Cysticercosis, a Potential Public Health Concern in Kuwait: A New Diagnostic Method to Screen *Taenia solium* Taeniasis Carriers in the Expatriate Population

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Highlights of the Study

- Detection of anti-*Taenia solium* taeniasis-specific antibodies in blood using taeniasis-specific rES33 antigen is a more efficient and sensitive tool than standard stool microscopy.
- Detection and deworming of *T. solium* taeniasis carriers can prevent transmission of *T. solium* infection and/or cysticercosis to the local population in countries where it is nonendemic.

Keywords

Taenia solium taeniasis · rES33 antigen · Anti-*T. solium* taeniasis-specific IgG · ELISA

Abstract

Objective: *Taenia solium* infection is not endemic to Kuwait, but several cases of cysticercosis have been detected in Kuwaiti nationals with no history of travelling to endemic countries. Infected domestic helpers/food handlers from endemic countries who may have escaped detection of infection by microscopy at the time of their arrival in Kuwait have been suspected as the possible source of infection. This study determined the seroprevalence of *T. solium* among domestic helpers/food handlers by screening their blood using a sensitive taeniasis-specific anti-rES33 antibody assay. **Subjects and Methods:** Newly arrived domestic helpers ($n = 500$) and food handlers ($n = 500$) from endemic countries were enrolled in the period 2015–2017. *T. solium*-specific rES33 anti-

gen was expressed and purified from human embryonic kidney (HEK)293-6E cells using the pTT5 mammalian expression vector. Stool samples were processed for microscopy, and blood samples were screened to detect anti-*T. solium* taeniasis-specific IgG antibodies by ELISA. **Results:** All stool samples were negative for *T. solium* parasite eggs by microscopy. However, 42 individuals (4.2%) tested positive for *T. solium* taeniasis-specific IgG antibodies. Though statistically not significant, the IgG seropositivity was higher in individuals with a lower education level, a low-income background, and a lower frequency of hand-washing. **Conclusions:** This is the first report from Kuwait and the Middle East on the detection of anti-*T. solium* taeniasis-specific serum IgG antibodies among the high-risk expatriate population. The results emphasize the importance of efficient and sensitive screening of *T. solium* carriers and thus the prevention of infection transmission and development of cysticercosis in the local population.

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Introduction

Though *Taenia solium* (pork tapeworm) causes mild intestinal infection (taeniasis) in humans via the accidental ingestion of larval cysts found in undercooked pork, accidental ingestion of *T. solium* eggs due to the contamination of food/drinks with human faecal material from *T. solium* taeniasis carriers may lead to cysts developing in the tissue (cysticercosis) [1] or central nervous system (neurocysticercosis, NCC). NCC is the most severe form of the disease and the leading cause of acquired epilepsy [2]. More than 80% of the world's 50 million people with epilepsy live in countries endemic for *T. solium* infection [3]. Cysticercosis is highly endemic in Latin America, Africa, and Asia, where domestic pig husbandry is practised [4]. Due to its public health impact, *T. solium* taeniasis/cysticercosis is one of the 20 neglected tropical diseases now prioritized by the World Health Organization [3]. The increasing incidence of NCC in more developed, industrialized countries in recent years has been attributed to increased immigration and international travel (imported cases) [5]. Early diagnosis of taeniasis is a crucial step in the control and elimination of cysticercosis and its associated complications. The current routine diagnostic test for screening taeniasis is done by stool microscopy, which has very low sensitivity and specificity. In addition, *Taenia* sp. eggs are shed intermittently and may be found in the faeces only once every few days [6]. An active infection may thus pass undetected if microscopy is performed on a single stool sample. To overcome these drawbacks, 2 taeniasis-specific excretory and secretory (ES33 and ES38) antigens were cloned and expressed; the recombinant antigens were then purified and used for the development of more efficacious diagnostic tests [7]. Serological diagnostic tests based on enzyme-linked immunoelectrotransfer blot assay (EITBA) were subsequently developed by using recombinant (r)ES33 and rES38 antigens; rES33 antigen exhibited very high sensitivity (>97%) and specificity (99%) for the diagnosis of taeniasis [8]. As this test is not available commercially, and as the ELISA format is the method of choice for large-scale epidemiological studies, we designed an ELISA-based assay for rapid screening of *T. solium* carriers.

The State of Kuwait, an Arabian Gulf country, has nearly 4.5 million inhabitants including 1.2 million Kuwaiti nationals and 3.3 million expatriates. There is limited information on the incidence of taeniasis and/or cysticercosis from the Middle East including Kuwait. Most of the domestic helpers and food handlers in restaurants in Kuwait come from countries in which *T. solium* infection is en-

demic [9]. Although stool specimens from these groups are routinely screened by microscopy for parasitic infections, only sporadic cases of *T. solium* infection have been detected. However, a total of 37 suspected cysticercosis cases (based on clinical and radiological findings and testing positive in immunoblot assay) were detected in Kuwait in the period 2014–2017; 21 of these cases were Kuwaiti nationals who had never consumed pork, and the source of the infection in most of these individuals remained unidentified (Iqbal, unpubl. data). The remaining 16 cases were expatriate workers originating from India, Nepal, and the Philippines. It is hypothesized that the Kuwaiti nationals acquired the infection from *T. solium* taeniasis carriers who were working as domestic helpers or food handlers in homes/restaurants, but this was not fully investigated. The objective of this study was to use stool microscopy and anti-*T. solium* taeniasis-specific IgG antibody detection in blood samples by using rES33 antigen as a screening tool for identifying taeniasis-carriers among newly arrived domestic helpers and food handlers in Kuwait, and to document it as a risk factor for cysticercosis in Kuwait.

Subjects and Methods

Study Design

A total sample size of 500 male food handlers and 500 female domestic helpers arriving from countries where *T. solium* infection is endemic were randomly selected in the period 2015–2017 based on 95% confidence interval (CI), 80% power, 5% margin of error, and a population size of 4.5 million individuals as determined by online statistical calculators. Relevant socio-demographics, social status, sanitation conditions, and lifestyle information about the registered participants were collected by using an approved structured questionnaire. A single stool sample and a 5-mL blood specimen were taken from each subject. The stool samples were screened for *Taenia* parasite eggs by microscopy at the Public Health Laboratory and the blood samples were screened for anti-*T. solium* taeniasis-specific IgG by enzyme-linked immunosorbent assay (ELISA). All blood samples were centrifuged at 4,000 rpm for 5 min and sera were stored at –70 °C until analyzed.

Detection of Taeniasis-Specific IgG Antibodies by ELISA

Seroprevalence of *T. solium* taeniasis was determined by using rES33 antigen as described previously by Levine et al. [8], except that the antibodies were detected by ELISA instead of EITBA. *T. solium*-specific rES33 antigen was obtained commercially from GenScript HK Ltd., Hong Kong, by following the same approach used by Levine et al. [8]. Briefly, rES33 antigen was expressed in human embryonic kidney (HEK)293-6E cells using the pTT5 plasmid. The expressed protein was purified by high-performance liquid chromatography using the glutathione sepharose affinity matrix. The final rES33 protein preparation had a purity of approximately 95% as determined by densitometry analysis of Coomassie Blue-stained SDS-PAGE gels. A *T. solium*-positive control serum obtained from a confirmed case of taeniasis (a generous gift from Dr. Sukwan Handali, Centers for Dis-

Table 1. Risk factors (socio-economic, education level, and lifestyle) and seropositivity of participants

Potential risk factor	Participants, <i>n</i>	Positive cases, <i>n</i> (%)
Gender		
Male (food handlers)	500	18 (3.6)
Female (domestic helpers)	500	24 (4.8)
Education level		
Primary: no school/elementary	26	3 (11.5)
Secondary: middle school/high	515	24 (4.7)
Higher: college/higher	459	15 (3.3)
Social status (income)		
Poor/low (USD 60–300/month)	783	37 (4.7)
Middle/high (USD 300–>1,500/month)	217	5 (2.3)
Hand-washing frequency		
<6 times/day	593	29 (4.9)
>6 times/day	407	1 (3.2)
Pork consumption		
Daily/weekly	594	24 (4.0)
Monthly/quarterly	87	4 (4.6)
None	312	2 (4.5)

ease Control and Prevention, Atlanta, Georgia, USA) was used as a positive control. Negative control serum samples were obtained from 50 Kuwaiti individuals who had never consumed pork and had never travelled to countries where taeniasis is endemic. A two-step checkerboard titration was used to determine optimal concentrations of rES33 antigen and primary and secondary antibodies.

For testing serum samples, Immulon 2 HB flat-bottomed 96-well microtitre plates were coated with *T. solium* rES33 antigen (0.25 µg/well) diluted in antigen-sensitizing buffer (0.05 M Tris/HCl [pH 8.0] + 1 M KCl + 2 mM EDTA) and incubated for 2 h. The plates were thoroughly washed and then blocked by adding 250 µL of blocking buffer (5% non-fat dry milk + 0.1% Tween 20 + PBS) and incubated on a shaker for 1 h. After decanting the blocking buffer, 100 µL of positive or negative control or test sera (diluted 1:25 in blocking buffer) were added in duplicate and incubated on a shaker for 1 h. After thorough washing, 100 µL of secondary antibody (goat anti-human IgG antibody-AP conjugated; Sigma Aldrich, USA) diluted in blocking buffer (1:1,000) was added to all wells and incubated on a shaker for 30 min. After washing, 100 µL of substrate (pNPP-1, MicroWell) was added and incubated in the dark on a shaker for 30 min. The reaction was stopped with 100 µL of Stop Solution (ImmunoChemistry Technologies, LLC). To identify serum samples positive for taeniasis-specific antibody reactivity, a stringent cut-off value of mean OD of 50 negative controls + 3 standard deviation (SD) was used [10, 11]. Serum samples from 46 domestic helpers and 46 food handlers were also tested with and without rES33 antigen to confirm that the OD values were not due to non-specific reactivity.

Statistical Analysis

Data collected from all participants was correlated with their corresponding OD values to determine the risk factors associated with *T. solium* taeniasis infection. Categorical variables were expressed as an absolute number. Statistical analysis was performed by using the χ^2 test, Fisher's exact test, one-way ANOVA, and post

hoc tests as appropriate, and $p < 0.05$ (two-tailed test) was considered statistically significant. Statistical analyses were performed by using WinPepi software v11.65 (PEPI for Windows, Microsoft Inc., Redmond, WA, USA).

Results

Demographic Data

A total of 500 female domestic helpers and 500 male food handlers were interviewed to collect socio-demographic information and screen for seroprevalence of anti-*T. solium* taeniasis IgG antibodies. The age of the participant ranged from 21 to 65 years (mean 33 years). Of 1,000 participants, only 459 had a college/higher education (Table 1). Most of the participants ($n = 783$, 78%) were from a poor-to-low income group. Hand-washing frequency of <6 times/day ($n = 593$, 59%) and daily/weekly pork consumption ($n = 594$, 59%) was reported by the majority of the participants (Table 1). At the personal interview, at least 3 subjects reported subcutaneous swelling, passing worm segments in stools, and/or abdominal discomfort.

Stool Microscopy and Seroprevalence of Taeniasis by ELISA

The microscopic examinations of stool samples from domestic helpers ($n = 500$) and food handlers ($n = 500$) were uniformly negative for gravid proglottids or eggs.

Fig. 1. Box and whisker plot showing ELISA results of 46 food handlers and 46 domestic helpers with and without rES33 antigen. The OD values were measured for domestic helpers (DH) and food handlers (FH) with rES33 antigen (w/Ag) and without rES33 antigen (w/o Ag).

Serum samples from negative controls yielded OD values of 0.15–0.45. When sera samples from domestic helpers ($n = 46$) and food handlers ($n = 46$) were tested without antigen in the ELISA, the OD values obtained from each sample were nearly the same (approx. 0.1) in both categories (Fig. 1).

The OD values for 1,000 serum samples ranged from 0.15 to 3.07, with 80% of the participants yielding values below background values ($OD \leq 0.45$) (Fig. 2). As described above, a stringent cut-off value for an ELISA positive control was set at $OD \geq 0.75$ which is 3 SD higher than negative controls (Fig. 2).

Based on the ELISA data, 42 participants (4.2%, 95% CI 1.1–7.3%) tested positive for anti-*T. solium* taeniasis IgG antibodies ($OD > 0.75$). Interestingly, 16 subjects yielded OD values $\geq$ the positive control ($OD = 1.2$) and all 3 subjects with a history of passing parasite segments in stools, subcutaneous swelling, and/or abdominal discomfort were strongly positive for the presence of anti-*T. solium* taeniasis IgG antibodies ($OD > 2.5$). Although not statistically significant, the percent positivity among fe-

male domestic helpers was higher (24/500, 4.8%) than in male food handlers ($p = 0.431$).

The percent positivity was highest (11.5%) among individuals with no education or only primary education, and declined in individuals with higher education status, but these differences were not statistically significant (Table 1). Similarly, the positivity was also higher, but not statistically significant, among individuals with a lower economic status than in those with a higher income ($p = 0.129$) or among subjects with a hand-washing frequency of < 6 times/day than in subjects with a hand-washing frequency of > 6 times/day ($p = 0.203$) (Table 1). Surprisingly, pork consumption had no obvious effect on seropositivity status, but most (24/42) positive individuals were found among the subjects who consumed pork on a daily/weekly basis (Table 1).

The highest number of seropositive subjects was from the Philippines, followed by India, Sri Lanka, Nepal, Ethiopia, Zimbabwe, and Ghana (Table 2).

Discussion

This study was initiated because several cases of cysticercosis were detected among Kuwaiti nationals who had never consumed pork, and most of whom had not travelled to any of the countries in which *T. solium* infection is endemic. We used (i) standard microscopy to detect *Taenia* parasite eggs/segments in the stool and (ii) rES33-based ELISA assay to detect anti-*T. solium* taeniasis-specific IgG antibodies in the blood of our study participants. For the ELISA-based assay, we obtained rES33 antigen from the same supplier that produced the antigen for the original study conducted by Levine et al. [8]. Other investigators have used EITBA, dipstick ELISA, and copro-antigen ELISA [12–15] which are more tedious to perform than ELISA.

In this study, 42/1,000 domestic helpers and food handlers, including 3 symptomatic subjects who had recently arrived in Kuwait tested positive for the presence of anti-*T. solium* taeniasis-specific IgG antibodies, showing a seroprevalence of 4.2% for *T. solium* taeniasis. None of the stool samples were positive on microscopy. The lack of a detection of parasite segments and/or eggs in symptomatic subjects was likely due to the poor sensitivity of stool microscopy or the intermittent shedding of parasite eggs/segments in stools [6]. Consistent with our data, an earlier study on 449 Hispanic residents in California, USA, showed a seroprevalence of 1.1% for *T. solium* taeniasis [16]. Interestingly, all the seropositive

Fig. 2. A scatter plot of OD values of 1,000 expatriate participants relative to background cut-off value and positive control. OD values were read at 405 nm. The cut-off value was calculated from the mean of control samples + 3 SD and corresponded to an OD of 0.75. The cut-off value using a positive control serum sample corresponded to an OD of 1.20. The x axis shows OD values and the y axis shows the number of expatriate participants whose blood was screened for anti-*T. solium* taeniasis IgG antibodies against rES33 antigen as measured by ELISA.

Table 2. Participant distribution, percent seropositivity, and OD range by nationality

Country of origin	Participants, <i>n</i>	Seropositive participants, <i>n</i> (%)	Seropositive participants by OD range, <i>n</i>			
			>0.75	>1.00	>2.00	>3.00
Philippines	578	24 (4.2)	12	8	4	0
India	276	7 (2.5)	3	4	0	0
Sri Lanka	67	5 (7.5)	3	2	0	0
Zimbabwe	10	1 (10.0)	0	1	0	0
Ethiopia	12	2 (16.7)	0	1	0	1
Nepal	33	2 (6.1)	1	1	0	0
Ghana	14	1 (7.1)	1	0	0	0
Others ^a	10	0	–	–	–	–

^a Cameroon *n* = 2; Togo *n* = 2; Uganda *n* = 2; Cote d'Ivoire *n* = 1; Gambia *n* = 1; Malawi *n* = 1; Senegal *n* = 1.

subjects in their study were also negative on stool microscopy, even though 1 subject subsequently reported passing worm segments following the treatment for taeniasis [16].

A serological field study in Peru to evaluate the EITB assay using rES33 antigen screened a total of 475 sera that included 203 *T. solium* taeniasis sera, 22 *T. saginata* sera, and 250 symptomatic neurological patient sera. Of the

203 *T. solium* taeniasis sera tested, 97% were positive, thus showing a sensitivity of 97% for the diagnosis of taeniasis. The test was also highly specific as all 272 non-taeniasis sera including 22 *T. saginata* sera were negative [5].

In our study, the percent positivity was higher among female domestic helpers than in male food handlers, in individuals with no education than in subjects with higher education, and in those with a lower economic status than in the higher-income group; however, these differences were not statistically significant. Although pork consumption had no apparent effect on seropositivity status, two-thirds of the seropositive individuals reported consuming pork previously when compared to individuals who had never consumed pork (28/42; 67% vs. 14/42; 33%) in our study. However, this data should be interpreted with caution as pork consumption is strictly prohibited in Kuwait, and many participants may not have disclosed their pork consumption habits for fear of legal consequences. Frequency of pork consumption and lower economic status are known risk factors for taeniasis. Two studies from India have reported significantly higher *T. solium* taeniasis infection rates among individuals who consumed undercooked pork [17, 18]. Three of 5 subjects seropositive for *T. solium* taeniasis in the Californian study also reported pork handling/consumption as the main risk factor since these subjects lived in a house with a pig [16].

The seropositivity of 4.2% (24 positive sera among 578 samples) was observed among individuals from the Philippines, the largest ethnic group in our study. Although the prevalence/seroprevalence of *T. solium* taeniasis in the Philippines has not been studied previously, a high seroprevalence (approx. 25%) of cysticercosis has been reported among Filipinos, attributed to poor sanitation and unpenned pig farming [19–21]. Surprisingly, we detected a higher seropositivity in Ethiopians (2/12, 17%) and expatriates from Zimbabwe (1/10, 10%) than in Filipinos (24/578, 4.2%; $p = 0.000$ and $p = 0.028$, respectively). These findings are consistent with previous studies which reported *T. solium* prevalence as a serious public health problem in East African countries including Ethiopia and in Southern African countries including Zimbabwe [22, 23].

Taeniasis carriers were rarely detected in Kuwait previously due to the poor sensitivity of stool microscopy. We observed that domestic helpers of Kuwaitis with cysticercosis detected in Kuwait were negative for *T. solium* infection on initial screening of their stool samples by microscopy. However, in 2014, 2 of the domestic workers suspected of transmission of the infection to their em-

ployers were found to pass *T. solium* parasite segments and eggs in their stools following treatment for taeniasis (Iqbal J, unpubl. data). Interestingly, the archived serum samples taken earlier from these 2 domestic helpers showed strong reactivity in ELISA (OD >2.5), thereby demonstrating the value of detection of anti-*T. solium* taeniasis-specific IgG antibodies over stool microscopy for identifying *T. solium* taeniasis carriers. Thus, the detection of anti-*T. solium* taeniasis-specific IgG antibodies by ELISA using rES33 antigen appears to be a more efficient screening test than stool microscopy among expatriates arriving from countries where *T. solium* is endemic. Our data suggest that domestic helpers and food handlers testing positive for anti-*T. solium* taeniasis-specific IgG antibodies should be dewormed with appropriate treatment with regular follow-up of their stool samples for parasite segments and/or eggs, and be allowed to work only when they are taeniasis-free.

Our study has a few limitations. We could not obtain repeat stool samples for microscopy after deworming from a number of seroreactive individuals who were either not traceable due to the highly dynamic nature of the expatriate population in Kuwait or refused to provide a specimen. As ELISA was used in place of EITBA to screen the serum samples, our results may have had lower specificity due to the inability of this procedure to determine the antigen size.

Conclusion

This is the first report from the Middle East on the detection of anti-*T. solium* taeniasis-specific IgG antibodies in the high-risk expatriate population by means of a highly sensitive ELISA using rES33 antigen. Our data may explain the source of infection in the series of cysticercosis cases detected previously among the Kuwaiti nationals who had never consumed pork or travelled to an endemic country. The findings have important public health implications as the screening of blood specimens from the suspected food handlers and domestic helpers with the more sensitive and efficient ELISA is expected to detect potential *T. solium* taeniasis carriers in Kuwait, who may then be appropriately dewormed to prevent the transmission of infection and the development of cysticercosis in the local population.

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Statement of Ethics

This study was approved by the Ethics Committee for the Protection of Human Subjects in Research, Kuwait University, and the Ministry of Health, Kuwait. A written informed consent was ob-

tained from all participants and they were assured of the privacy rights of their data and assay results. Personal details about individual participants were not disclosed in this study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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