

Short Communication

Seroprevalence of amoebiasis in Najran Saudi Arabia

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Received 29 June 2016; received in revised form 17 April 2017; accepted 18 April 2017

Abstract. Amoebiasis is still the cause of one of the major health problem and a common life-threatening parasitic disease affecting 12% of the world population. The present study was undertaken to evaluate the seroepidemiology of amoebiasis in Najran City, Saudi Arabia. A total of 455 blood samples taken from healthy persons and surplus serum from various hospitals serology laboratories, over a one year period, between September 2014 to September 2015 were screened for the presence of amoebic antibody in their blood serum using indirect hemagglutination assay (IHA). Out of the 455 samples of sera tested, 158 (40.7%) were found to be positive with a titers ranging from 1:32 to 1:512. The younger age groups had the highest t positive rates than the other groups ($p < 0.0001$). The prevalence of infection was more common in females (57.3%) than males (29.8%), and showed statistical significance ($P < 0.001$). There was a significant association on the presence of *Amoeba* antibodies in relation to the nationality of residence, and the seasonal dynamics of infection ($P < 0.05$). The present study noticed that stray cats are potential reservoirs of infections and should be considered important to public health. The present findings highlighted that *Entamoeba histolytica* parasite is prevalent in Najran area. The establishment of such data will be beneficial for the public health authorities in the planning and implementation of specific prevention and control strategies of this infection in Saudi Arabia.

INTRODUCTION

Amebiasis, caused by the intestinal parasite *Entamoeba histolytica*, is the third leading parasitic cause of death worldwide (Pham *et al.*, 2011). It is a very common human gastrointestinal parasitic disease which affected 50 million people worldwide and accounts for more than 100,000 deaths annually (Stauffer & Ravdin, 2003).

Entamoeba histolytica is unique among amebae because of its ability to disrupt the intestinal mucosa, causing intestinal disease, amebic colitis, and the capacity for hematogenous spread which may

result into potentially fatal abscesses and extraintestinal diseases (Sen *et al.*, 2007).

The diagnosis of amebiasis is based on microscopic detection of the parasite in both fresh stool samples and in culture at 37°C. Due to morphological similarities between *E. histolytica* and the non-pathogenic *E. dispar* these tests may be misleading (Jackson, 1998).

During the last decade, a number of serologic tests have been employed as laboratory aids in both developed and developing countries (Tanyuksel *et al.*, 2003; Samie *et al.*, 2010; Yang *et al.*, 2012) for diagnostic purposes and for sero-

epidemiological studies of amebiasis, particularly, amebic liver abscess and to a lesser extent in suspected amebic colitis. In a recent review article on the laboratory diagnosis of amebiasis, the indirect hemagglutination (IHA) assay showed very good sensitivity and specificity that ranged from 85 to 100% (Tanyuksel *et al.*, 2003).

To our knowledge, no data on *E. histolytica* infection in Saudi Arabia are available, except for information on fecal examinations obtained during the first national survey of human parasites (Al-Harthi & Jamjoom, 2007). The current study was conducted to estimate the seroprevalence of *E. histolytica* infection and the possible associated health risks among population in Najran area, the southern region of Saudi Arabia to reveal not only the true prevalence of *E. histolytica* infection, but also for the public health concerns about amoebiasis.

MATERIALS AND METHODS

1. The study site and Sampling

The present research was carried out in the southern region of Saudi Arabia where almost 60% of the population lives in rural areas. The city of Najran; wherein the study was conducted, is located at latitude of 17° 30' 20" and longitude of 44° 11' 3" and at altitude of 1264 m above sea level. Furthermore, this cross-sectional study includes all blood samples (n=455) from apparently healthy persons and surplus serum with completely informed data from routine serological tests, conducted for other serological examination received at different general hospitals serology laboratory, Najran, Saudi Arabia during the period from September 2014 to September 2015. Additionally, a data sheet was used to record variables such as age, sex, nationality, and date of collection.

Since the stray animals have an important role in the transmission of some dangerous zoonotic diseases, especially in Asia, consequently a total of twenty five discharged cats' fecal samples were collected by simple random selection from public places in Najran city including

squares and children playgrounds (Sowemimo, 2012). Samples were examined using salt flotation method (Pritchard & Kruse, 1982).

2. Serological methods

Blood samples were allowed to clot, tubes were centrifuged at 2,400 rpm for 20 minutes at 4°C within 1 hour of collection, and serum aliquots were maintained at -20°C until tested for antibodies against *Amoeba* parasites. Commercial indirect haemagglutination assay (IHAT) (Cellognost Amoebiasis, Siemens Healthcare Diagnostic Products GmbH, 35041 Marburg/Germany) was carried out for the detection of antibodies against *E. histolytica* according to the manufacturer's instructions. Briefly, the test procedure was as follows. Fifty microliters of a 1:20 initial dilution of each serum was subjected to further twofold serial dilutions, and 10µl of sheep red blood cells sensitized with *E. histolytica* antigen was added to each diluted sample. Positive and negative control sera and non-sensitized red blood cells were included in each test as controls for naturally occurring antibodies. After incubation for 2 h at room temperature the titer in the test serum was recorded as one dilution before that which yielded a clear, sharp dark spot similar to those in the negative control wells. Titers were expressed as reciprocal values. All sera were tested in duplicate. The seropositivity was defined as a titer of 1:32 or greater as recommended by the manufacturer.

3. Data analysis

The significance of differences was analyzed using chi-square (χ^2) using the Statistical Package for Social Science version 15.0 (SPSS Inc., Chicago, IL), and $p < 0.05$ was considered significant.

4. Ethical considerations

The research proposal was approved by the Research Ethics Committee of the University (NU/MID/14/047) and informed written consent was obtained from the subjects for blood sampling and information collecting.

RESULTS

Through this study, antibodies to *E. histolytica* were found in 185 of 455 (40.7%), with IHAT titers of 1:32 in 55 participants, 1:64 in nineteen, 1:128 in twenty three, 1:256 in thirty five and 1:512 in twenty six subjects.

Assessing the results with respect to age, the present investigation showed that the seroprevalence rate varied in different age groups, ranging from 78.1% among children less than 6 years of age to 21.7% among subjects 31–40 years of age. The correlation between age groups and percent of positivity is illustrated in Table 1, and this marked difference was found to be statistically significant ($\chi^2=80.72$, $p<0.0001$). Likewise, the seropositivity was more prevalent in females (57.3%) than in males (29.8%), with marked statistical significance ($\chi^2=22.52$, $p<0.0001$) as depicted in Table 1.

Besides, females' samples recorded higher seropositivity for all age groups (0–20 years, 21–40 years and >40 years) compared to their male counterparts; 78%, 36.1%, and 40% versus 76.5%, 22.5%, and 21.7% respectively. Furthermore, the difference between the rate of infection between the age groups of male subjects in this study is statistically significant ($\chi^2=62.49$, $p<0.0001$, OR=11.7, 95%CI: 4.50–30.36). Similarly, the seropositivity among the age groups of female counterparts is statistically significant ($\chi^2=15.94$, $p<0.005$, OR=6.29, 95%CI: 2.30–17.18).

In the present study, the prevalence of Amoebiasis was affected by nationality of the examined participants as it was significantly higher in Saudi residents (66.3%) in comparison to non-Saudi people (25.4%) as depicted in Table 2.

In regard to the seasonal dynamics, the seroprevalence of *Amoeba* antibodies was highest in autumn (50.4%), followed by winter and summer seasons where the infection rates were 38.5% and 27.1% respectively, while the least was recorded during spring season (15.8%) (Table 3). This marked difference was found to be statistically significant ($\chi^2=32.32$, $p<0.0001$). As well as the same table illustrated the monthly dynamics of the *Amoeba* antibodies seropositivity among participants in Najran area.

Table 4 summarized the frequencies of the available age groups and sex of the tested Saudi population. From this table, it was observed that female Saudis (87.5%) had a higher seropositivity than their male counterparts (56.9%). This marked difference was found to be statistically significant ($\chi^2=9.26$, $p<0.005$). Additionally, the same table declared that the young age groups of Saudi natives of both gender had the highest seropositivity with *Anti- Amoeba* antibody as compared to other age groups.

The current research also reported that the overall prevalence of amoebiasis in stray cats of Najran area via coprological examination was 4% (1 out of 25).

Table 1. Frequency of anti- *E. histolytica* antibody among different age groups and gender of participants

	Number examined	Positive	%	Negative	%	OR (95% confidence)	95% CI	P-value
Age range (years)								
< 6 years	64	50 ^a	78.1	14	21.9	12.87	6.35–26.11	
7–19 years	35	21 ^b	60	14	40	5.40	2.48–11.78	
20–30 years	154	42 ^c	27.3	112	72.7	1.35	0.80–2.28	<0.0001
31–40 years*	152	33 ^d	21.7	119	78.3	–	–	
> 40 years	50	12 ^e	24	38	76	1.13	0.53–2.42	
Gender								
Male*	273	111	29.8	262	70.2	3.16	1.94–5.17	<0.0001
Female	82	47	57.3	35	42.7			

a, b, c, d, e value with different superscript in the same column differ at $p<0.05$, *Reference category, OR - odds ratio, CI - confidence interval.

Table 2. Comparison of Saudi and Non- Saudi residents with antibody seropositivity to *E. histolytica*

Nationality	No. examined	Infected n (%)	Negative (%)	<i>P</i> - value
Saudi residence	104	69 (66.3%)	35 (33.7)	0.0001
Non- Saudi residence				
Egyptian	56	20 (35.7%)	36 (64.3)	
Yemeni	40	11 (27.5%)	29 (72.5)	
Sudanese	48	15 (31.3%)	33 (68.7)	
Ethiopian	22	7 (31.8%)	15 (68.2)	
Indian sub-continent	185	36 (19.5%)	149 (80.5)	
Total	351	89 (25.4%)	262 (74.6)	

Table 3. Monthly and seasonal dynamics of *E. histolytica* seropositivity

Month	No. examined	Infected n	%
January	39	37	94.9
February	96	15	15.6
Winter	135	52	38.5
April	43	8	18.6
May	52	7	13.5
Spring	95	15	15.8
July	61	17	27.9
August	35	9	25.7
Summer	96	26	27.1
November	33	26	78.8
December	96	39	40.6
Autumn	129	65	50.4

Table 4. Seroprevalence of anti- *E. histolytica* antibody among different age groups and gender of Saudi participants

Age range	Saudi natives					
	Male			Female		
	No. tested	No. positive	%	No. tested	No. positive	%
< 6 years	32	23	71.9	20	19	95
7–19 years	12	9	75	11	9	81.8
20–30 years	13	4	30.8	1	0	0
31–40 years	13	5	38.5	0	0	0
> 40 years	2	0	0	0	0	0
Total	72	41	56.9	32	28	87.5
<i>P</i> value	<0.005					

DISCUSSION

Amoebiasis is still considered as a major public health problem worldwide in tropical and subtropical regions with crowded population, poor hygiene and lower socio-economic levels. In developed countries, it was found only in travelers, immigrants, institutionalized persons and homosexuals. From a public health perspective, an overview on the prevalence of *E. histolytica* infection in Saudi Arabia could help predict future effects of *E. histolytica* infection on health care resources, as well as provide opportunities for evaluating the interventions.

Several coproscopic studies have been carried out in various regions of Saudi Arabia including Abha district and Al-Khobar (Abahussain, 2005), Al-Baha (Mohammad & Koshak, 2011), south Jeddah, Jeddah city (Hegazi *et al.*, 2013; Barnawi *et al.*, 2007) and Riyadh region (Eligail *et al.*, 2010) concerning female house keepers and food handlers. The results of the aforementioned surveys indicated that the prevalence of the disease ranged between 0.14% to 20.0%. Due to low sensitivity and specificity for detection of amoebiasis using microscopy these prevalence rates might be underestimates.

Conversely, because the serosurvey detected a sustained antibody response that persisted even after the infection (Abd-Alla *et al.*, 1998; Stanley *et al.*, 1998), it contributed to the higher prevalence of infection in the present study (40.7%) compared with other previous surveys carried out worldwide like China, 17.3% (Yang *et al.*, 2012), and Turkey, 8.5% (Kurt *et al.*, 2008). The seropositivity we found in the current study provides an overview of *E. histolytica* infection in Saudi Arabia, but does not provide information on the frequency of amoebiasis.

The present result was nearly consistent with previous studies carried out in Malaysia and Taitung City, Taiwan that reported the overall seropositivity of amoebiasis was 35.0% and 39.7%, respectively (Farhana *et al.*, 2009; Chu *et al.*, 1992). No data was available regarding the seroprevalence of amoebiasis in Saudi Arabia.

The findings of the current study showed that the age groups <6 years, and those in the 7 to 30 year age group had higher positivity rates compared with other age groups. This result was in line with previous worldwide reports (Bernal *et al.*, 2000; Sayyari *et al.*, 2005; Noor Azian *et al.*, 2007; Canete *et al.*, 2012; Reuben *et al.*, 2013; Mbae *et al.*, 2013). From our results and previous studies it is clearly indicated that *E. histolytica* infection is more prevalent in younger age groups. This could be explained on the basis that the younger people have lower resistance as compared to adults and because many of the crucial defense systems that help protect adults from disease are not fully developed in children, they are much more sensitive to parasites than adults. Furthermore, they do not take care of their personal hygiene, such as playing in contaminated outdoor environments, in and around disposal sites (which can certainly cause serious health problems), lack of fecal hygiene (Abu Mourad, 2004) and lack of washing hands before meals (Nematian *et al.*, 2004).

With regards to gender, the present study found a significant difference in the prevalence of amoebiasis between male and female participants. Female showed higher seropositivity than their males' counterparts. Similar findings of more prevalence were recorded in females (4.87%) than males (3.88%) in Mexico (Gonzalez *et al.*, 1995) and (9.46%) in females (8.15%) in males in Multan, Pakistan (Tasawar *et al.*, 2013). This could be due to hormonal fluctuation in females during various stages of reproductive cycle that may affect their immunity and help opportunistic parasites to establish (Mazigo *et al.*, 2010). Moreover, women do washing of clothes and cooking the more. This may increase exposure to waterborne diseases and may explain the increased prevalence of disease in women (Jamieson *et al.*, 2006). Female's personal hygiene like not washing hands before eating and after defecation, use of ordinary papers for cleaning after defecation and house hold practices are additional risk factors for the higher prevalence in female care takers (Amuta *et al.*, 2010).

Concerning the nationality, the present records showed that the rate of human amoebiasis was higher among Saudi residents compared to non-Saudi people. This variation could be explained due to variation in number of Saudi people tested compared to expatriates examined. Additionally, the increased prevalence of *E. histolytica* among Saudi natives might be related to the type of domestic water supply as the main source of water used from wells for drinking and tap water from home tanks for other purposes in our locality in the southern region of Saudi Arabia compared to other endemic areas. Similarly, this risk factor was proved to be the sole factor significantly associated with high prevalence rates of *E. histolytica* infection of 9.2% in south Jeddah, Saudi Arabia (Hegazi *et al.*, 2013).

When the data on monthly and seasonal incidence of amoebiasis was analyzed, it was observed that higher incidence of amoebiasis occurred in the autumn season (50.4%). This result could be explained due to the main important factors influencing the incidence of amoebiasis which are adequate temperature and moisture in the environment, which helped in hatching of cysts and development of rapid life cycle stages.

In the current study, Saudi females were more affected (87.5%) than their male counterparts (56.9%) with *E. histolytica* infection in Najran city. The infection patterns between women and men were significantly different. The high rate of female with *E. histolytica* infection in Najran is due to the fact that most Saudi families favor female housemaids over males for cultural, religious and traditional reasons (Abahussain, 2005). Also studies done on the expatriates working in Saudi Arabia reported different prevalence rates. It varies between 55.7% and 41.4% in Riyadh (Abdul-Hafez *et al.*, 1987) and 40.3% in Jeddah (Al-Fayez & Khogheer, 1989) and 46.5% in Abha (Al-Madani & Mahfouz, 1995).

The feces of stray cats in general environment are important as the potential source of gastrointestinal parasites from medical and veterinary point of view and play a major role in transmitting these parasites through fecal contamination of soil, food or

water. Therefore human health education is recommended in the communities through prevention measures should implement include control of stray cats and their exclusion from public places and children's playgrounds.

CONCLUSIONS

Gaining an overview of the seropositivity of *E. histolytica* infection in Najran city, the southern area of Saudi Arabia, provide a more accurate measure of incidence and prior probability of the prevalence of infection, thereby facilitating better resource allocation to protect the population from public health concerns. Moreover, the high prevalence of *E. histolytica* infection detected in this study warrants the implementation of actions directed toward health promotion and preventive measures. Further studies concerning the other associated personal and environmental factors such as level of education, income, contact with farm animals and method of sewage disposal are needed.

Recommendations

Diagnostic methods that are more sensitive and specific than light microscopy are required to establish the true distributions of *E. histolytica* and to reduce the rates of unnecessary treatment, thereby discouraging the development of drug resistance, precluding the risks of side effects, and reducing the costs of hospitalization.

Improved water supplies, together with obligatory inspection measures during transportation and distribution should be concerned.

Authors' Disclosures of Potential Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments. Authors are grateful to the Deanship of scientific research, Najran University for supporting and funding this research (NU/MID/14/047).

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