Evaluation of a newly established rapid and selective graphene-based kit for diagnosis of hydatidosis by using nano ELISA and nano-immunochromatography

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Abstract

Hydatid disease (HD) or hydatidosis, is a major public health problem that is spreading all over the world. The diagnosis of hydatid disease is considered to be challenging; however, the serological diagnosis provides an early protocol, and confirmation of the existence of the hydatid cyst microscopically is still a must. Enzyme-linked immunosorbent assay (ELISA) is one of the best diagnostic methods. Sandwich ELISA, nano-sandwich ELISA and immunochromatographic test (ICT) were conducted utilizing purified hydatid antigen for diagnosis of human hydatidosis. The analyzed sera comprised samples from patients infected with hydatidosis (n = 50) and sera of patients infected with helminthic parasites (20, 20 and 10 with *Schistosoma mansoni*, *Fasciola hepatica*, and *Ancylostoma duodenale*, respectively), and 50 healthy negative controls. The findings indicated that the sandwich ELISA detected positive results in 39 out of 50 hydatid-infected samples, yielding an assay sensitivity of 78%. Seven out of fifty parasite-infected groups yielded positive results, resulting in 93% specificity. In the nano-sandwich ELISA, 42 of 50 hydatid-infected samples yielded positive results, which produced an assay sensitivity of 84%. 7 out of 50 other parasite-infected groups had positive results giving 93% specificity. Using immunochromatographic test; Thirty-six out of fifty hydatid-infected samples yielded positive results, which gave an assay sensitivity of 72%. 16 out of 50 other parasite-infected groups had positive results, giving 80% specificity. Diagnostic specificity reached 93%, 93% and 80% by using sandwich ELISA, nano-sandwich ELISA and ICT techniques respectively. The use of nano-sandwich ELISA is one of the best methods for diagnosis of human hydatidosis.

Keywords: Echinococcosis, *Schistosoma*, *Fasciola*, *Ancylostoma*, Diagnostic Kit

1. Introduction

Hydatid disease, known as cystic echinococcosis (CE) is a significant zoonotic disease with a worldwide distribution, caused by the larval stage of the canine tapeworm. (Family Taeniidae; genus *Echinococcus*)**.** Endemicity of hydatid disease was clearly seen in western China, Siberia, South America, Australia, Central Asia, the Indian subcontinent, the Eastern Mediterranean region, Eastern and Northern Africa. (1).

The diagnosis of hydatid disease is challenging; however, efficient serological tests facilitate early intervention, post-treatment monitoring, and enhanced chemotherapy efficacy. Enzyme-linked immunosorbent assay (ELISA) is one of the well-known diagnostic methods to diagnose hydatid disease in humans and domestic animals**.** In the present, the diagnosis of hydatidosis relies on a combination of imaging techniques (ultrasound, X-ray, and computed tomography) and immunodiagnostic methods (2). Serological tests continue to exhibit insufficient diagnostic specificity, particularly in endemic regions. Purification and evaluation are necessary to enhance the sensitivity of these techniques for the detection and confirmation of the disease in its initial stages. The latex agglutination test is an effective and relevant diagnostic tool for hydatid disease, particularly when supplemented by confirmatory ELISA (3). Camel hydatid fluid, crude protoscolices, and antigens from sheep hydatid cysts may be beneficial for the accurate diagnosis of hydatidosis in humans (4).

When compared to the ELISA format, immunochromatographic testing (ICT) has shown good performance in the diagnosis of alveolar echinococcosis (5). Unlike traditional ELISA procedures, which call for specialized personnel and equipment that can be hard to locate in rural locations, ICT is a quick, easy, and dependable approach.

Nanomaterials based on graphene have become the focus of attention of scientists in nanotechnology research (6), because of the exceptional charge transport mobility and the thermal, optical, and mechanical properties of this compound. In terms of diagnosis of parasitic infections, graphene nanomaterial has been used to increase the sensitivity and specificity of some diagnostic tools (7).

The main objective of this proposal is to evaluate a novel test, which is rapid and selective for the diagnosis of hydatidosis by nano-sandwich ELISA and nano-immunochromatography compared with traditional sandwich ELISA technique.

2. Material and Methods

2.1 Preparation and characterization of Graphene oxides nanoparticles

The graphene oxide nanoparticles were obtained through purchase from NanoTech Co. (Gate 3, Dreamland, 6th October, Cairo, Egypt, https://www.nanotecheg.com). The size and morphology of graphene oxide were investigated by transmission electron microscopy (TEM) at Nano Tech Egypt Co (FEI Quanta 200 electron microscope). Graphene oxide nanoparticles were also characterized by Fourier-Transform Infrared Spectroscopy (Jasco FTIR 6100 Japan).

2.2 Animals

Two New Zealand white male rabbits, 1.5 kg weight and 2 months old, were acquired from the Rabbit Research Unit (RRU) at the Faculty of Agriculture, Cairo University. Prior to the commencement of the experiments, they were assessed and subsequently utilized in the antibody production process.

2.3 Parasites

Hydatid cysts were taken away from the livers and lungs of sheep and camels from an abattoir in Cairo Governorate and cysts were transferred to the parasitology lab of Theodor Bilharz Research Institute (TBRI) in Hanks' buffer solution to stimulate normal ion concentration under physiological tissue conditions.

2.4 Sera samples

To evaluate the sensitivity and specificity of the above-mentioned techniques, 50 blood samples from hydatid-infected patients were used; all of them were clinically asymptomatic. Also, sera samples infected with other parasitic diseases were used: 20 were infected with *Schistosoma mansoni*, 20 with *Fasciola hepatica*, and 10 with *Ancylostoma duodenale*. Approximately 50 blood samples were utilized as negative controls sourced from healthy individuals. Serum samples were separated, aliquoted, and stored at -20°C until utilization.

2.5 Preparation of antigen of parasite

Hydatid cyst fluids (HCF) were aseptically collected from hydatid cysts. HCF was subjected to centrifugation at 1,000 g for 30 minutes to eliminate protoscolices and larger materials. The protein content of the samples was determined.

2.6 Protein extraction in high salt concentration

Hydatid cyst fluid was homogenized in PBS buffer (pH 7.0) using an Ultrasonic Homogenizer 4710 (Cole-Palmer Instrument, III), with 30-second pauses incorporated during the process. The hydatid cyst fluid, after sonication, was subjected to centrifugation at 48,000 g for 30 minutes at 4°C. The resulting pellet was then resuspended in an equal volume of 20 mM Tris-HCl at pH 8. Supernatants were analyzed using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Comparable composition samples were pooled and concentrated

fourfold using ultrafiltration with a PM10 membrane (Amicon Inc., Mass.). The concentrated material was subjected to gel filtration chromatography (8).

2.7 Fabrication and purification of antibodies

Antibodies were produced using rabbits through intramuscular injection of 1 mg purified hydatid antigen suspended in Freund's complete adjuvant (Sigma) [1:1], four times, one week apart. The reactivity and sensitivity of anti-hydatid IgG polyclonal Ab were assessed using indirect ELISA**.** The anti- Hydatid Ab was labelled with Horseradish peroxidase (HRP) using periodates method, the labeled antibody was kept at -20 °C in small aliquots until used.

2.8 Conjugation of anti-hydatid polyclonal antibodies with graphene nanoparticles

Ten ml of prepared graphene oxide nanoparticle solution was supplemented dropwise into antibody (Ab) solution (20 mL, 1 mg/mL) and stirred for 2 h. Then, the solution was centrifuged to remove the excess free Ab (10,000 RPM, for 20 min.), and the pellet was redispersed in 5 ml of Milli-Q water. One ml of graphene oxide nanoparticles-conjugated antibodies was activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (25 µL), and stored at room temperature for 15 min. Then, the solution was centrifuged at 7000 RPM for 15 min to remove excess EDC and NHS. The pellet was resuspended in chilled phosphate buffer (PB), shake at 4 °C for overnight. Finally, the solution was centrifuged (7000 RPM, 15 min., 4 °C), and the final pellet was resuspended in sterile PB buffer.

2.9 Detection of hydatidosis in human sera by sandwich ELISA

The microtiter plates (Dynatec) were coated with 100 µl/well of 20 µg/ml anti-purified hydatid Ab. The plates were washed with buffer (0.1 M PBS/Tween, pH 7.4), three times. The free sites of the wells were blocked with 100 µl/well of 0.1% Bovine serum albumin (BSA)/0.1M phosphate buffer saline (PBS) and incubated at 37°C for 1 hour. Then, plates were washed 5 times with the washing buffer. 100 µl of serum sample (hydatid, other parasites, control samples) was added, then incubated for an hour at 37 ^{o}C. The plates were washed three times with the washing buffer. 100 µl/well of 1/20 peroxidase-conjugated Ab was added and incubated for an hour at 37 ^{o}C. Then, plates were washed with the washing buffer. 100 µl of substrate solution, comprising one tablet of o-phenylenediamine dihydrochloride (Sigma) dissolved in 25 mL of 0.05 M phosphate citrate buffer at pH 5, containing urea hydrogen peroxidase (Sigma). A solution was introduced into each well, and the plates were incubated in the dark at ambient temperature for 30 minutes. Fifty microliters of H_2SO_4 (8N) were applied to each well to terminate the enzyme-substrate

reaction. Absorbance was quantified at 492 nm utilizing an ELISA reader (Bio-Rad® microplate reader, Richmond, California, USA).

2.10 Detection of hydatidosis in human sera by nano-sandwich ELISA

Graphene-loaded anti-hydatid ELISA

The plates were treated with 100 µl/well of graphene nanoparticles-loaded anti-hydatid antibodies (10 ng/ml in carbonate buffer, pH 9.6) and incubated at room temperature overnight. Plates underwent three rinses with 0.1M PBS/Tween at a pH of 7.4. The remaining sites were blocked using 100 µl per well of 2.5 percent FCS/PBS/Tween and incubated at 37 °C for 2 hours. The plates were washed three times with PBS/Tween. Duplicate wells received 100 µl of sera samples and were incubated at 37°C for 2 hours. Following three washes, 100 µl/well of 1/160 peroxidase-conjugated polyclonal antibodies were introduced and incubated for one hour at room temperature.

2.11 Detection of hydatidosis in human sera by nano immunochromatography

On a plastic baking plate (300×80 mm), a conjugate pad was attached to the bottom of the membrane with 1–2 mm overlapping on the membrane, and the sample pad was attached to the bottom of the conjugate pad in a similar manner. The absorbent pad was attached to the top of the membrane with 1–2 mm overlapping on the membrane as well. The prepared master card was cut into 3.8 mm width strips using a CM4000 Cutter (Bio-Dot, CA, USA). The strips were then enclosed in the plastic box and sealed in the aluminum foil bag containing desiccant gel, then stored under dry conditions at room temperature until used. Eighty to 100 µL of sera samples were dripped into the sample holder of the test strip cell at the sample pad side and allowed the liquid to migrate for 5 min. The specific nano-graphene-labelled antibody was trapped by immobilization on the membrane, forming red test lines and further trapped by the goat anti-human IgG antibodies forming the control line, while the whole complex was migrating along the membrane. After 3–10 min, the test results were evaluated visually, or test lines were scanned with a Bio-Dot TSR3000 Membrane Strip Reader (Bio-Dot, CA, USA).

2.12 Statistical analysis

The data are expressed in terms of the mean and standard deviation (M±SD). The average values for each group were determined by analyzing the average values of individual patients. The groups' means were evaluated using analysis of variance. A One-way ANOVA test was performed, revealing a level of significance with $p < 0.05$. Sensitivity can be expressed as follows: the number

of true positive cases divided by the sum of true positive cases and false negative cases. Specificity is defined as follows: the number of true negative cases divided by the sum of true negative cases and false positive cases. The positive predictive value (PPV) is defined as the ratio of the number of true positive cases to the sum of true positive cases and false positive cases. The negative predictive value (NPV) is defined by the following formula: the number of true negative cases divided by the sum of true negative cases and false negative cases.

3. Results

3.1 Characterization of Graphene oxide nanoparticles

The morphology analysis of graphene oxide nanoparticles by TEM showed that the majority of particles were spherical and had smooth surfaces with homogeneous polydispersity. The diameters obtained by SEM range from 40 to 60 nm. The FTIR spectra of graphene oxide nanoparticles show ten characteristic absorption peaks observed at 786, 1268, 1390, 1540, 1650, 1780, 2265, 2876,3089, 3760 cm^{-1} (**Fig. 1**).

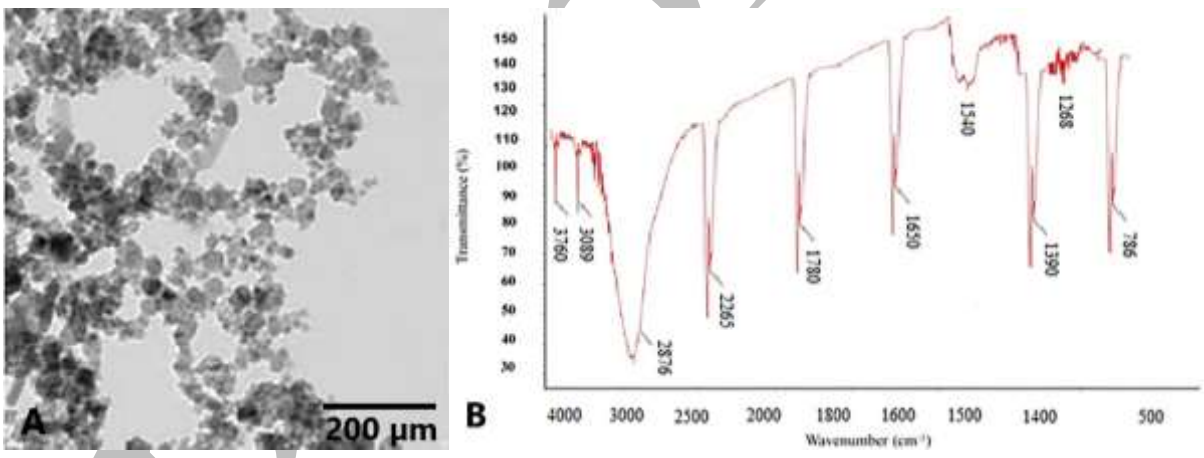

Figure. 1 A: The TEM of graphene oxide nanoparticles, **B**: FTIR spectra of graphene oxide nanoparticles show the characteristic absorption peaks.

3.2 Purification of hydatid antigen DEAE Sephadex G-50- ion exchange chromatography

The eluted antigen, purified by DEAE Sephadex G-50 ion exchange chromatography, shows an OD_{280} profile which was represented by 2 peaks at fraction no. 25 and fraction no. 30 with maximum OD value equal to 2.5 at fraction number 30 (**Fig. 2**).

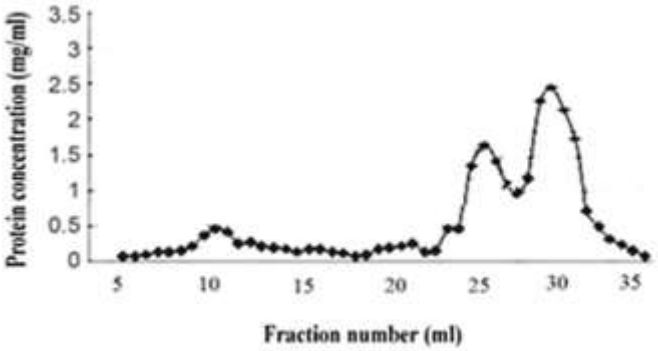

Figure. 2 The OD_{280} profile of the hydatid antigen fractions obtained following purification by DEAE Sephadex G-50 ion exchange chromatography.

3.3 Reactivity of hydatid antigens by indirect ELISA

The antigenicity of hydatid antigens was tested by the indirect ELISA technique. Serum samples of patients infected with hydatid gave a positive reaction against the purified hydatid antigen, and no cross-reactions were recorded with sera of patients infected with any other parasites, e.g., *Schistosoma*, *Fasciola*, and *Ancylostoma*. The strong reaction was detected against the purified hydatid antigen, with a mean optical density (MOD) reading equal to 1.11 ± 0.098 **(Table 1).**

Table. 1 Reactivity of hydatid antigens by indirect ELISA.

Serum samples	MOD readings at 492nm (± SD)
Hydatid	1.11 (± 0.098)
Schistosoma mansoni	0.326 (± 0.24)
Fasciola	0.314 (± 0.079)
Ancylostoma	0.322 (± 0.09)

MOD= Mean of optical density

3.4 Reactivity and specificity of polyclonal antibodies against purified hydatid antigens

The reactivity of hydatid polyclonal antibodies was tested by the indirect ELISA technique. Serum samples from hydatid-infected humans gave positive reactions against the purified *hydatid* polyclonal antibody, and no cross reactions were recorded with sera of patients infected with any other parasites, e.g., *Schistosoma*, *Fasciola* and *Ancylostoma*. The strong reaction was detected against the purified *hydatid* antibody, with MOD reading equal to 1.95± 0.06 (**Table 2**).

Table. 2 Reactivity and specificity of polyclonal antibodies against purified hydatid antigens.

Parasitic antigens	MOD readings at 492 nm (± SD)
Hydatid	1.95 (± 0.06)
Schistosoma	0.26 (± 0.03)
Fasciola	0.16 (± 0.04)
Ancylostoma	0.19 (± 0.13)

MOD= Mean of optical density

3.5 Measurement of hydatid antigen by sandwich ELISA

The level of hydatid antigen was measured by sandwich ELISA as mean (M) of OD of each group with standard deviation (±SD) at 492 nm. Sandwich ELISA was performed using optimal conditions reached after standardization. The plate was coated with 20 µg/ml of anti-*hydatid* IgG antibodies in 0.06 M carbonate buffer (pH 9.6). The concentration of peroxidase conjugated to anti-hydatid in 2.5% FCS/PBS/Tween was 1/200.

The OD value of the hydatid-infected group (1.30±0.25) was significantly higher than both the healthy control group (0.332±0.18) and other parasite groups (0.36±0.123). Eleven out of 50 *hydatid* patients showed false negative results, and the sensitivity of the assay was 78 %. All the 50 negative control sera patients were below the cut-off value, while 7 out of 50 of parasite-loaded groups were at the borderline of the cut-off value giving 93.0% specificity. The false positive cases in other parasite-infected patients were 3, 2, and 2 belonging to the subgroups infected with *Schistosoma, Fasciola, and Ancylostoma,* respectively (**Table 3**).

Table. 3 Measurement of *hydatid* antigen by sandwich ELISA.

Group	Positive cases			Negative cases		
	No.	MOD ±SD	% Positivity	No.	MOD ±SD	%Negativity
Health control (n= 50)	0	-----	---	50	0.332±0.18	100 %
Hydatid (n= 50)	39	1.30±0.25	78%	11	0.28±0.09	22 %
Other parasites (n= 50)						
Schistosoma (n= 20)	3	1.52±0.17	15 %	17	0.36±0.09	85 %
Fasciola (n= 20)	2	1.39±0.11	10 %	18	0.34±0.14	90 %
Ancylostoma (n= 10)	2	1.09±0.09	20 %	8	0.44±0.19	80 %

MOD= Mean of optical densities.

3.6 Measurement of hydatid antigen by nano-sandwich ELISA (Nano-ELISA)

Nano-graphene was performed using optimal conditions reached after standardization. The plate was coated with 20 µg/ml of anti-hydatid IgG antibodies in 0.06 M carbonate buffer, pH 9.6. The concentration of peroxidase conjugated to anti-hydatid in 2.5% FCS/PBS/Tween was 1/160. The OD values of the hydatid-infected group (1.73±0.46) were significantly higher than the corresponding values in both the healthy control group (0.309±0.145) and other parasite-infected groups (0.336±0.25). Eight out of 50 hydatid patients showed false negative results, and the sensitivity of the assay was 84 %. All 50 negative control sera were below the cut-off value, while 7 out of 50 other parasite-infected sera groups were at the borderline of the cut-off value, giving 93.0% specificity (**Tables 4 &12**). The false positive cases in other parasite-infected patients were 3, 2, and 2 belonging to the subgroups infected with *Schistosoma*, *Fasciola*, and *Ancylostoma*, respectively (**Table 4**).

Table 4 Measurement of hydatid antigens in sera by nano-graphene ELISA (Nano- ELISA)

Group	Positive cases			Negative cases		
	No	MOD ±SD	% Positivity	No	MOD ±SD	% Negativity
Health control (n= 50)	0	---	---	50	0.309±0.145	100 %
Hydatid (n= 50)	42	1.73±0.46	84 %	8	0.27±0.07	16 %
Other parasites (n= 50)						
Schistosoma (n= 20)	3	1.58±0.241	15 %	17	0.36±0.06	85 %
Fasciola (n= 20)	2	1.55±0.159	10%	18	0.35±0.08	90 %
Ancylostoma (n= 10)	2	1.41±0.35	20 %	8	0.30±0.11	80 %
	7			43		

MOD= Mean of optical densities.

3.7 Measurement of hydatid antigens by nano immunochromatography (Nano-ICT)

36 out of 50 hydatid-infected patients displayed positive results, with assay sensitivity of 72 %. 46 out of 50 negative control sera group showed negative results, while the remaining 4 ones showed false positive results. 16 out of 50 of the parasite-loaded group were at the borderline of the cut-off value, giving 80% specificity. Seven cases were associated with the *Schistosoma* group, five false positive cases were linked to patients infected with *Fasciola*, and four false positive cases were attributed to the *Ancylostoma*-infected group. **(Table 5) (Fig. 3).**

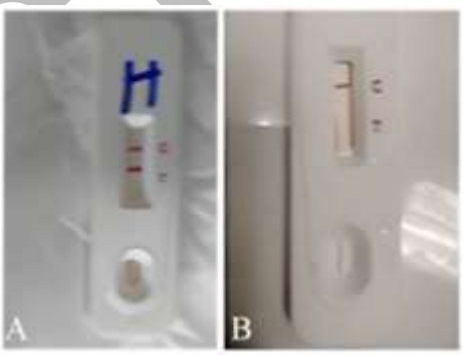

Figure. 3 Detection of hydatid antigens by nano immunochromatography, **A**: Positive, **B**: Negative

Table. 5 Number of positivity and negativity cases in examined groups by nano immunochromatography (Nano-ICT).

Group (No. of animals)	No. Positive cases (%)	No. of cases (Intensity of positivity)	No. Negative cases (%)
Health control (n= 50)	4	3 (+) 1 (++)	46 (92)
Hydatid (n= 50)	36	19(+) 8 (++) 9 (+++)	14 (28)
Other parasites (n= 50)			
Schistosoma (n= 20)	7 (35)	5 (+) 1 (++) 1 (+++)	13 (65)

Fasciola (n= 20)	5 (25)	4 (+) 1 (++)	15 (75)
Ancylostoma (n= 10)	4 (40)	3 (+) 1 (++)	6 (60)

4. Discussion

Echinococcosis is a cestode tapeworm of genus *Echinococcus* which is distributed at the worldwide, larval echinoccocosis were reported in the Eastern Mediterranean Countries. The initial diagnosis of *Echinococcus granulosus* using serological and immunological assays in humans and animals, and post-surgery and/ or medical treatment, but with different sensitivity and specificity that the immunodiagnosis remains a major problem (9).

Previously, numerous studies attempted to explore antigen detection tests for the diagnosis of CE (10). Parija et al. (1997) (11) documented the identification of hydatid antigen in the serum of 40 patients with CE through countercurrent immunoelectrophoresis (CIEP), achieving a diagnostic sensitivity of 45%. In a similar study, Sunita et al. (12) reported a sensitivity of 40% when utilizing rabbit hyperimmune serum in a sandwich ELISA.

In our study the sensitivity for detection of *hydatid* antigen in sera was 78 % using standard sandwich ELISA technique, while the application of nano-sandwich ELISA in this study the sensitivity reached 84 %. Khanbabaie et al. (13) indicated that the diagnostic sensitivity of the lateral flow antigen detection (LFD) was determined to be 77.14%, surpassing findings from several other studies (15-16, 18-19).

Ravinder *et al.* (14) showed a lower sensitivity used to detect antigen in serum samples (73%) using co-agglutination test (Co-A). Using the sandwich ELISA technique, diagnostic sensitivities of 25.7%, 52.5%, and 80% have been reported (15-16). Khanbabaie, *et al.* (13) showed that diagnostic specificity using antigen detection by sandwich ELISA test was 82.35%. Swarna and Parija (10) exhibited a comparable specificity of 83% using Dot-ELISA, whereas Bauomi et al. (15) reported a reduced specificity of 75% with ELISA.

In humans, the sensitivity of ELISA in detecting anti-protective antigen (anti-PA IgG) and circulating protoscoleces antigen (CPA) was 62.5 and 52.5%, respectively while the specificity of the assay was 66.7 and 75%, respectively29 (3, 15). In the current study, the ICT technique discovered 36 patients with lung cysts or with liver cysts from total (50 patients) with sensitivity of 72 %, which is higher than that stated in other studies (12-15). In the present study, an effort was made to develop an antigen detection rapid test in format of the ICT assay for CE.

The current investigation demonstrated a diagnostic specificity of 93%, 93%, and 80% utilizing sandwich ELISA, nano-sandwich ELISA, and ICT techniques, respectively. A comparable specificity (83%) was documented by Swarna and Parija (10) utilizing Dot-ELISA. Bauomi et al. (15) demonstrated a specificity of 75% when employing the ELISA technique.

The other studies of assays with higher specificity (> 90%) were reported by (10-12), however, the sensitivities of diagnostic assays liable on ELISA and CIEP were obviously much lower (25.7–45%) (11-12).

Notably, an ELISA constructed by Chaya and Parija (16) employing rabbit antibody to a 24 kDa human hydatid urinary antigen demonstrated a remarkable specificity of 92% alongside a sensitivity of 80%. Devi and Parija (17) demonstrated a specificity of 98% using LAT and Co-A, while another study (14) reported a specificity of 93.87% with the ELISA technique.

Other investigations that demonstrated high specificity utilized antigens derived from human sources to produce the antibodies (10, 14), as this could be a limitation for the sustainable production of such tests. Some studies showed that circulating antigen detection test could be used as a useful tool for post-treatment follow-up and monitoring of cyst activity (18). In the present study, cross-reactions were observed in 4 of 50 healthy individuals, and 16 of 50 patients with other parasitic infections using ICT technique. The cross-reactivity with serum of healthy individuals may be due to asymptomatic diseases other than CE since Egypt is not endemic for echinococcosis and all sera were found to be seronegative by sandwich ELISA technique, Sunita *et al.* (2011) (12) reported that cross- reactions with *Ascariasis* and cysticercosis by sandwich ELISA technique in sera, thus like what was observed in the present study.

Furthermore, cross-reactions have been documented with sera from patients suffering from schistosomiasis, fascioliasis, visceral leishmaniasis, neurocysticercosis, amebic liver abscess, tropical pulmonary eosinophilia, and partial seizures (12-13, 15-16). Nanotechnology may improve sensitivity, selectivity, speed, cost, and convenience of diagnostic tests (19).

Khanbabaie *et al.* (13) demonstrated that the LFD assay exhibited strong diagnostic accuracy in differentiating between positive and negative samples. Two rapid lateral flow antibody detection assays for the diagnosis of CE are currently available (20). Based on the existing literature, this report represents the first instance of an antigen detection test utilizing the rapid lateral flow assay format. The test is straightforward, rapid, and effective; it does not necessitate costly laboratory equipment and can be conducted in the field by individuals without laboratory training, making it advantageous for areas with limited resources (21). Graphene oxide (GO) is of great

interest due to its low cost, easy access, and widespread ability to convert to graphene. Scalability is also a much-desired feature (22).

Immuno chromatographic technique (ICT) is a card-based test which gives only qualitative results (23). Nanodiagnostic tests utilize nanotechnology in clinical diagnosis to enhance sensitivity, specificity, and early detection within reduced timeframes. The extensive surface area of nanomaterials facilitates the binding of numerous target-specific molecules, enabling ultrasensitive detection (24). This field has produced numerous unique and effective nanodiagnostics for infectious diseases. These nanotechnology-based systems have the potential to create portable, durable, and cost-effective point-of-care testing platforms for the detection of infectious diseases in developing countries (25).

EL-Lessy *et al.* (7) demonstrated that a novel antigen-capture immunoassay utilizing IgG pAb conjugated with graphene nanoparticles was employed for the detection of *Giardia* antigen in stool samples, marking the initial trial for diagnosing human giardiasis. The study found that both Dot ELISA and Nano graphene-based Dot ELISA exhibited identical sensitivity (91.8%) for detecting *Giardia* antigen in stool samples. However, Dot ELISA outperformed Nano Dot ELISA in terms of specificity (80% vs. 68.6%), positive predictive value (PPV) (88.9% vs. 83.6%), negative predictive value (NPV) (84.8% vs. 82.8%), and diagnostic accuracy (87.5% vs. 83.3%). Nonetheless, no statistically significant difference was observed between the two methods ($P > 0.05$). The Nano ELISA sandwich demonstrated a sensitivity of 92.6% in sera from MF patients and 47.6% within the chronic group, with a specificity of 95.1% (25). Ibrahim *et al.* (25) reported that the diagnosis of *W. bancrofti* using sandwich ELISA yields a quantitative result that correlates with adult worm load.

Finally, the present work's main objective is to evaluate and demonstrate a novel test, which is rapid and selective for diagnosis of hydatidosis by nano-immunochromatography compared with sandwich and nano-sandwich ELISA.

The results showed that by using sandwich ELISA; 39 out of 50 hydatid-infected samples showed positive results and the sensitivity of the assay reached 78%. 7 out of 50 other parasite-infected groups had positive results, giving 93% specificity. Using nano-sandwich ELISA; 42 out of 50 hydatid-infected samples showed positive results, and the sensitivity of the assay reached 84%. 7 out of 50 other parasite-infected groups had positive results giving 93% specificity. Using ICT; 36 out of 50 hydatid infected samples showed positive results, and the sensitivity of the assay was 72%. 16 out of 50 other parasite-infected groups had positive results, giving 80% specificity. The specificity of sandwich ELISA, nano-sandwich ELISA reached 93% and it was 80% by using

ICT techniques. The use of nano-sandwich ELISA is one of the best methods for diagnosis of human hydatidosis.

Acknowledgments
All thanks to the organization of the Science and Technology Development Fund (STDF) for funding this project, grant project no. "27778 P.I. Ibrahim Aly".

Author contributions
Study concept and design: SIR, ZRS, MS. Acquisition of data: AM, AMM, SIR, ZRS. Analysis and interpretation of data: AM, AMM, NA. Drafting of the manuscript: AM, AMM, NA. Critical revision of the manuscript for important intellectual content: SIR, ZRS, MS. Statistical analysis: AM Study supervision: SIR, ZRS, MS.

Funding
This study was supported by grant project no. "27778 P.I. Ibrahim Aly" from Science and Technology Development Fund (STDF).

Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All the experiment procedures were conducted according to the regulations of the TBRI-REC that operate in a manner consistent with good clinical practice (GCP) under the ICH guidelines and applicable national/local regulations. All approved research work complies with the world medical association codes of ethics (Declaration of Helsinki) for experiments in humans.

Consent for publication
The research work will be conducted on achieved sera samples from patients infected with hydatid, samples are stored at the Parasitology Department, TBRI, in a de-identified manner, ensuring the confidentiality and security of research and personal data.

Competing interests
The authors declare that they have no competing interests.

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