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THE PREVALENCE OF HUMAN INFECTION WITH WEST NILE VIRUS IN IRAN

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ABSTRACT

A total of 698 blood and serum specimens from residents of 13 Iranian communities were examined by plaque reduction neutralization test for antibodies to West Nile virus. In general, infection rates in the North of the country were low, while higher rates were observed among residents of Central and Southwestern Iran. The highest prevalence of infction was found among residents of Khuzestan Province, indicating that this region is one of endemic West Nile virus activity. The symptoms of West Nile virus infection in man are also discussed.

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A. *Introduction*

West Nile virus, a mosquito-borne flavivirus, was first isolated from a patient in Uganda in 1937 (1). Subsequent work has demonstrated that West Nile virus is widely distributed and infects humans in Africa, Europe, the Middle East, and Central and Southeast Asia (2). Previous serologic studies in Iran (3,4) have demonstrated the presence of West Nile antibodies among populatinos living in the Caspian coast region as well as in Khorassan and Khuzestan Provinces.

In order to gain a more complete picture of the frequency of human infection with this agent in Iran, we recently examined 698 blood and serum specimens from residents of 13 different communities in the country for neutralizing antibodies against West Nile virus. The present paper reports results of this study and presents new data on the distribution and prevalence of West Nile virus infection in Iran.

B. *Materials and Methods*

Specimens and populations tested : Figure 1 shows the location of the 13 communities sampled. Blood specimens from residents of Ali-Abad, Neishabor, Varamin, Deigi and Khorousi were collected by finger prick on filter paper discs (Schleicher & Schnell, No. 740-E) between June and August 1975. The blood-soaked discs were dried and stored at —20°C and subsequently were reconstituted in 1.0 ml of phosphate buffered saline, pH 7.2, containing 0.5% gelatin, to prepare an approximate serum dilution of 1 :20. Persons of all ages were bled in these communities, although the majority of donors were children.

Specimens from the remaining 8 communities were collected by venipuncture between 1971 and 1975 from persons attending government health and family planning centers. Most of these specimens were obtained from adults. The latter sera were stored at —20°C until tested and were examined at a dilution of 1 :10.

Virus : The West Nile virus pool used in neutralization tests was a fourteenth mouse brain passage of the Egypt 101 strain, originally obtained from Dr. Robert E. Shope, Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Neutralization test : Prior to testing, all specimens were heat-inactivated at 56°C for 30 minutes. Neutralization tests were performed in 24-well, micro-plate cultures of LLC-MK2 cells, by a standard plaque reduction neutralization methods described previously (5). Serum and blood specimens were tested at the dilutions noted above against a fixed virus dose (60 to 100 plaque forming units), using a single well per sample.Specimens producing $\geq$ 90% plaque inhibition were recorded at positive, indicating specific West Nile neutralizing anti-bodies. A subsample of 24 West Nile positive sera was also

screened at a 1 :10 dilution by the same method against yellow fever (17-D strain) and Japanese encephalitis viruses to eliminate the possibility of cross-neutralization by other Group B arbovirus antibodies. All serologic tests were performed at the Pacific Research Section, Honolulu, Hawaii, U.S.A.

C. *Results*

Table 1 summarizes neutralization test results obtained from the 13 communities sampled. Of a total of 698 sera and bloods examined, 186 (26.6%) had antibodies against West Nile virus. With the exception of Rasht, infection rates in communities located in the North of the country (Tabriz, Ali-Abad, Neishabor and Mashad) were low, while higher rates were observed in Central and Southwestern Iran. The highest prevalence of antibodies was found in the Dezful-Deigi area. In Deigi, 69 of 72 persons (95.8%) had West Nile neutralizing antibodies. As shown in Table 2, the three negative sera were from children 1 to 4 years af age. By the age of 5 years, 100% of the population sampled in this community had West Nile antibodies.

None of 24 selected West Nile positive sera neutralized yellow fever or Japanese encephalitis viruses, indicating that the antibodies were specific and were not due to cross-reaction with heterologous Group B arbovirus antibodies.

D. *Discussion*

Results of this serologic survey demonstrate that West Nile virus is widely distributed in Iran. The high prevalence of antibodies among residents of Kermanshah and Khuzestan Provinces suggests that the virus is endemic in these areas. Neutralization test results from the Dezful-Deigi area (Tables 1 and 2) indicate that this region is one of especially high virus activity. In recent years, this region has become a major agricultural area through the construction of an extensive system of irrigation canals. It seems probable that this irrigation water also provides suitable breeding places for large numbers of *Culex* mosquitoes, the presumed vector of West Nile virus. While the differences observed in the prevalence of West Nile virus antibodies between communities undoubtedly reflect the distribution and abundance of mosquito vectors within each area, the higher infection rates in Southwestern Iran may also be due to the warmer climate in this region and longer season of mosquito activity.

Despite the serologic evidence of its presence, West Nile virus has not yet been recovered in Iran; however many isolates have been obtained in neighboring countries of Egypt, Israel, Cyprus, USSR and India (2). Most of the reported West Nile virus isolates have been obtained from mosquitoes of the genus *Culex,* including *C. univittatus, C. antennatus, C. modestus, C. vishnui, C. fatigans, C. molestus* and *C. weschei* (2). All but the last species occur in Iran (6) and must be considered potential vectors of the virus.

In humans, West Nile virus infection produces a wide spectrum of clinical illness and its severity appears to be partially age dependent. Infants and young children generally experience a mild, non-specific febrile illness adolescents and adults usually develop a typical dengue-like disease (fever, headache, flushed face, muscular and orbital pain and muculopapular rash); in elderly patients, the central nervous system is frequently affected and occasionally encephalitis and death occur (7-10). The viremia associated with West Nile infection is usually low and diagnosis is based on demonstrating an antibody rise from acute to convalescent sera. Local physicians should consider this disease in their differential diagnosis of acute febrile illnesses, particularly in non-immune persons traveling or working in West Nile endemic areas of Southwestern Iran.

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TABLE 1

PREVALENCE OF WEST NILE VIRUS NEUTRALIZING ANTIBODIES IN 13 IRANIAN COMMUNITIES

Community-Province	Number /Total positive*/tested	Percentage positive
Tabriz (U)**, East Azerbaijan	0/50	0.0
Rasht (U), Gilan	6/51	1.8
Ali-Abad (R)**, Khorassan	2/50	4.0
Neishabor (U), Khorassan	0/50	0.0
Mashad (U), Khorassan	1/50	2.0
Tehran (U), Tehran	13/103	12.6
Varamin (R), Tehran	3/48	6.3
Isfahan (U), Isfahan	6/49	12.2
Kermanshah (U), Kermanshah	15/32	46.9
Deiqi (R), Khuzestan	69/72	95.8
Dezful (U), Khuzestan	34/38	89.5
Khorousi (R), Khuzestan	31/49	63.3
Abadan (U), Khuzestan	6/56	10.7

TABLE 2

PREVALENCE OF WEST NILE VIRUS NEUTRALIZING ANTIBODIES AMONG CHILDREN IN DEIGI, KHUZESTAN PROVNCE

Age group	Number/Total positive/tested	Percentage positive
1-2	6/8	75
3-4	11/12	92
5-6	12/12	100
7-8	18/18	100
9-10	10/10	100

* Sera producing $>$ 90% plaque inhibition

** U=Urban R=Rural

Figure 1 Map of Iran showing the location of 13 communities sampled for West Nile virus antibodies