Seroepidemiology of Zikavirus in Basrah, Southern Iraq

ShantASinbat ,[1]Alaa K Mousa[2,] Hassan J Hasony[1]

Department[1] of Microbiology, Dept[2] of Medicine, Basrah medical college

Abstract

Background: There were no laboratory-confirmed Zika virus (ZIKV) infection in southern Iraq., where Aedesmosquitoes are prevalent, has a close relationship with Southeast Asian countries. The aim of this serosurvey was to assess whether there was unrecognized ZIKV infections in Basrah.

Methods: A total of 282 serum samples collected in a cross-sectional seroepidemiologic study conducted during the end of the 2019 to December 2020, . Anti-ZIKV IgM and IgG were tested using commercial enzyme-linked immunosorbent assays (ELISAs).

Results: The mean age of the 282 participants was 45.0 years , and female was predominant (61.7%). Anti-ZIKV IgM and IgG were detected in 4 (1.4%) and 18 (6.3%) of the 282 participants, respectively.Seropositivity to ZIKV associated with increased age and Basrah district special habitat.

Conclusions: The results suggest that undetected indigenous ZIKV transmission might have occurred in Basrah. The findings also suggest that the threat of epidemic transmission of ZIKV in southern Iraq does exist due to extremely low-level of herd immunity.

Keywords: Zika virus, Seroprevalence ,Flaviviruses, southern Iraq,

INTRODUCTION

Zika virus disease is caused by a virus transmitted primarily by Aedes mosquitoes, which bite during the day.Symptoms are generally mild and include fever, rash, conjunctivitis, muscle and joint pain, malaise or headache. Symptoms typically last for 2–7 days. Most people with Zika virus infection do not develop symptoms[1].Zika virus infection during pregnancy can cause infants to be born with microcephaly and other congenital malformations, known as congenital Zika syndrome[2]. Infection with Zika virus is also associated with other complications of pregnancy including preterm birth and miscarriage.An increased risk of neurologic complications is associated with Zika virus infection in adults and children, including Guillain-Barré syndrome, neuropathy and myelitis .Zika virus infection can have an impact on fetal development and lead to severe neurodevelopmental abnormalities[3].

A number of seroprevalence studies have recently been conductedin affected countries among specific subgroups of populations and geographicalareas. ZIKV seroprevalence was found to be up to 73% in a cohort of individuals residing in Pau da Lima communityin the Salvador [4]. In Bolivia, ZIKV seroprevalencewas estimated in blood donors at 39% in Beni, 21.5% in SantaCruz de la Sierra and close to 0% in three different highlandregions (Cochachamba, La Paz and Tarija) [5]. InNicaragua, ZIKV seroprevalence was estimated at 36%, 46%,and 56% among participants of pediatric, household, and adultcohort studies, respectively [6]. These studieswere all performed in a small number of communities or specificpopulation subgroups in a constrained region. It is unclearwhether their findings are generalizable to the wider population.Such population-representative studies constitute the most reliablesource of information and often estimate seroprevalencerates that are lower but more representative than those obtainedin population subgroups [7,8].

In this cross-sectional study withinthe general population of Basrah aimed to characterize the seroprevalence ofZIKV and assess its association with sociodemographic and geographicalfactorsand to assess whether there was unrecognized ZIKV infections in Basrah.

MATERIALS AND METHODS

Study site

Basrah is located in southern Iraq,has an estimated population of ∼3.796.000. This study was conducted in foursites thatwere selected to represent almost all population. In Basrah, there is a likelihood of transmission to humans, from lakes and river in these areas that may serve as breeding sites for the vectors and a habitat for arboviruses reservoirs.

Study population

Serum samples of 282patients that live in Basrsh region were collected in the years 2019 and 2020. The samples were collected from patients during their visit to hospitals in Basrah city: the northern area(A)=Al-Mawanaateachinghospital, Al-Faihaateachinghospital, southern area (B)=Al-sader teaching hospital, Western area (C)=Al-basrah teaching hospital,Eastern are (D)= Basrahpaediatric hospital andOncology center. In this group, acute febrile illness(symptomatic) was the most common underlying disease (80% of the patients), followed by fever (70%), and joint pain (45%).Also healthy volunteers (Asymptomatic) were included to estimate the rates of exposure in Basrah community. The mean age of the study population was 45 years (range = 10–80). Male to female ratio was 1:1.6 (39.3% males and 60.6% females) of the total study population.

The study utilizes thosesamples from a hospital-based cross-sectional descriptive survey and retrospective data from an ongoing study that started in December 2019. To augment the collection samples of febrile illness patients from identified hospitals were collected with the assistance of clinical officers in the four respective study districts

A total volume of approximately 5 mL of blood was collected from the participants. Blood samples were incubated at room temperature for 1 hour, followed by 15 minutes centrifugation at 3,000 rpm and collection of the serum fraction. The serum samples were stored at −70°C until use.

Anti-ZIKVIgM antibodywas detected using commercial kits namely; MAC-ELISA Kit for of Arboviruses (ZIKV) IgM Capture (DxSelectTM, FOCUS Diagnostic, USA). An enzyme-linked immunosorbent assay (ELISA) specific for detection of Anti human IgG Immunoglobulin to ZIKV was used and the procedure followed according to the manufacturer's instructions(DxSelectTM, FOCUS Diagnostic, USA).

Statistics
Chi-squared test was used to compare the seroprevalence between genders and between age groups.

RESULTS

Table- 1: Area distribution of participants with serologic profileof ZIKV infections in Basrah governorate.

Area	Area of samples collection	No. tested(%)	IgM +ve(%)	IgG +ve(%)	IgM/IgG +ve(%)
A	North(Served by Al-Mawanae and Al-Faiha Teaching hospital)	150	3(2%)	10(6.7%)	13(8.7)
B	South(Served by AlSader T Hospital)	66	1(1.5%)	3(4.5%)	4(6%)
C	West(Basrah General Hospital)	51	0	3(5.9%)	3(5.9%)
D	East (Pediatric & Oncology hospital)	15	0(20%)	2(13.3%)	2(13.3%)
Total		282	4(1.4)	18(6.38%)	22(7.8%)

The overall seroprevalence of ZIKV was 7.8% among the participants in Basrah governorate (those with recent or old exposure; anti-ZIKV IgG and IgM). The northern part of the city (Area A) showed higher rates of seropositivity which was 2% for IgM and 6.7% for IgG antibodies. However, other areas showed variable rates of seropositivity (4.5% and 5.9% for areas B and C respectively).On the other hand, patient at areas D represent the highest rate of seropositivity (13.3%) as this group of patients are a pool of symptomatic cases (Table-1).

Table-2:   ZIKV seroprevalence in relation to residence

Residence	No tested N (%)	No(%) IgG/IgM	No (%) IgM +ve	No (%) IgG +ve
Rural	169	17(10%)	3(1.8%)	14(8.3%)
Urban	113	5(4.4%)	1(0.9%)	4(3.5%)
Total	282	22(7.8%)	4(1.4%)	18(6.4%)

* P …??

 Table-2 show that the exposure rate among rural population was higher (10%) than that for urban residents (4.4%). Similarly , the rate of recent infection (anti-ZIKV IgM) also higher among rural community (1.8%) compared to that among urban group (0.9%). However, the prevalence of anti-ZIKV IgG following the same trend where the rate greater in rural group(8.3%) compared to the urban group (3.5%). The differences is statistically significant (P<0.05).

Table -3: Symptomatic status of study population in relation to ZIKA seropositivity

Symptoms	No. tested(%)	ZIKV–IgM N(%)+ve	ZIKV–IgG N(%)+ve
Symptomatic	174(61.7)	1/174(0.6)	8/174(4.6)
Asymptomatic	108(38.3)	3/108 (2.8)	10/108 (9.3)
Total	282	4/282(1.4)	18/282(6.4)

Table-3 shows the symptomatic status of participants in relation to ZIKV seropositivity where 6.3% possess anti-Zika virus IgG and 1.4% with recent infections .The majority of enrolled individuals were symptomatic(61.7%) among them 4.6% were anti-ZIKV IgG positive which was less than that among asymptomatic individuals which was 9.3% .The differences in seropositivity between symptomatic and asymptomatic was statistically significant (P<0.05).

Table-4: Distribution of ZIKV seropositivity in relation to gender

Gender	No. tested (%)	ZIKV–IgM N(%)+ve	ZIKV–IgG N(%)+ve
Male	108(38.3)	1/108(0.9)	6/108(5.5)
Female	174(61.7)	3/174(1.7)	12/174(6.8)
Total	282	4/282(1.4)	18/282(6.38)

The distribution of ZIKV seropositivity in relation to gender is presented in table -4. There were slight increase in the anti-Zika virus IgG among female (6.8%) compared to that among male ((5.5%). The differences statistically not significant (P>0.05).

Table-5 Distributionof ZIKVseropositivity according to study population age groups

Age group	N. tested (%)	ZIKV–IgM N(%)+ve	ZIKV–IgG N(%)+ve
10-30	93(32.98)	0/93(0)	2/93(2.1)
30-50	135(47.87)	1/135(0.7)	6/135(4.4)
50-80	54(19.15)	3/54(6.6)	10/54(18.5)
Total	282(100)	4/282(1.4)	18/282(6.38)

The distribution of ZIKV seropositivity according to study population age presented in table-5. There were appositive relation between age of partipcipants and seropositivity to ZIKV as the highest rate of prevalence observed among those of > 50 years of age (18.5%) and the least was found among early age group of 10-30 years of age (2.1%). The differences in the prevalence among those of > 50 years and those with < 50 years of age was statistically significant (P<0.05)

Table-6: ZIKVseroprevalencein relation to clinical presentation of symptomatic and asymptomatic patient

Symptoms	Percentage	ZIKV–IgM	ZIKV–IgG
Fever (122)	70 %	3 (2.5%)	12(9.8%)
Headache (139)	80 %	0	0
joint pain (87)	50 %	2 (2.3%)	9(10.3%)
Vertigo (52)	30 %	0	0
severe malaise (49)	28 %	0	0

Chills (31)	18 %	0	0
Cough (28)	16 %	0	0
abdominal pain (24)	14 %	0	0
Drowsiness (17)	10 %	0	0
Total (174)		4(1.4%)	18(6.38%)

The main clinical presentation reported among symptomatic patients were fever (70%),headache (80%) and joint pain (50%).The prevalence of anti-Zika virus IgG was dominant among patient with fever(9.8%) and those with joint pain (10.3%).

DISCUSSION

Seroprevalence studies are an important tool to assessthe disease burden, epidemiology of viruses infectionsand herd immunity. Recently, a number of serosurveysof ZIKV infections have been conducted in Africa, Latin America, and the Caribbean. The seroprevalencewas high in Brazil(63.3%) [9], Nicaragua (36 – 56%) [10], Bolivia (0 –39%) [11], French Guiana (18.8%)[12], Saudi Arabia (12.68%) [13], and Nigeria (10%) [14],but was less than 10% in Laos (9.9%) [15], Zambia (6.1%) [16], Cameroon (5%) [17],Rwanda (1.4%) [18], and Kenya (0.24 – 7.11%) [19] In our study, 6.38% of the participantswere anti-ZIKV IgG positive using commercial ELISA kits,using highly specific monoclonal antibodies targeting specific zika virus epitope(E-protein) where the last group of the above studies is consistent with our finding in Basrah.However, the laboratory assays used were varied acrossthe studies. Some studies only used ELISA to identifyanti-ZIKV antibodies without performing neutralizationassays for further confirmation, and thus the falsepositiverate could be high due to cross-reactivity, especiallyin countries with endemic circulation of otherflaviviruses. Inaddition, the population selected and age distribution inthese serosurveys also differed significantly. Therefore,seroprevalences from different studies may not be directlycomparable [20].
The samplesize calculation was determined to obtain a sufficient point estimate
of prevalence but not to study risk factors of infection sothat we might lack power to ascertain them. Cross-reactionbetween viruses of the same family could have affected theinterpretation of seroprevalence results. Here we used anassay that minimizes the risk of cross-reaction by relying onthe E3 domain of the ZIKV. This means that the true proportionseropositive may be slightly higher than the real estimates.
However, only 1.4% were considered with recent ZIKV seropositive. Therefore, seroprevalence ofZIKV infection should be even lower in other parts of Iraq due to the climatic factors. Although the results suggest that seroprevalence in Iraq is low compared to other countries, this studyprovides serological evidence of unrecognized indigenous ZIKV transmission in Iraq. In addition, our findingsalso indicate that ZIKV outbreaks may occur in Iraqdue to the extremely low-level of herd immunity.

However, there was substantial heterogeneity in infectionrisk, with communities in the north of the governorate hardlyaffected at all, whereas up to half of the infected population was suffering from joint pain. Our seroprevalence estimates are slightly lower than mostprevious estimates from seroprevalence studies in South America [21,22,23] and in other parts of the world[24].This highlights the importance of conducting populationrepresentativestudies. Therefore, it has not been possible to generalizethe findings to the wider country.The future

risk of ZIKV in the area remains unclear. Our findings demonstratethat the majority of the population remains susceptible to ZIKV,which would potentially allow future successful reintroductionsof the virus. However, the observed differences in seropositivityacross the areas may partly reflect differences in the distributionof mosquitoes across the areas. Population movements,economic development, and urbanization that have facilitated the geographicalexpansion of *Ae.aegypti*and its implantation in almostall inhabited areas ofBasrah.

Differences in vector control activities implemented in the different geographical areas may also have contributedto variability in transmission intensities betweendistrict .Vector control activities include both indoor andoutdoor against adults andthe removal of breeding sites where these activities
rely on important logistical and human resources that canbe in some situations very difficult to apply uniformly in different geographical areas. This suggests that the individualrisk of infection is more deeply modulated by different
ecoenvironments related to geographic factors, urbanization level, and the related quality of sanitary infrastructures that mayhave an impact on the extent of vector infestation and, hence,viral circulation.

In some cases, Zika virus has spread from one person to another through blood products (blood transfusion). To reduce the risk of spread through blood transfusion, blood donation centers are required to screen all blood donations for the Zika virus[11,25].

 The proportion of asymptomatic ZIKV infection inZIKV-positive participants was estimated in previous generalpopulation cross-sectional studies at 43% in French Polynesia[24], 47% in Puerto Rico [26],we found that 38.3% (95% CI, 68.5%–79.8%) of the participantswith ZIKV IgG did not report symptoms confirming the highproportion of asymptomatic ZIKV infection. There was aconsiderable time delay between the symptoms' occurrence andthe time of the survey. Although, peoples may have considerably increased public awareness ofhealth risks related to arboviruses in a large majority of the population,awareness about Zika; cultural, social, and behavioralpractices; or previous expositions to diseases and parasites mayhave affected the reporting of presumptive ZIKV infection.

In conclusion, this study is the first serosurvey of ZIKV infection in Basrah. Results showed 18 (6.38%) of 282 participantshad positive anti-ZIKV IgG using commercial ELISAs but only 4 (1.4%) participant was considered to
be recent ZIKV infection defined by detection of IgM, suggestingthat unrecognized indigenous ZIKV transmission might have occurred silently in Basrah. The results also suggestthat the threat of epidemic transmission of ZIKV in southern Iraq does exist due to extremely low-level herdimmunity in general population and due to the prevalence ofAedes mosquitoes, and frequently available habitat of Southern governorates because of lakes and marshes that highly infested by mosquitoes . Our study also indicates that commercialELISA kits for anti-ZIKV IgM can be a gooddiagnostic test for acute ZIKV infection ; however, serological tests for ZIKVspecificIgG still remain a big challenge .

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