- Yu X, Xu H, Mu X, *et al.*, 2024. A multiplex PCR assay for simultaneous detection of *Giardia duodenalis*, *Cryptosporidium parvum*, *Blastocystis* spp. and *Enterocytozoon bienersi* in goats. *Veterinary Science* 11:448.
- Zhao Z, Cao S, Sun M, *et al.*, 2024. Rapid visual detection of *Giardia duodenalis* in faecal samples using an RPA-CRISPR/Cas12a system. *Parasitology Research* 123:176.