Am. J. Trop. Med. Hyg., 95(6), 2016, pp. 1469–1471
doi:10.4269/ajtmh.16-0174

Seroconversion to Causes of Febrile Illness in Mongolian Peacekeepers Deployed to South Sudan

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Abstract. Immediately before deployment (Fall 2012) and after deployment (Spring 2013) in support of United Nations peacekeeping operations, Mongolian Armed Forces medical personnel obtained serum samples from the first contingent of Mongolian peacekeepers deploying to South Sudan to monitor serologic evidence of exposure to diseases that cause acute febrile illness. A total of 632 paired samples were tested for IgG antibody for the following (number of seroconversions in parentheses): *Rickettsia* (spotted fever and typhus groups) (25), West Nile fever virus (WNV) (23), *Coxiella burnetii* (causative agent of Q fever) (12), dengue virus (8), leptospirosis (6), chikungunya virus (0), Congo–Crimean hemorrhagic fever virus (0), Japanese encephalitis virus (0), and Rift Valley fever virus (0). There was also evidence of exposure to WNV, *C. burnetii*, leptospirosis, and *Rickettsia* before deployment.

Historically, infectious disease represents the single most serious medical challenge to military operations. In recent years, the relative impact has decreased in comparison to trauma; however, deployments to unusual locations represent potentially new and/or elevated infectious disease risks.[1] Often, little information regarding disease risk is available from deployment destinations, since political instability and prevailing poor public health and medical networks tend to inhibit adequate disease surveillance. On the other hand, even when surveillance systems are functional, the accuracy of serosurveillance data from non-naive populations is questionable. Therefore, serological monitoring of presumably immunologically naive travelers represents an invaluable opportunity to better understand disease risks to enhance public health of the local population and manage the risk for future travelers.

According to Mongolian Armed Forces (MAF) health protection policy, serum samples from all deploying peacekeepers were collected and archived as a part of predeployment health screening approximately 2 weeks before travel while peacekeepers were housed at the deployment center, and then immediately upon return to Mongolia. There were no stopovers between South Sudan and Mongolia longer than a few hours. Therefore, there is a very high probability that seroconversions noted at screening have occurred during deployment in South Sudan and most probably represent circulating etiologies and disease exposure risks of South Sudan.

Before deployment, yellow fever, typhoid, hepatitis B, diphtheria, and tetanus vaccination was conducted on a unit basis; however, privacy regulations prevent any definitive conclusion whether all individuals were vaccinated, as well as the timing of any vaccination relative to blood draws. During deployment, peacekeepers did not have access to permethrin-treated uniforms, but did have mosquito repellent spray and cream. Also, weekly fogging was conducted in the camps, and mosquito nets were provided.

Mongolian troops operated mainly in Bentiu, Unity State in South Sudan, while smaller groups were deployed in Rumbek, Lakes State, and Wau, Western Bahr El Ghazal State. Still smaller groups were deployed in the Yida Refugee Camp in the northern part of Unity State. Note: the initial troops reaching South Sudan traveled from Juba to Bentiu by land.

While deployed, peacekeepers' water source was treated water from local wells. As such, treatment failures could allow for exposure to agents such as leptospirosis. The most common complaint reported by MAF medical personnel was influenza-like illness (no specific diagnosis made), but the second most common was rash. Approximately five cases of nephritis and two cases of hepatitis were noted during deployment.

In terms of existing knowledge about causes of acute febrile illness in South Sudan, dengue is known to occur in the region, but no specific information exists in the literature regarding dengue virus (DENV) circulation in South Sudan itself.[2,3] Congo–Crimean hemorrhagic fever (CCHF), West Nile virus (WNV), and chikungunya are known to occur in southern Sudan, on the border with South Sudan and Rift Valley fever (RVF) has occurred in South Sudan itself.[4–7] Importantly, RVF virus circulates sporadically or even cyclically, while most other agents of acute febrile illness circulate seasonally or continuously. Leptospirosis is known to occur in South Sudan.[8] Although circulation has been documented in the region, little is known about rickettsial disease or Q fever in South Sudan.[9,10]

In this study, peacekeeper's sera were separated and tested for the presence of IgG using the following commercial assays according to the manufacturer's instructions: WNV and CCHF indirect immunofluorescence assay (IFA) (Euroimmun, Luebeck, Germany), *Rickettsia* indirect IFA (Focus, Cypress, CA), leptospirosis and Q fever enzyme-linked immunosorbent assay (ELISA) (Serion/Virion, Würzburg, Germany), CCHF ELISA (VectorBest, Novosibirsk, Russia), and RVF recN IgG Indirect ELISA (BDSL, Johannesburg, South Africa). Sera were screened using hemagglutination inhibition (HAI) assay for Japanese encephalitis virus (JEV), DENV, and chikungunya virus and confirmed by plaque-reduction neutralization testing (PRNT) for JEV and DENV. Institutional review board (IRB) authorization for the Armed Forces Research Institute of Medical Sciences reference

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laboratory support work was obtained from the Walter Reed Army Institute of Research IRB.

HAI assays were performed using the adapted microplate method described by Clarke and Casals.[11] Two-fold serially diluted (heat inactivated and acetone extracted) samples were allowed to react with 8 hemagglutinating units of sucrose–acetone extracted DENV, JEV, or chikungunya virus antigens. After serum antibody and viral antigen reaction, residual hemagglutination was detected by subsequent addition of goose red blood cells. The highest dilution of sample that inhibited hemagglutination determined the HAI titer of the sample. A 4-fold rise in HAI titer between paired samples to any of the four DENV serotype, JEV or chikungunya virus antigens was defined as evidence suggestive of exposure, and any samples positive for any DENV serotype or JEV were tested by PRNT for antibody to JEV and all four DENV serotypes. However, neither the HAI nor the PRNT assays can determine DENV serotype of exposure.

To determine neutralizing antibody status, serum samples were tested by an in-house $PRNT_{50}$ using all four DENV serotypes and JEV as previously described.[12,13] A monolayer of *Macaca mulatta* kidney cells (LLC-MK2) was infected with 30–50 plaque-forming units of DENV in the presence of 4-fold serial dilutions (1:10 to 1:2,560) of a heat-inactivated sample on a 12-well plate. The assay was performed in three independent experiments (in duplicates). For each dilution, the number of virus plaques was counted and compared with the number of plaques in a control where no sample was added. Reference strains were as follows: DENV-1 (Thailand/16007/1964), DENV-2 (Thailand/16681/1984), DENV-3 (Philippines/16562/1964), DENV-4 (Thailand/C0036/2006), and JEV (0423 vaccine strain).

Seroconversion was defined as a 4-fold or greater rise in titer in a commercial assay, or a postdeployment sample positive by $PRNT_{50}$ where the predeployment sample was PRNT negative. Preexisting antibody was defined as any positive result in a commercial ELISA or IFA in the predeployment serum sample. The data were assessed in aggregate only, that is, there was no effort to link etiologies with clinical acute febrile illness of an individual patient during deployment.

There was no evidence of exposure to CCHF, RVF, chikungunya, or JEV; however, 25 peacekeepers seroconverted to *Rickettsia*, 23 to WNV, 12 to Q fever, eight to dengue, and six to leptospirosis during deployment. There was also evidence of exposure to WNV, Q fever, leptospirosis, and *Rickettsia* before deployment (Table 1). Although the manufacturer indicates a specificity of 98%, nonetheless, it is possible that the assay used for WNV may cross-react with some similar viruses, such as DENV, including the theoretical possibility that yellow fever vaccination could affect the results. However, of 23 samples positive for WNV seroconversion, less than half had any indication of antibody to the other flaviviruses evaluated in this study (JEV and DENV) based on the screening assay HAI. In addition, more than half of the samples that screened positive for another flavivirus were not IFA positive for WNV. Therefore, there is no apparent correlation of WNV positive results with positive results from other flaviviruses in this study.

Individual postdeployment samples often had fairly high HAI titers for JEV (1:80 to 1:640), whereas the DENV HAI titers for those same samples were often lower (1:160 or lower). However, JEV was never confirmed by PRNT, whereas most samples that screened positive for JEV by HAI instead proved to be positive for DENV seroconversion based on PRNT. Unfortunately, even DENV PRNT cannot identify specific serotype. Results from this study showed some PRNT cross-reactivity among the four serotype type strains used. Generally, the PRNT titer was substantially higher for either DENV-2 ($N = 3$) or DENV-3 ($N = 5$); however, it cannot be said that this necessarily represents evidence of seroconversion to any particular DENV serotype. On the other hand, the study provided definitive evidence of exposure to DENVs generally during deployment, as well as strong indication of exposure to Q fever, leptospirosis, WNV, and *Rickettsia*.

The study provided evidence that peacekeepers were exposed to tick-borne, mosquito-borne, waterborne, and airborne pathogens. The results indicate a continuing need for insect repellants, bed nets, and clean water for deploying peacekeepers. Also, antibiotics, especially doxycycline, should be available considering the possibility of cases of *Rickettsia* and Q fever in future deployments. The results do not indicate a need for any health follow-up of the peacekeepers themselves.

Preexisting antibody may have developed as a result of exposure to the agents either in Mongolia or during prior travel, including prior deployments; however, there is no statistical indication of prior exposure during other deployments. Some members of the unit were previously deployed to Sierra Leone, Chad, Darfur, Kosovo, Western Sahara, Afghanistan, and Iraq, but these deployments did not increase the frequency of seropositivity to any particular etiology. It is most likely that any prior exposures occurred in Mongolia.

Little is known regarding causes of acute febrile illness in Mongolia; however, illnesses consistent with rickettsiosis

TABLE 1

Serologic status of 632 paired (pre- and postdeployment) serum samples from Mongolian peacekeepers deploying in a United Nations Peacekeeping mission to South Sudan tested for IgG, hemagglutination-inhibiting or neutralizing antibody specific for various causes of acute febrile illness

	Predeployment seropositive (average titer of positives, if applicable)	Seroconversion (average titer of positives, if applicable)
Typhus group *Rickettsia* commercial IFA	130 (117.2)	25 (133.1)
SF group *Rickettsia* commercial IFA*	50 (121.6)	9 (128)
WNV commercial IFA	14 (307.7)	23 (413)
Q fever commercial ELISA	66	12
Leptospirosis commercial ELISA	15	6
DENV-1, HAI/PRNT	3 (16.7)/0	7 (26.7)/0
DENV-2, HAI/PRNT	6 (13.3)/0	7 (26.7)/3 (73.5)
DENV-3, HAI/PRNT	15 (18)/0	13 (41.7)/5 (92.2)
DENV-4, HAI/PRNT	15 (16)/0	14 (45.7)/0
JEV, HAI/PRNT	14 (42.9)/0	20 (108.4)/0

DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; HAI = hemagglutination inhibition (screening assay); IFA = immunofluorescent antibody assay; JEV = Japanese encephalitis virus; PRNT = plaque-reduction neutralization test (definitive assay); SF = spotted fever. HAI is a screening assay only and not considered to represent definitive evidence of serologic status. The number in the "predeployment seropositive" column for HAI assays represents all samples that had a measurable HAI titer. The number in the "seroconversion" column for HAI assays are those that showed a 4-fold or greater rise in titer. Samples shown as having an HAI 4-fold rise in titer represent 22 samples; many samples had an apparent rise in titer to various combinations of multiple DENV serotypes as well as JEV. Although positive results for PRNT for DENV were obtained with individual serotypes, there was evidence of DENV serotype-to-serotype cross-reactivity, and the results do not necessarily indicate evidence of exposure to a particular DENV serotype.

*Very rarely separate cases, nearly always positive for typhus group when positive for SF group.

have been noted clinically, and *Rickettsia* that could potentially cause rickettsial disease have been identified in ticks.[14] Also, clinical cases of spotted fever group *Rickettsia* have been identified in bordering areas.[15] Even less is known about Q fever in Mongolia, but it is reasonable to suspect that exposures are likely in light of the pastoral lifestyle of many residents.[16] Not surprisingly, leptospirosis has been identified in a wide variety of animal reservoir species, but little is known about leptospirosis in humans.[17] Also, little is known about arboviruses. The results of potential prior exposure reported here may also call for further research regarding causes of acute febrile illness in Mongolia itself.

The study illustrates that peacekeepers and travelers deploying to or visiting South Sudan are at risk for exposure to mosquito-borne arboviruses, as well as tick-borne, airborne, and waterborne diseases. Probably the most important result is definitive evidence of seroconversion to dengue in eight cases, without severe clinical manifestations. Repeated visits to the area could put travelers with a history of dengue infections at risk of severe dengue fever if they are subsequently exposed to a different DENV serotype.[18]

Received March 4, 2016. Accepted for publication July 20, 2016.

Published online October 24, 2016.

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