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Diagnostic Performance of Molecular and Immunological Techniques Alongside Cytokine Analysis for The Early Detection of *Echinococcus Granulosus* Infection

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ABSTRACT

In Iraq, cystic echinococcosis (CE) caused by Echinococcus granulosus is an important zoonosis, and improved early diagnostic methods are needed. The primary objective of this cross-sectional study was to evaluate and compare the diagnostic performance of conventional polymerase chain reaction (PCR), quantitative real-time polymerase chain reaction (qPCR), and enzyme-linked immunosorbent assay (ELISA) alongside pro-inflammatory cytokine profiling (tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8)) for the early detection of *Echinococcus granulosus* infection in an endemic Iraqi population. We performed a cross-sectional study among 90 participants, containing 45 confirmed CE patients, 15 suspected individuals with CE, and 30 healthy controls. PCR for COX1 and NAD1 genes, qPCR, and ELISA of anti-*E. granulosus* IgG was performed. Also, inflammatory cytokines (TNF-α and IL-8) were measured with ELISA. Statistical analyses, including diagnostic accuracy metrics (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) and receiver operating characteristic (ROC) curves, were implemented using SPSS v26.0 to establish optimal cut-off values via Youden's index. qPCR yielded the highest diagnostic performance with a sensitivity of 97.78% and specificity of 100%, followed by conventional PCR. ELISA demonstrated high sensitivity (100%) and specificity (86.67%). The ROC analysis indicated high diagnostic accuracy (area under the curve (AUC) = 0.975). Statistical analysis revealed that the mean age (38.7 ± 13.2 years for confirmed cases) and gender distribution (48.9% male, 51.1% female) exhibited no statistically significant variation across groups ($p > 0.05$), ensuring demographic comparability. The observed elevated levels for TNF-α and IL-8 of infected people also suggested a vigorous inflammatory response in the context of active infection. qPCR is the best diagnostic tool for CE, and ELISA is an efficient screening method for CE. An enhanced understanding of host immune response and potentially disease severity could be provided by cytokine profiling.

1. INTRODUCTION

Cystic echinococcosis (CE), or hydatid disease, is a chronic zoonotic parasitic infection caused by the larval phase of *Echinococcus granulosus*. The disease persists as a major public health concern in endemic locations like the Middle East, North Africa, Central Asia and portions of South America [1].

Transmission occurs via ingestion of parasite eggs excreted through the feces of infected dogs and livestock as intermediate hosts. Humans, as incidental intermediate hosts, deposit hydatid cysts into different organs. The liver is the organ most extensively involved (60–70% of all cases), followed by lungs (20–30%), but could be other organs as well. It is commonly asymptomatic for long periods, and it can vary by cyst size, location, and complications, and rupture or secondary infection may be the characteristic of incidence [2].

Delayed diagnosis is still a major problem in endemic areas of Hydatid disease today, leading to more morbidity and an even greater demand on health care systems. Most current methods used for the diagnostic process employ imaging modalities such as ultrasonography and computed tomography

(CT) that provide structural information. However, standard imaging modalities fail to definitively differentiate between viable (active) and non-viable (inactive) parasitic cysts, necessitating the integration of biological, serological, and molecular markers [1, 3]. Serology testing, including enzyme-linked immunosorbent assay (ELISA), supports laboratory testing. While ELISA has great sensitivity, it lacks specificity due to potential cross-reactions from other parasitic infections as well as persistence of antibodies post-treatment. The use of polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR) for the detection of parasite DNA represents significant improvements in specificity over traditional molecular diagnostics. Due to their high copy numbers, mitochondrial genes such as cytochrome c oxidase subunit 1 (COX1) and NADH dehydrogenase subunit 1 (NAD1) represent excellent candidates for detecting these pathogens. Additionally, qPCR allows for the measurement of the quantity of parasites loaded into the assay. As such, this technique could potentially increase the diagnostic accuracy of testing [4, 5].

Besides molecular or serological approaches, the immune reaction analysis based on inflammatory cytokines becomes more important. Cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8) are both crucial mediators of inflammation and are involved in the pathogenesis as well as the detection of disease processes and, potentially, disease severity [6, 7].

Comparison studies that incorporate a greater complement of molecular, serological, and immunological diagnostic tools in clinical practice among these segments of the population remain few, especially in endemic regions like Iraq. So, the aim of this study was to characterize standardized PCR, qPCR, ELISA, and cytokine profiling (TNF-α and IL-8) in the diagnosis of early CE and compare them to the confirmed, suspected, and healthy control groups [8-10].

2. MATERIALS AND METHODS

2.1 Study design

The present study was a cross-sectional diagnostic research carried out from 2024 to 2026 in an endemic area for CE in Iraq. The main aim was to compare and assess the results of conventional PCR, qPCR, ELISA, and inflammatory cytokine profiling in the initial diagnosis of *Echinococcus granulosus* infection. The work was designed to evaluate the diagnostic ability in real clinical scenarios under molecular, serological, and immunologic approaches. Confirmed cases provided the reference standard for the parameters for calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the diagnoses.

2.2 Study population

Ninety participants were sampled in the present study, and were divided into three groups according to clinical and diagnostic criteria: 45 patients with solid diagnosis (firm CE), 15 clinically suspected cases, and 30 healthy controls (Figure 1). No formal sample size calculation was performed prior to the study due to the exploratory nature of this diagnostic evaluation and the limited availability of confirmed clinical cases during the study period. The sample size of 90 participants was determined based on a convenience sampling approach of patients presenting to the participating centers between 2024 and 2026, which aligns with sample sizes utilized in similar published regional diagnostic studies. Confirmed cases were diagnosed on the basis of an imaging finding (ultrasound and/or CT) and, where applicable, surgical confirmation. Suspected cases consisted of people with clinical and radiological features suggestive of CE but without definitive confirmation when enrolling in the program.

Controls were randomized samples of healthy subjects with no clinical, radiological, or laboratory evidence of hydatid disease. During the study duration, all participants were recruited from hospitals and diagnostic centers in Iraq. All participants were asked to provide basic demographic and clinical information, such as age, sex, and clinical presentation.

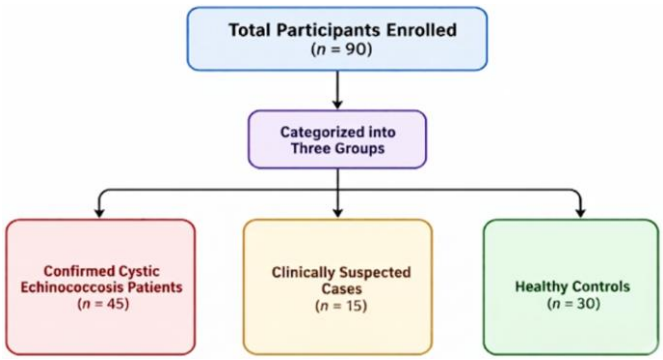

Figure 1. Study population

2.3 Ethical considerations

The study protocol was reviewed and approved by the Institutional Research Ethics Committee of the Northern Technical University, Mosul Medical Technical Institute (Approval No. NTU-REC-2024-114, dated January 15, 2024) in adherence to all national and international ethical guidelines. Written informed consent was obtained from all participants prior to sample collection. All procedures were undertaken according to the ethical principles of the Declaration of Helsinki. Participants were informed of the study's purpose, and personal data was kept strictly confidential throughout the study.

2.4 Sample collection

Under the principle of aseptic practice, peripheral venous blood samples were taken from all subjects. Each subject received about 5 mL of blood in 2 parts: 1 portion collected in ethylenediaminetetraacetic acid EDTA tubes for molecular analysis, and the other was collected in plain tubes for serum separation. The blood samples collected in plain tubes were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to yield serum. The separated serum was aliquoted and kept at −20 ℃ until further analysis, analysis for ELISA and cytokine measurements. Genomic DNA was extracted from EDTA-anticoagulated blood samples. DNA extraction was performed within 24 hours from the time the sample was gathered, or samples were kept at −20 ℃ until processing was finished to ensure that the DNA remains intact.

2.5 DNA extraction

Genomic DNA was extracted from EDTA anticoagulated whole blood samples using a commercially available silica column-based DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Germany, Cat. No. 51104), following manufacturer specifications. Blood samples were lysed with 20 µL of Proteinase K and 200 µL of Buffer AL, followed by incubation at 56 ℃ for 10 minutes to complete the cellular breakdown.

Selective DNA binding was enabled following lysis via a silica membrane column by adding 200 µL of ethanol (96–100%) and centrifuging at 8,000 rpm. Washing steps were performed using 500 µL of Buffer AW1 and 500 µL of Buffer AW2 to remove impurities and contaminants, and purified DNA eluted by the elution buffer was further added by adding 50 µL of Elution Buffer AE directly to the center of the membrane, incubating at room temperature for 5 minutes, and centrifuging at 8,000 rpm. Extracted DNA concentration and purity were determined spectrophotometrically using absorbance at 260 and 280 nm, respectively on a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA). DNA samples were then kept at −20 ℃ until molecular analyses.

2.6 Primer design and in silico validation

Mitochondrial gene sequences (COX1 and NAD1) for *Echinococcus granulosus* were retrieved from the NCBI GenBank database to facilitate target-specific primer design via the NCBI Primer-BLAST tool. In silico validation was performed against the NCBI non-redundant database to guarantee high specificity and eliminate potential cross-amplification with human genomic DNA or other related helminth species. Table 1 summarizes the sequences and characteristics of the design primers.

Table 1. Primers used for amplification of *Echinococcus granulosus* mitochondrial genes

Gene	Primer Name	Sequence (5′→3′)	Length (bp)	Tm (℃)	GC (%)	Amplicon Size (bp)
COX1	COX1-F	TTTTTTGGGCATCCTGAGGTTTAT	25	56	40	~450
COX1	COX1-R	TAAAGAAAGAACATAATGAAAATG	24	54	33	~450
NAD1	JB11-F	AGATTCGTAAGGGGCCTAATA	21	55	43	~450
NAD1	JB12-R	ACCACTAACTAATTCACTTTC	21	54	38	~450

Notes: GC (%): Guanine–Cytosine content. All primers were synthesized by a commercial provider and validated experimentally prior to use.

2.7 Conventional polymerase chain reaction amplification

Conventional PCR amplification was performed to identify *Echinococcus granulosus* DNA targeting the mitochondrial COX1 and NAD1 genes. PCR-based results were done using a final volume of 25 µL containing 12.5 µL of 2× GoTaq Green Master Mix (Promega, USA, Cat. No. M7122), 1 µL of each forward and reverse primer (final concentration of 0.4 µM; synthesized by Macrogen, South Korea), 3 µL of template DNA (~30 ng), and 7.5 µL of nuclease-free water. The amplification was executed using a Bio-Rad T100 Thermal Cycler (Bio-Rad, USA). The amplification protocol was an initial denaturation step at 95 ℃ for 3 minutes, followed by 35 cycles at 95 ℃ for 30 seconds, annealing at 55 ℃ (for both COX1 and NAD1) for 30 seconds, and extension at 72 ℃ for 40 seconds. A final extension step was performed at 72 ℃ for 5 minutes, followed by a hold at 4 ℃. PCR products were analyzed by electrophoresis on 1.5% agarose gel in 1× TAE buffer run at 90V for 50 minutes, stained with ethidium bromide (0.5 µg/mL), and visualized under ultraviolet (UV) illumination. The desired amplicon sizes (~450 bp) were determined by using a 100 bp DNA ladder (Promega, USA). Positive controls (DNA extracted from confirmed hydatid cyst fluid) and negative controls (no-template control) were included in each PCR run to achieve PCR amplification accuracy and reproducibility.

2.8 Quantitative real-time polymerase chain reaction

For *Echinococcus granulosus* DNA detection, qPCR was carried out in SYBR Green chemistry. Reactions were performed in a final volume of 20 µL containing 10 µL of 2× PowerUp SYBR Green Master Mix (Applied Biosystems, USA, Cat. No. A25742), 0.8 µL of each forward and reverse primer (final concentration of 0.3 µM), 2.5 µL of template DNA, and 5.9 µL of nuclease-free water. Amplification was carried out on an Applied Biosystems QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, USA) under the following cycling conditions: initial denaturation at 95 ℃ for 10 min., followed by 40 cycles of denaturation at 95 ℃ for 15 sec, and later annealing/extension at 60 ℃ for 30 s. Fluorescence measurements were taken at the end of each amplification cycle. Specificity of the PCR products was checked by a melting curve analysis, at the end of the amplification process, programmed from 60 ℃ to 95 ℃ with a continuous heating rate of 0.1 ℃/s, ensuring a single distinct peak matching the target product without non-specific primer-dimer amplification. If the cycle threshold (Ct) of the samples was lower than 38, positive for *Echinococcus granulosus* DNA was considered. All reactions were performed in duplicate, and both negative (no-template control) and positive controls (sequence-verified plasmid standard) were included in each run for assay accuracy and reliability. The amplification efficiency (E) was evaluated via a 5-point serial dilution standard curve (10^1 to 10^5 copies), calculated using the formula $E = -1 + 10^{(-1/\text{slope})}$. An efficiency between 92% and 105% with a correlation coefficient ($R^2 > 0.99$) was defined as the standard criteria for an acceptable assay run.

2.9 Enzyme-linked immunosorbent assay

Anti-*Echinococcus granulosus* IgG antibodies are present in serum samples using a commercially available ELISA Kit, as indicated on the manufacturer's guidelines. Briefly, we added the serum samples and control to the wells containing coated antigens; we then let them bind specifically for 30 minutes. We added enzyme-conjugated secondary antibodies next, and then substrate solution so that the color reaction can be seen. The optical density (OD) values of the samples were determined by reading at 450 nm with light from a microplate reader. Samples were run in duplicate to help validate accuracy and consistency. ROC Curve Analysis with Youden's Index was utilized to determine the best ELISA cut-off to declare the sample positive. microplate reader and then

utilized to determine cytokine concentrations via nonlinear regression. The reliability of each assay was demonstrated by the high correlation coefficients (R^2 values) that arose from standard curves. CE related to inflammation was assessed based on the levels of cytokines measured. It was vital to avoid variability as all the assays were carried out in standardized laboratory settings.

2.10 Cytokine analysis (TNF-α and IL-8)

The levels of TNF-α and IL-8, in terms of pg/mL, were analyzed through commercial ELISA kits as directed by the manufacturer. Each cytokine standard curve was generated from serially diluted samples that represented a known concentration indicated on the kit. The absorbance at 450nm was read utilizing a microplate reader and then utilized to determine cytokine concentrations via nonlinear regression of the respective standard curves. All samples were analyzed in duplicate to allow for accuracy and reproducibility. The reliability of each assay was demonstrated by the high correlation coefficients (R^2 values) that arose from standard curves. CE related to inflammation was assessed based on the levels of cytokines measured. It was vital to avoid variability as all the assays were carried out in standardized laboratory settings.

2.11 Statistical analysis

Statistical analysis was carried out with SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). Quantitative statistics were reported as mean ± standard deviation (SD). Group comparisons were carried out using an independent samples t-test for two groups and were considered statistically significant. Diagnostic test of conventional PCR, qPCR, and ELISA was performed based on sensitivity, specificity, PPV, and NPV using confirmed cases as a reference standard. The receiver operating characteristic (ROC) curve analysis was also used to evaluate the accuracy of ELISA diagnostics, and the optimal cut-off value was determined using Youden's index. The area under the curve (AUC) was used to determine the discriminating performance of the test.

3. RESULTS

3.1 Baseline characteristics

A total of 90 participants were included in this study and categorized into three groups: 45 patients with confirmed CE, 15 clinically suspected cases, and 30 healthy controls.

The demographic and clinical characteristics of the study population are summarized in Table 2. The mean age, sex distribution, and clinical presentation were comparable among the study groups. Liver involvement was the most common clinical manifestation among confirmed cases, followed by pulmonary involvement. Statistical comparison of demographic and baseline criteria revealed excellent homogeneity across the groups ($p > 0.05$). Specifically, the mean age did not differ significantly between confirmed (38.7 ± 13.2), suspected (40.5 ± 11.1), and control groups (37.9 ± 12.5; $p = 0.742$ via global ANOVA variance check, confirming that baseline characteristics remained homogeneous and cross-comparable).

Table 2. Demographic and clinical characteristics of study participants

Variable	Confirmed (n = 45)	Suspected (n = 15)	Control (n = 30)	p-Value
Age (mean ±SD)	38.7 ± 13.2	40.5 ± 11.1	37.9 ± 12.5	0.742
Male, n (%)	22 (48.9%)	8 (53.3%)	16 (53.3%)	0.973
Female, n (%)	23 (51.1%)	7 (46.7%)	14 (46.7%)	-
Rural residence, n (%)	29 (64.4%)	10 (66.7%)	12 (40.0%)	0.152
Liver cyst, n (%)	32 (71.1%)	9 (60.0%)	-	0.218
Lung cyst, n (%)	11 (24.4%)	4 (26.7%)	-	0.832
Albendazole therapy, n (%)	26 (57.8%)	5 (33.3%)	-	0.089

3.2 Conventional polymerase chain reaction findings

Table 3. Diagnostic performance of conventional polymerase chain reaction (PCR)

Parameter	Value
True Positive (TP)	43
False Negative (FN)	2
True Negative (TN)	30
False Positive (FP)	0
Sensitivity (%)	95.56
Specificity (%)	100
PPV (%)	100
NPV (%)	93.75

Notes: PPV: Positive predictive value; NPV: Negative predictive value.

Conventional PCR amplification of the mitochondrial COX1 and NAD1 genes of *Echinococcus granulosus* was accurately carried out for all these samples. A positive amplification was identified by the appearance of certain bands on agarose gel after electrophoresis for the anticipated amplicon sizes. Of the confirmed cases (n = 45), 43 samples were positive by conventional PCR, and 2 were negative. All control samples (n = 30) were negative, and no false-positive amplification was detected. Consistent with these results, the conventional PCR demonstrated a sensitivity of 95.56% (95% CI: 84.85% – 99.46%) and specificity of 100%, and the NPV was 93.75%. The analysis demonstrated that all positive clinical samples (Lanes L1, L2, and L3) yielded a single, highly dense, and distinct diagnostic band at the exact expected size of approximately 450 bp, matching the established molecular profiles of target mitochondrial marker genes (COX1 and NAD1) (Table 3 and Figure 2).

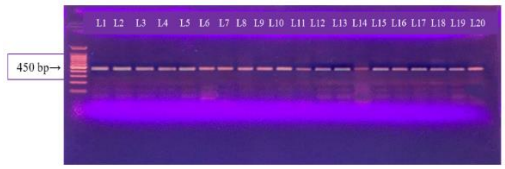

Figure 2. 2% agarose gel electrophoresis of conventional PCR products targeting *Echinococcus granulosus* mitochondrial genes (COX1 and NAD1)

Notes: Lane M: 100 bp DNA ladder. Bands at ~450 bp indicate the expected amplicon size.

3.3 Performance results of quantitative real-time polymerase chain reaction

Table 4 shows the data analysis of *Echinococcus granulosus*. qPCR exhibited the highest diagnostic capacity, successfully mapping 44 out of 45 confirmed clinical cases within a Ct threshold of < 38 cycles, leaving only 1 false negative. Conventional PCR correctly identified 43 true positive cases. Both molecular methods yielded a 100% specificity profile with no false-positive reactions within the healthy control population (n = 30). qPCR yielded a sensitivity of 97.78% and a specificity profile of 100% with no false-positive reactions within the healthy control population (n = 30). The improved sensitivity of qPCR may be attributed to its ability to detect low concentrations of parasite DNA.

3.4 Enzyme-linked immunosorbent assay and receiver operating characteristic curve analysis

Serum anti-*Echinococcus granulosus* IgG levels have been determined from ELISA as well. Results indicate that the confirmed cases possess a much higher OD than control subjects, in contrast. To assess the performance of ELISA with respect to diagnosis, a ROC curve was constructed. With an AUC of 0.975, the analysis showed superb discrimination capability. Youden's index is used to determine the optimum cut-off value for ELISA positivity, which is 0.184. ELISA had a specificity of 86.67% (95% CI: 69.28% – 96.24%) at this threshold and was sensitive at 100% (95% CI: 92.13% – 100.00%). False positives occurred in four control samples, while no false negatives were detected. These results show that ELISA is highly sensitive but has moderate specificity in contrast to molecular methods (Table 5 and Figure 3).

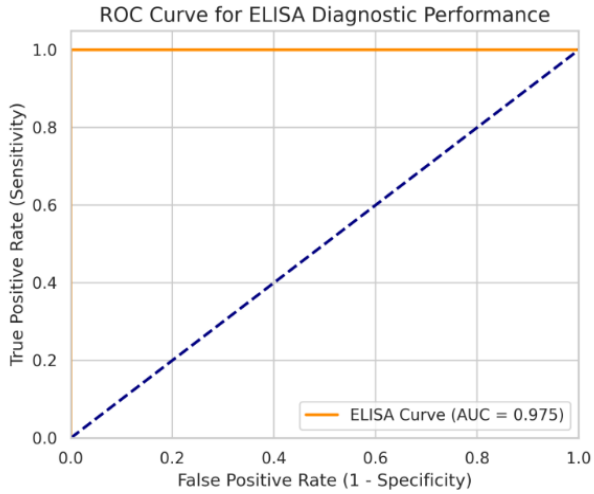

Figure 3. Receiver operating characteristic (ROC) curve displaying the diagnostic performance of the enzyme-linked immunosorbent assay (ELISA) technique for the detection of *Echinococcus granulosus* infection

Notes: The area under the curve (AUC) is exactly 0.975 (95% CI: 0.952–0.991, $p < 0.001$), indicating excellent diagnostic accuracy.

Table 4. Comparison of diagnostic performance between conventional polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR)

Method	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PCR	43	0	30	2	95.56	100	100	93.75
qPCR	44	0	30	1	97.78	100	100	96.77

Notes: TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; PPV: Positive predictive value; NPV: Negative predictive value.

Table 5. Diagnostic performance of enzyme-linked immunosorbent assay (ELISA)

Parameter	Value
True Positive (TP)	45
False Negative (FN)	0
True Negative (TN)	26
False Positive (FP)	4
Sensitivity (%)	100%
Specificity (%)	86.67%
PPV (%)	91.84%
NPV (%)	100%
AUC	0.975
Cut-off value	0.184

Notes: PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve.

3.5 Distribution of cycle threshold values

The distribution of Ct values in Figure 4 indicated differences between confirmed and suspected cases, with lower Ct values generally observed among confirmed infections. Although qPCR provides quantitative measurements of parasite DNA, the present study did not perform correlation analyses between Ct values and clinical characteristics, treatment status, ELISA OD values, or cytokine concentrations. Therefore, the findings support the diagnostic utility of qPCR, while further studies are needed to evaluate its potential role in estimating parasite burden and disease activity.

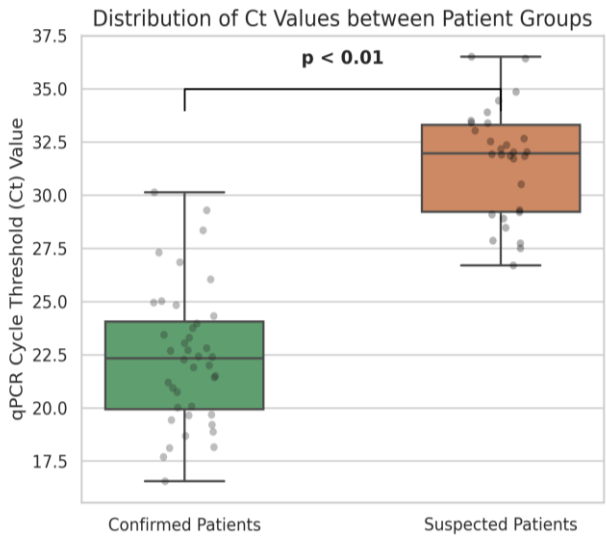

Figure 4. Box-and-whisker plot combined with individual data points showing the distribution of quantitative real-time polymerase chain reaction (qPCR) cycle threshold (Ct) values between confirmed and suspected patient groups

Notes: Confirmed patients exhibited significantly lower Ct values compared to suspected patients ($p < 0.01$).

3.6 Serum cytokine level (TNF-α and IL-8)

Figure 5 shows the serum levels of TNF-α and IL-8, which were quantified with an ELISA. Both cytokine standard curves exhibited a good degree of accuracy, based on their high correlation coefficient values (TNF-α: $R^2 = 0.987$; IL-8: $R^2 = 0.989$). Also, significant differences in TNF-α and IL-8 levels existed when comparing confirmed cases of CE to controls. Suspected cases presented cytokine levels that were greater than control values but less than confirmed case values. This increased presence of TNF-α and IL-8 suggests that these cytokines are elevated as a result of an active infection, which induces inflammation. Quantitative comparisons of cytokine levels among the trial groups are summarized.

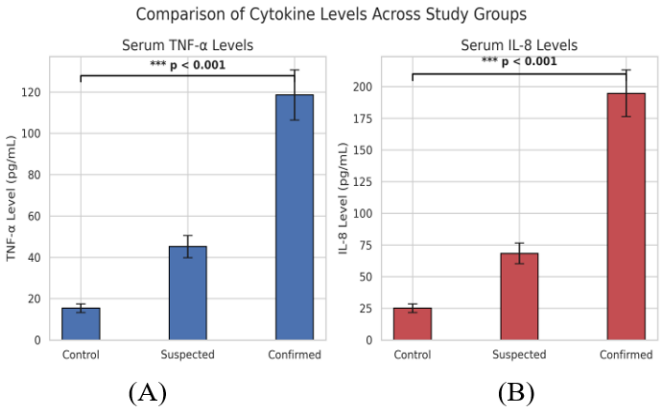

Figure 5. Comparative analysis of serum cytokine levels across the study groups (A) serum levels of TNF-α (pg/mL) and (B) serum levels of IL-8 (pg/mL) in the control group, suspected patients, and confirmed patients
Notes: Data are presented as Mean ± SEM (*** $p < 0.001$).

4. DISCUSSION

In this study, we also looked at how well three other tests worked to diagnose CE that occurs in a population with endemic cases: conventional PCR, qPCR, ELISA, and inflammation-based cytokine profiling. The data from these studies demonstrate that combining multiple types of methodologies, such as molecular diagnostics and serology/immunology-based diagnostics, provides wide-ranging and reliable methods to confirm CE diagnoses. Additionally, existing PCR methodology using primers targeted against the mitochondrial COX1 and NAD1 genes showed an excellent level of specificity (100%), and good sensitivity (95.56%) when used to detect DNA from *E. granulosus* [11, 12].

Although there was only a small number of false negative cases found, this may have been caused by either the amount of parasitic DNA present in peripheral blood being too low, or possibly because of restrictions on sampling. These findings align with previous research into how insufficient PCR is as a method to detect low-grade parasitic infection. qPCR performed better than other methods for detecting parasitic infection. It had a higher sensitivity (97.78%) and specificity (100%), compared to other methods [13, 14].

The increased sensitivity of qPCR is most likely due to both the use of real-time fluorescence measurement during PCR cycling as well as an increase in the overall efficiency of the amplification reaction. Additionally, the observed differences in Ct values between confirmed and suspected cases further support the high diagnostic performance of qPCR. However, the present study did not investigate correlations between Ct values and cyst location, treatment status, ELISA findings, or cytokine levels. Consequently, any potential relationship between qPCR measurements and parasite burden or disease activity requires further investigation in larger prospective studies [15, 16].

ELISA (100% sensitive vs. 86.67% specific) had many false positive results within control samples [17, 18].

The limitation of this test likely relates to potential cross-reactive antibodies against other parasites and the possibility that some antibodies may persist long enough post-exposure and/or treatment. In spite of its simplicity and high sensitivity, ELISA remains an effective method for identifying infected humans. Importantly, in addition to its excellent diagnostic capability as indicated by the area under the ROC curve (AUC = 0.975), this diagnostic method has been enhanced by including cytokine profiles as part of the diagnostic process within this investigation [19, 20].

A combination of molecular detection (e.g., qPCR), serology (e.g., ELISA), and immunological measures (cytokine levels; e.g., TNF-α and IL-8) offers a robust multi-faceted approach to detecting CE.

Confirmed cases with high levels of both TNF-α and IL-8 show evidence of an active infection through a strong pro-inflammatory response, consistent with the expected nature of this type of infection [21, 22].

TNF-α is involved in signaling for activation of the immune system, and IL-8 signals neutrophils to migrate into infected tissues, where they will help combat the pathogen by triggering an inflammatory response.

A combination of approaches (ELISA and PCR) can lead to a better rate of accurate diagnoses at diagnosis centers. It will also help us understand the progression of diseases within high-incidence regions that have an urgent need to diagnose at an earlier time. However, we should recognize some disadvantages [23, 24]. At this point in time, the sample size has been sufficient to evaluate diagnostic capabilities; however, it would be very difficult to generalize from this study [25].

Peripheral blood samples were analyzed using molecular techniques to detect parasite DNA. While they contained less parasite DNA than cyst fluid samples, there is still potential for detecting low levels of parasite DNA in peripheral blood. Therefore, it would be beneficial to use larger numbers of subjects and different types of sample materials in future studies [26]. The present findings indicate that molecular detection (qPCR), serological testing (ELISA), and immunological assessment (TNF-α and IL-8) provide complementary information for the evaluation of CE. However, because no combined diagnostic model or integrated ROC analysis was performed, the current study cannot determine whether their combined use improves diagnostic accuracy beyond the performance of the individual methods. In summary, the data collected here demonstrate the utility of qPCR as a confirmatory diagnostic method when coupled with ELISA for initial screening and cytokine profiling for monitoring disease activity and assessing the host's immune response [27, 28].

5. CONCLUSONS

In summary, the present study demonstrates that qPCR

provides excellent diagnostic performance for CE, whereas ELISA serves as a sensitive screening tool, and cytokine profiling offers additional information regarding the host inflammatory response. These approaches should be considered complementary, and future studies incorporating combined diagnostic models are warranted to determine their integrated clinical value.

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