

# Incidence of *Toxoplasma gondii*, *Entamoeba histolytica* and *Schistosoma mansoni* Infections Among Hepatitis C Virus Patients in Egypt

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## ABSTRACT

**Background & Objective:** Hepatitis C virus (HCV) infection badly affects the liver and represents a critical health problem in Egypt. The parasitic diseases such as schistosomiasis, toxoplasmosis, or amebiasis were highly distributed among Egyptian citizens and affect the liver status in various ways. Co-infections of these parasites with HCV are possible and accompanied by negative clinical consequences. The current study shed the light on the incidence of *Toxoplasma gondii*, *Entamoeba histolytica*, and *Schistosoma* infections among the HCV patients from Menoufia Province, Egypt.

**Materials & Methods:** One hundred and eighty HCV infected patients were examined for *T. gondii*, *E. histolytica*, and *Schistosoma* using ELISA or indirect haemagglutination test, and fecal examination was done to detect the *E. histolytica* cyst stage or *Schistosoma mansoni* ova.

**Results:** Overall incidence of *T. gondii* IgG and IgM was 65% and 3.89%, respectively. Moreover, the incidence of *E. histolytica*, and *Schistosoma mansoni* was (50% and 21.67) and (42.22% and 1.67%), using serological means and stool examination, respectively. Multiple parasitic infections (*T. gondii* and *E. histolytica*), (*T. gondii* and *Schistosoma*), (*E. histolytica*, and *Schistosoma*), and (*T. gondii*, *E. histolytica*, and *Schistosoma*) were 22.78%, 16.67%, 10.56%, and 10.56%, using serological means, respectively, among HCV-infected patients. Significant differences were demonstrated in the incidence of *T. gondii*, *E. histolytica*, and *Schistosoma* among HCV patients based on age, gender, and residence.

**Conclusion:** *T. gondii*, *E. histolytica*, and *Schistosoma* mono- or multiple infections highly exist among HCV patients from Menoufia Province, Egypt.

**Keywords:** *Toxoplasma*, *Entamoeba*, *Schistosoma*, HCV, Co-infection, Egypt



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## Introduction

In Egypt, HCV is still the most important public health problem, its prevalence in 2008 reached about 15% among Egyptians (1). The virus prevalence was the highest in Menoufia province (2).

On the other hand, schistosomiasis is an epidemic disease in Egypt (3). *Schistosoma mansoni*, which resulted in intestinal schistosomiasis, was among the main causes of chronic liver disease in Egypt (4). Clinical studies showed that HCV co-existence with schistosomiasis caused more severe liver disease complications than infection with HCV alone (5, 6).

Amebiasis is food or water-borne disease caused by acquiring the cyst form of the parasite *Entamoeba histolytica*, which is the cause of amebic colitis, and can migrate to the liver and result in liver abscesses (7). Mølle et al (2001) concluded that liver cirrhosis is the main risk factor for pyogenic liver abscesses (8). *E. histolytica* infection could result in liver injury and increase the production of the liver enzymes in the sera compared to parasite non-infected subjects (9, 10).

Besides the association of *Toxoplasma gondii* with abortion or neonatal mortalities, it can invade the liver and cause liver damage (11-13). Chronic HCV patients with related cirrhosis expressed a higher prevalence of *T. gondii* seropositivity (14).

All of these parasitic diseases schistosomiasis, toxoplasmosis, or amebiasis, were frequent among Egyptian citizens (3, 15-18). Co-infection of HCV and any of these parasites are possible and this can affect the liver status in various ways. Therefore, this study was conducted to investigate the incidence of *Entamoeba histolytica*, *Toxoplasma gondii*, or *Schistosoma* infection among HCV patients from Menoufia province.

## Materials and Methods

### Ethical Statement

The present study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice and approved by the Institutional Review

Board (IRB), Faculty of Medicine, Meunofia University, Meunofia Province, Egypt (approval code 00105/2017). The purpose and procedures involved in the current study were explained and written informed consent and a questionnaire including the demographic data was obtained from all participants.

### Study population

One hundred and eighty examined HCV-positive patient samples and data collected from private medical laboratories, Meunofia province, Egypt during the period between October 2017 and December 2018 were enrolled in this study. The patients included 105 males and 75 females, with age range 21-70 with a mean of (50.35±0.75) years.

### Detection of HCV antibodies and RNA

HCV antibodies were examined by ELISA using (Atlas Medical GmbH, Germany). PCR was done to assess qualitatively the HCV-RNA using the commercial kit (GeneProof diagnostic kit, India) according to the manufacturer's instructions.

### Serological detection of the parasite infections

*Schistosoma*-specific antibodies were determined by indirect haemagglutination test using the commercial kit (Fumouze Diagnostics Co., France). *T. gondii* specific antibodies (IgG and IgM) were detected using ELISA commercial kit (Pishtaz Teb Zaman Diagnostics, Iran). Finally, *E. histolytica* specific antibodies (IgG) were demonstrated using ELISA commercial kit (Creative

Diagnostics, NY, USA). All tests were done according to the manufacturer's instructions.

### Microscopic examination

Stool samples were collected in a twenty-five milliliter dry, clean plastic container. Gross examination was done for color, mucus, blood, consistency and adult parasites. Each sample was utilized to prepare slides for direct wet smear examination & formalin-ethyl acetate sedimentation concentration method (19).

### Statistical analysis

For statistical analysis, the SPSS (IBM SPSS Statistics for Windows, Armonk, NY) computer program was used. Binary logistic regression was used to assess significant differences of the examined parasites infection rates in HCV-infected patients of different age, residence, and sex;  $P < 0.05$  were considered to be statistically significant.

## Results

The examined parasitic infections among HCV Egyptian patients were summarized in [Table 1](#). The overall incidence of *T. gondii* IgG and IgM was 65% and 3.89%, respectively. Moreover, the incidence of *E. histolytica* and *Schistosoma* using (serology and fecal examination) was (50% and 21.67) and (42.22% and 1.67%), respectively.

**Table 1. Incidence of *Toxoplasma gondii*, *Entamoeba histolytica* and *Schistosoma* infection among HCV infected patients**

Parasite	Total	IgG	IgM
<i>Toxoplasma gondii</i>	180	117 (65.0%)	7 (3.89%)
	<b>Total</b>	<b>Serology</b>	<b>Fecal examination</b>
<i>Entamoeba histolytica</i>	180	90 (50.0%)	39 (21.67%)
<i>Schistosoma</i>	180	76 (42.22%)	3 (1.67%)

Data are expressed as: number (% among study population).

Regarding age, the higher incidence was recorded in older HCV patients infected with *T. gondii*, *E. histolytica*, or *Schistosoma* using serological tools than in younger patients ([Tables 2-4](#)). Similar results were obtained on the level of multiple parasitic infections ([Table 5](#)). Significant differences ( $P > 0.05$ ) were observed among the HCV older patients during the infection with *Schistosoma*, or the multiple infections with (*E. histolytica* and *Schistosoma*), (*T. gondii* and *E. histolytica*, and *Schistosoma*) when compared to younger patients. Without any significant changes, *E. histolytica* cyst detection in feces showed higher incidence in older HCV patients than in younger patients.

Gender-wise, a significant percentage increase ( $P > 0.05$ ) was observed among the HCV female patients during the acute infection with *T. gondii* (IgM positive) when compared to male patients ([Table 2](#)).

No significant changes were recorded during the infection with *E. histolytica* ([Table 3](#)). On the other hand, a significant increase ( $P > 0.05$ ) was observed among the HCV male patients during the infection with *Schistosoma*, or the multiple infections with (*T. gondii* and *Schistosoma*), (*T. gondii*, *E. histolytica*, and *Schistosoma*) when compared to female patients ([Tables 4 & 5](#)).

**Table 2. Socio-demographic characteristics and incidence of *T. gondii* infection among HCV infected patients**

Characteristics	Total	IgG	IgM
<b>Age</b>			
Less than(45)	49	26 (53.06%)	2 (4.08%)
45 and more	131	91 (69.46%)	5 (3.8%)
<b>Sex</b>			
Male	105	75 (71.43%)	0 (0.00%)
Female	75	42 (56.0%)	7 (9.33%)*
<b>Residence</b>			
Urban	108	72 (66.67%)	4 (3.70%)
Rural	72	45 (62.5%)	3 (4.16%)

Data are expressed as: number (% among study population). \*  $P < 0.05$  indicate significant difference.

**Table 3. Socio-demographic characteristics and incidence of *E. histolytica* infection among HCV infected patients using ELISA and fecal examination**

Characteristics	Total	IgG	Cyst
<b>Age</b>			
Less than(45)	49	17 (34.69%)	7 (14.29%)
45 and More	131	73 (55.73%)	32 (24.43%)
<b>Sex</b>			
Male	105	53 (50.48%)	23 (21.9%)
Female	75	37 (49.33%)	16 (21.33%)
<b>Residence</b>			
Urban	108	67 (62.04%)*	27 (25.0%)
Rural	72	23 (31.94%)	12 (16.67%)

Data are expressed as: number (% among study population). \*  $P < 0.05$  indicate significant difference.

**Table 4. Socio-demographic characteristics and incidence of *S. mansoni* infection among HCV infected patients using indirect haemagglutination test and fecal examination**

Characteristics	Total	IgG	Ova detection
<b>Age</b>			
Less than(45)	49	12 (24.49%)	1 (2.04%)
45 and More	131	64 (48.58%)*	2 (1.53%)
<b>Sex</b>			
Male	105	61 (58.09%)*	2 (1.90%)
Female	75	15 (20.0%)	1 (1.33%)
<b>Residence</b>			
Urban	108	37 (34.26%)	1 (0.93%)
Rural	72	39 (54.17%)	2 (2.78%)

Data are expressed as: number (% among study population). \*  $P < 0.05$  indicate significant difference.

**Table 5. Socio-demographic characteristics and incidence of mixed parasitic infections among HCV infected patients**

Characteristics	Total	Toxo+Enta	Toxo+Schi	Enta+ Schi	Toxo+Enta+ Schi
<b>Age</b>					
Less than(45)	49	7 (14.28%)	7 (14.28%)	2 (4.08%)	2 (4.08%)
45 and More	131	34 (25.95%)	23 (17.56%)	17 (12.98%)*	17 (12.98%)*
<b>Sex</b>					
Male	105	22 (20.95%)	25 (23.81%)*	13 (12.38%)	17 (16.19%)*
Female	75	19 (25.33%)	5 (6.67%)	6 (8.0%)	2 (2.67%)
<b>Residence</b>					
Urban	108	35 (32.41%)*	12 (11.11%)	11 (10.18%)	12 (11.1%)
Rural	72	6 (8.33%)	18 (25.0%)*	8 (11.11%)	7 (9.72%)
<b>Total</b>	<b>180</b>	<b>41 (22.78%)</b>	<b>30 (16.67)</b>	<b>19 (10.56%)</b>	<b>19 (10.56%)</b>

Data are expressed as: number (% among study population). \*  $P < 0.05$  indicate significant difference. Toxo: *Toxoplasma gondii*, Enta: *Entamoeba histolytica*, and Schi: *Schistosoma*.

Based on the residence, *E. histolytica* and (*T. gondii* and *E. histolytica*) incidence was significantly higher among urban area residents, 62.04% and 32.41% than rural area residents, 31.94% and 8.33%, respectively (Tables 3 & 5). Although a high percentage of *Schistosoma* was recorded among HCV rural residents compared to urban area residents, no significant difference ( $P > 0.05$ ) was detected (Table 4). On the other hand, the (*T. gondii* and *Schistosoma*) incidence was significantly higher among HCV rural area residents than urban area residents (Table 5).

Finally, mixed/multiple parasitic infections (*T. gondii* and *E. histolytica*), (*T. gondii* and *Schistosoma*), (*E. histolytica*, and *Schistosoma*), and (*T. gondii* and *E. histolytica*, and *Schistosoma*) were 22.78%, 16.67%, 10.56%, and 10.56%, respectively, in HCV patient's sera from Menoufia Province (Table 5).

## Discussion

In the current study, *T. gondii*, *E. histolytica*, and *Schistosoma* incidence in 180 HCV infected patients from Menoufia Province was detected. *T. gondii* IgG and IgM incidence were 65% and 3.89%, respectively. Moreover, *E. histolytica* and *Schistosoma* incidence using serological tools was 50% and 42.22%, their incidence using fecal examination was 21.67 and 1.67%, respectively. Multiple parasitic infections were determined among HCV patient's sera from Menoufia Province. Each parasite *T. gondii*, *E. histolytica*, or *Schistosoma* co-existence with HCV have been detected previously (6, 14, 20). El-Nahas et al. (2014), detected high *T. gondii* seropositivity 92.6% and 13.6% for IgG and IgM antibodies, respectively, among HCV cirrhotic patients and 76.9% and 12.8% for IgG and IgM antibodies, respectively, among HCV non-cirrhotic patients, who attended Mansoura University Hospital, Egypt (21). Moreover, using real-time

quantitative PCR, *T. gondii* parasitemia was positive in 31.4% of HCV-infected patients from Cairo, Egypt (14). Likewise, a high prevalence of 40.2% and 27.3% of both HCV and schistosomiasis were detected among Egyptian subjects (22, 23). Seventy-two cases out of 96 (75%) high positive anti-HCV antibodies Egyptian patients were anti- *Schistosoma mansoni* IgG positive (24). Previous studies reported high HCV antibody positivity among *Schistosoma mansoni* infected Egyptian patients (25, 26). A case study performed by Matsuo et al (1998), detected that *E. histolytica* subclinical infection in HCV female Japanese patient subjected to IFN therapy was developed into an amoebic liver abscess (20). While Abd-Alla and Ravdin (2002) demonstrated *E. histolytica* antigens in 43% using ELISA in Egyptian patients suffering from acute diarrhea from Cairo (27), Abozohra et al. (2020) detected *E. histolytica* in 14.7% of fecal samples using PCR in patients with gastrointestinal diseases in Behira, Egypt (18). According to our knowledge, this is the first report to detect multiple parasitic infections among HCV patients from Egypt.

In the current study, the sero-detection of *T. gondii*, *E. histolytica*, and *Schistosoma* mono-parasitic infection or multiple parasitic infections were increased among older HCV-infected patients. Similar patterns were obtained previously on the level of mono-parasitic infection among older HCV-infected patients (18, 24). Schistosome infection could happen at any age, by coming into contact with cercariae-containing freshwater (28). Al-Areeqi et al. (2017) reported that the age group of 18 to 40 years was considered among the major risk groups of amebiasis in Yemen (29). Moreover, *T. gondii* positivity was significantly observed more in older pregnant women than in the younger ones (17). Previous reports demonstrated the increase in *Toxoplasma* positivity with human age (30, 31).

In the present study, the incidence of the acute infection of *T. gondii* was increased among HCV female patients when compared to males. A previous report observed that Malaysian females from Pangkor Island in Peninsular were mostly infected with *Toxoplasma* compared to males (32). On the contrary, in the current study, the incidence of the acute infection of *Schistosoma* mono-parasitic or multiple parasitic infections was increased among HCV male patients when compared to females. Similar results were recorded among male HCV-infected patients infected with *Schistosoma* (24). A previous meta-analysis of thirty-two studies revealed that males were more often re-infected with different *Schistosoma* spp. (six and twelve months) after treatment with praziquantel than females (33).

Despite, several studies reported the high prevalence of *E. histolytica* in rural areas (18, 34), the current study demonstrated a significantly higher incidence among urban area residents, which suggest poor sanitation and unhygienic practices, especially in fast food shops/restaurants. In the present study, the incidence of *Schistosoma* and (*T. gondii* and *Schistosoma*) was higher among rural area residents. In these rural areas many activities such as crossing the contaminated water with bare feet, swimming in contaminated water streams, washing utensils and clothes using contaminated stream water, and fishing were among the major risk factors. In previous studies, similar findings have been demonstrated among rural residents (3, 35).

## Conclusion

*T. gondii*, *E. histolytica*, and *Schistosoma* infections are frequent among HCV Egyptian patients from Menoufia Province. Obtained results provide important data regarding the co-existence of HCV and *T. gondii*, *E. histolytica*, and/or *Schistosoma* in Egyptian patients and shed the light on the importance of screening of these parasites among HCV patients.

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

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