Original Article

Serological evidence of Flaviviruses infection among acute febrile illness patients in Afghanistan

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Abstract

Introduction: Current published reports on the causative agents of acute febrile illness (AFI) in Afghanistan are scarce, and the burden of disease due to flaviviruses is unknown.

Methodology: A hospital-based surveillance study for AFI was established in 2008 through 2010 to determine the seroepidemiology of West Nile virus (WNV), tick-borne encephalitis virus (TBEV) and dengue viruses (DENV) using commercial ELISA kits. Due to major logistical challenges, only acute sera were collected.

Results: Serological analysis for IgG were as follows: WNV 30.4% (277/913); TBEV 23.4% (214/913); DENV 19.7% (180/913). Single positive IgG reactions for WNV, TBEV and DENV were noted in 11% (100/913), 7.2% (66/913), and 5% (47/913), respectively. Reactivity for all three screened flaviviruses was detected in 44.5% (406/913) of sera. IgM positivity was uncommon, with only 0.5% (5/913), 2.2% (20/913) and 2.6% (8/312) of samples positive for WNV, TBEV, and DENV, respectively. Serological findings were confirmed in random positive samples by neutralization assay.

Conclusions: These serological results suggest circulation of WNV, TBEV, and DENV within Afghanistan, with evidence of current or prior infection noted in a significant proportion of patients seeking care for AFI. Obtaining additional information on the prevalence of these and other causes of AFI is paramount for improving the distribution of available limited syndromic treatment and improving the existing health protection policy in Afghanistan.

Key words: Flaviviruses; ELISA; acute febrile illness; Afghanistan.

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Introduction

For decades, Afghanistan has suffered from conflict, political instability and destruction of critical country assets [1,2]. While access to basic healthcare services is limited, the vast majority of the Afghans are vulnerable to a wide range of health threats and infectious diseases. Of these, acute febrile illness (AFI) is a prevalent syndrome that leads to significant levels of morbidity and mortality. It has been reported that 47% of hospital-admitted AFI cases in Afghanistan are due to typhoid fever, brucellosis, leptospirosis and rickettsioses [3,4]. Other causes include the *Flaviviridae*, a major group of arthropod-borne viruses (arboviruses) that involves more than 70 recognized virus members [5-7]. Serological antigenic analyses have classified flaviviruses into eight antigenic complexes: West Nile, tick-borne encephalitis (12 serotypes), Rio Bravo (6 serotypes), Japanese encephalitis (10 serotypes), Tyuleniy (3 serotypes), Ntaya (5 serotypes), Uganda S (4 serotypes), dengue (4 serotypes), Modoc (5 serotypes) and other unassigned categories [8,9]. Due to shared antigenic determinants within these viruses, cross-reactivities may occur, limiting the specificity of serologic testing.

While syndromic diagnosis of AFI is commonly used in Afghanistan, it is highly inaccurate and misleading. The need for considerable capacity restoration, high cost of laboratory diagnostics and availability of suitable expertise constitute serious challenges. The purpose of this study was to establish a hospital-based surveillance for AFI to delineate the seroepidemiology of West Nile (WN), tick-borne encephalitis (TBE) and dengue virus (DENV) viruses using commercial ELISA kits. The results of this surveillance could be the first to elucidate the burden

of *Flaviviridae* infections in Afghanistan and may help to improve prevention and control measures.

Methodology

The following case definition of AFI was utilized: "any person of any age who had a history of fever (T > 38ºC by oral route mainly) for 2 days or more - including undulant fever- without obvious clinical diagnosis. Excluded from study were cases clinically diagnosed with malaria, diarrhea, respiratory tract infection, urinary tract infection, visceral leishmaniasis, typhoid fever, brucellosis, leptospirosis or cellulitis.

Starting in 2008 through 2010, patients were enrolled from three provincial hospitals located in Uruzgon (Tarin Kowt), Helmand (Lashkar Gah), and Kandahar (Mirwais), and two quaternary hospitals in the capital city of Kabul (Indira Gandhi Children and Kabul Infectious Disease Hospitals). Provincial hospitals were selected because public health support in these provinces has been extremely limited and were selected as sentinel sites during ongoing public health surveillance. Hospitals in Kabul were selected to supply samples to the CPHL in order to exercise its newly established capacity and to compare results from the selected southern provinces. All patients meeting study criteria were offered enrollment after signing an informed consent. Study approvals were obtained through the Institutional Review Boards at the Afghan Public Health Institute (APHI), the Ministry of Public Health (MoPH), and the U. S. Naval Medical Research Unit No. 3 (NAMRU-3) in Egypt.

Two to three milliliter blood was collected in tiger top serum separator tubes from each individual at the time of admission. Convalescent samples were not obtainable due to patient access limitations and security concerns. Commercial ELISA kits were used for the detection of IgM and IgG antibodies against West Nile (WNV) (Focus Diagnostics, Inc., Cypress, CA, USA), DENV (PanBio/Inverness Medical, Princeton, NJ, USA) and tick-borne encephalitis viruses (TBEV) (IBL International GmbhH, Hamburg, Germany). The three tests were simultaneously performed for the three viruses according to manufacturers' instructions. Batch testing for each target was done at separate time points, and all samples available at the time of testing were included in the batch. Laboratory analysis was performed at the Central Public Health Laboratory (CPHL) in Kabul as part of capacity building. This capacity building effort included the provision of laboratory equipment, supplies and training of laboratory staff to improve disease diagnosis.

Single aliquots from almost all serum samples (n = 913) were received at NAMRU-3 for quality control testing and confirmation. Twenty-four WNV IgM- and/or IgG-positive samples were randomly tested for WNV-neutralizing antibodies by plaque reduction neutralization technique (PRNT) using 80% cut-off value. Sample size was determined, assuming continuous data distribution with a margin of error of 6%-7% and 95% confidence level [10]. Four additional samples of those positive for other antigens (TBEV and dengue) were included as negative controls.

Results

A total of 913 patients who showed negative bacterial blood culture and aged 20-59 years were enrolled. Females represented 46% of these patients. Table 1 shows the findings of serum testing against WNV, TBEV, and DEN.

Immunoglobulin G (IgG) was reactive to WNV only in 36.1% (100/277) of the samples, while many others were additionally coreactive with TBEV-IgG (15.9%, 44/277), DENV-IgG (10.5%, 29/277) or both (37.5%, 104/277). Likewise, 30.8% (66/214) of the sera showed TBEV-IgG reactions only, while some others were also coreactive with WNV-IgG (20.6%, 44/214). For DENV- IgG, only 26.1% of the sera (47/180) were reactive.

Overall, WNV-IgG was detected in 30.4% of the total samples (277/913), whereas TBEV-IgG and DENV- IgG were detected in 23.4% (214/913) and 19.7% (180/913) of the samples, respectively (Figures 1-3).

Immunoglobulin M (IgM) was detected in a small number of samples; 5 WNV, 8 DENV and 20 TBEV of which 4, 8, and 4, respectively were also IgG

Table 1. Summary of ELISA results

Pathogen	IgG	IgM
TBEV	214/943 (22.7%)	20/925 (2.2%)
DENV	180/937 (19.2%)	8/312 (2.6%)
WNV	277/913 (30.4%)	5/913 (0.5%)

reactive. While only 1.4% (13/913) serum samples were equivocal for WNV-IgG reactions, many showed indeterminate reactions in DENV 8.9% (81/913) and TBEV 23.5% (215/913). Difficulties with cross reactivity amongst flaviviruses limit unequivocal interpretation of the results. However none of the tested samples showed IgM for more than one pathogen of the tested flaviviruses. In the PRNT assay, WNV virus was neutralized in the 24 WNV serologically positive samples, regardless of antibody isotype involved or when those sera were IgG-positive for both WNV and TBEV. The other four samples - that were serologically positive for both TBEV and dengue- were negative for the virus in the assay.

Discussion

IgG antibodies to West Nile Virus were found in 11% (100/913) of the samples, suggesting prior exposure to infection. However, four samples of these showed positive IgM reactions, being the most efficient marker for detecting acute WNV infection. Another group was also positive for WNV/IgG along with antibodies for TBE (44/177, 24.8%), DEN (29/177, 16.4%) or both (104 /177, 58.8%), making a total of 277/913 (30.4%) positivity for WNV exposure. This is in agreement with earlier reports of WNV screening in Kunduz, Herat, Bamyan, and Helmand [11]. The presence of other IgG antibodies in WNV positive sera may indicate either a previous exposure or serological cross reactivity [12,13].

Single TBE IgG reactions were detected in 66/214 (30.8%) of the suspected AFI sera, suggesting previous exposure, whereas specific TBEV IgM was found in 2.2% (20/913) of the samples only; this is highly indicative of a recent or current infection [8,9]. Of these, eight samples harbored both TBE IgG and IgM. Most patients who developed IgM antibodies against TBEV reported consumption of raw milk, a possible method of infection declared earlier [14, personal communication]. This is the first report to suggest serologic evidence of TBEV infection in Afghanistan, although it has been historically observed in Central Asia and many of the former Soviet republics [15]. Limited cross reactivity of TBE IgM with other *flaviviridae* may occur as per the manufacturer of the employed TBEV kits. DENV IgG was noticed in 19.7% (180/913) of the AFI patient sera. This is similar to previous reports suggesting frequent exposure in Afghanistan [11] and surrounding countries [15]. The 16.1% (29/180) of the DENV IgG positive sera were also reactive to WNV IgG, indicating previous exposure to multiple diseases or

Figure 1. Percent of AFI patients with WNV antibodies in Afghan patients.

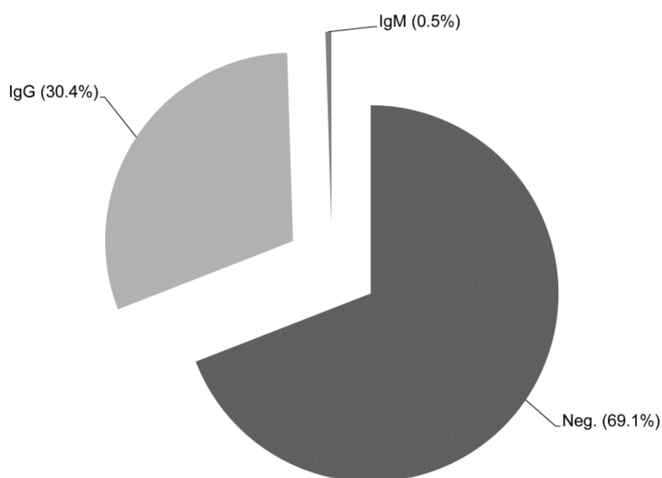


Figure 2. Percent of AFI patients with TBEV antibodies in Afghan patients.

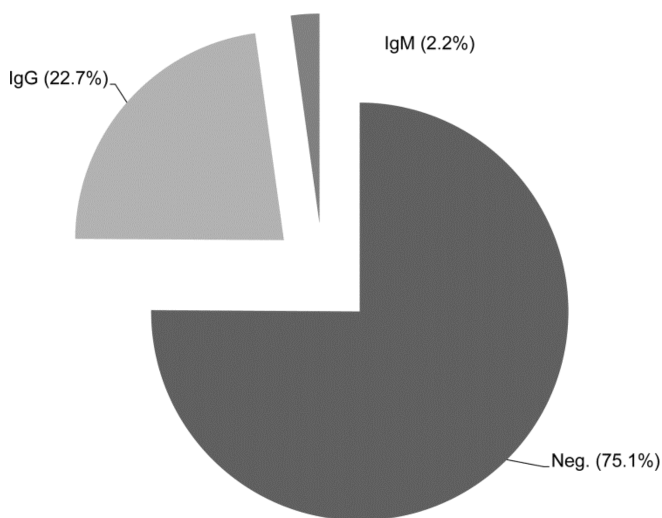
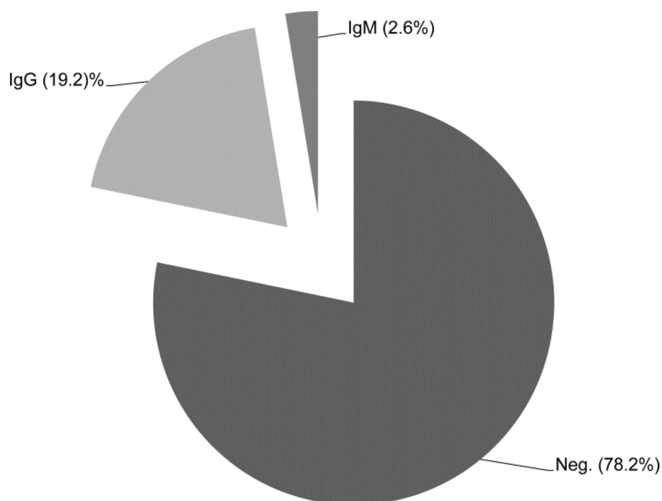


Figure 3. - Percent of AFI patients with DENV antibodies in Afghan patients.



relatively low assay specificity. However, the ELISA kits utilized in this study (PanBio/Inverness Medical, Princeton, NJ, USA) were reported to exhibit minimal or no cross reactivity to other related antigens. In the PRNT assay, it was confirmed that WNV virus was the cause of disease in serologically positive samples that were randomly picked. The test also reflects the specificity of ELISA used, since serological positivity for TBEV and dengue did not neutralize WNV. Anti-WNV antibody isotypes and the presence of other IgG against TBEV did not affect PRNT results, although it reflects previous exposure.

The data conclusively suggests the presence of single or multiple exposures to one or more of the screened flaviviruses. Most of the enrolled patients had either mild or moderate illness, probably expressing a late acute phase of these infections. More definitive confirmation of each specific disease may require screening of convalescent sera to document rising titers. Nevertheless, this was unfortunately not feasible due to limited hospital access, ongoing political conflicts and unstable security in Afghanistan.

The presence of disease vectors, especially mosquitoes was noticed in huge numbers (personnel communication and observation). Local health officials have to address the need for improving technical support and adopting better vector and disease control measures. This might contribute to increasing the accuracy of interpretation of the serological findings. Serological cross reactivity among closely related flaviviruses may hamper the discrimination of these etiologies, particularly when rising titers are difficult to attain. It is necessary to improve the health care infrastructure, develop and maintain enhanced surveillance to monitor and control these emerging diseases, and to help improve public health policy in Afghanistan.

Conclusions

Seroepidemiologic results suggest that dengue, West Nile, and tick-borne encephalitis viruses circulate within Afghanistan. Further information on the prevalence of these and other causes of acute febrile illness (AFI) is key to improving local syndromic treatment for AFI, and informing health protection advisories for travelers and people in the region.

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Human Use Statement

The study protocol number [OHDACA/CERP-01, IRB DoD#2007.0005, N-3 CPHS#:705] was approved by the U.S. Naval Medical Research Unit No. 3 Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

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