AN EPIDEMIOLOGICAL STUDY ON THE GENETIC RELATIONSHIPS OF FOOT AND MOUTH DISEASE VIRUSES IN EAST AFRICA

by

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Dedicated to my parents Sahle Fursa and Desta Sheferaw

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An epidemiological study on the genetic relationships of foot-and-mouth disease viruses in East Africa

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ABSTRACT

Within East African countries many of the known infectious diseases of animals occur commonly and are poorly controlled. Foot-and-mouth disease (FMD) is one of the contagious viral diseases that has great impact on economic development both in terms of direct and indirect losses. The epidemiology of the disease is complex due to the presence of six of the seven serotypes and the presence of large numbers of both wild and domestic susceptible animals in the region. Decision-making to determine the importance of FMD control relative to the economic consequences and what FMD control strategies should be applied based on the epidemiological information is required. In this regard the first step is to investigate the genetic relationships/variability of East African isolates and their phylogeographic distribution. These can provide base-line information for

designing control strategies by vaccination as well as the determination of the sources of infection.

Sufficient genetic information on the FMD serotypes O, SAT-1 and SAT-2 are lacking and therefore the number of viral lineages and genotypes or topotypes from East African countries could not be determined. Published studies on the relative occurrence and genotype distribution of FMD are largely confined to the southern and western part of the continent. In this study, the genetic profile of the 3 most prevalent serotypes (O, SAT-2 and SAT-1) recovered from outbreaks in East Africa between 1957 and 2003 was addressed. Phylogenetic analysis of partial and complete sequences of the 1D gene revealed the presence of distinct lineages and genotypes for East Africa as well as historical relationships of some of the genotypes with isolates from other regions. A great variation in the occurrence and distribution of these serotypes were found.

All the African and the Middle East/South East Asian isolates of serotype O included in this study clustered into one lineage having 8 distinct topotypes. These results indicated that between countries as well as inter-regional (east and west Africa) spread of viruses occurred in the past. Inter-regional spread of the virus between eastern Africa and western Africa was also confirmed for SAT-1 viruses. The fact that phylogenetic links are found with both serotypes implies that the spread of viruses was possibly associated with unrestricted animal movement due to nomadic movement in Africa. The phylogenetic relationships of SAT-1 viruses are more diversified in Africa. Eight lineages and 11 genotypes were identified when the optimal nucleotide sequence differences of $\geq$ 23% for lineages and $\geq$ 16% for genotypes were used as a cut-off values.

It was observed that viruses from Uganda are evolving independently from viruses elsewhere on the continent and clustered into 3 discrete lineages. In contrast, viruses from countries neighbouring Uganda, Kenya and Tanzania, clustered into one lineage. Uganda also harboured 3 topotypes of SAT-2 virus isolates, one is distinct for Uganda and the other are shared with Kenya and Zaire (DRC). This study highlighted distinct lineages found in Uganda and needs further investigation.

Within SAT-2, 67 isolates from 22 African countries and Saudi Arabia clustered into 5 lineages which consisted of 15 genotypes. Clustering of viruses into distinct genotypes (topotypes) according to year of isolation and geographical origin was observed showing countries with common boundaries shared common epizootics in the past. These results also showed a link between eastern and southern African countries.

Attempts were also made to investigate the incidence of FMD in Ethiopia using sera collected from cattle, small ruminants and wildlife. The results obtained from the liquid phase blocking ELISA and the 3ABC ELISA indicated the presence of SAT-1 and SAT-2 in buffalo populations in the southern part of Ethiopia while results from small ruminants and other wildlife were not indicative of any significant role in the epidemiology of FMD. Serological results also indicated that SAT-1 is present in cattle, although this serotype has not been previously identified.

The cumulative molecular epidemiological results from this and previous studies indicated that genetic variability of FMD viruses can be independently maintained within country/countries or regions as well as inter-regions of Africa. The serological results from buffaloes in East Africa are also suggestive of a possible reservoir of the SAT types FMD in the region which has a great impact on the control of the disease. Furthermore, the numerous lineages and genotypes of FMD virus isolates in Africa having distinct or overlapping distributions as well as the genetic linkage between regions will complicate the epidemiology of the disease. Therefore, it is strategically important to consider a regional approach and the use of a vaccine which contains a cocktails of antigens of FMD virus strains.

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LIST OF ABBREVIATIONS

BHK	Baby Hamster Kidney cells
bp	base pair
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
CFT	Complement fixation test
cDNA	Complementary deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
ed.	editor
edt.	edition
e.g	for example
ELISA	Enzyme-linked immunosorbent assay
EtOH	Ethyl alcohol
Fig.	Figure
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
g	Gram
x g	Unit of acceleration
GDP	Gross domestic production
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
IBRS-2	Instituto Biologico Rim Sunio
Lpb ELISA	Liquid phase blocking ELISA
kDa	Kilo dalton
masl	meter above sea level
mbsl	meter below see level
M	Molar
ML	Maximum likelihood
MM	Master mix
MOD	Maximum optical density
MOE	Ministry of Agriculture of Ethiopia
MP	Maximum parsimony
m/v	mass by volume
NaAc	Sodium acetate

NJ	Neighbour-joining
nt	nucleotide
OD	Optical density
OIE	Office International des Epizooties
OPD	o- phenelene diamine
PBS	Phosphate buffered saline
PBS-C	Phosphate buffer saline and casein
PBS-T	Phosphate buffer saline and twee-20
PK	Pig kidney
RT-PCR	Reverse transcriptase polymerase chain reaction
RGD	Arginine-Glycine-Asparic acid
Rnase	Human placental ribonuclease
S	Sedimentation coefficient
ssRNA	Single stranded ribonucleic acid
SAT	South African Territories
$TCID_{50}$	Tissue culture infective dose
UK	United Kingdom
UPGMA	Unweighted Pair-Group method using arithmetic average
VNT	Virus neutralization test
WRL	World Reference Laboratory