MOLECULAR CHARACTERIZATION OF FOOT AND MOUTH DISEASE VIRUSES ISOLATED FROM OUTBREAKS OCCURRED IN DIFFERENT PARTS OF ETHIOPIA

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INTRODUCTION

- FMD is a contagious and economically important viral disease of cloven-hoofed animal that is caused by FMDV.
- FMDV is +sense ss-RNA virus belongs to genus *Aphthovirus* in the family *Picornaviridae*.
- The viral genome is enclosed by four capsid protein includes VP1, VP2, VP3, and VP4.
- VP1-3 proteins are directly involved in antigenicity and exposed on viron surface, while VP4 is internal.

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- Four of the seven serotypes currently cause of FMD outbreaks in Ethiopia.
- Within each serotypes, many subtypes can be identified by molecular and immunological tools .
- **EA-3** and **EA-4** of FMDV serotype O and Africa topotype of **G-VII** and **G-IV** of FMDV serotype A were circulating in the country.
- The disease causes huge economic losses through morbidity and mortality and **hampering the export of livestock and livestock product** to different countries.
- The Egyptian trade ban of 2005, in which Ethiopia lost more than **US$14 million**, being a recent memory (Leforban, 2005).

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- It also cause reduction in milk and meat production which greatly affect especially **pastoralist** that rely heavily on milk for food and income generation.
- Reduction in milk supply would force households to either sell cattle or other assets to purchase grain for food.
- Clinically the disease is characterized by ropy salivation, lameness, loss of condition, pyrexia, and the appearance of vesicles and erosion on their oral cavity and interdigital space of feet and mammary gland.
- **Unrestricted animal movements** are an important mechanism by which FMD is spread from one place to the other place (Habiela *et al*., 2010).

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- Studies undertaken in Ethiopia revealed that the disease is still endemic and occur in different parts of the country mainly due to lack of effective vaccine, absence of livestock movement control and absence of systematic disease surveillance and epidemiological data and lack of molecular study (Chibssa, 2008).
- Lack of vaccination strategies and presence of free animal movement without certification are thus the main factors that could increase the spread of FMD.
- Determining the circulating serotype, studying the geographic relatedness and molecular evolution of the virus is needed for planning a proper control.

Therefore the Objective of this study were:

- To determine the serotype and isolate the virus.
- To detect and characterize the virus at molecular level.
- To compare the genetic relationship of the isolates in this study with the vaccine strain currently in use.

MATERIALS AND METHODS

Study areas

- The study was conducted in area where FMD outbreaks occurred in different parts of Ethiopia (Oromia, Amhara and Addis Ababa)

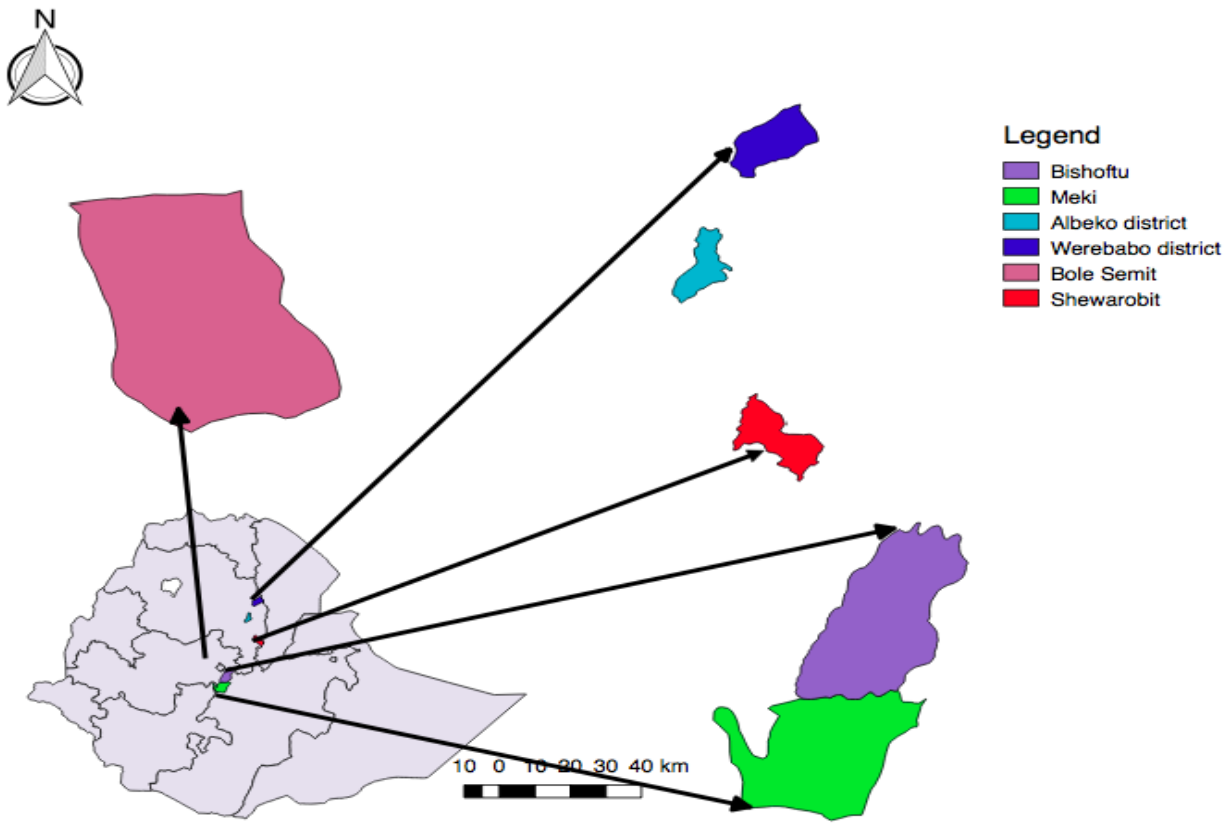

Figure 1. Map of study area

Study Design

- Based on reports of active cases of FMD outbreaks, a cross sectional study design were employed to collect samples.

Sample collection

- A total of 37 (25 EP and 12 Op) samples were collected using VTM for testing.
- Epithelial samples were collected aseptically from un-ruptured and freshly ruptured oral and foot lesions from suspected FMD infected cattle.
- OP samples were collected in VTM from previously suspected of FMD infected animals and asymptomatic cattle by passing a probang cup into oro-pharyngeal area.
- Samples were labeled and shipped frozen to NAHDIC, Sebeta.

Study methodology

Virus Isolation

- Virus isolation on BHK-21 cell was done following the procedure on OIE manual (OIE, 2012).
- Suspension was prepared by grinding the tissue sample in sterile sand in a sterile pestle and mortar with a small volume of tissue culture medium containing antibiotics.
- 1ml of clarified suspensions suspected to contain FMDV were inoculated to BHK-21 cells and monitored for CPE for 24hr-48hrs.
- When no CPE was observed, blind passaging of the inoculated cells was done up to three passages.

Serotype detection

- Antigen detection sandwich ELISA (IZSLER, Bresica, Italy) was used to detect FMDV serotypes according to manufacturer protocol.
- The kit was designed for typing of six FMDV serotypes such as type O, A, C, Asia 1, SAT 1 and SAT 2.
- OD values of the samples were interpreted by subtracting the OD value of each negative control from the OD value measured for test sample.

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- 50µl/well of each diluted sample were distributed to 8 wells of a column and 50µl of diluents per well were added in all wells .
- After discarding the fluid, 200µl of washing solution was added and repeated for 3x.
- 50µl/well of conjugates were added to each well and left at room temp. for 20m in the dark.
- The reaction was stopped by adding 50µl/well of stop solution (H_2SO_4).
- Immediately after stopping, reading the optical density (OD) of each well was done at 450 nm wavelength using micro plate reader.

DNA sequencing and phylogenetic analysis

- 18 representative samples were submitted to WRLFMD Pirbright Institute, United Kingdom for confirmation and sequencing.
- The 13 edited sequence (9 for O and 4 for A) were used to reconstruct the phylogenetic tree.
- BLAST in MEGA6 was used to collect additional sequences from GenBank NCBI.

Data management and analysis

- Sequence data were aligned by multiple sequence alignments using the Clustal W algorithm implemented in MEGA6 software.
- Amino acid variation b/n the vaccine virus and the current isolates were accessed by using BioEdit software.
- Phylogenetic tree were reconstructed using minimum evolution and maximum likelihood method using MEGA6.
- The robustness of tree was assessed with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

FMD virus isolation

- From 22 samples cultured on BHK-21 cell, CPE was observed on 15 (68.18%) samples.
- The CPE observed was characterized by
  - Fast destruction of mono-layer cell
  - Round and singly appearance of cell
  - Complete destruction of the cell
  - Cell detachment

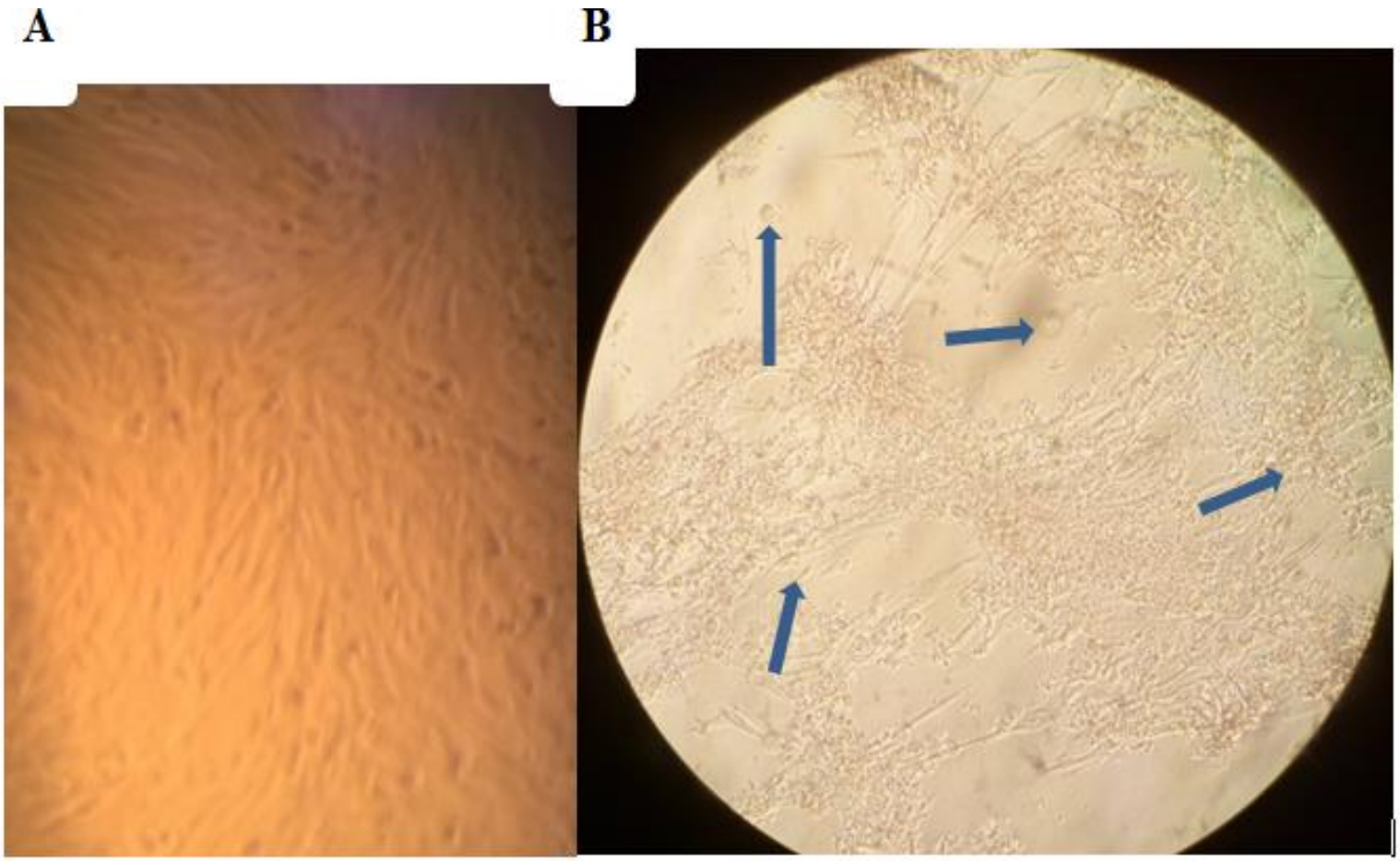

Figure 2: Picture showing FMDV isolation on BHK-21 cell line. **A:** un inoculated BHK-21 cell, **B:** CPE positive cell.

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- Most of the sample that didn't showed CPE were OP fluid samples.
- This could suggest that the chances of virus isolation from OP fluid samples were lower as a result of lower concentration of virus.
- OIE (2009) reported that the preferred sample for virus isolation and serotype detection is epithelial tissue samples than OP sample.

FMDV serotype identification

- From 37 sample subjected to Sandwich ELISA, 16 (43.24%) samples were typed serotype O and in 6 (16.22%) samples typed as serotype A.
- Serotype O from Shewarobit and South Wollo areas of Amhara regional state and Serotype A was from Meki district of Oromia regional state was detected.
- In this study serotype o was the dominant serotype detected from the two outbreak site.
- Menda *et al*. (2014) and Sulayeman *et al*. (2018) reported serotype O as the most prevalent and the dominant serotype causing outbreaks in different parts of the country.

Table 1: FMDV isolated from cattle from different outbreaks

Site of outbreak	Kebele	No. of sample inoculated	Type of samples	Virus isolated	Serotype detected
Meki	Sera Wekele	3	2Tissue and 1OP	ETH/02/18, ETH/07/18	A
	Tuchi	3	Tissue	ETH/11/18, ETH/13/18, ETH/14/18	A
Bishoftu	K05	2	OP	—	—
Addis Ababa	Bole Semit	1	Tissue	—	—
Shewarobit	Yelen	4	3Tissue and 1OP	ETH/17/18, ETH/21/18, ETH/23/18	O
	Kewet	3	Tissue	ETH/24/18, ETH/27/17	O
South Wollo	Albeko	2	Tissue	ETH/35/18, ETH/36/18	O
	Werebabo	4	Tissue	ETH/38/18, ETH/39/18, ETH/41/18	O
Total		**22**		**15/22 (68.18%)**	

ETH, Ethiopia; OP, Oro-pharyngeal fluid

Molecular characterization

Phylogenetic analysis of FMDV serotype O

- Assessment of phylogenetic analysis of 639 nucleotide sequences of VP1 gene of FMDV serotype O isolates from Shewarobit and South Wollo were clustered in **East African topotype-3 (EA-3).**
- Similarly, Ayelet *et al*. (2009) and WRLFMD (2016) reported EA-3 lineage in different parts of Ethiopia.
- Isolate from both outbreak sites had also **99.3% nucleotide similarity** with each other which indicates the **origin of the outbreak is the same.**

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- FMD viruses with <5% of nucleotide differences in the VP1 sequence are considered as closely related & believed to originate from the same epizootic (Gorna *et al*., 2014, Slager-Bastos, 2001).
- The isolates also had 96.6% nucleotide similarities with Sudan isolates of (O/SUD/3/2008, O/SUD/5/2008, O/SUD/4/2008).

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- The isolates differs by
- 15.7% with **EA-1** (O/UGA/5/96, O/KEN/83/79), 17.1% with **EA-2** (O/KEN/5/2002, O/UGA/3/2002), 21.5% with **EA-4** (O/ETH/58/2005, O/ETH/59/2005) 22.1% with **WA** (O/BKF/1/92, O/BKF/2/92) as indicated.

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Phylogenetic analysis of serotype A

- Serotype A isolates from Meki districts were belonged to **genotype G-I within the Africa topotype.**
- This is the first report for the existence of **G-I** in Ethiopia.
- Africa topotype (G-VII) (WRLFMD, 2016) and Africa topotype (G-IV) (Sulayeman *et al*., 2018) of FMDV serotype A were reported in the country.

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- The current isolates showed >97% nucleotide similarity with isolates of Kenyan (A/KEN/28/2008 and A/KEN/22/2009) and Tanzanian (A/TAN/47/2009 and A/TAN/42/2009) which are grouped in G-I.
- But it was dissimilar with Ethiopian isolate (A/ETH/7/92) with 17% nucleotide level which is previously grouped under genotype G-VII as indicated.

..\Phylogenetic tree for FMDV serotype A.docx

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- Both serotype O and A in this study have high nucleotide sequence similarity with isolates of the virus in neighboring countries.
- This suggested that the outbreaks due to those isolates were most probably spread by uncontrolled trans-boundary animal's movement.
- Such uncontrolled cattle movements have a big risk on the transmission of the virus across the border in both directions.

Sequence based comparison of Serotype O isolates with the vaccine strain (O/ETH/38/2005)

- A total of 22/ 213 (10.3%) amino acid variations were observed in different sites of the VP1 gene .
- The variations were predominantly occurred G-H loop (aa positions 133–158) and the C-terminus (positions 194–213), known to be the main immunogenic sites of VP1.

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- aa substitutions at different positions are:
  - **133 asparagine (N) → serine (S)**
  - **138 valine (V) → glutamate (E)**
  - **140 serine (S) → arginine (R)**
  - **141 valine (V) → alanine (A)**
  - **158 proline (P) → threonine (T)**
  - **197 serine (S) → alanine (A) and**
  - **198 glutamate (E) →glycine (G)**

..\AA variablity.docx

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- The presence of limited genetic variation in the immunodominant regions can alter the antigenic specificity of FMD viruses isolates (Sahle *et al*., 2004).
- Thus, in this study, the existence of amino acid variation especially at immunodominant region indicates the need for in-vitro vaccine matching studies.

CONCLUSION

- In present study, serotype O and A was identified by Antigen detection ELISA and isolated on BHK-21 cell.
- Serotype O was clustered in **EA-3** while serotype A was grouped in **G-I of African topotype.** G-I of serotype A FMD virus is observed in Ethiopia for the first time.
- The presence of high genetic similarity in both serotype with FMDV isolates in neighboring countries indicates the presence of unrestricted trans-boundary animal movement at bordering area.
- A number of amino acid variation especially in immmunodominant region VP1 gene sequence were noted indicating the presence of genetic heterogenecity b/n isolate vaccine strain in use.

Recommendations

- Research on serotyping and molecular characterization on FMDV should be conducted check the introduction and circulation of new serotype and topotypes .
- *Invitro* Vaccine matching test were should be conducted for both serotype O and A isolates in order to assess the protection potential of the vaccines in use.
- Restriction of free animal movement with neighboring countries and
- Establishment of quarantine station around the border area is necessary.
- Government FMD control strategy through regular vaccination by matching vaccine should be started.

Thank You!!!