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The Presence and Seroprevalence of Arthropod-Borne Viruses in Nasiriyah Governorate, Southern Iraq: A Cross-Sectional Study

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Introduction

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Arthropod-borne viruses (arboviruses) cause several emerging and reemerging diseases. They are spread by blood-feeding arthropods such as mosquitoes, ticks, and sandflies. Taxonomically, arboviruses are found in diverse virus families and genera, such as the families *Togaviridae* (genus *Alphavirus*), *Bunyaviridae* (genera *Orthobunyavirus*, *Phlebovirus*, and *Nairovirus*), and *Flaviviridae* (genus *Flavivirus*). Although arbovirus infections are often asymptomatic or present with mild flu-like manifestations, they may also cause more severe disease manifestations ranging from rash and arthritis to encephalitis and hemorrhagic fever.

Most arboviruses are spread through transmission cycles between the invertebrate vectors and an enzootic vertebrate reservoir. The transmission to humans occurs occasionally via a bridge-vector that feeds both in human and the enzootic (amplification) host. In addition to the enzootic-cycle, an urban epidemic cycle in which humans have become the primary amplifying host has been established for some arboviruses, particularly dengue virus (DENV) and chikungunya virus (CHIKV).

The epidemiological patterns of arbovirus-induced diseases typically reflect the biology of the zoonotic reservoir host and the

file:///C:/...thropod-Borne%20Viruses%20in%20Nasiriyah%20Governorate,%20Southern%20Iraq_%20A%20Cross-Sectional%20Study.html[4/15/2018 8:44:03 AM]

vector. Climate, geography as well as socioeconomic, environmental, and ecological factors also contribute to the emergence of arbovirus-induced diseases. There is only limited recent information about human exposure to arboviruses in Iraq. In this cross-sectional study, our aim was to determine the seroprevalence of IgG antibodies against selected arboviruses in genera *Flavivirus* (West Nile virus [WNV], Usutu virus [USUV], tick-borne encephalitis virus [TBEV]), *Phlebovirus* (sandfly-borne Sicilian virus serocomplex, sandfly-borne Naples virus serocomplex), *Alphavirus* (Sindbis virus [SINV], CHIKV), and *Orthobunyavirus* (California serogroup: Tahyna virus [TAHV], Inkoo virus [INKV], Chatanga virus [CHATV]; Bunyamwera serogroup: Batai virus [BATV]; and Simbu serogroup: Sathuperi virus [SATV]) (Table 1) in the adult human population visiting health-care facilities in Nasiriyah, Iraq. The study site is located in southern Iraq in the vicinity of both Mesopotamian marshes (a large wetland area located at the delta of the river Euphrates and Tigris) and more arid terrain. The marshland provides a habitat for many amplification hosts (e.g., birds and rodents) and vectors (e.g., mosquitoes and ticks) for zoonotic pathogens, whereas arid and semiarid regions are likely to provide habitats for other arbovirus vectors, such as sandflies. Therefore, arboviruses can be expected to have a considerable impact on human health in this region.

Table 1

The virus species included in the study

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Genus	Serogroup	Species
<i>Alphavirus</i>	Semliki Forest serogroup	CHIKV
	Western equine encephalitis serogroup	SINV
<i>Flavivirus</i>	Japanese encephalitis serogroup	WNV
		USUV
	Tick-borne flavivirus	TBEV
<i>Orthobunyavirus</i>	California serogroup	TAHV
		INKV
		CHATV
	Bunyamwera serogroup	BATV
	Simbu serogroup	SATV
<i>Phlebovirus</i>	Sandfly fever Sicilian serocomplex	SFSV
	Sandfly fever Naples serocomplex	SFNV

^{tfn1}BATV = Batai virus; CHATV = Chatanga virus; CHIKV = chikungunya virus; INKV = Inkoo virus; SATV = Sathuperi virus; SFNV = sandfly fever Naples virus; SFSV = sandfly fever Sicilian virus; SINV = Sindbis virus; TAHV = Tahyna virus; TBEV = tick-borne encephalitis virus; USUV = Usutu virus; WNV = West Nile virus.

Materials and Methods

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Study site.

The study site, Nasiriyah, is located in southern Iraq (31°03'N 46°16'E) ([Figure 1](#)). It is the capital of the Dhi Qar Governorate and has an estimated population of ~8,60,000. Because of its central location, the health-care facilities of Nasiriyah city are used also by the neighboring rural communities.



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Figure 1.

Map of Iraq.

Study population.

Serum samples of 399 adult volunteers that live in Nasiriyah region were collected in the years 2012 and 2013. Of these, 200 samples were collected from healthy individuals (medical staff, blood donors, and university students) and 199 samples were from individuals with chronic diseases not associated with virus infection. For the latter group, the samples were collected from patients during their visit to one of the two major university hospitals in Nasiriyah city: Al Hussain General Teaching Hospital or Bint Al Huda Maternity and Children Teaching hospital. In this group, diabetes was the most common underlying disease (45.5% of the patients), followed by cancer (27.5%), renal failure (11%), hypertension (10.5%), and rheumatoid arthritis (8.5%). Informed consent was obtained from the participants.

The mean age of the study population was 45.1 years (range = 10–82). For the healthy individuals, the mean age was 40.7 years (range = 14–71) and for the patients with chronic underlying disease, the mean age was 49.5 (range 10–84). Male to female ratio was 1.44:1 (59% males and 41% females) in the total study population, 1.90:1 (65.5% males and 34.5% females) in the healthy group, and 1.13:1 (53.0% males and 47.0% females) among the individuals with chronic underlying disease.

Study design.

Since antigenic cross-reactions are known to occur among most of the studied virus groups, the serum samples were first screened using an in-house immunofluorescence assay (IFA) that has been designed to detect representatives of different virus groups. Thereafter, the positive samples and the samples that showed ambiguous results were subjected to neutralization test

(NT) and/or hemagglutination inhibition (HI) test to confirm the result and/or to differentiate between distinct pathogens within a serogroup.

Sample collection.

A total volume of approximately 5 mL of blood was collected from the participants. The blood samples were incubated in 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 −40°C and shipped frozen to the University of Helsinki, Finland, where the samples were stored at −70°C.

Viruses.

The virus strains WNV Eg101, USUV, SINV EgAr339, CHIKV, INKV KN3641, 1 TAHV Bardos strain 92, BATV Calovo strain (no. 184), CHATV Möhkö strain (M07-1)2 were used in IFA and NTs. SATV was kindly provided by Richard Elliott (MRC-University of Glasgow). Sandfly fever Sicilian virus (SFSV) and sandfly fever Naples virus (SFNV) were kindly provided by Sirkka Vene (Public Health Agency of Sweden).

Immunofluorescence assay.

IgG antibodies against selected arboviruses were detected using IFA as described previously.3 In brief, virus-infected Vero E6 cells were detached with trypsin, mixed with uninfected Vero E6 cells (a background control) in a ratio of 1:3, washed with phosphate-buffered saline (PBS), and air-dried on slide spots. The glasses were fixed with acetone and stored dry at −70°C. The serum samples were diluted 1:20 in PBS, added to slides, and incubated in a humid chamber for 30 minutes at 37°C. The slides were washed three times with PBS and once with distilled water, followed by 30 minutes incubation with fluorescein isothiocyanate–conjugated goat antihuman IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted 1:100 in PBS. The slides were washed again and examined under a fluorescence microscope.

Hemagglutination inhibition assay.

HI assay was performed as described previously. 4,5 In brief, the sera were diluted in borate (pH 9) + 0.6% bovine serum albumin (Sigma-Aldrich, St. Louis, MO), pre-adsorbed with an equal volume of 25% kaolin (Fluka Chemika) (in borate pH 9.0) and goose erythrocytes and tested subsequently at 2-fold dilutions, starting at 1:10. Tween-ether-treated TBEV and WNV antigens at four hemagglutinating units and goose erythrocytes at 0.2% suspension were used.

Microneutralization test.

Aliquots of the sera were inactivated at +56°C for 30 minutes. Serial 4-fold dilutions of sera were mixed with ~50 plaque-forming units of virus, incubated at +37°C for 1 hour, and transferred into 96-well cell culture plates of Vero-E6 cells and incubated at +37°C in a 5% CO2 atmosphere for 2–3 days. The highest dilution completely inhibiting the viral cytopathic effect was regarded as the end point titer of the serum.

Statistics.

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

Results

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To assess the presence and seroprevalence of arboviruses in Nasiriyah District, two serum panels representing healthy adult volunteers (*N* = 200) and individuals with chronic underlying disease (*N* = 199) were studied for antibodies against flaviviruses, alphaviruses, orthobunyaviruses, and phleboviruses, as specified below. Altogether, 137 of 399 (34.3%) samples were positive for at least one of the studied viruses. There were no statistically significant differences between the healthy volunteers and those with chronic illnesses in the seroprevalence for any of the studied viruses. Therefore, the combined data representing both groups (*N* = 399) are presented in Table 2 .

Table 2

The IgG seroprevalence of the studied arboviruses

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Genus	Taxonomic group	No. of positive samples/no. of samples tested (%)	95% CI
Flavivirus	Mosquito-borne flavivirus	66/397 (16.6)	13.2–20.6
	WNV	46/396 (11.6)	8.8–15.1
	USUV	2/396 (0.5)	0.1–1.8
Alphavirus	SINV	6/399 (1.5)	0.7–3.2
	CHIKV	2/399 (0.5)	0.1–1.8
Orthobunyavirus	TAHV-like	8/399 (2.0)	0.9–4.1
	BATV	0/399 (0)	–
	SATV	0/399 (0)	–
Phlebovirus	SFSV	72/396 (18.2)	14.6–22.3
	SFNV	31/396 (7.8)	5.5–11.0

[†]BATV = Batai virus; CHIKV = chikungunya virus; CI = confidence interval; SATV = Sathuperi virus; SFNV = sandfly fever Naples virus; SFSV = sandfly fever Sicilian virus; SINV = Sindbis virus; TAHV = Tahyna virus; USUV = Usutu virus; WNV = West Nile virus.

Genus *Flavivirus*.

The sera were first screened for the presence of antibodies against flaviviruses using broadly reactive in-house WNV IFA. A subset of samples that were positive or had borderline positivity for WNV in this assay were also subjected to IFA test for USUV, DENV, Japanese encephalitis virus (JEV), and TBEV at 4-fold dilutions. In all of these, the IFA titers were highest for WNV or USUV (Table 3). Thereafter, to distinguish between mosquito- and tick-borne flaviviruses, all IFA positive and borderline positive/ambiguous sera were subjected to HI test for WNV and TBEV. Finally, to distinguish between WNV and other mosquito-borne flaviviruses, the samples confirmed positive for WNV by HI and/or IFA test were subjected to microneutralization test (MNT) using WNV and USUV, both of which are members of the Japanese encephalitis antigenic complex.⁶

Table 3

Flavivirus IFA, HI, and NT titers for a subset of samples

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	IFA					HI		NT	
	WNV	USUV	JEV	DENV-2	TBEV	WNV	TBEV	WNV	USUV
WNV pos co	320	80	20	20	20	ND	ND	ND	ND

Sample 1	80	80	< 20	< 20	< 20	320	< 20	ND	20
Sample 2	< 20 *	< 20	< 20	< 20	< 20	40	< 20	20	20
Sample 3	20	< 20	< 20	< 20	< 20	40	< 20	80	20
Sample 4	20	< 20	< 20	< 20	< 20	80	< 20	80	20
Sample 5	20	80	< 20	< 20	< 20	20	< 20	0	20
Sample 6	80	320	< 20	< 20	< 20	320	< 20	80	20
Sample 7	80	20	< 20	< 20	< 20	320	< 20	80	80
Sample 8	< 20 *	< 20	< 20	< 20	< 20	80	< 20	20	20
Sample 9	80	80	< 20	< 20	< 20	320	< 20	320	20

^{†††}DENV = dengue virus; HI = hemagglutination inhibition; IFA = immunofluorescence assay; JEV = Japanese encephalitis virus; NT = neutralization test; TBEV = tick-borne encephalitis virus; USUV = Usutu virus; WNV = West Nile virus; pos co = positive control; ND = not determined.

*Borderline positivity.

Altogether, 14.9% (59/397; 95% confidence interval [CI]: 11.7–18.7) of the serum samples were positive and 9.6% (38/397) had ambiguous result for WNV with IFA. Of these, 60 samples (seroprevalence: 15.1%; 95% CI: 11.9–19.0) were positive for WNV in HI test, whereas none of the samples were positive for TBEV. Out of the samples positive for WNV in HI test and/or IFA test (*N* = 68), 46 samples (seroprevalence: 11.6%; 95% CI: 8.8–15.1) had an over 4-fold higher neutralizing titer for WNV than for USUV (Table 2). Two samples had an over 4-fold higher neutralizing titer for USUV, whereas 10 samples had equal titers (> 20) for both viruses. Four samples showed no neutralization and no HI despite the positive IFA result. Six samples had ambiguous results in NT, however, all of these were confirmed flavivirus positive by HI and IFA tests.

In summary, 16.6% (66/397; 95% CI: 13.2–20.6) of the sera were confirmed as positive for mosquito-borne flaviviruses (i.e., IFA+ or ± and HI/NT+), whereas 11.6% (46/396; 95% CI: 8.8–15.1) were WNV positive and 0.5% (2/396; 95% CI: 0.1–1.8) were USUV positive in NT (Table 2).

There were no statistically significant differences between males and females (seropositive for mosquito-borne flavivirus: females 28/162, males 38/234 [χ^2 = 0.01; *P* = 0.92]; seropositive for WNV: females 20/162, males 26/234 [χ^2 = 0.03; *P* = 0.86]) or between age groups in the mosquito-borne flavivirus or WNV seroprevalence, although for both, seroprevalence was highest in the highest age group (> 50 years). Similarly, there were no gender-specific differences between age groups.

Genus *Alphavirus*.

Using in-house IFAs, 2.0% (8/399) of the samples were positive and 1.8% (7/399) had an ambiguous result for SINV, whereas 0.5% (2/399) of the samples were positive and 0.8% (4/399) had an ambiguous result for CHIKV (Table 2). The presence of neutralizing antibodies was confirmed in six of eight SINV IFA–positive samples (seroprevalence: 1.5%; 95% CI: 0.7–3.2) and none of seven ambiguous samples. For CHIKV, none of the IFA positives and two of four ambiguous samples was confirmed specific for CHIKV with MNT (seroprevalence: 0.5%; 95% CI: 0.1–1.8). All SINV- or CHIKV-positive or ambiguous samples were also subjected to NT for Semliki Forest virus (SFV). None of the samples had neutralizing antibodies against SFV.

Genus *Orthobunyavirus*.

Orthobunyaviruses have wide antigenic cross-reactivity within serogroups but not between serogroups.⁷ Therefore, the seroprevalence of orthobunyaviruses was first screened using an in-house IFA for the representatives of California (INKV), Bunyamwera (BATV), and Simbu (SATV) serogroups. The samples that showed positivity or ambiguous reactivity against any

of these viruses were subjected to MNT for INKV, TAHV and CHATV (California serogroup), BATV, and SATV. Altogether, 14 of 399 samples (INKV 8/399, BATV 5/399, and SATV 6/399) were positive or gave an ambiguous result for orthobunyaviruses with IFA. In the MNT, eight of the samples (8/399; seroprevalence: 2.0%; 95% CI: 0.9–4.1) had neutralizing antibodies. The antibody titers were highest for TAHV (over 4-fold higher than for the others) (Table 4). No neutralizing antibodies were detected against SATV or BATV.

Table 4

Neutralization titers for the orthobunyavirus IFA-positive samples

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Gender	Age (years)	California serogroup			BATV	SATV
		INKV	TAHV	CHATV		
Female	23	< 20	1,280	80	< 20	< 20
Female	23	40	1,280	320	< 20	< 20
Female	48	< 20	80	20	< 20	< 20
Female	72	< 20	1,280	80	< 20	< 20
Male	31	< 20	1,280	20	< 20	< 20
Male	51	< 20	1,280	80	< 20	< 20
Male	54	< 20	320	20	< 20	< 20
Male	72	< 20	1,280	80	< 20	< 20

^{tfn5}BATV = Batai virus; CHATV = Chatanga virus; IFA = immunofluorescence assay; INKV = Inkoo virus; TAHV = Tahyna virus; SATV = Sathuperi virus.

Genus *Phlebovirus*.

As to genus *Phlebovirus*, we focused on those phleboviruses that are spread by sandflies. The overall seroprevalence was 18.2% (95% CI: 14.6–22.3) and 7.8% (95% CI: 5.5–11.0) for SFSV and SFNV, respectively. There were no statistically significant differences between males and females (seropositive for SFSV: females 30/162, males 42/234 ($\chi^2 = 0$; $P = 1$); seropositive for SFNV: females 14/162, males 17/234 ($\chi^2 = 0.1$; $P = 0.75$)). SFSV seroprevalence was approximately equal in all age groups, whereas for SFNV, the seroprevalence was higher among individuals aged > 30 years than among those aged < 30 years (3.1% versus 8.7%). However, this difference was not statistically significant ($\chi^2 = 1.71$; $P = 0.19$).

Discussion

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No comprehensive studies on arbovirus circulation in Iraq have been conducted for decades. In this study, we determined the arbovirus exposure of the adult population in Nasiriyah, by testing a sample of 399 sera taken in 2012 and 2013 for antibodies against a comprehensive panel of arboviruses potentially circulating in the region.

The results showed a considerably high (17%) seroprevalence of mosquito-borne flaviviruses in Iraq. Flaviviruses are divided into four major groups: tick-borne, mosquito-borne, insect-specific, and no known–vector flaviviruses (TBFV, MBFV, ISFV,

and NKV, respectively) (not monophyletic). The major human pathogens among flaviviruses are found in two major clades, those transmitted by mosquitoes and those transmitted by ticks. The most mosquito-borne viruses pathogenic for humans are further subdivided into three antigenic complexes of closely related viruses: yellow fever, dengue, and Japanese encephalitis antigenic complexes. The viruses used in this study, WNV and USUV, are members of the Japanese encephalitis antigenic complex.^{6,8}

Most of the mosquito-borne flavivirus IgG-positive samples were further identified as WNV positive. The high seroprevalence of WNV in Iraq (11.6%) is in accordance with reports from neighboring countries such as Turkey (17%),⁹ Jordan (8%),¹⁰ and Iran (0–95.8%, depending on the location).^{11,12} Furthermore, some of the major vector species for WNV such as *Culex pipiens* and *Culex quinquefasciatus* have been detected in Iraq.¹³ Altogether, these results indicate intensive WNV circulation in the Middle East region.

CHIKV (genus *Alphavirus*) is another arbovirus that has recently expanded its geographic distribution. Although the results of this study suggest limited or no CHIKV circulation in Iraq at present, the recent CHIKV epidemic in Yemen^{14,15} highlights the circulation of CHIKV in the Middle East region. Importation of CHIKV to Iraq is therefore possible. To our knowledge, there are no reports on the presence of the vector species of CHIKV, *Aedes albopictus* and *Aedes aegypti* (*Stegomyia aegypti*), in Iraq.¹³ However, more comprehensive studies would be needed to rule out their presence, since *Ae. albopictus* is present in Lebanon and Syria¹⁶ and *Ae. aegypti* has been detected in Yemen.¹⁷ Given the invasive nature of *Ae. albopictus*, the potential introduction of CHIKV to southern Iraq should be monitored.

Another alphavirus, SINV induces disease manifestations similar, albeit milder, to CHIKV.¹⁸ SINV has been detected in a wide geographical region including Africa, Europe, China, southeast Asia, and Australia.¹⁹ Curiously, the incidence of SINV-induced disease is highest in South Africa and northern Europe.^{20,21} The enzootic transmission cycle of SINV occurs between birds and mosquitoes. Several bird and mosquito species (mainly *Culex* and *Culiseta*) are involved in the transmission cycle. The data presented in this study suggest that SINV also circulates in Iraq, and humans are exposed to this virus. Previously, a single case of SINV seroconversion has been detected among U.S. Army troops in Iraq,²² and the virus has been isolated from mosquitoes in Saudi Arabia,²³ as well as from mosquitoes and a European turtle dove (*Streptopelia turtur*) in Israel.²⁴ The seroprevalence of SINV in Iraq was rather low (1.5%) as compared with those of SINV-endemic regions in northern Europe (Finland: 5.2% of the total population, reaching 25.7% in North Karelia²⁰; Sweden: 3.6% in central Sweden, 2.9% in northern Sweden^{25,26}) and China (seroprevalence: 19%²⁷).

The family Bunyaviridae contains four genera that cause infections in humans and other animals: *Orthobunyavirus*, *Phlebovirus*, *Nairovirus*, and *Hantavirus*. Of these, orthobunyaviruses are transmitted mainly by mosquitoes, phlebo viruses by phlebotomine sandflies, and nairoviruses by ticks, whereas hantaviruses are rodent borne. In this study, we assessed the seroprevalence of selected orthobunyaviruses and phleboviruses.

Since Bunyamwera and California serogroups of orthobunyaviruses are geographically widely distributed in the Old World and capable of causing human infections, we assumed that these viruses might also circulate in the Middle East region. The members of Simbu serogroup cause diseases mainly in animals (cattle). The representative of this serogroup (SATV) was included in the study to assess the potential human exposure to arboviruses in cattle. Evidence of California serogroup, namely TAHV, infections was found in the study population. TAHV has been associated with febrile disease.^{28,29} Mosquito vectors for TAHV include several species, many of which (such as *Aedes vexans*, *Ochlerotatus caspius*, *Culiseta annulata*, *Cx. pipiens*, and *Anopheles hyrcanus*) have also been found in Iraq.¹³ Principal vertebrate hosts of this virus include lagomorphs, hedgehogs, and rodents. The TAHV IgG titers detected in the current study were over 4-fold higher than those of the other studied orthobunyaviruses in the California serogroup (INKV and CHATV). This is consistent with the view that TAHV is widespread in Europe,³⁰ Russia,³¹ Africa,^{32–35} and Asia (southern Siberia and the Far East, Turkey, Armenia, Azerbaijan, Tadjikistan, Uzbekistan, and China),^{30,36–38} whereas INKV and CHATV may be restricted to northern Europe and Asia.^{2,30,39}

In the endemic region of Europe, approximately 5% of the general population are seropositive for TAHV,⁴⁰ and very high frequency (60–80%) of neutralizing antibodies have been reported in elderly persons in the endemic foci.³⁰ In this study, we detected eight TAHV seropositive individuals (2% of the study population) in southern Iraq, suggesting that the virus also

circulates in the Middle East region, even though the human exposure to this virus may be lower than in the central Europe.

As to other orthobunyaviruses, one sample was IFA positive but NT negative for SATV. Given that SATV is an animal pathogen, it is more likely that this individual had been infected by some other virus of the Simbu serogroup, most likely the Simbu virus, which is the only member of this serogroup that has been associated with human infections in the Old World.^{41,42} No BATV (Bunyamwera serogroup)–positive samples were detected in this study, although BATV and related Bunyamwera serogroup viruses are widely distributed in Europe, southeast Asia, Japan, Asian Russia (Irkutsk, Far East), and Africa (Ilesha virus).³⁰

A relatively high seroprevalence of sandfly-borne phleboviruses was detected in Iraq. These viruses may cause a transient febrile illness or more severe neurological manifestations. The sandfly-borne phleboviruses found in the Old World comprise of three antigenically distinct serocomplexes: sandfly fever Naples serocomplex, Salehabad serocomplex, and sandfly fever Sicilian serocomplex. In this study, 7.5% and 17.0% seroprevalence were detected for Naples and Sicilian serocomplexes, respectively. For the Naples serocomplex, this is similar to that observed in Massayeb-Al-Kabir, Iraq, in 1972 and 1973 (7.5%),⁴³ whereas for the Sicilian serocomplex, a considerably higher seroprevalence (17.0% versus 2.5%) was detected in this study. However, a high seroprevalence of Sicilian serocomplex has been detected previously in the neighboring countries Iran and Saudi Arabia,^{43,44} suggesting that there may be local differences in the exposure of humans to these pathogens. The Sicilian virus also caused an outbreak in U.S. Army troops in central Iraq in 2007,⁴⁵ and SFSV seroconversions were detected among U.S. troops in Al Asad, Iraq.²² Altogether, these results suggest active circulation of sandfly-borne phleboviruses in the region.

For most of the viruses studied, there were no sex- or age-specific differences in the seroprevalence, although for WNV and SFSV, the seroprevalence were highest among those aged > 50 years. In the case of stable endemic transmission, an additive risk of exposure would be expected over time and subsequently an increasing seroprevalence toward higher age groups. The relatively stable seroprevalence in all age groups in Iraq might therefore suggest an increase in the transmission over time. More detailed studies with higher number of participants are needed to test this hypothesis. No children and very few individuals under the age of 20 years ($N = 10$) were included in this study. Therefore, the age of seroconversion could not be determined for most of the viruses studied. A notable exception for this was the Naples serocomplex that showed clear increase in the seroprevalence for those aged >30 years. The higher age of seroconversion for the Naples serocomplex as compared with that of the Sicilian serocomplex is consistent with the earlier study conducted in the Isfahan Province in Iran.⁴⁴

In conclusion, the results of this study suggest a high seroprevalence for mosquito-borne flaviviruses and sandfly-borne phleboviruses in southern Iraq. The high exposure rates suggest that these viruses should be considered as potential causative agents in patients with febrile disease and/or neurological manifestations in this region. In addition, TAHV-like orthobunyavirus and SINV most likely circulate in southern Iraq, and humans are occasionally exposed to these viruses.

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