I S T C

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PARTNER PROJECT AGREEMENT #G-2101p

BETWEEN

THE INTERNATIONAL SCIENCE AND TECHNOLOGY CENTER,

THE NATIONAL CENTER FOR DISEASE CONTROL AND PUBLIC HEALTH
AND

US Department of Health & Human Services / US Centers for Disease Control and Prevention

Emerging zoonotic pathogens in Georgian bats

Operative Commencement Date: February 1, 2014

Article 16 - Annexes

As specified in Article 1.2, the Annexes are an integral part of the Agreement. They are:

Annex I	Work Plan
Annex II	General Conditions
Annex III	Formats for Progress and Cost Reports
Annex IV	Disclaimer

Article 17 - Entry into Force of the Agreement

The Agreement shall enter into force on February 1, 2014.

Prepared in Moscow in the English language.

For the Center	For the Recipient	For the Partner
David Cleave Acting Executive Director ISTC	Amiran Gamkrelidze General Director National Center for Disease Control and Public Health	Eduardo Gomez Biosecurity Coordinator US Department of Health& Human Services/US Centers for Disease Control and Prevention
23.01.2014 (date)	11.02.2014 (date)	3 Feb 2014 (date)

ANNEX I

Work Plan

I. Summary Project Information

1. Project Title

Emerging zoonotic pathogens in Georgian bats

2. Project Manager

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3. Participating Institutions

3.1 Leading Institution

Short reference:	NCDC		
Full name:	National Center for Disease Control and Public Health of Georgia (NCDC&PH)		
Street address:	9 M. Asatiani str		
City:	Tbilisi	Region:	Tbilisi
ZIP:	0177	Country:	Georgia
Name of Signature Authority:	Amiran Gamkrelidze		
Title:	MD, PHD	Position:	Director General
Tel.:	995 32 231 17 55	Fax:	+995 32 231 17 55
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Governmental Agency:	Ministry of Health		

3.2 Other Participating Institutions

None

4. Foreign Collaborators/Partners

4.1. Collaborators

Institution: Centers for Disease Control and Prevention	
Street address: 1600 Clifton Rd NE	
City: Atlanta	Region/State: Georgia
ZIP: 30030	Country: USA
Person: Dr. Andres Velasco Villa	
Title: Microbiologist	Position: Lead, Genomics and Diagnostics Development Unit, Rabies Team, Poxvirus and Rabies Branch
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4.2 Partners

Institution: U.S. Centers for Disease Control and Prevention	
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Project Coordinator: David Bull	
Title: PhD	Position: BTEP Program Manager
Tel.: 1(404) 639-0536	Fax: +1(404) 235-0292
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5. Project Duration

18 months

6. Project Location and Equipment

Institution	Location, Facilities and Equipment
Funding Institution	Tbilisi, Georgia, National Center for Disease Control and Public Health
	CPHRL building SSLI, BSL 2 Facilities
	Room numbers: 2131; 2213; 2215; 2221,
	Available main equipment
	Biological Safety Cabinet, Class II, type 2A/B3, Incubators, CO_2 Incubators, Refrigerators, -80° C Freezers, Fluorescent microscope, Thermo cyclers, Techno, Light Cycler Roche 2.0, Thermo-mixer, Eppendorf, Ultracentrifuge, Rotina-Gesa 3000 x, Centrifuge Horizontal

	and vertical electrophoresis equipment
	Gel documentation and analysis system, Genetic Analysis System CEQ-8000 Beckman Coulter, Sequencer - ABI 3130XL; WGS - Illumina MiSeq platform, PCR Workstations

II. Specific information

1. Introduction and Overview

Bats as Important Reservoirs of Zoonotic Diseases

Bats are increasingly recognized as reservoirs of emerging zoonotic pathogens. The importance of bats as a reservoir of infectious viral agents potentially transmissible to humans and other animals has become more evident with the passage of time. For example, West Caucasian bat virus (WCBV) was isolated from an insectivorous bat (*Miniopterus schreibersii*) in the Russian part of the Great Caucasian Ridge ~300 km away from the Georgian border. This virus, and related viruses Ikoma and Lleida Viruses, is currently the most divergent member of *Lyssavirus* genus. Commercially available rabies biologics do not provide protection against WCBV. The virus is pathogenic for laboratory animals (such as mice, and hamsters), but its significance for public health is unknown, since fast rabies cleanouts in Georgia, similar to other countries of Eastern Europe, is typically carried in the majority of cases, and is not supplemented by virus identification.

The National Centers for Disease Control and Public Health (NCDC&PH) in collaboration with the U.S. CDC conducted bat surveillance in five regions of Georgia during June, 2012. Eight species of bats were identified among captured 236 bats in total. Samples from these bats are stored at NCDC&PH and in September 2013 will be examined by direct fluorescent antibody (DFA) testing to detect Lyssaviruses. During the survey approximately 40% of collected bats were *Rhinolophus spp*. Accordingly, there is the possibility of existence newly emergent CoV in Georgian bats.

The data obtained from the above mentioned survey triggered initiation of this project. The objectives of the project will be: characterization of DFA positive Lyssaviruses by cell-culture methodologies; study prevalence of coronaviruses in Georgian bats; comparison of human and bat Lyssaviruses.

Lyssaviruses

Rabies virus is a genus of *lyssavirus*, and is listed as a priority pathogen by the Georgian ministries of Health and Agriculture. Even with limitations of the existing national surveillance system, 40-100 animal cases, and 6-12 human cases of rabies are documented in the country every year. No recent rabies virus (RABV) isolates have been characterized thoroughly at the molecular or antigenic level, and information on their circulation patterns, host ranges and distribution is lacking.

In frame of the project it is planned to carry out the following: PCR confirmation and sequencing of Lyssaviruses from DFA positive samples; collection of fecal material and rectal swabs from bats for further coronavirus investigation by molecular methodologies; comparison of sequence data of Lyssaviruses from human and bat samples.

Coronaviruses

Among the different virus families hosted by the bats, much attention has been paid to the coronaviruses which demonstrate considerable variability in these hosts. This is also associated with the recent role of Chiroptera as a source of coronaviruses closely related to the SARS virus which, no more than ten years ago, caused a serious global epidemic in humans.

In view of this, there have been numerous studies in recent years with the goal of finding new species of coronaviruses in bats in order to monitor the viral evolution, to study the possible origin of human SARS virus, and to predict possible new outcomes as outbreaks in humans.

On the other hand, the local surveillance of the emerging coronavirus will suspect the Caucasus region for the possible outbreak of the SARS-related CoV and a novel beta (β)-CoV which cause fatal respiratory diseases.

The 2002 severe acute respiratory syndrome (SARS) epidemic in China resulted in 8,098 laboratory-confirmed human cases with 916 fatalities. The causative agent was a newly emerged coronavirus (CoV) that may have originated from a similar virus, SARS-related CoV, discovered in Chinese horseshoe bats (*Rhinolophus spp.*). The recent implication of a novel beta (β)-CoV as the cause of fatal respiratory disease in the Middle East emphasizes the importance of surveillance for CoVs (MERS-CoV) that may potentially jump species from bats into humans. During the first bat survey Georgia ~40% of collected bats were *Rhinolophus spp.* accordingly, there exists the possibility of identifying newly emergent CoV in Georgian bats. These data will serve as preliminary data to support second survey consisting of the collection of fecal material and rectal swabs from bats for further coronavirus investigation by molecular methodologies. The potential risk of a new group of bat coronaviruses as a reservoir for human infections is suspected.

Benefits of Work

Implementation of this study will help in the future to identify the infection source and pathogens transmission path from the bats to humans in compliance with different epidemiological tools. Under the project diagnostic infrastructure is established that will lead to the early detection and prediction of emerging and reemerging diseases in Georgia.

The accomplishment of the project will provide Georgian researches involved in the project with the possibility to gain experience and establish international connections for performing fundamental, as well as applied studies within the framework of international science and technology programs in the field of biology and medicine.

The proposed project intends to research the emerging infectious diseases, specifically lyssavirus and coronavirus, and knowledge of the antigenic and genetic properties of lyssaviruses will be important for selecting further vaccine candidates.

In addition, although the significance of bats as reservoirs of zoonotic diseases in the Caucasus region is unknown, we do know that West Caucasian bat virus circulates in *Miniopterus schreibersii* bats in the area. The same bat species was recently implicated as reservoir of a novel *filovirus* in Spain. After second bat sampling, which will be provide under this project, will be collected different tissues from the bats, for further harvesting for several emerging pathogens. By information from other parts of Europe and world, bats from Caucasus are expected to maintain circulation of important zoonotic.

2. Expected Results and Their Application

The proposed study will be the first investigation of lyssavirus and coronavirus condoned in Georgia. Implementation of this study will help in the future to identify the infection source and pathogens transmission path from the bats to humans in compliance with different epidemiological tools.

The project will allow detection of novel emerging and re-emerging pathogens, like coronavirus, as well as a better understand of their host range, pathobiology, distribution and circulation patterns, and the existing and potential threat for veterinary and human public health, as well as for the bat populations, which perform an essential ecological role.

Project will supply the laboratory with reagents necessary for providing reliable results that are important for timely responses to outbreaks of emerging pathogens.

Finding new isolates of WCBV or novel lyssaviruses and coronaviruses in bats will provide new insights on the diversity of rabies and acute respiratory syndrome causative agents in the region. This information can be of

further use for vaccine development and for the implementation of focal prevention and control strategies to prevent emerging infections diseases in humans.

No recent lyssa and corona viruses have been investigated, characterized at the molecular level and information on their circulation patterns, host ranges and distribution is lacking. Improvements in laboratory capacity and staff training can accomplish this goal.

Phylogenetic analysis of lyssavirus strains isolated in the frame of the project will provide us with findings for understanding lyssavirus evolution.

Antigenic and genetic properties of lyssaviruses may be important for selecting further vaccine candidates and immunoglobulin preparations.

Decision of the project is to establish laboratory diagnostic infrastructure that will lead to the early detection and prediction of emerging and reemerging disease in Georgia.

The incidence of rabies post-exposure prophylaxis (PEP) in Georgia is highest in Europe region, 876/100,000. Expected that West Caucasian bat virus (WCBV), circulate at Georgia. Commercially available rabies biologics do not provide protection against WCBV.

Implementation of this study will help in the future to identify the infection source and pathogens transmission path from the bats to humans in compliance with different epidemiological tools.

3. Meeting ISTC Goals and Objectives

The development of the project will provide Georgian researches that are involved in the project with the possibility to gain experience and establish international connections for performing fundamental, as well as applied, studies within the framework of international science and technology programs in the field of biology and vaccinine. It also will allow the establishment and organization of productive international activities at the National Center for Disease Control and Public Health.

4. Scope of Activities

Task 1

Task description and main milestones		Participating Institutions
Bat surveillance for taking different types of samples		1- NCDC
	Description of deliverables	
1	Ordering equipment and capacities for bat survey, working on the protocols – develop protocols based on experience of CDC, USA. Planing of survey	
2	Collecting bats from 5 regions of Georgia: Imereti and Kakheti and taking tissues for Lyssavirus investigation brains and tissues for corona virus collection, archival fecal material and nasal swabs. Handling, ringing, and sampling, dissection by taking tissues lung, kidney, liver, spleen, intestine for further investigation on different viruses	
3	Bat Samples arrangement and storage for further investigation	

Task 2

Task description and main milestones	Participating Institutions
Strengthening of laboratory capacity for detection and characterization of Rabies etiological agents	1. NCDC
Description of deliverables	
1.	Purchasing of supplies and reagents for project needs. Development and implementation of standard operating protocols for the detection and genetic characterization of etiological agents of rabies
2.	Screening of bat's brain samples for the detection of Lyssavirus antigen by Direct fluorescent antibody test (DFA)
3.	Genetic characterization of all Lyssavirus positive samples by end point Reverse Transcription-PCR (RT-PCR) coupled to nucleotide sequence and phylogenetic analysis of the partial and complete N-gene sequences.
4.	Reconstruction of the evolutionary and dissemination dynamic history of recent and historical lyssaviruses found in this study

Task 3

Task description and main milestones	Participating Institutions
Laboratory PCR diagnostic for Coronavirus	1- NCDC
Description of deliverables	
1.	Ordering reagents for coronavirus PCR diagnostic, implementation of a pan-coronavirus RT-PCR assay in bat's feces and organ samples
2.	Coronavirus positive specimens by RT PCR will be further genetic characterization by sequencing and phylogenetic reconstructions.
3.	Combination of all obtained laboratory results with epidemiological data will be used to construct some predictive models which will be used to enhance disease surveillance strategies and better implement prevention and control activities.

Task 4

Task description and main milestones	Participating Institutions
laboratory PCR diagnostic for Corona virus	1. NCDC

	Description of deliverables
1.	Analysis data quarterly
2.	Comparison obtained results with different study data
3.	Working with epidemiologists for identification the infection source and pathogens transmission path from the bats to humans in compliance with different epidemiological tools.

5. Role of Foreign Collaborators/Partners

Provide training and transfer adequate technology for the detection and genetic characterization of Lyssavirus and coronavirus circulating in the bats populations of the Georgian Caucasus.

Provide sequences of primers and reagents for the detection of Lyssaviruses and coronaviruses

Provide guidance on the safety and good laboratory practices to handle Lyssaviruses and coronaviruses

6. Technical Approach and Methodology

The conducted work will include following methodologies:

- Bat Surveillance, which will be conducted 2 regions of Georgia: Imereti and Kakheti during first quarter of project. Bats, will be sampled randomly, manually or using nets from different roosts in compliance with CDC protocols. The numbers per roost and species will approved by expert zoologists to avoid harmful consequences for bat populations.
  - The Bats will be anesthetized by a 0.05 - to 0.1 mg intramuscular injection of ketamine hydrochloride, measured, identified to species, weighted, tagged, recorded, and subjected to euthanasia via cardiac bleeding out. Afterward, oral and rectal swabs, and the following tissue samples were obtained: brain, lung, kidney, liver, and spleen, intestine. Will be collected a rabies fecal material. All specimens will be held in dry ice (after collection) and later placed in -80°C.

    Lyssaviruses
  - Lyssavirus screenings will be conducted in brain samples with a Lyssavirus pan- reactive cocktail of fluorescent monoclonal antibodies.
  - RT-PCR coupled with sequencing analysis will be used to characterize Lyssavirus positive specimens genetically and to conduct the phylodynamic studies.

    Coronaviruses
  - RT-PCR coupled with sequencing analysis will be used to screen and characterize coronaviruses from bat fecal samples.

    Modeling
  - GPS collection data together with sequence data and abiotic variables will be used to construct predictive models for disease occurrence for both Coronaviruses and Lyssaviruses.