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FMD virus isolates: The candidate strains for polyvalent vaccine development in Ethiopia

G. Ayelet[a,d,*], M. Soressa[b], T. Sisay[b], A. Belay[a], E. Gelaye[a], S. Jembere[a], E. Skjerve[c], K. Asmare[c,d]

[a] National Veterinary Institute, P.O. Box 19, Debre-zeit, Ethiopia
[b] School of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre-zeit, Ethiopia
[c] School of Veterinary Medicine Hawassa University, P.O. Box 5, Hawassa, Ethiopia
[d] Centre for Epidemiology and Biostatistics, Norwegian School of Veterinary Science, P.O. Box 8146, 0033 Oslo, Norway

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ABSTRACT

The study was conducted on foot-and-mouth disease (FMD) viruses with the aim of selecting appropriate vaccinal strain to control of FMD in Ethiopia. The study was conducted in two-dimensional virus neutralization assay to determine the antigenic relationship 'r' value between the candidate vaccine strains and field isolates. A total of 21 serotype O, 7 serotype A, and 8 serotype SAT 2 FMD viruses, which were isolated from cattle and swine. A couple of isolates from each serotype were identified as vaccine candidates in the trial (O-ETH/38/2005, O-ETH/58/2008, A-ETH/7/2008, A-ETH/6/2000, SAT2-ETH/76/2009 and SAT2-ETH/64/2009). The finding revealed all the vaccine candidate depicted high antigenic similarity, above the mean "r" value, to their own serotypes in the studied serotype population except for one serotype A field isolate, A-ETH/13/1981, with "r" value = 0.14 and 0.25) which is significantly lower than the minimum requirement. In general, the result indicated that these candidate vaccinal strains can be used for polyvalent vaccine production in the country.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed domestic and wild animal species (OIE, 2008). The disease has considerable economic consequences which can be attributed to both direct and indirect costs. The direct effects of the disease are loss of milk production, loss of draught power, retardation of growth, abortion in pregnant animals, death in calves and lambs, while indirect losses can be attributed to the disruption in trade of animals and derivative products (Paton et al., 2005; James and Rushton, 2002; Bayissa et al., 2011).

Clinically, the disease is characterized by fever, salivation and vesicular eruptions on the feet and mouth. Morbidity has been reported to reach as high as 100% in susceptible animal populations but mortality is low in adults. Infected animals show a spectrum of responses to FMD ranging from unapparent infection to severe disease and death. FMD is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae* (OIE, 2008). The virus has got seven serotypes, namely, O, A, C, SAT 1 (South African Territories), SAT 2, SAT 3 and Asia 1 (OIE, 2008; Sahle, 2004; Knowles and Samuel, 2003). Within each serotypes, many subtypes can be identified by molecular and immunological tools (OIE, 2008; Knowles and Samuel, 2003). The presence of the seven serotypes, multiple subtypes, and variants has added difficulty in control of the disease using vaccine and laboratory diagnosis (Domingo et al., 2003). Antibody generated by infection or vaccination against one serotype fails to cross-protect against other types. Furthermore, antigenic differences within a serotype may be so great that there is little or no cross-protection between strains of the same serotype (Araujo et al., 2002; Mumford, 2007).

The protective capacity of FMD vaccine could be evaluated through vaccine matching using indirect serological methods (Paton et al., 2005; Rweyemamu, 1984; Rweyemamu and Hingley, 1984) or alternatively on the calculation of the relatedness between the field isolate and available vaccine strains using *in vivo* challenge tests (Brehm et al., 2008; Goris et al., 2008). Viruses for vaccine matching can be selected based on epidemiological information, including stages of an epidemic, geographical locations or range of host species (Alonso et al., 1993; Paton et al., 2005) by incorporating a minimum of two isolates per outbreak (OIE, 2008).

FMD is endemic to most of sub-Saharan Africa, except in a few countries in southern Africa, where the disease is controlled by separation of infected wildlife from susceptible livestock as well as

* Corresponding author at: National Veterinary Institute, P.O. Box 19, Debre-zeit, Ethiopia. Tel.: +251 114338411; fax: +251 114339300.
E-mail address: gelagayayelet@yahoo.com (G. Ayelet).

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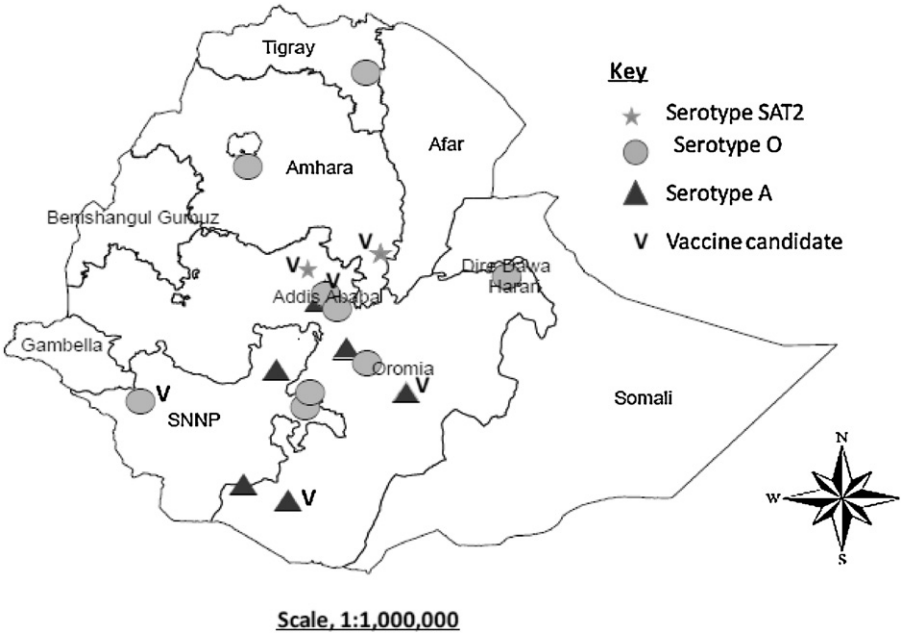

Fig. 1. A map showing the origin of the vaccine strains and field isolates used for the study.

by vaccination (Sahle, 2004; James and Rushton, 2002). Studies in Ethiopia have shown that five of the seven serotypes of FMDV were recorded as a cause of FMD outbreaks for the last decades. The presence of serotypes O, A, C and SAT2 were reported by Roeder et al. (1994) and Sahle (2004). Serotypes O, A and SAT2 were reported by Gelaye et al. (2005). Sebsequently, serotypes O, A, C, SAT1 and SAT2 were reported (Ayelet et al., 2009; Negusssie et al., 2010). However, Serotype O is the most predominant strain circulating in the country followed by serotype A and SAT 2. Serotype C was not isolated in any of the outbreak investigation since 1983 and there is only a single isolation report of SAT 1 in 2007 (Ayelet et al., 2009). The presence of multiple serotypes of FMD and lack of cross protection among serotypes and subtypes warrants the need for development of a polyvalent vaccine containing strains that confer broader protection. Therefore, this study was designed to determine FMD vaccine strains appropriate for serotypes/topotypes circulating in Ethiopia.

2. Materials and methods

2.1. FMD virus candidate strains for polyvalent vaccine

The trial experiment was conducted on foot-and-mouth disease virus isolated from different parts of Ethiopia (Fig. 1) between 1981 and 2011. Field isolates collected at NVI, Ethiopia were molecularly characterized at the World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) United Kingdom. Accordingly a total of 21 serotype O, 7 serotype A and 8 SAT 2 FMD virus isolates were identified. Among these field isolates, candidate strains for possible polyvalent vaccine production were selected from each serotype considering their topotype/subtype, geography, source species and period of occurrence (OIE, 2008). Moreover, vaccine strain selection considered additional biological characteristics, including good growth and the ability to elicit an antibody response which is broadly cross-reactive within a subtype (Doel, 2003). Accordingly, two isolates from each of the circulating serotypes, namely, O, A, and SAT 2 were selected. The isolates used in the trial were O-ETH/38/2005, O-ETH/58/2008, A-ETH/7/2008, A-ETH/6/2000, SAT2-ETH/76/2009 and SAT2-ETH/64/2009 (Table 1). The remaining nineteen O, five A, and six SAT2 FMDV serotype field isolates were evaluated for antigenic similarity with candidate vaccine strains (Table 2) using in vitro viral neutralization test (VNT).

2.2. Vaccine preparation

Inactivated monovalent FMD vaccine was prepared for the selected vaccine strain candidates using standard protocol of vaccine production recommended by OIE (2008) manual in the FMD vaccine production laboratory of the NVI. The virus strain was inoculated to a monolayer cell culture of BHK-21 cells and the resulting preparation was clarified, inactivated withethyleneimine and adjuvanted with aluminum hydroxide and saponin. The vaccines prepared were kept at +4 °C until used.

2.3. Antiserum preparation in cattle

A total of thirty young cattle (6–12 months old) were used after being screened for the presence of FMD specific antibodies using 3ABC-ELISA kit (Ceditest FMDV-NS, Cedi-diagnostics, The Netherlands). FMDV specific antibody negative animals were selected, quarantined in a separate barn, ear-tagged and vaccinated with 2 ml subcutaneously in dewlap region with the vaccine prepared from each candidate vaccine strain. For each vaccine strain five animals were vaccinated. Sera were collected following separately for each vaccine candidate strains on day 21. The titer of antibody to the vaccine strain was established for each serum. Sera with strong titer were selected excluding low responders and stored at −20 °C for the intended vaccine matching.

2.4. Titration of FMD virus

Both FMDV vaccine strains and field isolates were titrated by tenfold serial dilution starting with 10^{-1} by mixing 1 ml of virus in 9 ml of diluents (2% MEM) and subsequent transfer of 1 ml from previous virus dilution to the next using sterile micro pipette. Fifty microliter of each virus dilution (10^{-1} to 10^{-8}) was distributed into the wells of their respective rows on microtiter plates containing established cell layers of baby hamster kidney (BHK-21). Then 100 μl/well minimum essential medium (MEM) was added