

TADR ACTIVITY PROJECT (TAP)
Georgia 712020
Project Proposal

Latest Revision: 31 October 2013

Project Title

Epidemiology of Febrile Illnesses caused by Dengue viruses and Other Arboviruses in Georgia

Project Summary

Circulation of dengue viruses and the incidence of disease caused by these and other arboviruses are not well characterized in Georgia. Epidemiological characteristics, geographic range, and contribution of arboviruses to human febrile illness in Georgia are also poorly understood with most cases of febrile illness or hemorrhagic fever remaining undiagnosed or misdiagnosed.

The overall goal of this proposal is to study the ecology of dengue viruses and other arboviruses (West Nile virus (WNV), Tick-borne encephalitis virus (TBEV), Chikungunya virus (CHIKV), hemorrhagic fever with renal syndrome (HFRS) causing hantaviruses, and Crimean Congo hemorrhagic fever virus (CCHFV)), the epidemiology of fevers caused by these pathogens, and to implement laboratory-supported studies to identify dengue virus and other arboviruses as possible etiologic agents associated with febrile illness or hemorrhagic fever. Implementation of modern diagnostic tests will provide the Georgian scientific community with approaches that could improve disease monitoring capabilities and enhance disease control efforts by Public Health system of Georgia.

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Detailed Project Information

I. Project Description (Introduction and Overview)

Arboviral infections can result in mild to moderate febrile illness, as well as severe disease, such as hemorrhagic or neurological manifestations. Most cases of febrile illness and hemorrhagic fever currently remain undiagnosed in Georgia. With the Georgian climate trending toward warmer temperatures over the last two decades, an increase in the population of disease vectors is likely, which will no doubt correlate with increased incidence of arboviral disease.

Dengue viruses. Dengue virus (DENV) is a positive-sense, single-stranded RNA flavivirus, and is the etiologic agent of dengue fever and dengue hemorrhagic fever (1). This virus occurs as four antigenically distinct serotypes with cross-reactive antibodies. Sequential infection is characterized by severe disease, which complicates efforts for vaccine development (2). Dengue fever is prevalent worldwide, especially in tropical and subtropical climates (3), with most cases occurring as subclinical or self-limiting febrile disease. Regions in which multiple serotypes circulate are at an increased risk for severe disease;

however viral distribution has not yet been investigated in Georgia. *Aedes* species, currently found in Georgia (historical data), are responsible for viral transmission, and sporadically imported cases of dengue fever in individuals travelling overseas have been registered (unpublished data).

Chikungunya virus. Chikungunya virus (CHIKV) is a positive-sense, single-stranded alphavirus also transmitted by *Aedes* mosquitoes (4). This virus is considered a serious threat to temperate areas colonized by *Aedes* spp., such as Europe and the Americas (5). The epidemic potential of this arboviral disease has been demonstrated by outbreaks in both Italy and France (6, 7), and warrants investigation in Georgia.

West Nile virus. West Nile virus (WNV), a positive-sense, single-stranded RNA flavivirus, can cause encephalitic disease and death in birds, humans, and horses. This mosquito-borne virus has been documented in Europe and Russia for decades (8, 9, 10). In Georgia, although WNV has not been detected in nature, evidence of WNV circulation has been suggested by detection of Immunoglobulin G (IgG) antibodies during seroprevalence studies. During the 1980s and 1990s, the NCDC conducted several sero-epidemiological studies using Soviet kits and reagents to test human and insect samples for the presence of WNV, and many samples were positive for WNV antigens (unpublished data).

Tick-Borne Encephalitis virus. Thousands of cases of severe central nervous system infections caused by tick-borne encephalitis virus (TBEV), a positive-sense, single-stranded RNA flavivirus, are reported annually throughout Central and Eastern Europe and Russia (11). Tick sampling and antigen testing has demonstrated that many *Ixodes* species carry the TBEV in Georgia. Two recent and severe human cases of TBE in Georgia have highlighted this growing public health concern. There is also evidence of the asymptomatic carrier state in humans (unpublished data). Currently, laboratory diagnosis of this infection is limited to the serological detection of specific antibodies. Thus, there is an urgent need for improving the ability to detect and identify the TBEV in Georgia.

Hantavirus. Hantavirus infections causing hemorrhagic fever with renal syndrome (HFRS) occur throughout most of Europe and Russia (12). HFRS has been registered in the South Caucasus region since before Soviet times. In Georgia, serological analysis of rodent samples collected during the early 1980s and 1990s revealed the existence of HFRS viral antigens in 1-5% of all samples obtained from various ecological niches. Limited information is available about Hantavirus infection in the Caucasus, though laboratory-confirmed cases of hantavirus infection in Georgia have been described (13).

Crimean-Congo hemorrhagic fever. Crimean-Congo hemorrhagic fever (CCHF) is a tick-born disease widely distributed in the southwestern regions of the former Soviet Union, the Balkans, the Middle East, western People's Republic of China, and Africa (14). CCHF is a substantial public health threat due to its high mortality rate, limited treatment options, lack of effective vaccine, and potential for human-to-human transmission (15). In Georgia, antigens of the CCHF virus have been detected during serological surveys of rodent populations and ixodid ticks. Additionally, a laboratory confirmed case of human hemorrhagic fever caused by CCHF virus in Georgia has been published (16).

Currently, epidemiological characteristics, geographic range, and contribution of arboviruses to human febrile illnesses in Georgia are poorly understood. Therefore, laboratory-supported studies to identify arboviruses as possible etiologic agents of febrile illness and hemorrhagic fever urgently need to be implemented. The investigation of multiple arboviruses that cause febrile illnesses with similar symptoms will contribute to the development of differential diagnostic capabilities.

In order to study the epidemiology of febrile illnesses caused by dengue viruses and other arboviruses, field materials and clinical specimens from undifferentiated febrile patients and from patients exhibiting hemorrhagic fever will be investigated. Laboratory investigations will be conducted using molecular and serological methods. Molecular investigations will include identification of specific viral genes by polymerase chain reaction (PCR) or real time (RT)-PCR in field collected specimens, and in acute-phase clinical samples including serum, plasma, or cerebrospinal fluid. Infection can be serologically confirmed by the detection of Immunoglobulin M (IgM) antibodies to the virus in acute phase clinical samples. Also, rising titers of specific IgG antibodies can be used to confirm convalescent serum specimens by Enzyme-Linked Immunosorbent Assay (ELISA).

Based on the results of the proposed study, differential diagnosis algorithms for febrile illnesses and hemorrhagic fevers will be developed in addition to algorithms for further ecological and epidemiological investigation of arboviral infections.

II. Scientific Goals

This project is designed to provide data on the ecology of dengue viruses and other arboviruses, and the epidemiology of diseases caused by these pathogens in Georgia. Information gained from this project will help to develop more focused and in depth studies in the future, and will enable the Georgian Public Health system to adequately respond to re-emerging biological threats.

Our research focuses on the following objectives:

- (1) To study the epidemiology of febrile illnesses caused by dengue viruses and other arboviruses in Georgia
- (2) To study the seroprevalence of selected arboviruses in Georgia as a discovery investigation
- (3) To develop differential diagnosis algorithms for febrile illnesses and their hemorrhagic manifestations

The proposed research meets CBEP goals and addresses several important issues that will have an impact on laboratory and epidemiologic surveillance capacities in Georgia. The proposed research focuses on viruses. The project will provide education and training for Georgian scientists, lead to important developments in detection and diagnostic systems for EDPs in Georgia, and support the integration of Georgian scientists into the international scientific community.

III. Technical Approach and Methodology

The duration of this project will be one year. This project will emphasize the implementation of modern techniques for diagnosis of arboviral infections causing febrile or hemorrhagic illnesses in humans and will obtain initial data on the ecology and epidemiology of arboviruses in Georgia.

Objective 1: To study the epidemiology of febrile illnesses caused by dengue viruses and other arboviruses in Georgia.

The project will consist of sampling expeditions for mosquitos and ticks during which samples will be collected from various locations throughout Georgia. Insects will be collected by NCDC and the USAMRIID/WRAIR Vector Surveillance team from NCDC.

Mosquitoes. Mosquitoes will be collected in both Eastern (Tbilisi) and Western Georgia (Batumi, Poti). Sampling expeditions to Western Georgia will be conducted twice in the period July to September with each trip lasting 10 days (details are found in the Form B). In Tbilisi, mosquitoes will be collected three times in the period from July through to September. Mosquito collection will employ baited miniature light traps in an effort to collect both host-seeking and gravid females (17). In order to achieve the collection of approximately 10,000 female *Aedes species*, a total of approximately 100,000 mosquitoes will need to be collected. Field teams will be responsible for identifying insects to species and pooling female *Aedes species* into pools of 10 females per pool based on sampling site (18, 19). A total of 100 pools will be tested for the presence of arboviruses (19, 20). Insect research will not be conducted in this project.

Ticks. As part of the already approved CBR GG-19 project, ticks will be collected in South Georgia in regions with endemic bunyaviruses. However, this project will also collect ticks and expeditions to South Georgia will be conducted twice, each for seven days, during spring and summer (details are found in the form B). Ticks will be collected using “drag sampling” and carbon dioxide-baited sticky traps. Drag sampling of ticks is a commonly used method to estimate the abundance of free-living ticks. The technique involves dragging a blanket over the vegetation for a fixed time or distance and counting the number of ticks found clinging to the blanket (21, 22). Carbon dioxide-baited sticky traps are based on the CO₂ signal that attracts host-seeking ticks (22). The trap consists of a covered ice bucket or Styrofoam container with several holes in the sides near the bottom through which gaseous CO₂ could emit. Dry ice is placed in a container attached to a rectangular plywood base. The upper perimeter is covered with a double-sided carpet tape to provide a sticky surface. Ticks will be pooled in groups of 2-10 by species, sex, collection date, and site (15). A total of 100 pools will be tested for the presence of arboviruses. Tick sampling will also be conducted by NCDC in cooperation with the WRAIR-USAMRIID Vector Surveillance teams under CBR GG-19. Samples, after they are utilized by GG-19 teams, and inactivated, will be used in this TAP project. Insect research will not be conducted in this project.

Rodents. Field collected materials from rodents collected under CBR GG-19 will be utilized by the NCDC lab. Aliquots of field samples will be made and screened using appropriate methods for each targeted virus, including ELISA and real time (RT)-PCR. A database for all samples will be compiled containing the samples ID numbers, sources, initial locations and investigation results. Sample logging, transfer, and sample handling are all conducted using DTRA-approved SOPs. Rodent sampling will be conducted by NCDC in cooperation with WRAIR-USAMRIID Vector Surveillance teams under CBR GG-19. Samples, after they are utilized by GG-19 teams, and inactivated, will be used in this TAP project.

Objective 2: To study the case identification of selected arboviruses in Georgia

Patients presenting with febrile illness or hemorrhagic fever at clinical study sites will be investigated. Blood specimens will be utilized from hospitals that are currently under investigation through the CBR GG-19 and GG-21 projects. The project information / data on each sample will be provided to the Project Manager, Dr. Kutateladze in order to conduct epidemiological data analyses. These samples will then be used for laboratory testing. A total of 200 samples will be used for testing. In addition, any information gained from the standardized questionnaires will be used to study the epidemiology of the diseases caused by arboviruses.

The samples obtained will be tested for dengue, Chikungunya, West Nile, Tick-borne encephalitis, hantavirus, and Crimean-Congo hemorrhagic fever viruses. It is important to note, that in this part of the study, patient samples will have already been collected from previous studies, including CBR projects GG-19 and GG-21. Clinical protocols and informed consent for these projects specifically state that

patient serum could be used for future research efforts. Therefore, no new clinical protocols, IRB, and SOPs or procedures will be required to complete this part of the study. Patient samples will have been stored at -20 °C and will be thawed for use in this investigation. All serum and blood samples will be heat inactivated for ELISA or already prepared for PCR under CBR GG-19 and/or CBR GG-21 and then used for this project. Laboratory investigations will be conducted using molecular and serological methods. Molecular investigations will include isolation and purification of viral RNA from acute-phase clinical samples including blood or serum, and identification of specific viral genes by RT-PCR. Serological investigations will be completed using ELISA assays specific to each pathogen. Viral research will not be conducted in this project.

Diagnostic Assays

<i>Pathogen</i>	<i>Available Assay</i>	<i>Source</i>
Dengue viruses	Platelia NS1 ELISA (23), IgM, IgG ELISA RT-PCR kit	Standard Diagnostics DTRA Assay
Chikungunya virus	ELISA	NovaTec
	RT-PCR kit	DTRA Assay
West Nile virus	ELISA	Focus Diagnostics
	RT-PCR kit	DTRA Assay
Tick-borne encephalitis virus	ELISA	NovaTec
	RT-PCR kit	DTRA Assay
Hantavirus	ELISA	Focus Diagnostics
	RT-PCR kit	DTRA Assay
Crimean-Congo hemorrhagic fever virus	ELISA	Vector-Best
	RT-PCR kit	DTRA Assay

IV. Expected Results

The successful implementation of this project will enhance the pathogenic virus detection capabilities of NCDC, will provide data on arboviral ecology and epidemiology of febrile illnesses in Georgia, and will contribute to the development of algorithms for differential diagnosis of febrile illnesses and hemorrhagic fevers. Experimental findings could be used for further pathogen-specific research in Georgia. We will consider the development of new algorithms for the testing and detection of these viruses as a final deliverable of this project.

V. Tasks

Task Description			
Project management, reporting, and administration			
	Description of Deliverables	Responsible Party	Schedule for Completion
1	Materials & equipment procurement	CH2M HILL	Q1
2	Training on entomology and lab techniques	NCDC	Q1-Q2
3	Sample transfer from the CBR GG-19 and GG-21 projects	NCDC	Q1
4	Collection of field materials	NCDC	Q2-Q3
5	Laboratory investigations	NCDC	Q2-Q4
6	Data analysis	NCDC	Q4

* Note: Schedule for Completion starts with initiation of the project

VI. Project Management

This project will be managed by NCDC. The project manager will communicate through BTRIC Georgia with U.S. Collaborators via e-mail or conference calls and will be responsible for preparation of monthly reports and project presentations for international conferences. The project manager will also be responsible for budget management in collaboration with BTRIC Georgia. Duties of the project manager are detailed below.

Project reporting

- Consolidation of monthly reports from participating institutes (assemble and quality check deliverables);
- Preparation of a short executive summary for the monthly report;
- Leadership in preparation of project presentations, abstracts for conferences and international meetings;
- Publications in peer review journals;
- Regular communication with the U.S. Collaborators;
- Participation in monthly project calls;
- Organization of the meetings of the TAP project participants (all sub-projects) to discuss the project progress;
- Participation in international conferences and meetings.

Team Member Responsibilities:

- Team members must take ownership of all project activities;
- Along with the project manager, they are directly responsible for the successful completion of the project;
- Ensure that they have the time required for the project;
- Are familiar with the science work plan and project SOPs and protocols;

- Record the project work in a laboratory book/journal;
- They must rely on each other and work well as a team;
- They must be technically competent in the area of their responsibility;
- Their skills must complement others in the group - overlapping skills allow for changes in assignments at a later date.
- Participation in international conferences

VII. Meeting Goals and Objectives of CBR Program

EDPs remain a significant public health threat in Georgia. Development and application of modern diagnostics could lead to the improvement of disease monitoring capabilities and enhance disease control efforts in Georgia.

Our proposal focuses on viruses which are among EDPs and addresses the following CBEP goals:

1. Enhancing detection and diagnostic capabilities for especially dangerous pathogens
2. Providing background data for further pathogen specific research studies
3. Integrating host nation scientists and institutes with expertise in research into the international scientific community.

VIII. Pathogens

Pathogen	Biosafety Level (Diagnostic Quantities)			
	1	2	3	4
<i>Environmental samples potentially containing inactivated arbovirus</i>		X		
<i>Human serum samples potentially containing inactivated arbovirus samples</i>		X		

All specimens will be inactivated before investigation, and lab work will be carried out at BSL-2 using BSL-3 practices. All specimens collected in this project, or, transferred to this project, will be destroyed at the completion of the project. Please note that some required biosafety items are not listed in the Form B. This omission of biosafety items from the Form B is accommodated through sharing of boots and field biosafety items with CBR GG-19. All biosafety items are accounted for based on a review conducted on 29 October 2013.

IX. Approximate Budget

Form B contains all of the relevant budgetary information.

X. Relevance to DoD Program Objectives

This study directly supports DoD requirements and will facilitate the establishment of a biological baseline of pathogenic organisms in the environment. This study will increase knowledge of the epidemiology and ecology of several viral EDPs in Georgia and will promote involvement of Georgian scientists in the international scientific community for coordinating research on human disease agents.

XI. Relationship to Other On-Going or Planned BTRP Projects

This work will be continuation of cooperative studies with U.S. specialists. CBR Project GG-18 demonstrated the presence of EDPs in environmental and animal samples. Within the current project, we plan to study cases of viral EDPs in environmental and clinical samples. In addition, this project reuses and utilizes costly human clinical samples from previous projects to extrapolate additional information and disease baseline data for Georgia. Further, unconfirmed studies have shown that dengue may be present in Georgia; this is the first comprehensive study to address this question, which could lead to scientific publications and international scientific debate on dengue migration into the Caucasus.

XII. References

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