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Research Note

Some epidemiological and serological studies on schistosomiasis in Najran area,
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Summary

Schistosomiasis is a major cause of morbidity and mortality and has been estimated to infect over 200 million people. Diagnosis of schistosomiasis by detection of specific antibodies is likely to be more sensitive than the traditional method of diagnosis by detection of eggs in stool or urine. Therefore the present study was the first attempt to highlight the seroepidemiology of schistosomiasis among the general population of Najran City, southern of Saudi Arabia, as well as to achieve the performance of the diagnostic tests used. A total of 180 participants attending King Khaled hospital in Najran Province, Saudi Arabia, over a one year period, from September 2013 to September 2014 were screened for the presence of *Schistosoma* antibody in their blood serum using an indirect hemagglutination assay (IHA). Specific immunoglobulin (Ig) G antibody was evaluated using an enzyme-linked immunosorbent assay (ELISA). Out of the 180 samples of sera tested using IHA, 32 (20 %) were found to be positive with a titer ranging from 1:160 to 1:1280, while 42(23.2 %) revealed *Schistosoma* IgG. A positive relationship was found between the seroprevalence of schistosomiasis and age of tested participants, especially in the age group of 20-40 years old. Additionally, prevalence of infection was more in males (36 %) than females (7.5 %), and showed statistical significance ($P < 0.001$). Similarly, there was significant association between the presence of *Schistosoma* antibodies and the nationality of residence, and education of participants ($P < 0.05$). The current investigation reveals an alarmingly high prevalence of schistosomiasis among participants in Najran, southern region of Saudi Arabia and this supports an urgent need to re-evaluate the current control measures and implement an integrated, targeted and effective schistosomiasis control measures.

Keywords: Indirect hemagglutination assay (IHA); Enzyme-linked immunosorbent assay (ELISA); seroepidemiology; schistosomiasis; Saudi Arabia

Introduction

Schistosomiasis is one of the oldest parasitic infections of man, yet it remains a serious public health concern throughout the world's tropical and subtropical regions, affecting more than 200 million people (WHO, 2002; King *et al.*, 2005). One factor contributing to high disease prevalence is the scarcity of effective diagnostic

methods to detect infections and response to drug treatment and to assess the success of control measures (Bergquist *et al.*, 2009). The traditional gold standard for the diagnosis of schistosomiasis includes parasitological detection, commonly done via the use of Kato-Katz thick smears (Katz *et al.*, 1972). The sensitivity of this diagnostic technique depends on the rate of egg excretion (Engels *et al.*, 1996), which can be very low or even absent when para-

site transmission is low or when intestinal fibrosis has occurred. Hence, a major disadvantage of parasitological detection as a diagnostic technique is that it exhibits low sensitivity in areas of low endemicity (Lin *et al.*, 2008; Zhang *et al.*, 2009), labor-intensive and time-consuming. This approach is not suitable for large-scale disease surveillance and improvement in diagnosis is needed. Immunodiagnostic detection of schistosome antibodies is more sensitive and less time consuming than parasitological diagnosis and has thus become a more attractive option for the diagnosis of schistosomiasis (Zhu, 2005). These alternative diagnostic techniques have proven particularly useful for diagnosing atypical forms of schistosomiasis (e.g., infections with low parasite burdens) and in disease surveillance (Doenhoff *et al.*, 2004; Cesari *et al.*, 2005; Sorgho *et al.*, 2005; Sulahian *et al.*, 2005; Enk *et al.*, 2008). In Saudi Arabia, the prevalence of schistosomiasis was 2.2, 2.9, and 2.78/100,000 in 2000, 2004, and 2008 respectively, according to the Saudi Arabia Ministry of Health Statistic Book, (<http://www.moh.gov.sa/statistics/1425/Default.html>).

Furthermore, the highest schistosomiasis rates were recorded in Aseer (39%), Jazan (27.6%), Bishah (19.3%), and Al-Bahah (9.8%), while the least rate was observed in Riyadh (0.2%) and Tabouk (0.2%) (Shati, 2009). Although a real hope for morbidity control of schistosomiasis emerged with the discovery of the highly efficacious and broad-spectrum drug praziquantel in the 1970s (Cioli & Pica-Mattocchia, 2003), the epidemiological situation in some countries remains alarming (Engels *et al.*, 2002). In addition, there is scarce information regarding schistosomiasis in Saudi Arabia, particularly in the Najran area. Therefore, the objectives of the present study are to determine the seroprevalence of schistosomiasis among participants in Najran city and identify the risk factors for the infection within the population in order to understand the disease epidemiology, and consequently to formulate a suitable integrated strategy for control. Furthermore, the performance of serological tests used was evaluated.

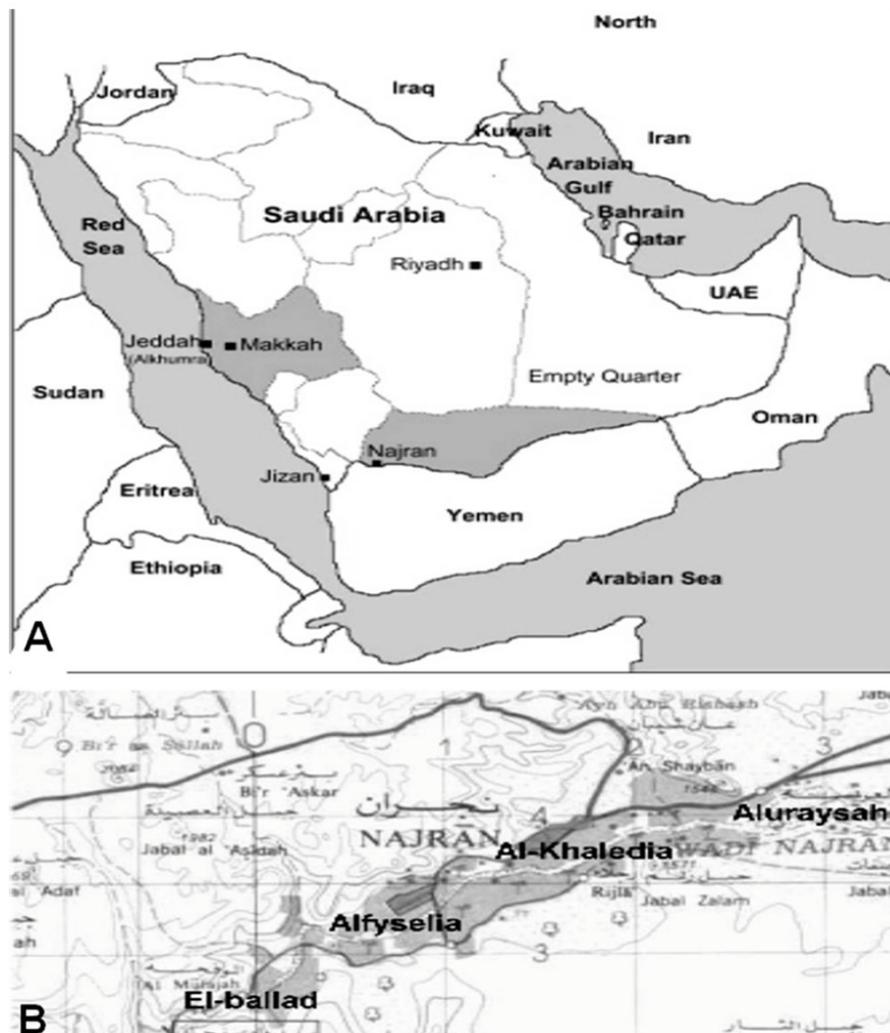


Fig. 1. Map of Saudi Arabia showing: A – Najran city, B – The four study districts in Najran

Table 1. Indirect haemagglutination assay (IHA) and enzyme-linked immunosorbent assay (ELISA) seroprevalence of *Schistosomiasis* in Najran region, Southern Kingdom of Saudi Arabia

Tests	Number of total samples	Number of positive samples (%)	Titer				
			1:1280	1:640	1:320	1:160	1:1280
IHA	180	36 (20)	14	6	4	12	14
Eliza	180	42(23.3)	-	-	-	-	-

P-value= 0.44

Material and Methods

I- Study area

This study was conducted in the Southern region of Saudi Arabia, particularly in the Najran city, which lies between 17°30'20" North and 44°11'3" East, near the border with Yemen. Almost 60 % of the population lives in rural areas in close contact with livestock.

II- Patients and collection of samples

This laboratory-based study includes all blood samples from apparently healthy persons screened for periodic health check, received at the King Khaled hospital serology laboratory, Najran, Saudi Arabia during the period September 2013 to September 2014. Four districts were selected for this study namely, Aluraysah, Al-Khaleidia, Alfyselia and El-ballad (Fig. 1). During this cross-sectional survey, a single blood sample (5ml of blood by venipuncture) was taken from each participant (n=180) under aseptic conditions. A questionnaire on activities and knowledge concerned with schistosomiasis was answered by respondents aged 10 – 19, 20 – 40 and >40 years of age. Sera were prepared after centrifugation and a trace of sodium azide was added. Sera were stored frozen at -20 °C and transported on dry ice to the Department of Applied Medical Sciences, Community College, Najran University, Najran, Saudi Arabia.

III- Indirect haemagglutination (IHA)

The IHA test kit sold by Fumouze Laboratories (*Schistosomiasis* fumouze, FUMOUE Laboratories, Levallois- Perretcedex, France) was used 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 10 µl of sheep red blood cells sensitized with *S. mansoni* 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 results were evaluated with a cut-off titer of 1:160 as recommended by the manufacturer.

IV- Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) was also used for the evaluation of anti-*Schistosoma mansoni* IgG antibody with ELISA set (RE 58711, IBL international GMBH, Flughafenstrasse 52a D-22335, Hamburg, Germany). Antibody levels were evaluated by following the manufacturer's instructions at the laboratory of the Department of Applied Medical Sciences, Community College, Najran University, Najran, Saudi Arabia.

V- 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. Additionally, the degree of agreement between the results from the 2 tests was quantified using κ statistics and the accuracy of the ELISA test in detecting exposure to *Schistosoma* was evaluated in comparison to the IHA, and measured using the relative sensitivity and specificity.

Table 2. Seroprevalence and frequency of anti-*Schistosoma mansoni* IgG antibody (ELISA Test) among different age groups of participants from the Najran region, Southern Kingdom of Saudi Arabia

Age range (years)	No. tested	No. positive	%	No. negative	%	OR (95% confidence)	95% CI	<i>P</i> -value
10 – 19*	60	6 ^a	10	54	90			
20 – 40	60	24 ^{b,c}	40	36	60	6.0	2.232 – 16.132	<0.001
> 40	60	12 ^c	20	48	80	2.25	0.784 – 6.456	

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

Table 3. Distribution of anti-*Schistosoma mansoni* IgG with socio-demographic variables, among examined participant from the Najran region, Southern Kingdom of Saudi Arabia

Variables	Schistosomiasis		P- value
	No. examined	Infected n (%)	
Sex			
Male	100	36 (36)	<0.001
Female	80	6 (7.5)	
Site of residence			
Aluraysah	66	16 (24.2)	0.516
Al Khaledia	30	6 (20)	
Alfyselia	48	14 (29.2)	
El-ballad	36	6 (16.7)	
Fathers' educational levels			
Primary	60	24 (40)	<0.001
Secondary	60	15 (25)	
University	60	3 (5)	

Results

The obtained results showed that antibodies to *Schistosoma* species were found in 36 out of the 180 participants (20 %) with the IHA test. Twelve samples were positive with a 1:160 titer; 4 with a 1:320 titer; 6 with a 1:640 titer and 14 with a 1:1280 titer. On the other hand, 42 (23.3 %) samples of sera were found to be seropositive for schistosomiasis (*Schistosoma mansoni*), whereas 138 (76.7 %) were seronegative using ELISA as displayed in Table 1. There was no significant difference detected between positive and negative results when comparing IHA and ELISA results with chi-square test ($p=0.44$) and the strength of agreement between the 2 tests was considered to be very good. Therefore, not only did the ELISA test using anti-IgG antibody give a 100 % specificity but it also exhibited a 85.7 % sensitivity for identifying infected individuals as illustrated in Table 5.

Evaluating the results with respect to age, the distribution of positive serum samples among different age groups for anti-*Schistosoma* IgG showed that participants in the age group of 20-40 years had the highest percentage (40 %) of positive results followed by the age group over 40 years (20 %), while the age group of 10-19 years showed the lowest percentage (10 %). The correlation between age groups and percent of positivity is illustrated in Table 2, and this marked difference was found to be statistically significant ($\chi^2=15.65$, $p<0.001$). Similarly, the frequency of infection was higher among the male participants (36/100) 36 % compared to the female counterpart (6/80) 7.5 % ($p<0.001$; Table 3). Based on the study results, the highest seroprevalence was observed in Alfyselia (29.2 %), while the lowest rate was found in participants serum obtained from El-ballad district (16.7 %). This marked difference was not found to be statistically significant ($\chi^2=0.865$, $p=0.644$) as shown in Table 3.

Table 4. Comparison of Saudi and Non- Saudi residents with IgG seropositivity to *Schistosoma mansoni*

Nationality	No. examined	Infected n (%)	P- value
Saudi			
Male	30	4 (13.3) ^a	<0.005
Female	30	0 (0) ^b	
Total	60	4 (7)	
Non- Saudi			
Male	70	32 (46) ^c	
Female	50	6 (12) ^d	
Total	120	38 (32)	

^{a, b, c, d} value with different superscript in the same column differ at $p<0.05$

Table 5. Diagnostic performance of indirect hemagglutination assay (IHA) and enzyme-linked immunosorbent assay IgG (ELISA IgG) of participants (n=180) with antibodies against schistosomiasis

Variables	ELISA IgG		Sensitivity (Se)	Specificity (Sp)	κ value	SE of kappa
	-VE (Negative) n=138	+VE (Positive) n=42				
IHA(+VE)	0	36	0.857(0.714 – 0.945)	0.1 (0.973 – 0.1)	0.902	0.039
IHA(-VE)	138	6				

Numbers in parentheses are 95 % confidence interval, note-sensitivity=true positives detected/(true positives + false negatives); specificity=true negatives detected/(true negatives + false positives), SE - standard error

With regards to fathers' education level, the current study showed that the prevalence of schistosomiasis was significantly higher among participants of primary educated fathers (40 %) compared to the other education levels as illustrated in Table 3.

In the present study, the prevalence of schistosomiasis was affected by nationality of the examined participants as it was significantly higher in non-Saudi residents (32 %) in comparison to Saudi people (7 %) as depicted in Table 4.

Discussion

Schistosomiasis is a water-borne disease which is considered the second most important parasitic infection after malaria in terms of public health and economic impact. Concern has grown since schistosomiasis may progress to chronicity in the absence of any signs and symptoms of disease in individuals who are repeatedly or occasionally exposed to contaminated water, for example, travelers (WHO, 2009)

The present study was the first attempt to explore the seroprevalence of schistosomiasis in Saudi Arabia, particularly the southern region.

According to the various studies conducted in Saudi Arabia, these results indicated that the disease was recorded in 12 regions at a prevalence ranging from 5 % to 20 % via coproscopic examination (Ashi *et al.*, 1989). Recently, details of human infection were delineated in a study done in 2004; results showed that Saudis accounted for 61.2 % of total infected cases and infection peaked at 15 – 39 years, providing evidence about the endogenous source of infection. In addition males were four times more infected than females (Barakat *et al.*, 2014).

Arfaa in 1976 reported that transmission of schistosomiasis is limited only to a few foci in rural areas, and the disease is rare in large cities, e.g., Riyadh, Jeddah, Mecca, Taif, and Tabouk. He concluded that the snail habitats – which consist of wells, small canals, cisterns, small swamps, interrupted streams and ponds – create a special type of transmission which can be defined as “oasis transmission,” making control of the disease both simple and practical. However, the agricultural irrigation projects and construction of new dams have led to creation of permanent breeding habitats for

the snail intermediate hosts over a wide region of KSA (Ghandour *et al.*, 1986; 1990).

Through this study, the seropositive rates of IHA were 20 %, and of ELISA were 23.3 %, which is considered alarmingly high. This difference of positive rates may have resulted partly from the different antigenic epitope recognized by IHA and ELISA, and partly from a difference in the limits of sensitivity of both tests (Song *et al.*, 2005). Furthermore, this prevalence was nearly consistent with other previous studies carried out in some neighboring Arab countries like Yemen (Raja'a *et al.*, 2000; Raja'a *et al.*, 2001; Al-Shamiri *et al.*, 2011). No data was available regarding the seroprevalence of schistosomiasis in Saudi Arabia. Therefore, the present investigation was the first to consider them.

The findings of the current study also showed that participants aged 20 – 40 years were significantly more prone to be infected than younger and older ones. This result was in agreement with previous reports worldwide (Raja'a *et al.*, 2000; Gryseels *et al.*, 2006; Mafuyai *et al.*, 2006; Matthys *et al.*, 2007; Ugbomoiko *et al.*, 2010; Deribe *et al.*, 2011). This could be explained by the excessive mobility of people at this age and they may become more exposed to infected water while swimming/playing or fetching water for domestic purposes or helping in agriculture activities. Additionally, Galvani (2005) in his field study reported that schistosomes and the major intestinal nematodes *Trichuris trichiura* and *Ascaris lumbricoides* repeatedly demonstrate that the intensity and prevalence of infection exhibit marked dependency on host age.

With regards to gender, the present study found a significant difference in the prevalence of schistosomiasis between male and female participants. These are consistent with many other reports worldwide (Garba *et al.*, 2010; Ahmed *et al.*, 2012). Males usually have higher prevalence rates of schistosomiasis than females and this was attributed to religious and cultural reasons or to water contact behavior (El-Khoby *et al.*, 2000; Haidar, 2001; Matthys *et al.*, 2007; Deribe *et al.*, 2011). However, significantly higher infection rates among females compared to their male counterparts have been also reported elsewhere (Satayathum *et al.*, 2006; Rudge *et al.*, 2008). In Saudi Arabia, and many other Islamic countries, females are prohibited from bathing in open water sources whereas the males frequently play and swim during their leisure time.

Hence, community-based drug distribution should also be considered together with the school-based control in order to reach this group and reduce the transmission in the entire communities.

The observed differences between districts that have been attributed to the differences in size and methodology of studies could partly explain the observed findings. Also, the wide range of infection prevalence rates among districts in our study illustrates the focal distribution characteristic of schistosomiasis.

We have also identified participants' educational level as a significant predictor of schistosomiasis. However, previous studies among rural communities in some neighboring countries like Yemen found no association between the prevalence of schistosomiasis and the fathers' or participants' educational status (Raja'a *et al.*, 2000; Nagi, 2005). On the contrary, in Cote d'Ivoire and Nigeria, the higher education level of the head of family was identified as a protective factor against schistosomiasis (Matthys *et al.*, 2007; Ugbomoiko *et al.*, 2010).

With respect to nationality, the present figures showed that the rate of human schistosomiasis was higher among non-Saudi residents (nationals of Egypt, Pakistan, Bangladesh and India) compared to Saudi people. This likely due to the fact that most of workers in farming and other activities require contact with water are usually expatriates from such foreign countries. Additionally, uncontrolled population movement from the highly endemic neighborhood in Yemen and the creation of multiple irrigation projects.

One of the major challenges for control of schistosomiasis worldwide is the animal reservoir host. In Saudi Arabia, a population of several hundreds of thousands of the hamadryas baboons (*Papio hamadryas*) inhabit an extensive range from the Yemen border to the southwest area of Saudi Arabia. They live in close association with human and use the same water sources in their habitat where human schistosomes have been recorded (Yamane *et al.*, 2003). Therefore, it is worth mentioning that schistosomiasis has been eradicated from Japan despite widespread animal reservoir hosts contributing to over 80 % of transmission (Wang *et al.*, 2005). Also, irrigation projects can be constructed to hinder breeding places and to limit human access for domestic activities; China is a good example where control of schistosomiasis is being maintained in spite of vast expansion of irrigation projects through installation of concrete irrigation canals and piping systems for the water supply in order to eliminate the infection sources and *Oncomelania* snails. In addition, mollusciciding and environmental modification were carried out at an early stage (Fenwick *et al.*, 2006).

In conclusion, this survey is important because it can be considered as a baseline for further studies. Furthermore, the current investigation reveals an alarmingly high prevalence of schistosomiasis among participants in Najran, southern region of Saudi Arabia and this supports an urgent need to re-evaluate the current control measures and implement an integrated, targeted and effective schistosomiasis control measures. Besides periodic drug distribution, health education regarding good personal hygiene and good sanitary practices, provision of clean and safe drinking water, in-

troductory of proper sanitation are imperative among these communities in order to curtail the transmission and morbidity caused by schistosomiasis.

Ethical considerations

The research proposal was approved by the Research Ethics Committee of the University and informed written consent was obtained from the subjects for blood sampling and information collecting.

Conflict of Interests

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

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