



ANNUAL REPORT OCT 2020 TO SEPT 2021			
Project Title:	Development of Diagnostic tests and sub unit vaccine for <i>Pestes des petits ruminants</i> (PPR)		
KCSAP livestock Applied	Value chain: Red Meat	Duration: 18 Months	Start Date: Oct 2020
Lead Institution:	Kenya agricultural and livestock organization (KALRO) – BioRI, Kabete		
PI and contacts:	Dr. Yatinder Singh Binepal yatinder.binepal@kalro.org ; ybinepal@yahoo.com		
Collaborators and their contacts: Angela M’kiinda; Leonard Ateya - ateydel@gmail.com			
Background <i>Peste des petits ruminants</i> (PPR) is a viral disease of small ruminants such as sheep and goats. Cattle and pigs are susceptible to infection by the virus but do not exhibit clinical signs and do not transmit the disease to other animals (Bayry, 2017). The annual economic loss is estimated to be in excess of KES 1 Billion (Banyard <i>et al.</i> , 2010). The PPR virus was first isolated in 1962 in sheep culture and observed under the electron microscope in 1967. It has an incubation period of 2 to 7 days (Kumar <i>et al.</i> , 2014). Nigeria 75/1 and Sungri 96, are the two vaccines used in PPR endemic areas with great success (Sen <i>et al.</i> , 2010). Commercially available diagnostic ELISA kits with high specificity and sensitivity to detect antibodies against the N or the H proteins of the virus, are available to assess seropositivity within animal populations (Balamurugan <i>et al.</i> , 2014). However, no tools currently exist that enable serological Differentiation between Infected and Vaccinated Animals (DIVA). To this end, marker vaccines are a potential solution to the DIVA concept that may play an important role in the reduction of the disease in endemic regions and the success of any future eradication campaign. PPR virus is endemic across the East African region with PPR antibodies being detected in Kenya and Uganda. PPR was first detected in Kenya in 2006 in Turkana District after which it spread to 16 districts with mortality rates varying between 10%-100% depending on the age of the infected animal. Young animals were most affected with mortality rates of 100%. It was estimated that between 2006 and 2008 more than 5 million animals were affected across the 16 Kenyan districts with death occurring in more than half of the affected animals. Inadequate funding, limited stock of vaccine, unavailability of adequately trained staff as well as the constant mobility of pastoral communities have made the effort to control the spread of the disease quite challenging (Banyard <i>et al.</i> , 2010).			
Objectives: 1. To develop a rapid and cheap pen-side diagnostic kit for PPR virus. 2. To develop a subunit vaccine for PPR based on Matrix and Fusion genes. 3. To validate diagnostic kit and vaccine in live animal models.			
Expected Outputs 1. Rapid, cheap pen –side diagnostic tests developed. 2. At least one sub – unit vaccine for PPR developed			

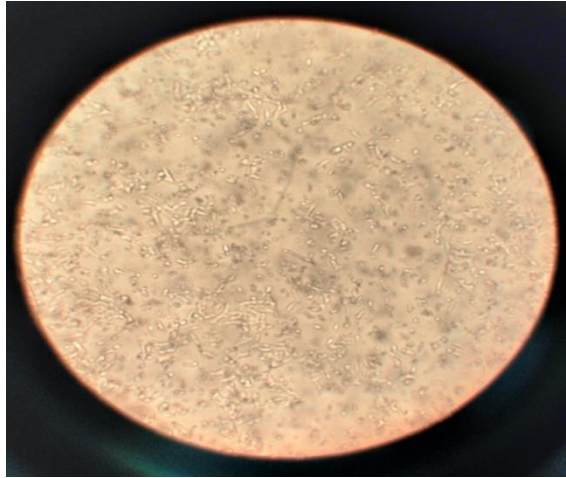
3. Sub – unit vaccine and diagnostic tests validated for commercialization.

I ACHIEVEMENTS

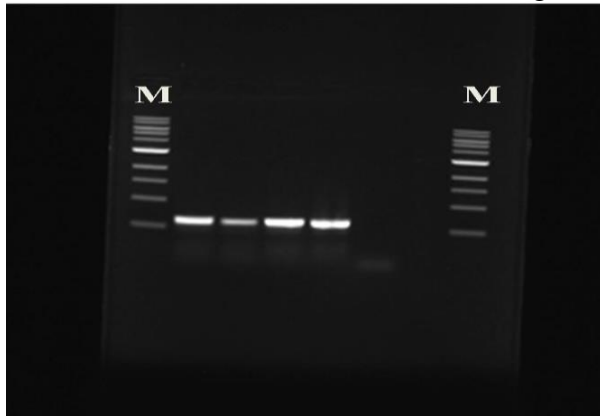
Objective 1: To develop a rapid and cheap pen-side diagnostic kit for PPR virus.

Activity 1.1: Amplify Fusion and Matrix genes from PPR virus

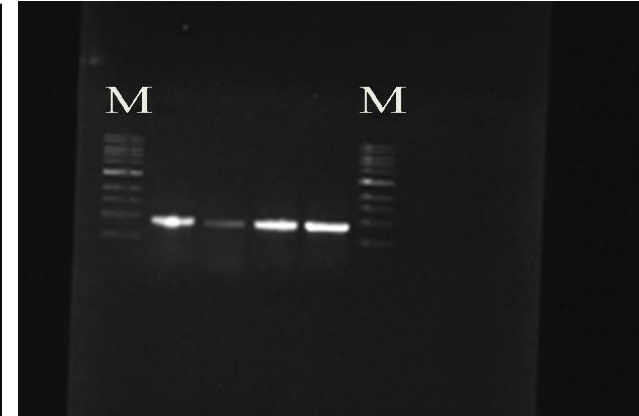
PPR Virus samples have been grown, RNA extracted and RT-PCR carried out for both Matrix and Fusion genes



Vero cells showing CPE for PPRv



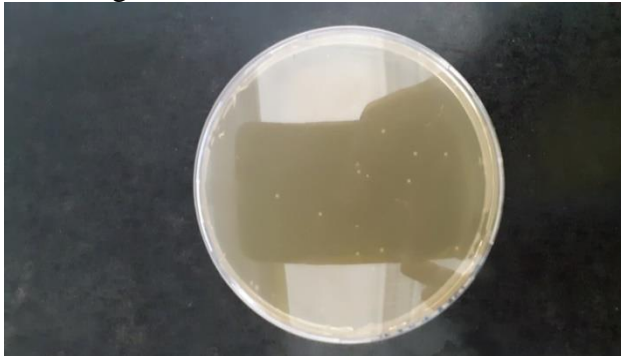
Matrix gene (590bp)



Fusion gene (842bp)

Activity 1.2: Clone RT- PCR products into expression Vector

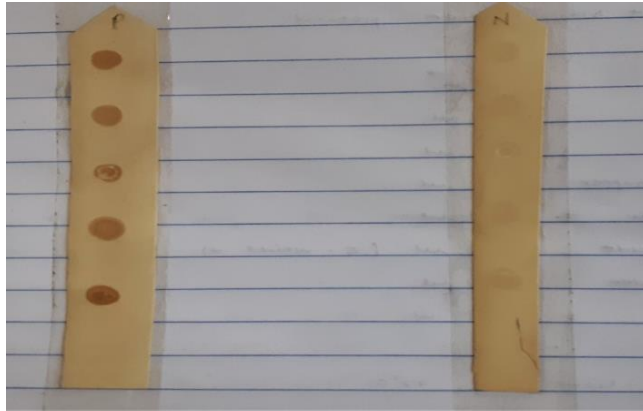
Matrix and Fusion genes have been transformed and cloned into *E. coli*



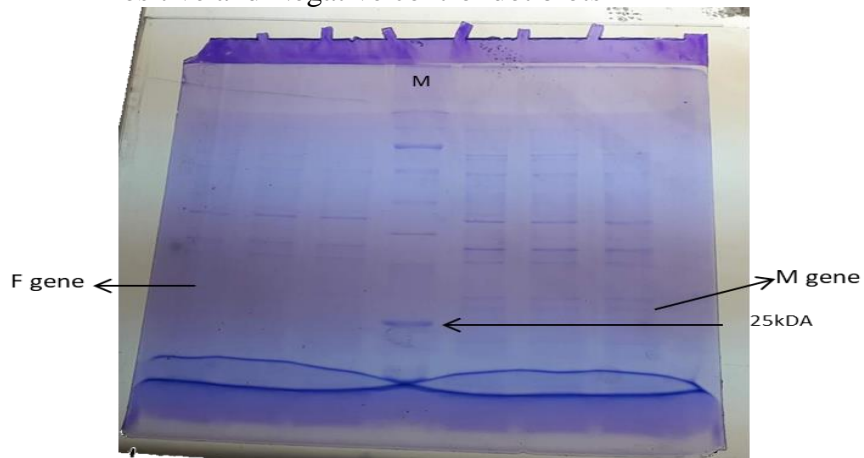
Colonies of transformed genes

Activity 1.3: Check Expression of Genes

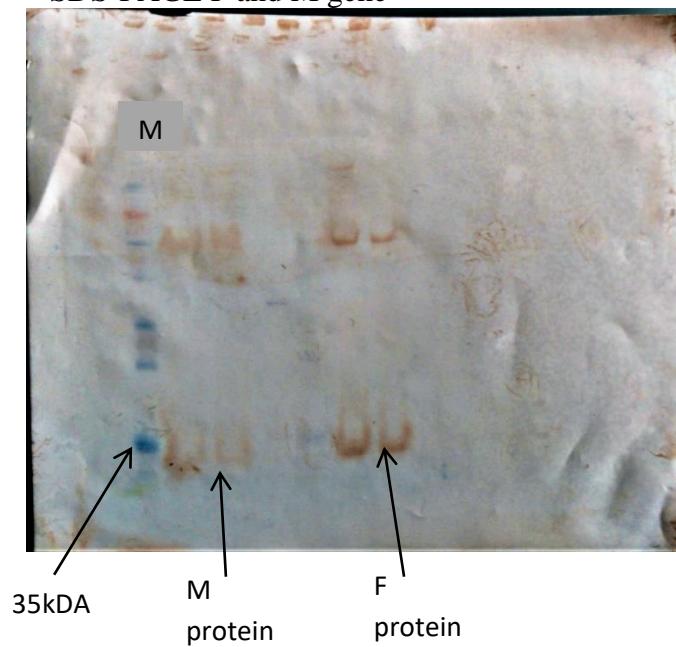
1. Expression and expression analysis has been done in SDS-PAGE, dot blots and western blots.



Positive and Negative control dot blots



SDS-PAGE F and M gene



M and F protein Western blot

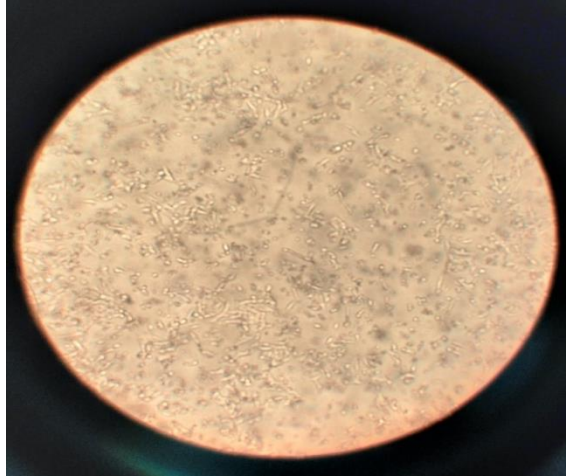
Activity 1.4: Develop tests – antibody detection test & antigen detection tests

- Not yet done

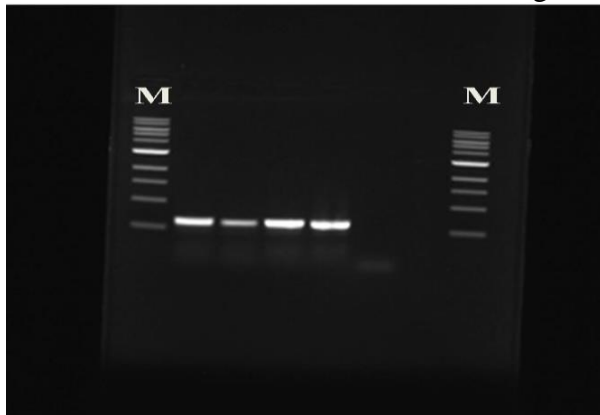
Objective 2: To develop a subunit vaccine for PPR based on Matrix and Fusion genes.

Activity 2.1: Amplify Fusion and Matrix genes from PPR virus

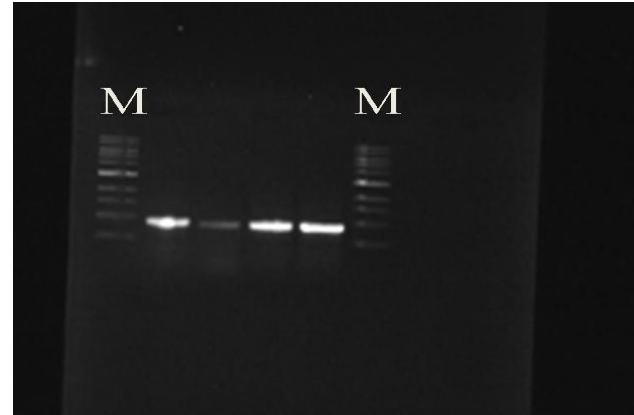
PPR Virus samples have been grown, RNA extracted and RT-PCR carried out for both Matrix and Fusion genes.



Vero cells showing CPE for PPRv



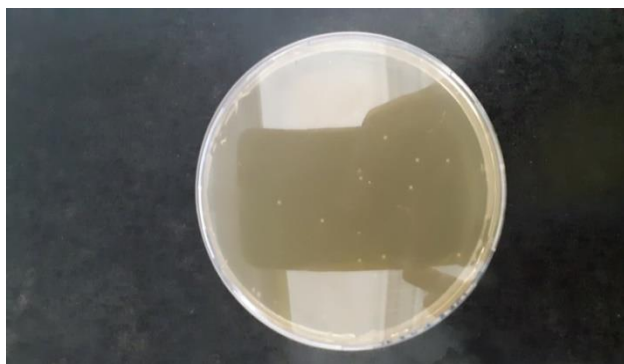
Matrix gene (590 bp)



Fusion gene (842bp)

Activity 2.2: Clone RT- PCR products into expression Vector

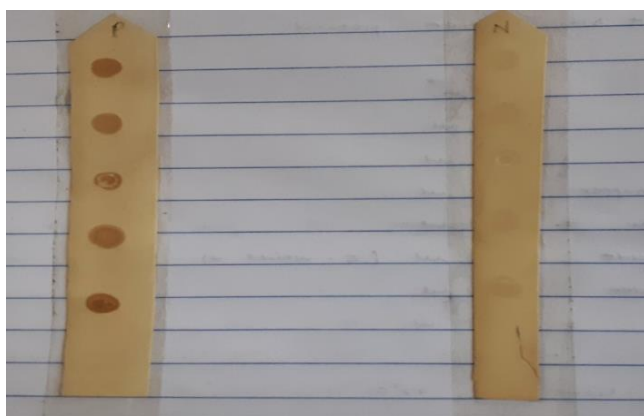
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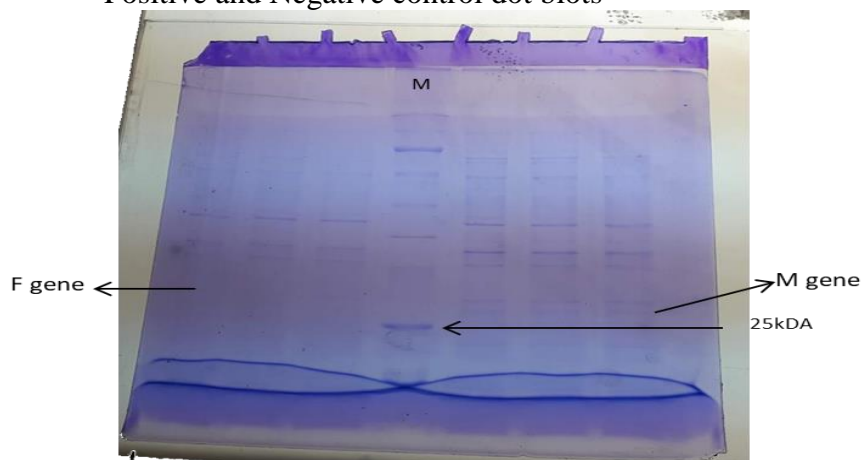
Colonies of transformed genes

Activity 2.3: Check Expression of Genes

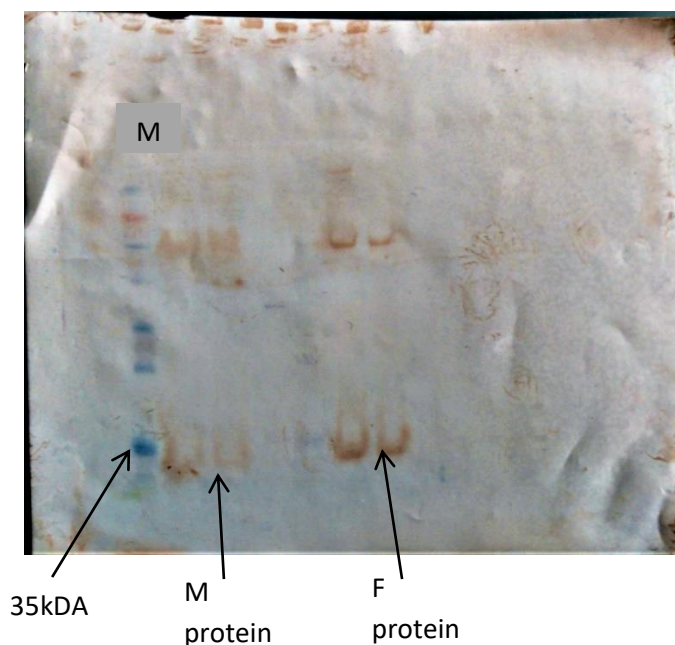
1. Expression and expression analysis has been done in SDS-PAGE, dot blots and western blots



Positive and Negative control dot blots



SDS-PAGE F and M gene



M and F protein Western blot

Activity 2.4: Evaluate Expressed protein as A Sub – unit vaccine in Goats

- Not yet done

Output 3: Sub – unit vaccine and diagnostic tests validated for commercialization.

Activity 3.1: Purchase PPR antibody free goats – Not yet done

Activity 3.2: Test expressed protein in goats in confined isolation unit – Not yet done

Activity 3.3: Test sub – unit vaccine in goats in confined field. – Not yet done

II Other achievements

N/A

III Constraints and how they were overcome

1. The laboratory has been under renovation since October 2021 and thus many of the lab activities have been put on hold until this is done. Some of the lab activities were moved to an adjacent laboratory as temporary host.
2. The time taken to receive reagents is quite long due to the procurement process. No molecular biology supplier in Kenya stores products in – house, all have to be imported. Unfortunately, this takes 3 – 6 weeks from the time an L.P.O. is issued. This has been compounded by having to clear all imports with Poisons board. *(This has not been overcome).*

IV Summary of funds received, accounted for and balance

Project Amount (KES)	Amount Received (KES)	Amount accounted for (KES)	Balance (KES)
5,007,450	1,923,650/-	576,200/-	3,083,800

IV Way Forward

1. Upscale Expressed protein to infect rabbits and guinea pigs and produce antibodies (Objective 1).
2. Develop Diagnostic tests (Objective 1).
3. Upscale expressed protein to infect Goats for sub – unit vaccine evaluation (objective 2).
4. Purchase and infection of live animals with the expressed proteins in confined field trials. (Objective 3).
5. Validation using ELISA and Serum Neutralization tests. (Objective 3).