Project Title:	Development of subunit vaccine against Rift Valley Fever		
Annual Report	Period Covered: 12 Months		
KCSAP livestock Applied	Value chain:	Duration: 18 Months	Start Date: Oct 2020
Lead Institution:	Biotechnology Research Institute-Kabete		
PI and contacts:	Dr. Yatinder Binepal Email: ybinepal@yahoo.com		
Collaborators and their contacts: 1. Eugine Mukhaye Email: euginemukhaye17@gmail.com 2. Francis Chaka Email: chakawekesa@gmail.com 3. Leonard Ateya – ateydel@gmail.com			
Background: Rift Valley Fever (RVF) is a transmissible zoonotic disease capable of very serious and rapid spread irrespective of national border. The disease has serious socio-economic and public health consequences. During an outbreak, the infection is characterized by an abortion storm in pregnant animals and death among animal population with mortality rates as high as 90% in young animals and 30% in adults. This leads to drastic loss of herds and flocks, resulting into food insecurity and loss of revenue not only to the farmers but also to the traders, butchers and a country. The disease has potential to cause death among naïve human population with recent statistics showing a mortality rate of up to 30%. The virus affects wild animals, domestic animals and humans. Outbreak of RVF usually occurs whenever there are heavy rainfall and flooding and a synchronous generation of large numbers of infected mosquitoes. Animal infection is characterized by abortion, fever, lack of appetite, mucopurulent nasal discharge, bloody diarrhea and death. Humans get RVFV as a result of bites from infected mosquitoes and through exposure to blood, body fluids, or tissues of infected animals. Direct exposure to infected animals can occur during handling and slaughter or through veterinary and obstetric procedures. Laboratory technicians are at risk of acquiring the disease by inhalation of infectious aerosols generated from specimens. Fever, chills, severe headache, diarrhoea and vomiting characterize the disease in humans. In most cases, the disease lasts for 4 to 6 days with complete recovery, but in some cases, it can lead to further complications such as encephalitis, retinitis, blindness, hepatic necrosis, or fatal hemorrhagic fever. The disease has no cure and control is by vaccination with live-attenuated and formalin-inactivated vaccines. This project is developing a safe, efficacious and cheap subunit vaccine to confer protection against virulent strain of RVFV.			

Objectives
1. Clone and express RVFV NS/NC genes **2.** Characterize expressed RVFV NS/NC genes **3.** Evaluate a subunit vaccine candidate against RVFV.
Expected Outputs
1. Escherichia coli expression system expressing the RVF NC and NS genes generated **2.** Recombinant RVFV NS/NC proteins developed and characterized **3.** Subunit vaccine candidate against RVFV evaluated.

ANNUAL REPORT
I ACHIEVEMENTS

Objective 1: Clone and express RVFV NS/NC genes
Activity 1.1
  i. Primer design
  ii. Animal cell culture and infection of cells with RVFV strain G64 and 1883
  iii. Replication of RVFV in LT cells.
Achievement 1.1
  i. Primers specific to RVFV NC and NS genes developed
  ii. Total RNA from cells infected with RVFV strain G64 and 1883 successfully extracted
  iii. RVFV successfully replicated in LT cells.

Activity 1.2
  i. Extraction and characterization of total RNA extracted from infected animal cells
  ii. Reverse transcriptase PCR
  iii. Cloning and expression of RVFV NC and NS genes in BL21 strain of E coli.
Achievement 1.2
  i. The correct size of NC and NS genes correctly amplified
  ii. Total extracted RNA characterized successfully
  iii. Six clones of *E coli* expressing recombinant NC/NS glycoprotein developed.

Summary of achievements under objective 1:
i.   Primers specific to RVFV NC and NS genes developed
ii. Total RNA from cells infected with RVFV strain G64 and 1883 successfully extracted
iii. The correct size of NS and NC genes correctly amplified
iv. Six clones of E coli expressing recombinant NS/NC protein developed.

Objective 2: Characterize expressed RVFV NS/NC genes
Activity 2.1
  i. Dot blot, Western blotting and Gel filtration
  ii. Quantification of purified recombinant protein.
Achievement 2.1
  i. The recombinant proteins positively reacted with RVFV antiserum on dot blot and western blot.
Summary of achievements under objective 2
  i. The recombinant proteins were extracted

ii. The recombinant reacted positively with RVFV antiserum on dot blot
iii. The recombinant protein were successfully infiltrated from the gel.

Objective 3: Evaluate a subunit vaccine candidate against RVFV
Activity 3.1
  i. Immunize and challenge mice with virulent strain of RVFV
  ii. Serum neutralization test
  iii. Development of a subunit vaccine candidate against RVFV.

Achievement 3.1
  i. Immunization and immunized mice with virulent strain of RVFV of mice was done
  ii. Serum neutralization test from immunized mice was done
  iii. Progress on the development of a subunit vaccine candidate against RVFV is promising

II   Other achievements
  i. Presentation of the project to the BioRi journal club.
III   Constraints and how they were overcome

  i. Covid-19 pandemic and delayed supply of reagents (4-6 months lead time)
    • acquired a new supplier of the reagents
  ii. NC clones not working
    • Changed the expression system from DH5α to BL21.
IV   Summary of funds received, accounted for and balance

Project Amount (KES)	Amount Received (KES)	Amount accounted for (KES)	Balance (KES)
5,086,850	2,465,350/-	563,600/-	2,621,500

IV   Way Forward
  i. Quantification and purification of recombinant proteins (objective 3)
  ii. Immunization of mice (objective 3)
  iii. challenging of immunized mice with virulent strain of RVFV (objective 3)
  iv. Serum neutralization test (objective 3)
  v. Development of a sub unit vaccine candidate against RVFV (objective 3).