

**Statement of Work Between APHIS, SENASA and IICA**  
**Project: Characterization of *M. bovis* strains from Costa Rica**  
(Reference Cooperative Agreement 20-9419-0570)

**Background**

*Mycobacterium bovis*, a member of the MTBC, is an important livestock and human pathogen. Understanding the epidemiology and movement of the pathogen is critical to the control of the disease. The advent of whole genome sequencing (WGS) allows an unprecedented method for better characterizing outbreaks and recognizing when and where spillover into humans, or potentially spill back into livestock occurs.

Whole genome sequencing (WGS) of *Mycobacterium bovis* and other animal adapted strains (zoonotic tuberculosis or zTB) is replacing traditional genotyping methods in laboratories across the world. The resolution of WGS is startling and can provide great benefit to disease investigations and research. However, the method is only as good as the database of isolates used for comparison. The addition of sequences from other countries, especially from the same lineages can add important perspective to the interpretation of local outbreaks and understanding research results. Consequently, international collaboration and cooperation among animal health ministries and researchers is needed to fully utilize this new high resolution technology.

A pillar of disease control is correct and timely diagnosis, using standard validated methods. The national reference laboratory in Costa Rica (Laboratorio Nacional de Servicios Veterinarios-LANASEVE) plays an essential role in the surveillance of bovine tuberculosis (bTB), supporting field veterinarians and animal health authorities. Consequently, it is of great importance to maintain up-to-date knowledge on the necessary diagnostic procedures. A way to achieve this is through collaboration with other laboratories. The U.S. Department of Agriculture, through the Animal and Plant Health Inspection Service (APHIS) and the National Veterinary Services Laboratories (NVSL) conducts capacity-building activities and training for foreign animal health laboratories to harmonize disease diagnostics. Given the importance of bTB worldwide, international efforts must take place in order to achieve successful eradication of the disease.

To fulfill this necessity for collaboration, the USDA APHIS, under Cooperative Agreement 20-9419-0570CA, will provide Federal financial assistance to SENASA (Servicio Nacional de Salud Animal) to characterize the dominant *M. bovis* strains circulating in Costa Rica. This will be done by collecting representative samples (both historical and recent) to isolate MTBC bacteria and subsequently perform WGS for strain characterization. At the same time, the USDA APHIS will provide laboratory and field support through training that focuses on the needs and priorities of SENASA to improve bTB diagnostics and surveillance. Consequently, USDA APHIS seeks to collaborate with IICA-Costa Rica (Inter-American Institute for Cooperation on Agriculture) to act as the financial operator of this project and manage, administer and execute the obligated funds.

## Objectives

Our primary objective is to collect MTBC isolates from cattle (samples from swine and wildlife would also be acceptable) for genotypic characterization, through whole-genome sequencing, from endemic countries in the Americas. The goal will of this particular project will be to sample as broadly as possible to characterize the dominant *M. bovis* strains occurring in Costa Rica. Secondary objectives include: building laboratory capacity; providing training to collect, isolate and identify *M. bovis* from tissue; and provide local scientists an opportunity to publish the findings.

## Activities

**Sample Collection and Analysis:** The local laboratory over the course of the year, will collect tissues from cattle and other susceptible animal species in formalin and sodium borate or fresh/frozen at abattoirs and during necropsies. The goal will be to sample as broadly as possible to characterize the dominant MTBC strains occurring in Costa Rica.

**Laboratory Testing:** Bovine tissue samples will be sent to the local laboratory for culture/isolation and identification of *M. bovis* using a variety of media. This information will be used to determine the areas of training emphasis after the initiation of the project. Once isolates have been recovered, they will be heat killed and/or DNA extracted according to standard protocols and then the heat killed cells and/or DNA will be shipped with the appropriate permit to NVSL for whole genome sequencing and strain characterization. Results of the sequencing and the raw data will be provided to the collaborators as soon as they are available.

**Training:** A two-phase training process will take place.

- a. Two laboratory personnel will travel to NVSL in Ames for one week to be trained in tissue processing and molecular diagnostic protocols for *M. bovis*. NVSL will provide SOPs and laboratory subject matter experts fluent in Spanish for consultation as needed.
- b. Two USDA APHIS personnel (one NVSL bacteriology expert and one APHIS-IS TB expert) and one USDA APHIS ORISE will travel to Costa Rica to provide field and laboratory training.

**Analysis and Publication:** Sequences along with the agreed upon metadata will be shared internally between the recipient and USDA APHIS as soon as the results are available and then will be deposited after 12 months (or earlier if both parties agree) to The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). Any resultant publications will be approved by both parties.

IICA (Inter-American Institute for Cooperation in Agriculture, Agricultural Health and Food Safety Program, will act as the financial operator, handling and administering the funds for this project.

## Period of performance

This agreement shall become effective December 1, 2020 and shall continue through September 30, 2021, subject to continuation in writing by mutual agreement of the parties. Further, this Agreement may be amended at any time during the effective period by mutual agreement of the parties in writing. It may be suspended or terminated following provisions of 2 CFR Part 200.339-342.

## Budget

The funds available for this project total \$20,350.00 USD.

<b>FY2020 FINANCIAL PLAN</b>	
<b><i>M. bovis</i> strain characterization from Costa Rica</b>	
<b>Costa Rica Animal Health Authorities (SENASA and LANASEVE)</b>	
<b>ITEM</b>	<b>TOTAL BUDGET</b>
<b>PERSONNEL:</b>	
1 USDA APHIS NVSL personnel	
1 USDA APHIS ORISE personnel	
1 USDA APHIS IS TB expert personnel	
<b>Subtotal</b>	<b>\$0.00</b>
<b>TRAVEL:</b>	
2 Costa Rica LANASEVE personnel to NVSL in Ames, IA for lab diagnostics training [1 week]	\$5,000.00
<b>Subtotal</b>	<b>\$5,000.00</b>
<b>EQUIPMENT:</b>	
Mini-Beadbeater-24 (BioSpec Products)	\$5,000.00
<b>Subtotal</b>	<b>\$5,000.00</b>
<b>SUPPLIES:</b>	
Laboratory supplies	\$4,500.00
Supplies and material for field training (in Costa Rica)	\$3,000.00
<b>Subtotal</b>	<b>\$7,500.00</b>
<b>OTHER:</b>	
Shipping	\$1,000.00
<b>Subtotal</b>	<b>\$1,000.00</b>
<b>TOTAL DIRECT COSTS</b>	<b>\$18,500.00</b>
<b>Overhead Expenses</b>	<b>\$1,850.00</b>
<b>TOTAL PROJECT COSTS</b>	<b>\$20,350.00</b>

## **Quantitative projection of Accomplishments to be achieved**

Provide Federal Financial Report quarterly and submit Request for Advance or Reimbursement (SF-270) as needed. You can send a scanned copy of Financial Reports to: [Connie.J.Osmundson@usda.gov](mailto:Connie.J.Osmundson@usda.gov)

Quarterly accomplishment reports will be submitted to NVSL as required using a standard, agreed-upon format. The report should include a list of accomplishments of agreed upon work functions in objectives including the progress and activities related to the project. The report will contain narrative explanations for any deviations from projected norms. The report will be submitted to [Connie.J.Osmundson@usda.gov](mailto:Connie.J.Osmundson@usda.gov) within the time frames indicated the Notice of Cooperative Agreement award. When accomplishments cannot be quantified by activity or function the Cooperator will provide the target date for each function and an estimate of what capabilities will be.