Laboratory strategies for selection of effective virus strains to support the control of Foot and Mouth disease in Kenya.

By

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Presentation

A proposal submitted to University of Nairobi in partial fulfillment of requirements for Doctor of Philosophy in [Applied Veterinary Microbiology (Virology Option)]
Introduction

- Foot-and-Mouth Disease (FMD) - highly contagious and acute viral disease of domestic and wild cloven-hoofed animals
- Complex disease caused by FMDV - genus *Aphthovirus* in the family *Picornaviridae*
- Seven serotypes of FMDV - O, A, C, SAT1, SAT2, SAT3 and Asia type 1
- No cross-protection between serotypes
- Single stranded RNA virus - 8.5kb genome
Clinical signs

- Characterized by fever and vesicular eruptions in the mouth, nares, muzzle, feet and on the mammary glands which later become erosions
- This leads to lameness, salivation and unwillingness to feed, high fever, and sometimes a fatal myocarditis in juveniles
- Some animals like cattle, buffaloes, sheep and goats remain carriers of FMDV after infection
Clinical signs.....

Salivation

Vesicles on feet
Clinical signs.....

Ematiation

Mouth lesions
Economic loses of FMD

• Foot and mouth disease probably has the greatest economic impact on livestock than any other disease.

• Serious socioeconomic consequences occur such as loss of production of milk and meat, retarded growth rates, infertility as well as deaths in young animals.

• Economic disease due to resultant trade restrictions and subsequent loses in income.
Global distribution of FMD virus 2013
FMD in Kenya

• Endemic - >100 reported outbreaks per year.
• Four serotypes are currently in circulation in Kenya i.e. O, A, SAT1 and SAT2.
• Serotype C last encountered in 2004 in Kenya (Koibatek Sub-County)
• Estimated to cause economic loss of >KES 7 billion (82 million US$) every year (direct and indirect).
• Kenya is in the process of implementing the Progressive Control Pathway (PCP) for FMD in line with the GF-TADs.
This study will inform the process and augment the commitment already embedded in the Constitution, the Kenya Vision 2030, the Agricultural Sector Development Strategy, the National Livestock Policy and the Veterinary Policy.
Confirmed FMD Outbreaks in Kenya by serotypes in the last 4 years

Year

Number of confirmed outbreaks

- O
- SAT 1
- SAT 2
- A
- C

2010: 70 (O), 60 (SAT 1), 10 (SAT 2), 5 (A), 2 (C)
2011: 50 (O), 40 (SAT 1), 15 (SAT 2), 10 (A), 5 (C)
2012: 40 (O), 30 (SAT 1), 20 (SAT 2), 10 (A), 5 (C)
2013: 30 (O), 25 (SAT 1), 15 (SAT 2), 15 (A), 5 (C)
2014 (Up to April): 30 (O), 20 (SAT 1), 15 (SAT 2), 10 (A), 5 (C)
Justification

• According to the FMD control strategy of Kenya, vaccination will play a major role in the control of this disease

• Current vaccine strains in use were developed over 30 yrs ago

• Like other RNA viruses, the FMD virus continually evolves and mutates, thus one of the difficulties in vaccinating against it is the huge variation between, and even within, serotypes.
Justification

- This means FMD vaccines must be highly specific to the strain
- Evidence of possible vaccine failure due to introduction of new strains of SAT 1 was experienced in Kenya in 2009 to 2010
- Field strain was 10% divergent from the vaccine strain SAT 1 T 155/71
- However Vaccine matching was not carried out to ascertain that they are antigenically unrelated.
Justification….

• The laboratory approaches dealing with in vitro tests like the ones proposed in this study will therefore support the control strategy and improve the effectiveness of the vaccines produced leading to effective FMD control.

• To choose the most effective vaccine for use and to monitor on an ongoing basis the suitability of vaccines maintained in vaccine antigen reserve
Objectives of the study

Main Objective
To determine the genetic and antigenic relationships of circulating FMDV field strains with the current vaccine strains and selection of candidate vaccine strains for effective control of FMD
Specific Objectives

• Genetic characterization of recent, current and past field strains of four serotypes O, A, SAT1 and SAT 2 in relation to vaccine strains

• To determine and quantify the FMD vaccine strains (vaccines ‘O’K 70/78, ‘A’K 5/80, SAT 1 T 155/71 and SAT 2 K 52/84) relationship with the field strains through Virus Neutralization Test and Liquid Phase Blocking ELISA vaccine matching tests
Materials and methods

A. Genetic characterization

- Genetic profiling by PCR amplification of VP1, sequencing and sequence analysis
- Make use of published sequences (Genbank)
- Analyze divergent and closely related strains, matrix relationship
- Select strains for vaccine matching from various clusters among the generated phylogenetic trees
B. Antigenic characterization.....

Vaccine matching experiments

• Virus strains selected from genetic characterization will be reselected based on their ability to adapt to cell cultures

• Utilize a vaccine reference serum of pre-determined homologous antibody titre at 100 TCID 50 (100 tissue culture infective units of the virus) calculated by regression against the same dose of various heterologous field isolates.
Vaccine matching.....

- To determine how antigenically similar the heterologous virus is to the antibodies generated by vaccine homologous virus.

- Main samples to be used are those that have been collected within the last three years and those that will be encountered in subsequent outbreaks during the course of the research so as to make the isolates as current as possible.
Test procedures

- Two test procedures will be used, Virus Neutralization Test (VNT) and Liquid Phase Blocking ELISA (LBPE)
- Simple One way relationship testing (r1)
- To be determined by comparing the ratio of Bovine Vaccinal titre against the isolate to the titre against the vaccine strain.
- The is carried out by two dimensional neutralization and it makes use of an antiserum raised against the vaccine strain.
VNT Test

- Heterologous titre \( x \) – homologous titre \( y = x-y \)
- Reciprocal log of \( (x-y) \) is the \( r \) value
- Serological relationship “\( r \)” value between the homologous and heterologous virus which fall in the range of \( 0.3-1.0 \) is indicative of a reasonable level of cross protection.
The end point is defined as the reciprocal of the final dilution at which half the wells show 50% inhibition or more.

This will be divided by 2 to give the 50% threshold value.

\[ r_1 = \text{heterologous serum titre (field isolate)} / \text{homologous serum titre (vaccine strain)} \]

\[ r_1 = 0.4-1.0. \] Suggests that there is a close relationship between field isolate and vaccine strain.
c. Vaccine potency reports analysis

• Determination on how well it can stimulate a strong immunity (potency)
• Retrospective analysis of these results from lots of animals that have been tested in five years will be done.
• At least 7 lots per year for 5 years (35 lots for all serotypes and at least 10 per serotype)
Data analysis

• Genetic profiles, serological vaccine matching data and vaccine potency data will be analysed using descriptive statistics.
• A map of Kenya will be created using Google Earth 7.1 and imported into ArcGIS 10.2. and genotyping results displayed by serotype showing location of different topotypes and strains.
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References


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Acknowledgements

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