

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/apjtm.apjtm_80_24

Fasciola worm and egg−derived antigens: Exploring their diagnostic potential for urogenital schistosomiasis in resource−limited endemic regions

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ABSTRACT

Objective: To evaluate the immunodiagnostic potential of crude *Fasciola gigantica*-worm (FWA) and egg antigen (FEA) in detecting anti-*Schistosoma (S.) haematobium* antibodies in sera and urine samples.

Methods: This is a cross-sectional diagnostic study. Employing an indirect ELISA, antibodies against these antigens were assessed in samples from infected and non-infected individuals in both schistosomiasis endemic (NE) and non-endemic (NNE) areas, using microscopy as the diagnostic standard.

Results: FWA-sera exhibited excellent diagnostic accuracy with an area under the curve (AUC) of 0.957, a sensitivity of 93.75%, and a specificity of 85.42% for discriminating between infected and non-infected individuals in non-endemic areas. FWA-urine also demonstrated robust performance, achieving AUC>0.95, sensitivity>97.0%, and specificity>85.0% in both NE and NNE categories. Notably, *S. haematobium*-specific antibody levels against FWA were significantly elevated in infected individuals in both endemic and non-endemic areas. FEA-sera exhibited outstanding diagnostic performance with sensitivity exceeding 90% and an AUC of 0.968 in non-endemic samples but not in FEA-urine.

Conclusions: FWA-based ELISAs, applicable to both sera and urine, emerge as promising tools for *S. haematobium* diagnosis in resource-limited settings, offering advantages of high sensitivity and specificity with shared antigens with *Fasciola*. The superior diagnostic metrics of urine samples suggest their potential as a non-invasive biological sample for diagnostic purposes.

KEYWORDS: *Schistosoma haematobium*; Non-invasive; Worm antigen; ELISA; Immuno-diagnosis

1. Introduction

Schistosomiasis is recognized as one of the neglected tropical diseases, impacting some of the world's most impoverished communities and often escaping the attention of the global health community[1]. It is a waterborne illness that can lead to infections in the urinary tract or intestines. Untreated cases may result in damage to the liver, kidney failure, infertility, and bladder cancer[2].

Significance

The study investigated the potential of utilizing crude antigens derived from *Fasciola* for diagnosing *Schistosoma haematobium* infection. The findings demonstrated outstanding diagnostic efficacy of *Fasciola* egg and worm antigens in diagnosing urogenital schistosomiasis. Moreover, the remarkable performance of *Fasciola* worm antigens with urine samples indicates its suitability for non-invasive diagnostic methods. The incorporation of *Fasciola* antigens in serological schistosomiasis diagnosis presents a valuable solution to the accessibility challenges posed by limited resources in *Schistosoma*-endemic areas.

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How to cite this article: Adesida A, Oriade T, Sulaiman K, Afolayan F, Auta T, Akanbi I, et al. *Fasciola* worm and egg-derived antigens: Exploring their diagnostic potential for urogenital schistosomiasis in resource-limited endemic regions. Asian Pac J Trop Med 2024; 17(11): 501-507.

Article history: Received 31 January 2024 Revision 11 November 2024
Accepted 19 November 2024 Available online 26 November 2024

Urogenital schistosomiasis is predominantly prevalent in sub-Saharan Africa, with approximately 93% of all schistosomiasis infections occurring in this region[3]. Nigeria is identified as the most endemic country for schistosomiasis in Sub-Saharan Africa, with a reported 29 million people infected and an estimated 101.3 million people at risk of infection[4]. This disease contributes significantly to high absenteeism among school children due to nutritional deficiencies, growth retardation[5], and impediments to cognitive development[6]. Nevertheless, adults bear the most profound consequences of chronic infection[7]. A broad spectrum of urogenital clinical manifestations, such as granulomatous inflammation, fibrosis, urinary tract infections, and bladder cancer, is linked to the presence of trapped eggs of *Schistosoma* (*S.*) *haematobium* in the human bladder[8,9].

It is widely acknowledged that haematuria, often accompanied by dysuria, serves as a crucial morbidity marker for diagnosing urogenital schistosomiasis[10,11]. Additionally, the heightened risk of bladder cancers associated with chronic urogenital schistosomiasis emphasizes the pressing need for accurate and early diagnosis[12]. Definitive diagnosis typically involves the microscopic detection of parasite eggs in urine, and treatment primarily hinges on the administration of the anti-helminthic drug, praziquantel[13]. As per the WHO Roadmap for 2021–2030, the development and implementation of accurate and standardized diagnostic techniques suitable for various prevalence settings are urgently required for the effective management and elimination of urogenital schistosomiasis[14]. These advancements aim not only to reduce morbidity but also to lower overall programming costs associated with the disease.

The traditional methods for diagnosing schistosomiasis, such as stool and urine microscopy, have inherent limitations[15]. These approaches may struggle to identify low-intensity infections, particularly in regions with low endemicity[16]. Additionally, in cases of female genital schistosomiasis, where patients may be asymptomatic or not excrete eggs in urine or stool, these conventional methods are ineffective for diagnosis[17].

An alternative diagnostic pathway involves the detection of specific antibodies, providing increased sensitivity, especially in cases with minimal or absent egg excretion. In situations of imported infections, antibody detection may be the sole method for diagnosing schistosomiasis[18]. Furthermore, the adoption of antibody detection has been suggested as a more reliable and cost-effective diagnostic approach, particularly in instances of light infections where traditional light microscopy shows limited sensitivity.

While the use of antigens for antibody detection in diagnosing urogenital schistosomiasis shows potential, significant obstacles arise due to the lack of a widely accepted laboratory animal model for the completion of the life cycle of *S. haematobium*. The relatively small size of the parasite further complicates matters, particularly in high-prevalence regions like Nigeria[19]. For instance, the absence of laboratory hamsters for cultivating *S. haematobium* in Nigeria and the limited availability, maintenance challenges, and high cost of alternative animal models like Balb/c mice pose significant difficulties. This study explored the application of enzyme-linked immunosorbent assay (ELISA) to diagnose urogenital schistosomiasis. The focus was on detecting urine and serum immunoglobulin (Ig) G antibodies that react to the soluble egg and worm antigens of *S. haematobium* and *Fasciola* (*F.*) *gigantica*. Our objective was to assess the immunodiagnostic potential of *Fasciola* egg and worm-based antigens in the serological diagnosis of urogenital schistosomiasis.

This study is supported by the possible identification of immunological similarities and cross-reactive antigens between *F. gigantica* and *S. haematobium*, both trematode parasites. This relationship is grounded in their shared ancestry and close phylogenetic alignment, as demonstrated in previous studies[20–22]. The strategic use of *Fasciola* antigens derived from *F. gigantica*, a prevalent ruminant parasite in the study's geographical context, presents a sensible approach. This is reinforced by the distinct advantage offered by the larger size, accessibility, and availability of *F. gigantica*, potentially addressing challenges associated with the diminutive size of *S. haematobium* and the complexities of its culturing.

2. Subjects and methods

2.1. Ethics statement

Ethical approval was obtained from the University of Medical Sciences/Teaching Hospital Research Ethics Committee (NHREC/TR/UNIMED-HREC-Ondo St/22/06/21) and the Ethics Committee of the Katsina State Ministry of Health (MOH/ADM/SUB/1152/1/515). Participants and their guardians were duly informed about the study before sampling and written informed consent was obtained from all participants. All *Schistosoma*-infected children were administered a single dosage of 40 mg/kg praziquantel.

2.2. Study population and samples

Urine and serum specimens were sourced from the sample repositories of individuals infected with *S. haematobium* in Kurfi and Safana Local Government Areas (LGAs) within Katsina State, Nigeria. These samples were stored at the Seeding Graduate Laboratory, University of Medical Sciences, Ondo. The specimens were collected from 'Almajiri' children aged 5 to 19 years in the specified LGAs. Prior to this study, microscopic examination

confirmed the presence of *S. haematobium* in urine and other helminths in stool samples. Although mass drug administration (MDA) has been conducted in the LGAs, the 'Almajiri' children are often excluded from preventive treatment with praziquantel. The prevalence of *S. haematobium* among these children was 33.2% (Supplementary Table 1).

For the immunoassay, 48 samples from a pool confirmed *S. haematobium*-infected by microscopy (from the total of 500 samples originally sampled) and 48 negative samples from the two LGAs were used to detect anti-*S. haematobium* IgG in urine and sera. Additionally, 48 samples from adult residents of Ondo City, a non-endemic area for schistosomiasis, were included in the analysis. These individuals tested negative to *S. haematobium* using microscopy. All samples that tested positive for one or more helminths through microscopic stool examination were excluded.

2.3. Collection of the F. gigantica parasites and antigen preparation

Following the routine examination of all slaughtered cattle' livers from the Central Abattoir at Akinyele, Ibadan for evidence of *Fasciola* infection by the meat hygiene service at Akinyele, positive cases were identified. Adult liver flukes were then collected from naturally infected ruminants brought to slaughterhouses. At the Ibadan Municipal Abattoir in Nigeria, the on-site veterinary inspector identified infected livers, carefully opened the main bile ducts, and extracted the adult flukes[23]. The specimens were placed in a collection bottle, preserved in ice, and transported to the laboratory.

The adult worms underwent washing in phosphate-buffered saline (PBS) and homogenization under cold conditions. The homogenate was passed through two sieves (180 μm and 35 μm). The washed fraction, containing *Fasciola* eggs, was collected and subjected to centrifugation. The obtained eggs were washed, crushed in ice-cold PBS, and then centrifuged at 3900 ×*g* at 4 ℃ for 1 hour. The resulting supernatant constituted the *Fasciola* egg antigen (FEA). A similar procedure was followed for the preparation of the worm antigen (FWA), except that the homogenized worms were not filtered. The protein concentrations of the crude antigens, FEA and FWA, were determined using a Bicinchoninic acid (BCA) protein assay kit.

2.4. Indirect enzyme-linked immunosorbent assay (ELISA)

The assessment of the anti-*Fasciola* IgG antibody followed the protocol outlined by Oyeyemi *et al.*[24] with slight modifications. In summary, each well of the microtiter plate (APS Lifetech West Africa) was coated with 100 μL of FEA and FWA, both diluted at 10 μg/mL in carbonate-bicarbonate buffer, and incubated overnight at 4 ℃. Subsequently, the plates were washed with PBS (1) containing 0.05% tween 20. The unbound proteins were then blocked with 300 μL per well of 2.5% skim milk diluted in washing buffer, followed by a 3 h incubation at 25 ℃. After another round of washing, 100 μL of each human serum sample (diluted 1:100) in PBS (1) was added to each well, and the plates were incubated at room temperature for 1 h. Following a subsequent wash, the plates were incubated at room temperature for 1 h with anti-human-IgG horseradish peroxidase-conjugated antibody (Sigma-Aldrich, St. Louis, MO, USA), diluted at 1:10000 in washing solution. After another round of washing, 100 μL of substrate solution (3,3′,5,5-tetramethylbenzidine) (Invitrogen, Grand Island, USA) was added to each well. The reaction was halted after 10 min of incubation by introducing 100 μL per well of 2N sulfuric acid. Absorbance values of the antigen-antibody reactions were obtained by reading the plates in a microplate reader at 450 nm. Antibody production against FEA and FWA was determined in *S. haematobium*-infected and non-infected individuals in endemic and non-endemic areas.

2.5. Statistical analysis

Data analysis was conducted using GraphPad Prism®, version 8.0. To assess the performance of FEA and FWA IgG-ELISA, a reference standard was established with microscopy diagnosis. Student's *t*-test was employed to determine significant differences in IgG levels between *S. haematobium*-infected and non-infected individuals, with a significance threshold set at $P<0.05$.

Receiver operating characteristics (ROC) curves, the area under the curve (AUC), and diagnostic cut-off points for *S. haematobium*-positive and negative groups were determined using GraphPad Prism. The absorbance data of the positive and negative samples obtained from ELISA were entered into the GraphPad Prism software in two separate columns (positive *vs.* NE; positive *vs.* NNE). The data were analyzed to determine diagnostic cut-off values. The cut-off value corresponding to the optimum sensitivity and specificity was selected as the diagnostic threshold for each antigen. The mean absorbance value was thereafter divided by the cut-off and values ≥1 and <1 denote positive and negative ELISA results respectively[25]. A true positive is indicated by both a positive ELISA result and positive microscopy (the reference standard). A false positive is shown by a positive ELISA result and negative microscopy. A false negative is represented by a negative ELISA result and positive microscopy, while a true negative is indicated by both a negative ELISA result and negative microscopy. The sensitivity of the immunoassay was computed using the formula; (number of true positives)/(number of true positives+false negatives). The specificity was computed using the formula; (number of negatives)/(number of true negatives+false positives). Accuracy was determined using the formula: (number of true positives+number of true negatives)/(number of true positives+

true negatives+number of false positives+number of false negatives). The AUC value serves as a measure of the probability of accurately identifying true positives, allowing differentiation between tests with no discrimination (AUC=0.5), acceptable discrimination (0.78-0.8), excellent discrimination (0.8-0.9), and highly outstanding or perfect discrimination (AUC ≥ 0.9) as defined by Hosmer & Lemeshow[26].

3. Results

Table 1 shows the diagnostic potential of FWA and FEA. All 48 samples selected from endemic areas and used in the ELISAs were confirmed positive for *S. haematobium* by microscopy, while all 48 samples from the non-endemic area tested negative by microscopy. The sensitivity and specificity of FWA were 97.92% and 85.42%, respectively when urine samples from *S. haematobium*-infected individuals were mixed with non-infected individuals in endemic areas. Similarly, an outstanding diagnostic performance was recorded for infected urine samples mixed with negative samples from *Schistosoma* non-endemic areas. With sera samples, the diagnostic performance of FWA with sensitivity (93.75%) and specificity (85.42%) was excellent in *S. haematobium*-infected individuals mixed with non-infected individuals in non-endemic areas but was generally poor when infected sera samples were paired with non-infected samples from endemic areas. Excellent diagnostic performances (sensitivity 93.75%, specificity 95.83%) in infected sera samples mixed with negative samples from a *Schistosoma* non-endemic area, and acceptable diagnostic performances (sensitivity 79.17%, specificity 60.42%) in infected sera mixed with negative sera from endemic areas were recorded for FEA.

Figure 1 shows the receiver operating characteristics (ROC) curve of FWA-ELISA immunoassay of urine of sera and urine samples. The area under the ROC curves for FWA-ELISA for negative non-endemic and endemic sera samples mixed with infected sera samples were 0.957 (0.914–1.00) and 0.530 (0.413–0.646), respectively. For urine samples, the area under the ROC curves were 0.967 (0.929–1.00) and 0.968 (0.929–1.00) for negative non-endemic and endemic samples, respectively (Figure 1). The AUC-ROC curves for negative non-endemic and endemic urine samples were poor but were either excellent 0.968 (0.933-1.00) or acceptable 0.744 (0.646-0.842) with FEA as the captured antigen (Figure 2). Although an acceptable ROC curve value 0.861 (0.770-0.952) was recorded for FEA in the *S. haematobium*-infected individuals mixed with non-infected in *Schistosoma* non-endemic area using urine, the result generated by the statistical software did not produce a true reflection of the diagnostic performance of the antigen.

Table 1. Comparative potential of Fasciola worm and egg-based antigens in diagnosis of urogenital schistosomiasis.

Antigens	Samples	Diagnostic cut-off	Accuracy % (95% *CI*)	Sensitivity % (95% *CI*)	Specificity % (95% *CI*)	Positive predictive % (95% *CI*)	Negative predictive (95% *CI*)	AUC
FWA								
Urine	Infected + negative (non-endemic)	0.193	89.57 (81.66-94.88)	97.92 (89.10-99.89)	85.42 (72.83-92.75)	76.94 (62.70-86.89)	98.80 (92.20-99.83)	0.967 (0.929-1.000)
	Infected + negative (endemic)	0.197	90.96 (83.36-95.85)	97.92 (89.10-99.89)	87.50 (74.75-95.27)	79.56 (64.79-89.18)	98.83 (92.37- 99.83)	0.968 (0.929-1.000)
Serum	Infected + negative (non-endemic)	0.768	88.18 (79.99-93.88)	93.75 (82.80-98.69)	85.42 (72.24-93.93)	76.16 (61.61-86.41)	96.49 (90.13-98.81)	0.957 (0.914-1.000)
	Infected + negative (endemic)	1.129	55.61 (45.1-65.75)	70.83 (54.67-80.05)	31.25 (19.95-45.33)	31.69 (20.15-46.02)	66.13 (60.01-71.76)	0.530 (0.413-0.646)
FEA								
Urine	Infected + negative (non-endemic)	0.449	6.92 (2.75-13.98)	16.67 (7.48-30.22)	2.08 (0.05-11.07)	7.80 (4.30-13.75)	4.79 (0.72-26.00)	0.861 (0.770-0.952)*
	Infected + negative (endemic)	0.179	59.0 (48.49-68.94)	72.92 (59.00-83.43)	52.08 (38.33-65.53)	43.06 (34.96-51.56)	79.46 (69.33-86.88)	0.607 (0.490-0.724)
Serum	Infected + negative (non-endemic)	0.6351	94.44 (87.80-98.09)	93.75 (83.16-97.85)	95.83 (86.02-99.26)	88.40 (71.78- 95.8)	97.84 (92.09-99.44)	0.968 (0.933-1.000)
	Infected + negative (endemic)	0.8985	72.94 (62.91- 81.51)	79.17 (65.74-88.27)	60.42 (46.31-72.98)	59.04 (44.24-72.37)	80.10 (73.38-85.45)	0.744 (0.646-0.842)

*: AUC computed was not a true reflection of diagnostic performance as absorbance in negative non-endemic urine samples was higher than in positive samples.

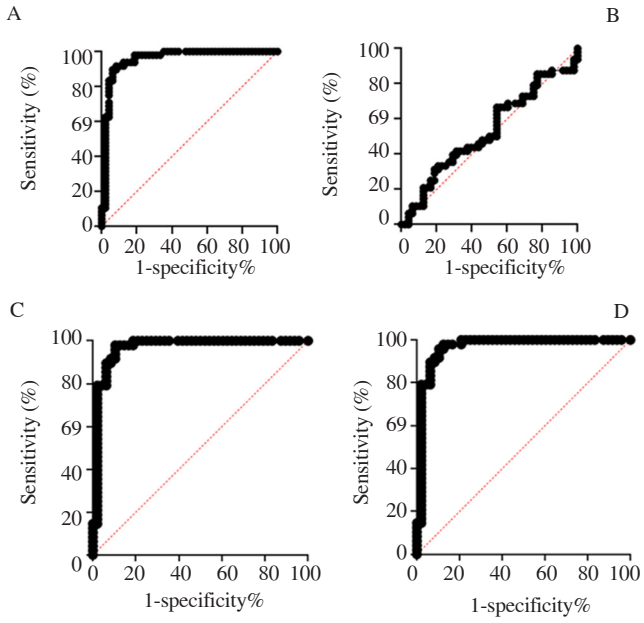

Figure 1. Receiver operating characteristics (ROC) curve of FWA-ELISA immunoassay of urine of sera and urine samples. A. NNE/sera; B. NE/sera; C. NNE/urine; D. NE/urine. NNE: negative non-endemic; NE: negative endemic; FWA: *Fasciola* worm antigen; FEA: *Fasciola* egg antigen.

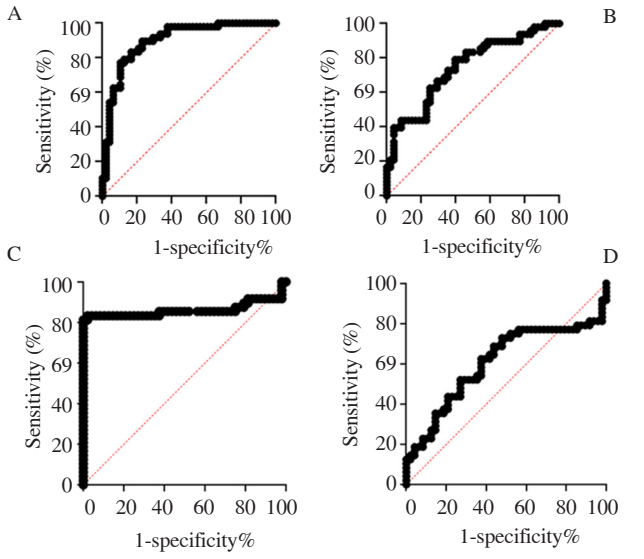

Figure 2. Receiver operating characteristics (ROC) curve of FEA-ELISA immunoassay of urine of sera and urine samples. A. NNE/sera; B. NE/sera; C. NNE/urine; D. NE/urine.

Antibody production against *S. haematobium* infection was significantly lower in non-infected urine in endemic (NE, OD: 0.173±0.082) and non-endemic (NNE, OD: 0.173±0.082) areas compared to infected urine samples (OD: 0.482±0.258) when FWA was employed for the diagnosis (P<0.001). Specific anti-*Schistosoma* antibody production did not show significant difference between endemic negative sera samples (OD: 1.043±0.157) and infected sera samples (OD: 1.038±0.175) (P>0.05) but the antibody production in the non-endemic negative sera samples OD: 0.649±0.149) was significantly lower than the infected sera samples (P<0.001). Anti-*Schistosoma* antibody production was significantly lower in endemic and non-endemic negative sera (NE, OD: 0.745±0.201; NNE, OD: 0.422±0.174) compared to the *Schistosoma* positive sera samples (OD: 0.937±0.181) when FEA was used as the captured antigen (P<0.001). However, antibody production was significantly higher in non-endemic negative urine samples (OD: 0.648±0.148) compared with infected urine samples (OD: 0.347± 0.292) (Figure 3).

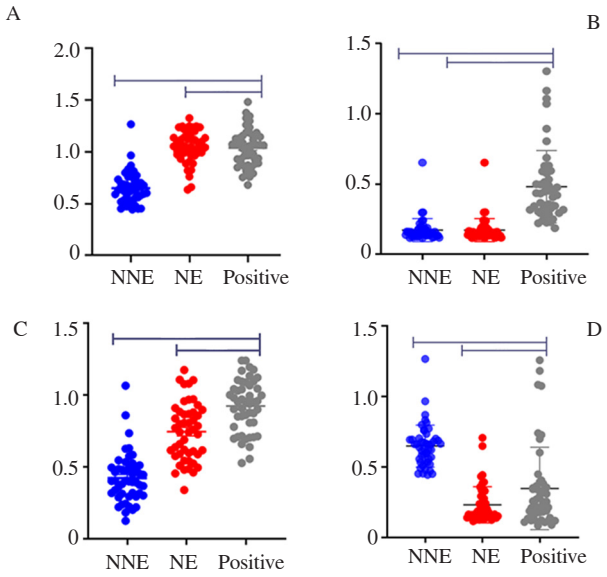

Figure 3. Anti-*Schistosoma* IgG production in relation to diagnostic antigens and biological samples. A. FWA / sera; B. FWA/urine; C. FEA/sera; D. FEA/urine.

4. Discussion

Accessing materials from the *S. haematobium* parasite to prepare capture antigens for ELISA diagnostics is challenging, limiting its use in resource-limited labs. Nigeria, with the highest prevalence of schistosomiasis, lacks readily available animal models for cultivating the parasites. Relying on eggs from infected human urine is unsustainable, providing limited diagnostic antigens. A practical alternative is using a different parasite as a sustainable antigen source with excellent diagnostic potential.

The antigenic community, or the presence of immune-triggering molecules across species, leads to antigenic cross-reactivity due to shared molecules among various genera, families, or phyla[27]. This is promising for diagnostics, treatment, and vaccine development. Studies by Vichasri-Grams *et al.*[20], Le *et al.*[21], and Oettle *et al.*[22] highlight that *Fasciola* spp. and *Schistosoma* spp., both digenetic trematodes, exhibit shared immunological similarities and cross-reactive antigens due to their common evolutionary origin.

F. gigantica, primarily affecting livestock, can yield more diagnostic proteins from a single adult worm than from cultivating *S. haematobium* in 10 hamsters or Balb/c mice. Given the shared proteins between these parasites, *F. gigantica* is a more feasible and cost-effective antigen source. This study explores using FWA and FEA for diagnosing *S. haematobium* infections *via* ELISA, evaluating their performance in serum and urine samples.

The evaluation of diagnostic performance in this study relied on metrics such as the area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, and specificity. Both FWA-serum

and FWA-urine demonstrated exceptional diagnostic accuracy, evident from their high AUC values surpassing 0.95, along with excellent sensitivity and specificity. These attributes make FWA a suitable capture antigen for diagnosing patients in non-endemic areas with imported cases, monitoring infections post-intervention, and identifying chronic infections in endemic regions.

The outstanding diagnostic accuracy of FWA-serum observed in our study aligns with Sarhan *et al.*'s[28] findings, where serum dilutions of 1/100 and 1/50 exhibited 100% sensitivity. In comparison, *Schistosoma* worm antigen (SWA) showed specificity levels of 76.47% and 64.7%. Notably, our study surpassed Kinkel *et al.*'s[29] results, which reported sensitivity values ranging from 21.4% to 71.4% in diagnosing imported schistosomiasis using eight serological assays, including commercial kits.

The exceptional diagnostic performance of FWA-urine, with a sensitivity exceeding 97%, makes it a promising non-invasive diagnostic tool. It is comparable to the circulating anodic antigen (CAA) test and outperforms urine CCA[30,31]. CAA also demonstrated higher sensitivity than traditional methods using microhematuria and urine filtration in low-endemic settings[30]. On the other hand, FEA-urine exhibited inconsistent performance with low sensitivity and specificity, raising doubts about its reliability as a standalone diagnostic tool for schistosomiasis. This is even more concerning for non-infected individuals in endemic areas, as it poses a significant diagnostic challenge and particularly limits its potential to evaluate specificity. These findings underscore the need for further investigation to unravel the underlying mechanisms and comprehend their implications for diagnostic accuracy. The inconsistent performance and unforeseen patterns in antibody response associated with FEA underscore the necessity for additional research to enhance its diagnostic potential and comprehend its limitations. It is crucial to optimize sample types, explore alternative assays, and investigate influencing factors to achieve this.

Significantly higher *S. haematobium*-specific antibody levels against FWA were found in infected individuals compared to non-infected controls, confirming FWA's immunogenicity and ability to induce a specific immune response. Notably, FWA-urine triggered a stronger antibody response in infected individuals in non-endemic areas than FWA-serum, suggesting its potential as a more sensitive marker. Conversely, FWA-serum antibody levels showed no significant difference between infected and non-infected individuals in endemic areas, likely due to chronic exposure or cross-reactivity with other helminth infections.

Our findings strongly support the viability of FWA as a diagnostic tool for *S. haematobium* infections. FWA-urine demonstrates exceptional accuracy and sensitivity in detecting active infections. However, variations in antibody response in endemic areas highlight the need for further research to refine diagnostic methods for diverse contexts. This includes exploring alternative FWA preparations, combinations with other antigens, supplementary markers like cell-mediated immunity, and conducting extensive studies in various endemic regions to validate and enhance these findings.

The use of *Fasciola* crude antigens for immunodiagnostic development faces significant limitations. Historically, crude antigen preparations, such as those used for schistosomiasis, have exhibited issues with reproducibility and batch-to-batch variability. This study lacks basic antigen characterization *via* 1 or 2-dimensional gel electrophoresis and does not address strategies for ensuring reproducibility, crucial for consistent diagnostic performance. Although the study highlights the advantages of cross-reactivity among closely related helminths with shared diagnostic markers, this method might not be suitable for less related helminths, as cross-reactivity of the antigens could otherwise hinder diagnostic accuracy. Additionally, sourcing *Fasciola* antigens from naturally infected cattle could raise concerns about the economic impact and human fascioliasis risk should we prioritize the accessibility of biological materials for diagnostic development over intervention in livestock. The use of microscopy as the diagnostic reference standard may not accurately reflect infection status in chronic conditions, as eggs can become trapped within the host.

In conclusion, this study provides evidence supporting FWA's promising potential as a diagnostic tool for *S. haematobium* infections. FWA-urine, specifically, emerges as a highly accurate and non-invasive option with widespread applicability. While challenges persist in adapting diagnostic approaches for FEA, further research guided by these insights has the potential to pave the way for more effective schistosomiasis control strategies.

Conflict of interest statment

We declare that there is no conflict of interest.

Acknowledgments

We thank the Seeding Labs for acceptance as an Instrumental Access awardee for participation in the 2019 programme year.

Funding

This work was supported by the National Research Foundation –Tertiary Education Trust Fund (TETF/DR&D/CE/NRF2020/SETI/105).

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

OO, FA, RIN, ABO: the conception and design of the study. OO, AA, TO, AB, TA, IA, MA: data collection. OO, AA, TO, AB, TA, IA: data analysis and interpretation. AA, OO: drafting of the article or revising it critically for important intellectual content. All approved the final draft before submission.

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Edited by Lei Y, Pan Y, Zhang Q

Supplementary Table 1. Prevalence of urogenital schistosomiasis and intestinal helminths among the children.

Infection	LGA	No. examined	No. positive	Prevalence (%)
S. haematobium	Safana	277	87	31.4
	Kurfi	223	79	35.4
		500	166	33.2
S. mansoni	Safana	277	1	0.4
	Kurfi	223	0	0.0
		500	1	0.2
Ascaris lumbricoides	Safana	277	2	0.7
	Kurfi	223	0	0.0
		500	2	0.4
Strongyloides stercoralis	Safana	277	1	0.4
	Kurfi	223	1	0.4
		500	2	0.4