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RESEARCH ARTICLE

Molecular and Serological Diagnosis of *Strongyloides stercoralis* Infection Among Cancer Patients at Hospital Universiti Sains Malaysia

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ABSTRACT

Strongyloidiasis, caused by *Strongyloides stercoralis*, is a neglected disease with a worldwide prevalence, particularly in tropical and subtropical regions. Most people have chronic asymptomatic infections, which may transform into potentially fatal hyper- or disseminated infections when immunosuppressed. Cancer patients on corticosteroids are at an increased risk of developing severe forms of the infection due to their impaired immune status. The present study used molecular, serological, and parasitological methods to detect *S. stercoralis* infection in cancer patients on corticosteroids. Using faecal and serum samples from 99 individuals, real-time PCR demonstrated the highest detection rate (27.3%), followed by the *Strongyloides* IgG4 rapid test (IgG4-RDT or SsRapid) (22.2%) and a commercial IgG-ELISA (4.0%). Agar plate culture performed on 88 of 99 stool samples was negative. There was no significant difference in detection prevalence between the IgG4-RDT and real-time PCR ($p = 0.413$), and the agreement between them was slight (kappa coefficient, 0.108). Using a composite reference standard (CRS), 41 of 99 samples (41.4%) were classified as positive for *Strongyloides* infection. Based on the CRS, PCR demonstrated higher sensitivity (65.9%) than IgG4-RDT (53.7%), while both assays exhibited 100% specificity and positive predictive value (PPV). The negative predictive value (NPV) was greater for PCR (80.6%) than IgG4-RDT (75.3%). McNemar's test indicated no significant difference between the two assays ($p = 0.49$). Notably, combining results of the real-time PCR and IgG4-RDT increased the detection rate to 41%, which was significantly higher than that of PCR alone (27%, $p = 0.036$) or IgG4-RDT alone (22%, $p = 0.0036$). The combined results showed substantial agreement with PCR ($\kappa = 0.693$) and moderate agreement with IgG4-RDT ($\kappa = 0.576$). In conclusion, the combination of real-time PCR and IgG4-RDT offers a more reliable approach for detecting *S. stercoralis* in cancer patients undergoing corticosteroid therapy than either assay alone.

Keywords: *Strongyloides stercoralis*; cancer patients; real-time PCR; *Strongyloides* IgG4 rapid test (IgG4-RDT or SsRapid); commercial IgG-ELISA.

INTRODUCTION

Strongyloidiasis is a soil-transmitted helminth infection mainly caused by the roundworm *Strongyloides stercoralis.* It is estimated to infect 600 million people globally, especially in tropical and subtropical countries (Buonfrate *et al*., 2020). In 2021, the World Health Organization (WHO) listed it as among the neglected tropical diseases (NTDs) under the soil-transmitted helminth group, requiring control actions in endemic areas (Czeresnia & Weiss, 2022).

Humans commonly become infected when they come into contact with contaminated soil, where infective larvae penetrate the skin. Infected immunocompetent individuals are often asymptomatic or experience mild gastrointestinal, pulmonary, and cutaneous symptoms, accompanied by or without fever. However, chronic strongyloidiasis may turn into severe hyperinfection and fatal disseminated infection in immunosuppressed/immunocompromised patients. They may be under immunosuppressive treatment, especially corticosteroids, or with diseases such as human T-lymphotropic virus type 1 infection, diabetes, malignancies, and HIV (Segarra-Newnham, 2007). A common trigger for *Strongyloides* hyperinfection syndrome among the immunosuppressed population is the use of corticosteroids, which has been associated with cases as short as six days and doses as low as 20 mg prednisone per day (Wurtz *et al*., 1994; Ghosh & Ghosh, 2007).

Routine diagnosis for strongyloidiasis involves microscopic examination of stool samples; however, this method has low sensitivity due to low and intermittent stool larval output and thus often leads to misdiagnosis. Performing the Baermann technique or agar plate culture (APC) prior to microscopy enhances the detection rate. Serology is a common used as a complementary method in diagnosing strongyloidiasis in clinical and epidemiological settings. However, IgG detection assays, especially those using *Strongyloides* native antigen, may suffer from possible cross-reactions with antibodies to other helminths and may persist for some time after cure (Montes *et al*., 2010). Molecular assays, such as PCR and real-time PCR, are available but are commonly restricted to reference or research laboratories.

To improve serological diagnostic accuracy, recombinant antigens have been developed. One such antigen is the recombinant NIE protein (rNIE), a 31-kDa larval antigen that has demonstrated high sensitivity and specificity in *Strongyloides* antibody-based assays (Ramanathan *et al*., 2008; Rascoe *et al*., 2015; Yunus *et al*., 2019). An IgG4-based rapid diagnostic test (IgG4-RDT or SsRapid) is a point-of-care test (POCT) utilising rNIE, which enables faster turnaround and reduced cross-reactivity. In two evaluation studies, it demonstrated ~82% sensitivity and ~96% specificity in Thailand, and 97% sensitivity and 90% specificity at the Swiss Tropical and Public Health Institute (Noordin *et al*., 2021a; Nickel *et al*., 2024).

In immunosuppressed patients, antibody responses may be impaired, resulting in variable serodiagnostic accuracy. This concern is particularly relevant in cancer patients, who are immunosuppressed not only due to their underlying disease but also because they are frequently treated with corticosteroids, a significant risk factor for *Strongyloides* hyperinfection. Accurate and early diagnosis is therefore crucial to prevent progression to severe and potentially fatal disease.

The present study applied molecular, serological, and parasitological methods to detect *S. stercoralis* infection in cancer patients on corticosteroids. Notably, the serological methods include the above-mentioned IgG4-RDT prototype test.

MATERIALS AND METHODS

Sample collection

The study samples were obtained from patients at the Hospital Universiti Sains Malaysia (USM) with various malignancies who received chemotherapy, including corticosteroids, such as dexamethasone, prednisolone, and hydrocortisone. We used samples from 99 patients who contributed faecal and serum samples. Most (72%) had haematological malignancies, 21% had breast cancer, and 7% had other cancers. After collection, the samples were cultured on agar plates or stored at -20°C for real-time PCR. Ethical approval for this study was obtained from the USM Human Research Ethics Committee, No. USM/JEPem/20050254.

Agar plate culture (APC)

The detection of *S. stercoralis* was conducted using two variations of the agar plate culture (APC) method, conventional and modified, to increase the likelihood of obtaining positive culture results. Conventional APC was prepared with beef extract, peptone, and Difco™ nutrient agar, while the modified APC utilised Oxoid™ nutrient agar supplemented with Lab-Lemco powder, yeast extract, peptone, and NaCl; both agar preparations were adjusted to pH 6 (Kaewrat *et al.,* 2020). A fresh faecal sample (2 – 4 g) was applied to the agar centre; the plate was sealed with parafilm tape and incubated at 29–30°C for 3–5 days. On days 2, 3, 4, and 5, the plates were examined under a stereomicroscope to observe the presence of furrows/tracks of moving larvae. At the end of 5 days, all plates were washed with 10 ml formalin and centrifuged at 40,000 x g for 10 minutes, and the sediment was examined under a light microscope.

DNA isolation

Approximately 100 mg of unpreserved faeces were suspended in 200µl phosphate-buffered saline (PBS) containing 2% polyvinylpolypyrolidone (PVPP; Sigma, Steinheim, Germany). After heating for 10 minutes, the DNA was extracted using the QIAamp DNA Stool Mini Kit (Hilden, North Rhine-Westphalia, Germany) following the manufacturer's instructions. The yield and purity of the extracted DNA were determined using the NanoDrop ND-1000 (Thermo Fisher Scientific, Wilmington, Delaware, USA) and stored at -20°C. A dedicated set of pipettors was used for the DNA extraction to avoid cross-contamination.

Real-time PCR assay

The real-time PCR assay used an established and widely used set of primers and probes (Verweij *et al*., 2009). The sequences were: Stro18S-1530F 5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3' (101 bp), Stro18S-1630R 5'-TGCCTCTGGATATTGCTCAGTTC-3', and Stro18S-1586T FAM-5'-ACACACCGGCCGTCGCTGC-3'-BHQ1. The amplification profile was as follows: initial denaturation for 3 minutes at 95°C, followed by 40 cycles of 10 seconds at 95°C, and 1 minute at 60°C using the AriaMx Real-time PCR System and AriaMx software for data analysis (Agilent, Santa Clara, California, USA).

The real-time PCR experiments were performed meticulously. All experiments were conducted in triplicates, with a standard deviation (SD) below 0.2, including positive and negative controls. If the SD exceeded 0.2, the process was repeated from sample DNA extraction to real-time PCR. A dedicated set of pipettors was also used for the real-time PCR. Two types of positive controls were included, i.e., plasmid and genomic DNA. They were derived from clinical samples containing *S. stercoralis* third-stage larvae. Meanwhile, the negative control consisted of a non-template control. The cut-off value of the PCR assay was set at 35. It was determined using a standard curve data set derived from serial dilutions of positive plasmid (10 pg to 10 ag) with a reaction efficiency of 100% and an R^2 value of 0.998. We observed inconsistent results of sample replicates with Ct values beyond 35. For samples with Ct values between 33.9 and 35.4, the steps from DNA extraction to real-time PCR were repeated.

Serological assays

We used two serological assays: an IgG-ELISA and a lateral flow IgG4 rapid test. The former is a commercial *Strongyloides* IgG-ELISA (Euroimmun, L beck, Schleswig-Holstein, Germany). The latter is a prototype *Strongyloides* IgG4-RDT, also called SsRapid elsewhere (Noordin *et al*., 2022; Anuar *et al*., 2023; Nickel *et al*., 2024; Wongphutorn *et al*., 2024). The IgG-ELISA kit contains wells coated with *Strongyloides papillosus* larvae antigen. The test was performed according to the manufacturer's instructions, and absorbance measurements were recorded at 405 nm using the Thermo Scientific Multiskan™ FC Microplate ELISA Reader (Waltham, Massachusetts, USA).

The IgG4-RDT is POCT with rNIE as the test line and anti-human IgG4 as the detector antibody. It has been used in studies conducted in various countries and exhibited high diagnostic sensitivity (94% to 86%) and reasonable diagnostic specificity (100% to 74%) (Tamarozzi *et al*., 2022; Noordin *et al*., 2022; Anuar *et al*., 2023; Nickel *et al*., 2024; Wongphutorn *et al*., 2024). As previously described, the serum sample (35 µL) was placed in the sample well and allowed to flow up the nitrocellulose membrane strip. If antibodies to NIE are present, they bind to the rNIE on the test line. Three drops of buffer were added to the top oval well to reconstitute the gold-conjugated anti-human IgG4. The conjugate flows down the membrane strip and binds to goat anti-mouse IgG at the control line and the antibody-antigen complex at the test line. After 15 minutes, two red lines (control and test) indicate a positive result, while one red control line indicates a negative result (Noordin *et al*., 2022).

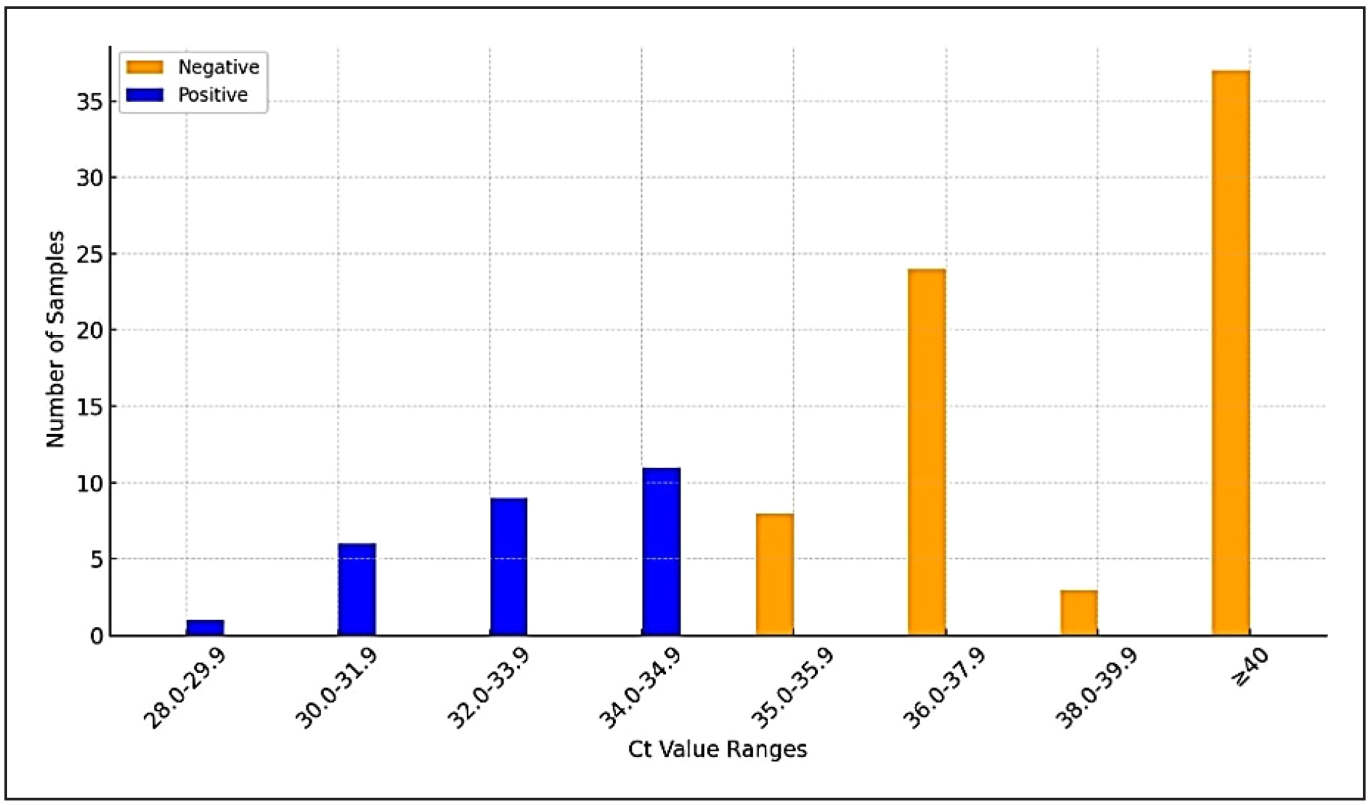


Figure 1. Histogram showing the frequency distribution of Ct values for all tested samples. Ct values > 40 are considered as "No Amplification".

The technologist conducting the IgG4-RDT was blinded to both clinical and molecular results, while researchers handling the PCR were blinded to both clinical and rapid test outcomes. Additionally, the primary developer of the rapid test, Rahmah Noordin, was not involved in the performance or interpretation of the IgG4-RDT or real-time PCR.

Statistical analysis

Data analysis was performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, California, USA) and IBM SPSS Statistics version 28.0 (Armonk, New York, USA). Diagnostic accuracy measures, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated using "combined detection" (PCR or IgG4-RDT positive) as the reference standard. Agreement between assays was assessed using 2×2 contingency tables and Cohen's kappa coefficient, interpreted according to established benchmarks (values <0 = no agreement; 0.00–0.20 = slight agreement; 0.21–0.40 = fair agreement; 0.41–0.60 = moderate agreement; 0.61–0.80 = substantial agreement; 0.81–1.00 = almost perfect agreement). McNemar's test was applied to evaluate differences in discordant pairs between assays. A p-value <0.05 was considered statistically significant.

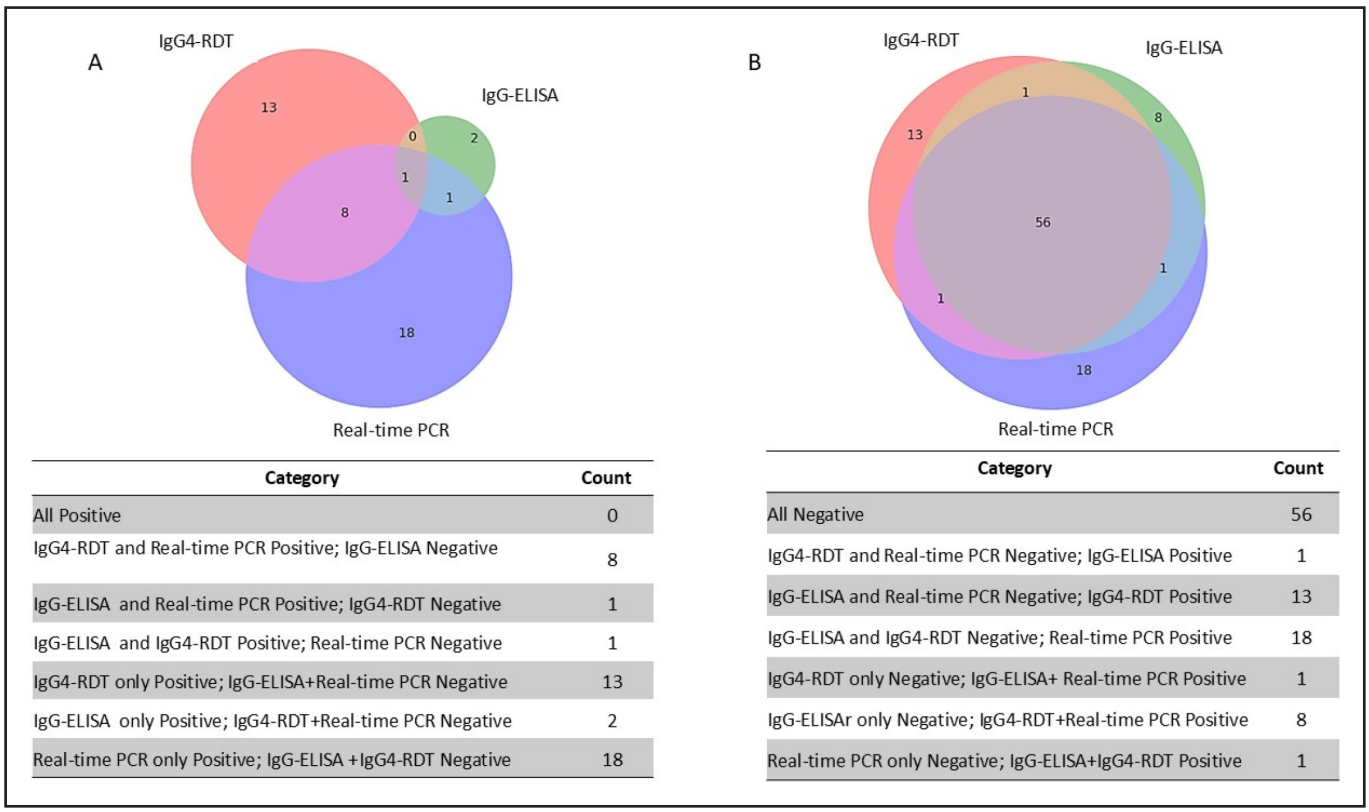
RESULTS

Ninety-nine patients provided faecal and serum samples for this study. Conventional and modified APCs were performed on 88 samples; the remaining 11 samples could not be cultured due to insufficient stool volume or unsuitable consistency, e.g., watery or highly mucoid. All 88 APCs yielded negative results. DNA was successfully extracted from all samples, enabling PCR analysis to be performed on the complete set of 99 specimens. For the molecular assay, the DNA concentration and purity of the extracted stool samples ranged from 103 to 182 ng/µL, with an A260/A280 ratio of 1.85 to 1.94. The Ct values of the positive samples ranged from 28.00 to 34.49. The Ct values of positive controls, both plasmid and DNA, ranged from 26.6 to 27.15. The distribution of Ct values across positive and negative samples is presented in a histogram (Figure 1). The results demonstrate a clear separation between positive and negative detections. All positive samples clustered within the Ct range of 28.99–34.49, with the highest frequency observed in the 34.0–34.9 range (11 samples). Negative samples began at a Ct value of 35.56 and extended to Ct>40 ('No Amplification'). A total of 12 positive samples fell within the range of 33.9–34.9, while none were detected between 35.0 and 35.4. The samples with Ct values between 33.9 and 35.4, which underwent repeated DNA extraction and PCR, showed reproducible results.

The highest detection rate was obtained using real-time PCR, i.e., 27.27% (27/99), followed by IgG4-RDT (22.22%; 22/99), and the lowest detection rate was achieved by commercial IgG-ELISA (4.04%, 4/99) (Table 1). There was no significant difference in detection rates between the IgG4-RDT and real-time PCR (p = 0.413). The detection rates across the three assays were also examined using Venn diagrams (Figure 2). Real-time PCR detected the highest number

Table 1. Summary of detection of *Strongyloides* infection by different assays in cancer patients (n = 99)

Assay	Positive (n)	Negative (n)	Detection Rate (%)	Notes
Real-time PCR	27	72	27.27	–
IgG4-RDT	22	77	22.22	–
IgG-ELISA	4	95	4.04	–
Agar Plate Culture (APC)	0	88	0	Only 88/99 samples tested due to stool availability or quality
Real-time PCR and IgG4-RDT	41	58	41.41	Positive by either PCR or IgG4-RDT



Category	Count
All Positive	0
IgG4-RDT and Real-time PCR Positive; IgG-ELISA Negative	8
IgG-ELISA and Real-time PCR Positive; IgG4-RDT Negative	1
IgG-ELISA and IgG4-RDT Positive; Real-time PCR Negative	1
IgG4-RDT only Positive; IgG-ELISA+Real-time PCR Negative	13
IgG-ELISA only Positive; IgG4-RDT+Real-time PCR Negative	2
Real-time PCR only Positive; IgG-ELISA +IgG4-RDT Negative	18

Category	Count
All Negative	56
IgG4-RDT and Real-time PCR Negative; IgG-ELISA Positive	1
IgG-ELISA and Real-time PCR Negative; IgG4-RDT Positive	13
IgG-ELISA and IgG4-RDT Negative; Real-time PCR Positive	18
IgG4-RDT only Negative; IgG-ELISA+ Real-time PCR Positive	1
IgG-ELISAr only Negative; IgG4-RDT+Real-time PCR Positive	8
Real-time PCR only Negative; IgG-ELISA+IgG4-RDT Positive	1

Figure 2. Venn diagrams depicting the overlap of *Strongyloides* detection by real-time PCR, IgG4-RDT, and IgG-ELISA. (A) Positive detection: Real-time PCR detected the most unique positives (n=18), followed by IgG4-RDT (n=13) and IgG-ELISA (n=2), with the most overlap between PCR and IgG4-RDT (n=8). (B) Negative detection overlap. Most samples (n = 56) yielded negative results across all assays.

of unique positives (n = 18), followed by the IgG4-RDT (n = 13) and the IgG-ELISA (n = 2). The best concordance was observed between PCR and IgG4-RDT (n = 8), while overlaps involving IgG-ELISA were minimal (n = 1 each). Most samples (n = 56) yielded negative results across all assays. Due to its very low detection yield, IgG-ELISA results were excluded from further analyses.

To further assess diagnostic agreement, a 2×2 contingency table was constructed for real-time PCR versus IgG4-RDT (Table 2). Both assays identified eight overlapping positives, while real-time PCR and IgG4-RDT each detected additional unique positives (19 and 14, respectively). The overall concordance between IgG4-RDT and real-time PCR results was moderate (66.67%), with a kappa coefficient of 0.108 (95% CI: –0.098 to 0.314), indicating slight agreement between the two assays.

Using a composite reference standard (CRS) based on the results of both real-time PCR and IgG4-RDT, 41 of 99 samples (41.4%) were classified as positive (Table 3). The IgG4-RDT correctly identified 22 of these 41 positives, yielding a sensitivity of 53.7%, whereas real-time PCR detected 27 of 41 positives (65.9% sensitivity). Both assays showed 100% specificity, as all 58 negative samples were correctly classified. The positive predictive value (PPV) was 100% for both assays, while the negative predictive value (NPV) was higher for real-time PCR (80.6%) than for IgG4-RDT (75.3%). McNemar's test revealed no significant difference between real-time PCR and IgG4-RDT (χ^2 (1) = 0.49, p = 0.49), indicating comparable overall performance when evaluated against the CRS.

The combination of real-time PCR and IgG4-RDT increased the overall detection rate to 41%, compared with 27% by real-time PCR alone and 22% by IgG4-RDT alone. The differences in detection rates between the combined results and single assays were statistically

Table 2. 2×2 contingency table of the results of real-time PCR versus IgG4-RDT (n = 99)

	IgG4-RDT Positive	IgG4-RDT Negative	Total
Real-time PCR Positive	8	19	27
Real-time PCR Negative	14	58	72
Total	22	77	99

Note: Concordance between IgG4-RDT and real-time PCR results was moderate (66.67%), with a kappa coefficient of 0.108 (95% CI: –0.098 to 0.314), indicating slight agreement.

Table 3. Diagnostic performance of real-time PCR and IgG4-RDT using a composite reference standard (CRS) (n=99)

Test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %	Accuracy %
Real-time PCR	65.9 (50.1–79.5)	100 (93.8–100)	100	80.6	85.86
IgG4-RDT	53.7 (38.6–68.2)	100 (93.8–100)	100	75.3	80.8

PPV: Positive Predictive Value; NPV: Negative Predictive Value.

CRS is based on the results of both real-time PCR and IgG4-RDT. McNemar's test comparing PCR and IgG4-RDT based on CRS was not significant: χ^2 = 0.49, p = 0.49.

Table 4. Contingency table for real-time PCR vs. composite reference standard (CRS) (n = 99)

	Combined Positive	Combined Negative	Total
PCR Positive	27 (TP)	0 (FP)	27
PCR Negative	14 (FN)	58 (TN)	72
Total	41	58	99

TP=True Positive; FP=False Positive; FN=False Negative; TN=True Negative; PP=Positive Predictive Value; NPV =Negative Predictive Value.

- Sensitivity = 27 / (27 + 14) = 65.9%
- Specificity = 58 / (58 + 0) = 100%
- PPV = 27 / (27 + 0) = 100%
- NPV = 58 / (58 + 14) = 80.6%
- Accuracy = (27 + 58) / 99 = 85.86%

Table 5. Contingency table for IgG4-RDT vs. composite reference standard (CRS) (n = 99)

	CRS Positive	CRS Negative	Total
RDT Positive	22 (TP)	0 (FP)	22
RDT Negative	19 (FN)	58 (TN)	77
Total	41	58	99

TP=True Positive; FP=False Positive; FN=False Negative; TN=True Negative; PP=Positive Predictive Value; NPV =Negative Predictive Value.

- Sensitivity = 22 / (22 + 19) = 53.7%
- Specificity = 58 / (58 + 0) = 100%
- PPV = 22 / (22 + 0) = 100%
- NPV = 58 / (58 + 19) = 75.3%
- Accuracy = (22 + 58) / 99 = 80.8%

significant (p = 0.036 and p = 0.0036, respectively). Concordance between the combined assays and real-time PCR was high (85.86%), with substantial agreement [κ = 0.693, 95% CI: 0.552–0.835]. Similarly, concordance between the combined assays and IgG4-RDT was high (80.81%), with moderate agreement [κ = 0.576, 95% CI: 0.421–0.731]. Detailed 2×2 contingency tables are provided as Table 4 and Table 5.

DISCUSSION

Autoinfection is a unique phenomenon in the life cycle of *S. stercoralis*. Some larvae produced in the human small intestine re-enter the peripheral blood circulation at the colon or the perianal skin. It causes a low-level asymptomatic infection that can persist almost lifelong. When the person is immunosuppressed, the infection may produce active proliferating larvae, leading to potentially fatal hyperinfection and disseminated disease (Hamze *et al*., 2023). The prevalence of severe and complicated strongyloidiasis has significantly risen due to the expanding population of immunosuppressed individuals (Keiser & Nutman, 2004; Mirzaei *et al*., 2021). *Strongyloides* hyperinfection has been reported after the administration of corticosteroids for the treatment of malignancies, transplants, COVID-19 pneumonia, rheumatoid arthritis, and other conditions (Krishnamurthy *et al*., 2007; Lier *et al*., 2020; Marchese *et al*., 2021). Other immunosuppressive drugs have also been associated with cases of *Strongyloides* hyperinfection syndrome (Czeresnia & Weiss, 2022).

In the context of cancer, this issue is particularly critical, as patients frequently receive corticosteroids either as part of chemotherapy regimens or supportive care. Corticosteroid therapy is a well-established trigger for *Strongyloides* hyperinfection and delayed or missed diagnosis in this group can rapidly lead to life-threatening outcomes. Therefore, strengthening diagnostic strategies in cancer patients is of high clinical importance.

Molecular, serological and parasitological methods measure different parameters and thus may not be directly comparable. A good reference ('gold standard') test for strongyloidiasis is also unavailable (Weitzel *et al*., 2024). However, analysing differences in detection rates using various methods helps select the best approach for diagnosing *Strongyloides* infection in different populations, including cancer patients.

Relying on stool parasitological detection may be inadequate, as it is insensitive and may require multiple stool samplings on different days due to intermittent larval shedding. According to our study results, the parasitological method yielded negative results despite using the more sensitive APC. It may be attributable to low larval output and only single stool sampling. Other contributing factors could be dead larvae in stool samples due to delayed transport, improper storage, medication effects, or immune system interactions. Since timely diagnosis is essential to avoid complications in cancer patients, more than one detection method, often including serology, should be performed. Furthermore, difficulty in providing stool samples due to constipation is a common problem for many individuals with cancer, occurring in almost 60% of patients (Wickham, 2017).

Several reported primers and probes for *Strongyloides* real-time PCR have been reported (Verweij *et al*., 2009; Pilotte *et al*., 2016; Chan & Thaenkham, 2023). In the present study, we used primers and probe that are widely recognised and commonly used and have been clinically validated for their high sensitivity and specificity (Repetto *et al*., 2016; Watts *et al*., 2019; Chan & Thaenkham, 2023). We performed real-time PCR meticulously to maximise its reliability and reproducibility. Although molecular diagnosis is generally highly sensitive and specific, detecting *Strongyloides* in stool may require an increased sampling frequency due to intermittent larval output or the presence of asymptomatic patients (Chan & Thaenkham, 2023). Noteworthy is a meta-analysis of the diagnostic accuracy of molecular diagnostics for *Strongyloides*, which revealed that the accuracy of molecular diagnosis is 61.85% using either parasitological or immunological techniques as the reference (Buonfrate *et al*., 2018).

PCR is considered an advanced faecal test since it detects the larvae (alive or dead) or the larvae's DNA in the faeces. In published reports, it is used as one of the reference standard tests for strongyloidiasis (Tamarozzi *et al*., 2022, 2023; Nickel *et al*., 2024). It is considered highly specific; thus, a positive result is usually taken as a true positive, especially when the sample is from a patient with cancer. For high-risk individuals, such as cancer patients, the consequence of a hyperinfection stemming from an accelerated autoinfection is likely to be devastating. Thus, unless the patient has been recently treated with an anti-*Strongyloides* drug, a PCR-positive result should be considered a current infection and treated appropriately. In a recently published paper, 30 Strongyloides-positive patients were followed up post-treatment. The IgG4-RDT results revealed a significant difference between the pre- and post-treatment groups. In some patients, the rapid test results changed from positive to negative after treatment, while in others, there was a reduction in the intensity of the test line scores (Ashiri *et al*., 2021). Thus, it showed that the rapid test detects active infection in those patients.

Numerous studies have evaluated the diagnostic performance of the IgG4-RDT cassette test, demonstrating good overall performance. The test showed high diagnostic sensitivity of 93.9%, 97%, 95% and 86% (Tamarozzi *et al*., 2022; Noordin *et al*., 2022; Anuar *et al*., 2023; Nickel *et al*., 2024; Wongphutorn *et al*., 2024). The diagnostic specificity ranged from 100% to 74%; the lower specificity was in a study that used serum samples from individuals potentially infected with *Strongyloides* cryptic infection (Tamarozzi *et al*., 2022).

The IgG4-RDT's high specificity was further validated by a serum absorption study using several recombinant antigens (Noordin *et al*., 2021b). The IgG4-RDT has also been applied in studies among children in Ecuador (Tamarozzi *et al.,* 2023). Additionally, in its initial 'crude' dipstick format, it was evaluated in Thailand and Malaysia (Yunus *et al*., 2019; Noordin *et al*., 2021a) and used in detecting *Strongyloides* infection in immunocompromised patients in Malaysia (Osman *et al*., 2022). A recent paper on diagnostics for the WHO *Strongyloides* control program compared the Baermann method (as reference), LFA-NIE, and Bordier ELISA. The LFA referred to in the paper was the cassette IgG4-RDT used in the present study. They recommended the IgG4-RDT as a cost-efficient alternative to the Baermann method for making program decisions, as it minimises the risk of incorrect policy decisions (Kazienga *et al*., 2025). Although the study's use case was for a control program and not for patient diagnosis, it acknowledges that, to date, the cassette IG4-RDT is the rapid test with the most published data from both laboratory and field studies. More data is needed on its performance in detecting *Strongyloides* infection in immunosuppressed patients; thus, the present study addresses this gap.

Several studies have used multiple diagnostic assays in populations of immunosuppressed patients. Most studies have compared parasitological methods with ELISAs (Luvira *et al*., 2016; de Souza *et al*., 2016; Ahmed *et al*., 2019; Ashiri *et al*., 2021). Two studies included molecular diagnostics on stool samples, and they found a higher prevalence by PCR than ELISAs (Zueter *et al*., 2014; Paula *et al*., 2016). Our study also showed a higher prevalence by PCR than IgG-ELISA, but a comparable prevalence to IgG4-RDT. However, the agreement between the PCR and IgG4-RDT was slight, as indicated by the kappa coefficient. To date, only one other study has evaluated an early prototype of a lateral flow IgG4 rapid dipstick test in HIV and cancer patients, comparing it with a commercial IgG ELISA (Osman *et al*., 2022). Our present IgG4-RDT is a rapid cassette POCT (an advanced prototype version) on cancer patients treated with corticosteroid, and it was compared to a commercial IgG ELISA and real-time PCR. The seroprevalence, as determined by the IgG4 rapid test in both studies (using dipstick or cassette tests), was higher than that determined by the IgG ELISA. The present study provides new information on the performance of the POCT IgG4-RDT compared to other diagnostic assays using samples from immunosuppressed patients.

Serological methods generally show low sensitivity in immunocompromised patients because their general antibody production is suppressed (Noordin *et al*., 2021b). Thus, in cancer patients treated with corticosteroids, diagnosis of strongyloidiasis by serology may not be prioritized. Also, the commonly reported serological tests used in patients with immunosuppression or immune dysfunction are IgG-based assays (Luvira *et al*., 2016; Osman *et al*., 2022). In our study, the detection rate using the IgG4-RDT was relatively high, similar to that of real-time PCR, whereas the commercial IgG-ELISA yielded a poor detection rate. Thus, despite the general antibody suppression, the level of specific IgG4 was sufficient to be detected by the IgG4-RDT.

Using combined rather than single methods is recommended for *Strongyloides* diagnosis (Hailu *et al*., 2022). However, it usually involves combining parasitological and molecular methods or parasitological and serological methods. However, a few reports combined serological and molecular assays to diagnose *Strongyloides* infection (Zueter *et al*., 2014; Paula *et al*., 2016; Erdem Kivrak *et al*., 2017). Notably, a study on children in Ecuador reported that the IgG4-RDT's sensitivity was 79.4% if used alone and 91% if combined with PCR, and the specificity was 94%. Similarly, in the same study, combining PCR with either an established commercial ELISA (Bordier Affinity Products, Switzerland) or a prototype ELISA (Strongy Detect ELISA, InBios International, USA) also produced significantly higher diagnostic sensitivity than using single assays (Tamarozzi *et al*., 2023).

Consistent with the results of the above Ecuador study in children, our study also showed that combining results of serological and PCR assays significantly increased the detection of *Strongyloides* infection in cancer patients. The detection rate increased from 22 or 27% using single assays (IgG4-RDT or real-time PCR, respectively) to 41% when both assay results were combined. The low kappa coefficient (slight agreement) between the results of real-time PCR and the IgG4-RDT indicated that many samples did not show concordant results. Intermittent larvae output in the stool may explain the samples with negative real-time PCR and positive IgG4-RDT results. Thus, the larvae may not be present when the stool was sampled for the PCR. Meanwhile, samples with positive real-time PCR and negative IgG4-RDT results may be from individuals whose stool contained *Strongyloides* larvae; however, the IgG4 antibodies were not detectable, probably due to their suppressed immune response.

Although all patients were receiving corticosteroid treatment, the degree of antibody suppression varied among individuals. Several factors can influence the extent of the humoral immune response, including the type of cancer, stage of disease, prior or concurrent chemotherapy, amount of corticosteroid use, and the time elapsed since the last treatment. Consequently, some patients may retain partial B-cell function and still produce detectable levels of IgG4 antibodies, whereas in others the antibody response may be too weak to be detected despite ongoing infection. This variability likely explains why the IgG4-RDT detected certain PCR-positive individuals, but not others.

The main limitation of this study is the relatively small sample size (n = 99), which was determined by the number of available consenting patients rather than a priori power calculation. Additionally, only single stool samples were collected, which has reduced the sensitivity of parasitological methods. Another key limitation is the lack of systematically collected clinical data, including symptoms and laboratory parameters such as eosinophil counts, which restricted our ability to correlate diagnostic findings with clinical status. Future studies should incorporate formal sample size planning, multiple stool collections, and comprehensive clinical assessments to evaluate diagnostic performance accurately and strengthen clinical correlations.

In conclusion, our study highlighted that the real-time PCR and IgG4-RDT results complement each other. Thus, performing both assays and combining their results improved the detection of *Strongyloides* in cancer patients. It also showed that the IgG-ELISA was an insensitive diagnostic tool for cancer patients.

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Conflict of interest statement

Rahmah Noordin and Nor Suhada Anuar developed the prototype IgG4-RDT.

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