

Challenges and prospects for the control of foot-and-mouth disease: an African perspective

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Abstract: The epidemiology of foot-and-mouth disease (FMD) in Africa is unique in the sense that six of the seven serotypes of FMD viruses (Southern African Territories [SAT] 1, SAT2, SAT3, A, O, and C), with the exception of Asia-1, have occurred in the last decade. Due to underreporting of FMD, the current strains circulating throughout sub-Saharan Africa are in many cases unknown. For SAT1, SAT2, and serotype A viruses, the genetic diversity is reflected in antigenic variation, and indications are that vaccine strains may be needed for each topotype. This has serious implications for control using vaccines and for choice of strains to include in regional antigen banks. The epidemiology is further complicated by the fact that SAT1, SAT2, and SAT3 viruses are maintained and spread by wildlife, persistently infecting African buffalo in particular. Although the precise mechanism of transmission of FMD from buffalo to cattle is not well understood, it is facilitated by direct contact between these two species. Once cattle are infected they may maintain SAT infections without the further involvement of buffalo. No single strategy for control of FMD in Africa is applicable. Decision on the most effective regional control strategy should focus on an ecosystem approach, identification of primary endemic areas, animal husbandry practices, climate, and animal movement. Within each ecosystem, human behavior could be integrated in disease control planning. Different regions in sub-Saharan Africa are at different developmental stages and are thus facing unique challenges and priorities in terms of veterinary disease control. Many science-based options targeting improved vaccinology, diagnostics, and other control measures have been described. This review therefore aims to emphasize, on one hand, the progress that has been achieved in the development of new technologies, including research towards improved tailored vaccines, appropriate vaccine strain selection, vaccine potency, and diagnostics, and how it relates to the conditions in Africa. On the other hand, we focus on the unique epidemiological, ecological, livestock farming and marketing, socioeconomic, and governance issues that constrain effective FMD control. Any such new technologies should have the availability of safe livestock products for trade as the ultimate goal.

Keywords: vaccine, foot-and-mouth disease virus, vaccine matching, new-generation vaccine, diagnostic tests

Introduction

Foot-and-mouth disease (FMD), of which FMD virus (FMDV) is the causative agent, is a contagious viral disease which affects cloven-hoofed animals such as cattle, pigs, sheep, goats, and other artiodactyl species.¹ FMD ranks as one of the most economically important infectious diseases of animals according to the World Organisation for Animal Health (OIE). The occurrence of the disease not only affects international trade in livestock and animal products but also results in damaging consequences for the

livelihoods of local farmers due to impacts upon productivity, food security, and losses of income. The disease is widely distributed in the developing world, in particular Africa, Asia, and South America. In these regions, livestock farming forms the backbone of rural economies that supports approximately 70% of the world's poor. FMD outbreaks particularly affect vulnerable individuals such as women and children since approximately 75% of livestock in Africa are raised under the communal smallholder, communal-grazing, or pastoral systems that sustain livelihoods of these groups.²⁻⁴ The lack of veterinary infrastructure, human resources, movement controls, and appropriate vaccines render many developing countries particularly exposed to the spread of FMD.⁵⁻⁷

The epidemiology of FMD in Africa is influenced by two different patterns (ie, a cycle involving wildlife), in particular the African buffalo (*Syncerus caffer*), and an independent cycle maintained within domestic animals.⁸⁻¹² Another unique feature of FMD epidemiology in Africa is the presence of the three Southern African Territories (SAT) serotypes (ie, SAT1, SAT2, and SAT3), which are maintained within the African buffalo populations.¹²⁻¹⁵ The presence of large numbers of African buffalo provides a potential source of sporadic infection to domestic livestock and other wildlife species.¹⁶⁻¹⁸ Although the precise mechanism of transmission of FMD from buffalo to cattle is not well understood, it is facilitated by direct contact between these two species. Once cattle are infected they may maintain SAT infections without the further involvement of buffalo.^{12,19-21} Sub-Saharan Africa is endowed with an abundance of wildlife, which has been preserved within national parks and game reserves.²² In communities neighboring these parks, the livestock/wildlife interface presents unique challenges to livestock disease control.^{12,23,24} In addition, the creation and expansion of transfrontier conservation areas in southern and eastern Africa presents a particular challenge to the management of FMD.

In response to this unique epidemiological situation, certain southern (eg, Botswana, Namibia, and South Africa) and North African (such as Egypt) countries²⁰ have invested in regular livestock vaccination programs to manage the disease and facilitate access to international and regional trade markets in livestock and livestock products. However, in southern Africa, the incidence of the disease has increased appreciably over the last decade, and since the eradication of the disease in Africa is unlikely in the near future, more flexible ways of managing FMD are required to obviate clashes between conservation-based and livestock-based initiatives aimed at rural development. Despite systematic

use of vaccination, numerous outbreaks of FMD have been recorded, and there is evidence of sustained virus circulation in vaccinated cattle populations in southern and eastern Africa since 2000,^{25,26} as well as in North Africa where exotic incursions of FMD (serotypes A, SAT2, and O) have caused widespread outbreaks.^{20,27}

Effective control and prevention of FMD relies largely on the implementation of strategies such as physical separation of wildlife and livestock, repeated vaccination of cattle herds exposed to wildlife, control of animal movements, and careful assessment of the risk of FMDV introduction into disease-free areas.^{12,23,25} The current inactivated vaccines have proven effective in reducing clinical disease in FMD-endemic areas and have been critical to the success of FMD control programs in South America and Europe.²⁸ In Africa, the diversity of circulating field strains of FMDV makes the selection of sufficiently cross-protective FMD vaccines a challenge. Therefore, local and regional programs of surveillance to monitor FMDVs circulating in wildlife and livestock populations are a crucial component of vaccine control, to provide vaccine matching data and access to appropriate viral strains that can be used in the development of new vaccines. There is need also for risk-based surveillance to be able to determine primary endemic areas and factors that influence disease dissemination, to assist the design of targeted, area-wide, or ecosystem-based disease control strategies, as African regions embark on the Food and Agriculture Organization of the United Nations (FAO)-OIE Progressive Control Pathway (PCP) for the Control of FMD.²⁹ The success of any FMD control campaign ultimately depends on the abundant supply of vaccine of the appropriate strain composition and proven potency, adequate vaccine coverage, rapid vaccine development, overall planning and management by a well-resourced veterinary service, and the involvement and cooperation of the livestock farmer.³⁰

In summary, animal diseases, in particular transboundary animal diseases such as FMD, severely constrain the development of competitive livestock enterprises in developing countries.^{31,32} The aim of this review is to identify the current limitations that are experienced in the control of FMD in endemic settings in Africa caused by gaps in knowledge of epidemiology, vaccinology, and diagnostics. We also emphasize the progress that has been achieved in the development of new technologies, including research towards improved tailored vaccines, vaccine matching, and diagnostics, and how this relates to the conditions in Africa. Furthermore, we advocate applied research into vaccination and disease

control strategies to enable fit-for-purpose approaches to FMD control in Africa.

Epidemiological patterns in Africa

The current global burden of FMDV infection is maintained within three continental reservoirs in Asia, Africa, and South America, which can be further subdivided into seven major virus pools of infection.^{29,33} Each of these contains at least three serotypes of virus, and because virus circulation is mainly within these regional reservoirs, strains have evolved which are specific to the region and which often (in the case of type A and SAT viruses) require tailored diagnostics and vaccines for control.³⁴

In Africa, the FMDV serotypes are not uniformly distributed, and each serotype results in different epidemiological patterns. The cumulative incidence of FMDV serotypes show that six of the seven serotypes of FMD (O, A, C, SAT1, SAT2, and SAT3) have occurred in Africa.^{35,36} The distribution of five serotypes and the different topotypes are shown

in Figure 1A–E. Based on the genetic characterization of the virus and antigenic relationship of FMDV in Africa, the virus distribution has been divided into three virus pools: namely, pool 4 covering East and North Africa, with predominance of serotypes A, O, SAT1, and SAT2; pool 5 restricted to West and northern Africa, with serotypes O, A, SAT1, and SAT2; and pool 6 restricted mainly to South Africa, with SAT1, SAT2, and SAT3 serotypes. Recent studies in East and southern Africa have revealed genetic differences between viruses isolated at different times and places.^{37–39} Periodically, there have been incursions of types SAT1 and SAT2 from Africa into the Middle East, probably as a result of animal movement.^{35,36} The most recent reports include the spread of viruses of the SAT2 serotype to Yemen in 1990, to Kuwait and Saudi Arabia in 2000, and to the Palestinian Autonomous Territories and Bahrain in 2012.⁴⁰ Overall, the geographic and genetic clustering of FMDVs suggest ecological adaptation and/or separation, but in many endemically affected areas, the temporal and spatial dynamics of infection still

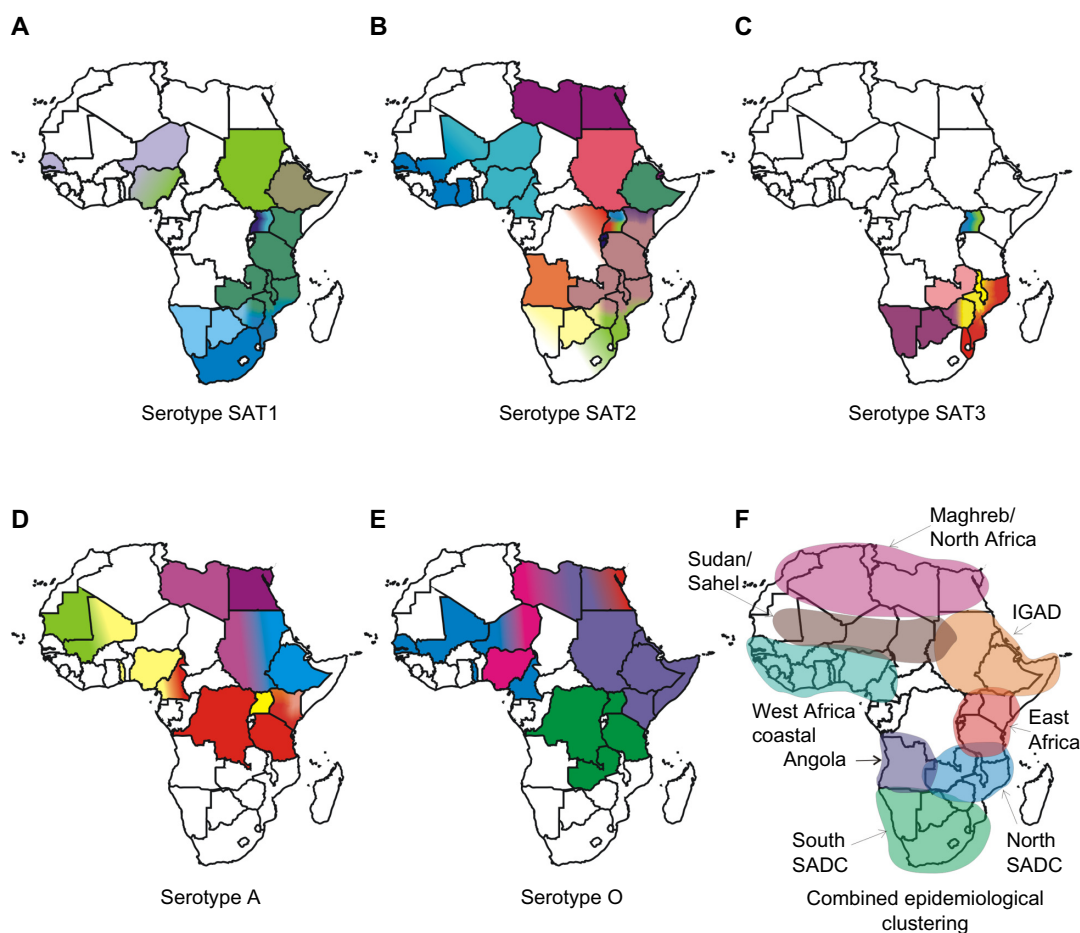


Figure 1 Maps of Africa showing the serotype and topotype distribution.

Notes: The topotypes are color coded. The epidemiological clustering is indicated. The epidemiological clusters shown in the maps (A–F) do not necessarily indicate political borders of the countries.

Abbreviations: IGAD, Intergovernmental Authority on Development; SADC, Southern African Development Community; SAT, Southern African Territories.

need to be much more accurately determined by analysis of host animal distributions and contact opportunities, serosurveys to estimate weight of infection, and use of the latest available techniques in genetic tracing of FMDV incursions into disease-free regions.⁴¹ Generally, many of these factors are driven by climatic factors and socioeconomic changes centered on human behavior. Also, findings regarding the epidemiology of FMD involving wildlife within a particular ecosystem of Africa may not be applicable to other ecosystems because of ecological, host, and viral variability differences.⁴² Understanding of how these risk factors are clustered and associated in space and time may assist in effective disease control planning.^{43,44}

To understand the complexity of FMD epidemiology in Africa and to assist decision making and improve the continental control of FMD, it is important to further divide the virus pools into epidemiological clusters.²⁹ Rweyemamu et al²⁹ proposed eight epidemiological clusters for Africa (Figure 1F) based on the distribution of serotypes and topotypes in different regions in Africa (Figure 1A–E), animal movement patterns, impact of wildlife, and farming systems. Here, we revisited the epidemiological clusters in light of the latest information regarding outbreaks in the region. The epidemiological clusters for Africa have the following characteristics.

Indian Ocean Island Countries (Madagascar, Mauritius, and Seychelles) are free of FMD, with a recognized status of FMD freedom without vaccination.

The South Southern African Development Community (SADC) cluster includes Swaziland, Lesotho, South Africa, Botswana, and Namibia, the southern and western part of Zimbabwe, and the southern part of Mozambique. The commercial livestock sectors of South SADC countries, with the exception of Zimbabwe and Mozambique, are free from FMD and meet the conditions of the OIE for zonal or country freedom from FMD without vaccination. Over the last 5 years, the region has suffered from an increasing number of outbreaks in cattle, most of which has been caused by SAT2 viruses. Cross-border epidemiological events have occurred on a number of occasions in South SADC, and in some cases, outbreaks were caused by viruses from different topotypes (Figure 1). The epidemiology of FMD in this region is characterized by virus circulation between the wildlife host, the African buffalo, and domestic animals, as well as spread among domestic animals, without the involvement of wildlife.¹⁸ In some of these countries, there are segregated wildlife areas that harbor African buffalo known to be infected, asymptotically, with FMDV serotypes SAT1,

SAT2, and SAT3. These wildlife parks are segregated from livestock through a system of game-proof fencing and vigorous surveillance. In these countries, game ranching or other wildlife conservation activities with FMD-infected African buffalo are not allowed within FMD-free zones. However, for this epidemiological cluster, the primary source of FMD seems to be the risk posed by the wild buffalo herds,^{45,46} as evidenced by many outbreaks in or near transfrontier conservation areas (TFCAs), such as the Kavango-Zambezi TFCA.⁴⁷

The North SADC cluster comprises the northern part of Zimbabwe, Zambia, northern Mozambique, Malawi, and southern Tanzania. The North SADC cluster countries have to deal with at least four serotypes of the virus (A, O, SAT1, and SAT2), and maybe even five (SAT3), each with multiple subtypes in the region (Figure 1). This may require the incorporation of more than one strain of a given serotype into a single vaccine to allow effective control in this region. Viral diversity and thus antigenic diversity is a complicating factor in effective vaccination against FMD in this cluster. Cross-border spread of the disease is common, and SAT1 and/or SAT2 outbreaks in Mozambique, Malawi, and Zambia between 2002 and 2013 were either because of outbreaks spreading from neighboring countries or to internal buffalo–cattle contact. Northern Malawi and Northern Zambia are under constant threat of FMD spread from southern Tanzania.^{29,38,39,48}

The Angola cluster may also include the western Democratic Republic of Congo (DRC). Very little is known about the true incidence of FMD within this cluster, and no official information is available on the isolation of FMDV from Angola since 1974. However, an FMD outbreak has been recorded in Angola in 2009, although no virus could be isolated. The southern part of Angola forms part of the Kavango-Zambezi TFCA, and it may be appropriate to include it within the South SADC cluster.

The East African Community cluster is comprised of Tanzania, Uganda, Kenya, Rwanda, and Burundi, plus the eastern part of the DRC (Figure 1). In addition to large livestock populations, this cluster has the highest concentration of wildlife in the world. The transmission and maintenance of FMD in this region is complex, as farming practices, trade, and wildlife contribute to the maintenance and spread of the virus. Farming is dominated by agro-pastoral and pastoral communities and is characterized by communal grazing and migrations. Eastern DRC is heavily dependent on trade in livestock from Uganda, Tanzania, Rwanda, and Burundi. The cluster probably contains several FMD primary

endemic foci, and cross-border epidemiological events suggest that animal movement plays an important role in virus dissemination.³⁹ At least four serotypes (A, O, SAT1, and SAT2) are endemic in this cluster,^{37–39,49–51} with serological evidence for a fifth serotype (C)⁵² (Figure 1). A sixth serotype (SAT3) was isolated in wildlife (African buffalo) in Uganda in 1970,¹⁰ although it has never been isolated from livestock in this cluster. SAT3 was also reported in Uganda in 1997⁵³ and in the DRC in 2005, but was not genotyped.⁵⁴ Isolates of serotypes A, O, SAT1, and SAT2 from Tanzania and Kenya (2004–2009) were genetically related.^{38,39} Similarly, viruses from Uganda and Kenya (1998–2008) were related.⁵¹ FMDV isolates belonging to serotypes SAT1 (topotype IV) and SAT2 (topotype X) have been isolated from African buffalo.¹⁵ As discussed above, there are also wide genetic and antigenic variations in the virus strains in this epidemiological cluster. The role of the African buffalo in the maintenance and transmission of FMD serotypes (eg, A and O)⁵⁵ that occur in this cluster has not been systematically studied.

The Intergovernmental Authority on Development (IGAD) cluster comprises Sudan, South Sudan, Eritrea, Ethiopia, Djibouti, Somalia, Northern Kenya, and Northern Uganda (Figure 1). Similar to the East African Community cluster, this cluster probably harbors major FMD primary endemic foci. Ethiopia and Sudan have the highest cattle populations in Africa.²⁹ Historically, isolates of serotypes A, O, SAT1, and SAT2 from Sudan and Ethiopia were genetically related to isolates from Uganda, Kenya, and Tanzania, most likely as a result of cross-border movement, a situation that has not changed.

The Soudan/Sahel cluster comprises Western Sudan, Niger, Chad, Burkina Faso, Mali, Northern Nigeria, Senegal, and Mauritania. The farming system in this ecosystem is predominantly pastoral, characterized by long-distance movement of livestock due to either transhumance or trade. This cluster probably also contains important FMD primary endemic areas, and at least four serotypes (A, O, SAT1, and SAT2) of the virus have been found. Furthermore, it may be an important disease-corridor cluster, linking the IGAD cluster with West Africa and probably West Africa with North Africa. The 1999 FMD strain in Algeria was found to be related to the West African type O topotype.²⁹ Similarly, isolates of serotype O from Niger (2007) and Nigeria (2007 and 2009) were genetically related to viruses found in Eritrea (2004 and 2011), Ethiopia (2005, 2006, 2008, and 2010–2012), and Sudan (2005, 2008–2011).⁵⁶ Viruses belonging to serotype A were isolated from cattle samples from Togo (2005), Nigeria (2009) in West Africa, and

Cameroon (2005) in Central Africa, which had close genetic relationships with viruses from Eritrea (1998) and Sudan (2006 and 2011) in East Africa^{56,57} (Figure 1).

Although the epidemiology of FMD in the coastal belt countries of West and Central Africa has not been deeply studied, it seems that this cluster probably gets infected from the Soudan/Sahel cluster. It could therefore be described as secondary endemic.

North Africa/Maghreb cluster countries Morocco, Algeria, and Tunisia have not reported FMD since 1999, most likely because of routine preventive vaccination and other measures. Libya and Egypt have sporadic FMD, and take routine preventive vaccination. Libya reported a SAT2 outbreak in 2003 (topotype VII) (Figure 1), probably as a result of live animal introductions from neighboring countries to the south, breaching the Sahara barrier. The virus was genetically related to outbreaks in cattle in Saudi Arabia in 2000 and Eritrea in 1998.³⁵ In 2012, Libya experienced another SAT2 outbreak (topotype VII), this time genetically related to isolates from Sudan (2007) and Nigeria (2008). Egypt also reported a SAT2 outbreak in 2012, the first occurrence of this serotype since 1950, and at least three sub-lineages (one Libyan and two Egyptian)²⁰ were identified. Egypt also reported African type A viruses in 2006, 2007, 2009, and 2012, as well as ME-SA (Middle East–South Asia) types O and A. Yemen reported EA (East Africa)-3 type O in 2007 and 2009. Thus, North African countries will remain at risk from the south and east, but across the majority of their territories, and at-risk populations should effectively maintain FMD freedom.

Antigenic diversity of FMDV: implication for the selection of vaccine strains

The selection of FMD vaccine candidates is complicated by the wide spectrum of genetic and antigenic variability of the FMDV and the continuous emergence of new mutants from populations that escape the host immune response.^{58–61} Most of the impacts of this variation derive from changes within the three major surface-exposed capsid proteins of the virus (ie, VP1, VP2, and VP3). At least 30%–50% of the residues that constitute the capsid proteins are exposed on the virus surface, many of which encompass neutralizing epitopes.^{62–67} It has been shown that the majority of FMDV-neutralizing antibodies are directed against conformational epitopes located on the β -barrel connecting loops projecting from the surface of the virus, especially the highly mobile β G– β H loop in VP1.^{62,68–70} It is important to recognize that

the SAT1 and SAT2 viruses display greater antigenic variation compared with the Euro-Asian serotypes (O, A, C, and Asia-1).^{61,66,71} The variation is not random, but tends to be concentrated at the surface-exposed β -barrel connecting loops. Therefore, knowledge of the amino acid residues that comprise the antigenic determinants of FMDV, and those that function as protective epitopes in particular, will greatly improve our understanding of virus neutralization in vivo.^{72,73}

Several studies have been carried out to delineate the neutralizing antigenic sites of representative viruses from serotypes A,^{74–76} O,^{68,77} C,⁷⁸ and Asia-1.^{79,80} In these studies, monoclonal antibodies (MAbs) have been pivotal in identifying critical amino acid residues of the different neutralizing antigenic sites. In addition, mapping the topography of the mutations on the X-ray crystallographic structure of FMDV^{62,63,67} and predicting B-cell epitopes using computational algorithms⁸¹ have resolved several other antigenic sites on the capsid of FMDV. Although the overall role and importance of these antigenic sites in induction of protective immunity and cross-reactivity in target species is still poorly understood, it appears that animals vaccinated with FMDV do not elicit a predominant antibody response against a single antigenic site, but rather utilize a broad repertoire of epitopes on the viral capsid.^{82–84}

In a study to investigate the antigenic structure of FMDV serotypes SAT1 and SAT2, unique critical MAb contact residues were found on VP3 and VP1 (site VI), VP2 and VP1 (site VII), or VP1 residue 111 (site VIII) of a SAT1 virus.⁸⁵ Similarly, for a SAT2 virus, two epitopes have been mapped, one encompassing the β G– β H loop of VP1 and the other involving residue 210 in the C-terminus of VP1.^{85–87} These antigenic sites are summarized in Figure 2. The residue positions where substitutions occurred in MAb-resistant mutants are variable, with high entropy or uncertainty in SAT1 and SAT2 capsid protein alignments (Figure 2). For both serotypes, the variable regions were clustered around the fivefold and threefold axes of symmetry of the virion. A single amino acid change can lead to the abrogation of antibody-binding, which emphasizes the structural flexibility of the surface-exposed loops.

Vaccine matching: the selection of vaccine strains for specific geographic, endemic regions

The two important determinants that will affect the efficacy of a vaccine and determine whether it will protect or not are 1) the ability of the vaccine strain to elicit antibodies that will cross-react and protect against the field or outbreak virus in question (defined as the vaccine or antigenic match),

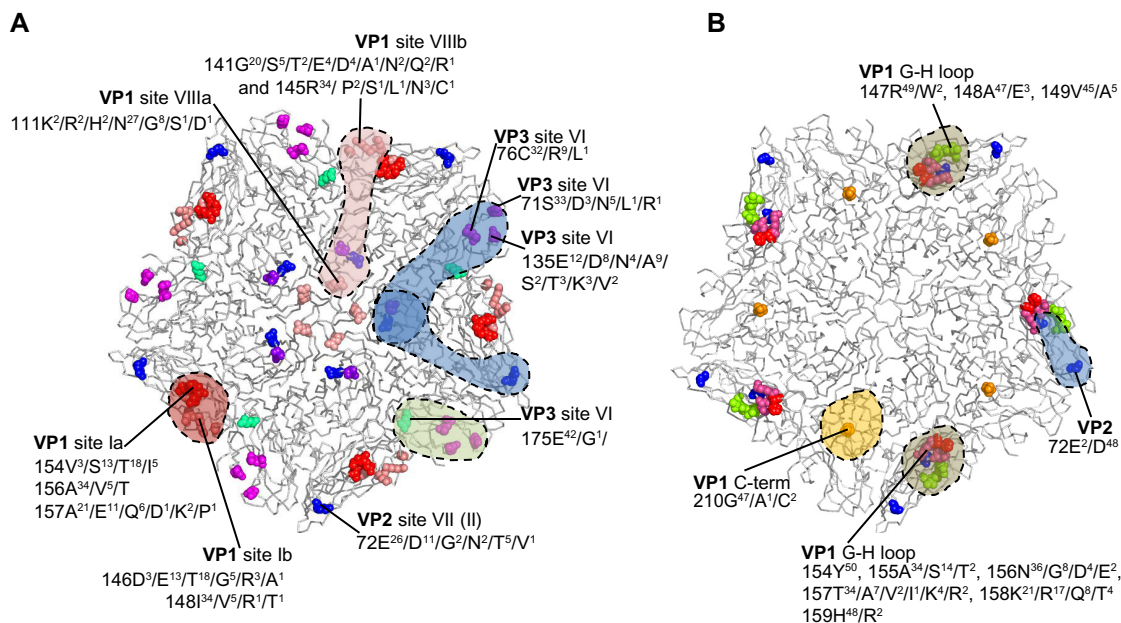


Figure 2 The antigenic structure of foot-and-mouth disease SAT1 (A) and SAT2 (B) viruses are depicted.

Notes: The amino acid substitutions observed in SAT1 and SAT2 monoclonal antibody-resistant (MAR) mutants are shown as spheres on the grey ribbon backbone of the pentamer (five copies of VP1, VP2, and VP3) unit, and the potential antibody footprints are stipulated. The variation in amino acid residues at each antigenic residue position, in a complete capsid protein alignment of 43 SAT1 and 50 SAT2 viruses available on the genetic sequence database (GenBank) are indicated. The viruses in the alignments include isolates from South Africa, Zimbabwe, Mozambique, Namibia, Botswana, Zambia, Angola, Malawi, Tanzania, Kenya, Uganda, Rwanda, Zaire, Nigeria, Senegal, Ghana, Eritrea, Saudi Arabia, and Sudan. Adapted from Grazioli S, Moretti M, Barbieri I, Crosatti M, Brocchi E. Use of monoclonal antibodies to identify and map new antigenic determinants involved in neutralization of FMD viruses type SAT 1 and SAT 2 In: European Commission for the Control of Foot-and-Mouth Disease: International control of Foot-and-Mouth disease: Tools, Trends and perspectives. Paphos, Cyprus: Food and Agriculture Organization; 2006:287–297.⁸⁵

Abbreviation: SAT, Southern African Territories.

and 2) the potency of the vaccine to elicit a strong and long-lasting immune response. The quality and quantity of the antigen in the vaccine as well as the formulation of the vaccines and inclusion of immune-stimulating adjuvants are all factors that will influence and contribute to the overall potency of the vaccine.⁸⁸ In addition to vaccine efficacy, the number of animals vaccinated in the target population during a vaccination campaign will determine effective protection at herd level and should be taken into consideration. Basic capability to undertake vaccine matching tests on a routine basis in diagnostic laboratories in African countries is severely limited, and therefore current advice regarding the selection of the best vaccine to be used in these settings is normally provided by regional (ARC-Onderstepoort Veterinary Institute, South Africa; Botswana Veterinary Institute, Botswana) and international reference centers.

The OIE/FAO FMD Reference Laboratory Network reports over the last five years have revealed a gap in the vaccine strains available to match against circulating SAT1 and especially SAT2 viruses. The urgent requirement for the development of new SAT vaccine strains with good immunogenicity for use in Africa was also highlighted at the recent Global FMD Research Alliance congress (Arusha, Tanzania in October 2013). For the African continent (FMD endemic pools 4, 5, and 6), at least five vaccine strains are available for SAT1, and seven vaccine strains are described for SAT2 viruses. However, not all these vaccine strains are of recent derivation or are currently used in production, and it is therefore imperative that outbreak samples are properly matched to the vaccine strains that are available for use in control programs.

The direct assessment of the degree of protection induced by a vaccine against the field virus is performed using animal challenge studies and is the most accurate way to determine whether a vaccine will cross-protect against a particular field virus.^{82,89,90} However, routine heterologous challenge studies are rarely performed, as this is time consuming, requires access to expensive bio-containment facilities, and raises serious animal welfare issues when large numbers of animals need to be vaccinated and challenged. If small groups of animals are used, these challenge studies can suffer from low precision.⁹¹

In South America, a variation of this direct method has been developed, based on the probability that cattle will be protected against a challenge of 10,000 infective doses of test virus following a single or boosted vaccination.⁹² In order for the expected percentage of protection method to be effective, the vaccine needs to be extensively tested in many (hundreds) cattle by previous challenge tests with homolo-

gous virus.⁹² Although this method has been widely used in South America,^{90,93} the availability of good datasets and sera and the need for diverse vaccine strains are limiting factors for use of this approach in the African context.

Therefore, *in vitro* alternatives to measure cross-reaction between sera elicited by a vaccine and a particular field/outbreak isolate is recommended, supporting the need to accurately predict vaccine matching without the involvement of animals.⁹⁴ Traditionally, antigenic characterization of a field or outbreak virus is performed indirectly using the *in vitro* virus neutralization test (VNT), which measures the ability of sera from vaccinated animals to cross-react with the field virus.^{61,82,89,90} The neutralization titers are used to calculate r_1 -values to determine antigenic relationships.⁹⁵ However, interpretation of the test is plagued by limitations, including the uncertainty as to how well the *in vitro* matching data actually correlates to *in vivo* cross-protection, the impact of vaccine potency on protection, and the availability of reference reagents.⁸⁸ Furthermore, the use of r_1 -values to estimate cross-protection relies on having sufficient repeated measures to overcome the inherent variability of the neutralization titers.⁹⁶ In a recent study with SAT1 viruses, we found a number of factors to impair reproducibility in one-way relationships, such as the operator, batch variability of reagents, day-to-day variation in the cells, and variation in individual cattle sera. It is also known that measuring the titer ratio to a known control is not sufficient to eliminate the inter-experiment variability, highlighting the necessity for time-consuming duplicate tests to be undertaken on separate and independent occasions to compensate for day-to-day variations.⁹⁶ A novel way to quantify and visualize antigenic relationships is antigenic cartography.^{97,98} However, the combination of genetic sequencing and antigenic profiling of the outbreak virus are still useful methods to identify newly emerging or re-emerging virus strains and whether available vaccine strains are likely to provide protection against the outbreak virus or not.

Using this *in vitro* approach in vaccine matching, we have shown that the SAT1 vaccine strains SAR/9/81 and KNP/196/91 are antigenically relevant for South Africa, Zimbabwe, Mozambique, Botswana, Namibia, Zambia, and Tanzania.⁶¹ The r_1 -values determined in assays with antisera from cattle that had been vaccinated with SAT1/KNP/196/91, and a panel of SAT1 viruses, was greater than 0.3 for 75% (n=30) of viruses (Figure 3A). This result suggests that efficient cross-protection may be induced in animals following vaccination with these strains. Similarly, a vaccine based on NIG/5/81 could most likely protect against many strains circulating in West Africa (pool 5 viruses), while a

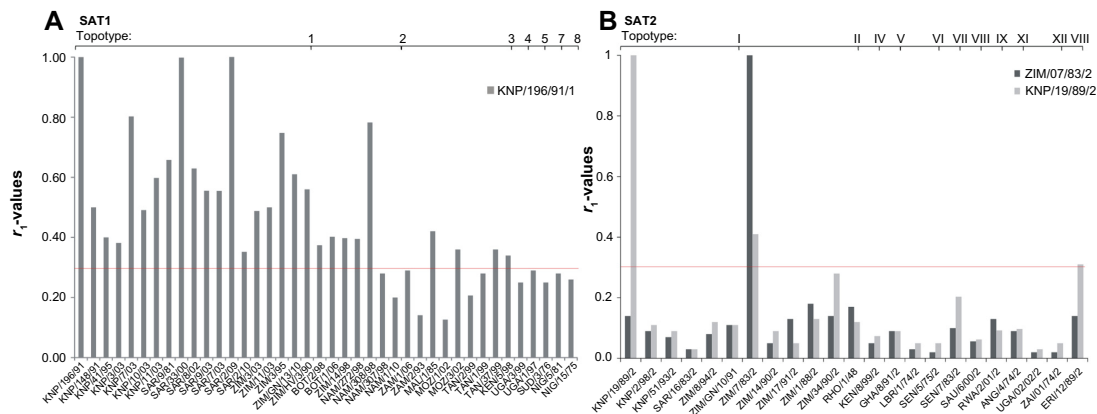


Figure 3 Chart to indicate the r_1 -values of various SAT1 and SAT2 foot-and-mouth disease virus isolates from different toptypes compared with antiserum prepared from the vaccine strains (A) SAT1/KNP/196/91 (topotype I) and (B) SAT2/KNP19/89/2 (topotype I) and SAT2/ZIM7/83 (topotype II).

Notes: The antigenic relationships of the field isolates were determined using cross-neutralization assays on IB-RS-2 cells, carried out as described by the OIE.²⁰⁷ The r_1 -values were calculated as: $r = \text{serum titer against heterologous virus} / \text{serum titer against homologous virus}$, and interpreted as proposed by Samuel et al.¹⁰⁴ Viruses from the Kruger National Park (South Africa) (KNP), South Africa (SAR), Ghonera Zhou National Park (Zimbabwe) (GN), Zimbabwe (ZIM or RHO), Botswana (BOT), Namibia (NAM), Mozambique (MOZ), Angola (ANG), Zambia (ZAM), Malawi (MAL), Tanzania (TAN), Kenya (KEN), Uganda (UGA), Rwanda (RWA), Sudan (SUD), Zaire (ZAI) Ghana (GHA), Nigeria (NIG), Senegal (SEN), Eritrea (ERI), Saudi Arabia (SAU) were included in the study.

Abbreviations: OIE, World Organisation for Animal Health; SAT, Southern African Territories.

vaccine based on BOT/1/06 virus alone or in combination with another strain from East Africa will effectively cross-react to control most pool 4 viruses.⁹⁹ Thus, a high potency or bivalent SAT1 vaccine could most likely be effective across toptypes. However, there is a dearth of knowledge about the antibody response of animals vaccinated with a multivalent vaccine containing a combination of intra-serotype strains to cross-react against field viruses. Furthermore, it has been shown for serotype A viruses that a high potency vaccine provides protection against heterologous challenge, despite low r_1 -values.¹⁰⁰ In the past, the combination of SAT1/SAR/9/81 and SAT1/KNP/196/91 in a tetravalent vaccine, containing also a SAT2 and a SAT3 strain, were able to protect against SAT1 outbreaks in southern Africa.¹⁰¹ The antigenic relationship of SAT2 viruses against two SAT2 vaccine strains showed poor cross-reactivity, indicative of low level of cross-neutralization in vivo (Figure 3B). Notably, a single type O vaccine strain was efficacious in controlling the circulation of different toptypes in Kenya, even though temporal fluctuation in the prevalence of a particular type was observed.⁵⁰

Alternatively, serological cross-reactivity can be estimated using a liquid-phase blocking enzyme-linked immunosorbent assay (ELISA) procedure for vaccine matching reviewed by Paton et al.⁸⁸ and Kitching et al.¹⁰² In common with the VNT method, this approach is based on the reactivity of bovine serum raised against the available vaccine strains to the field virus and the homologous vaccine strain.¹⁰³ The r_1 -value is calculated as the heterologous titer divided by the homologous titer and interpreted as proposed by

Samuel et al.¹⁰⁴ The r_1 -value calculated by the two assays differ, because the VNT approach is based on the ability of the antisera to neutralize the virus, whereas the liquid-phase blocking ELISA method measures binding of the antisera to the virus or viral components. Therefore, there is still a need for a faster and more reliable method that is as effective as the VNT at evaluating vaccine matching.

Since the antigenic variability, reflected in the VNT titers, is a measure of whether the sites with which the immune system reacts to neutralize the virus remain sufficiently similar between a homologous and heterologous virus to be recognized,¹⁰⁵ we have described a new approach, using linear mixed-effect models, to estimate antigenic matching.⁶⁶ In principle, the amino acid variation and in vitro cross-protection titers from VNTs were combined with crystallographic structural data to generate antigenic matching information indirectly.⁶⁶ Examining SAT1 and SAT2 viruses, we identified a correlation between genetic distance and antigenic relatedness (r_1 -values), but more significantly, we could identify areas on the surface of the capsid where mutations were strong predictors of antigenic distance. These were consistent within serotypes, and were found to match some of the independently identified antigenic sites in other serotypes. Applying the linear mixed-effect model to SAT1 viruses, two surface-exposed regions of the capsid (ie, VP1 β G- β H (132–174) and VP3 β H- β I (191–202) loops) were identified as better predictors of cross-reactivity between the field virus and vaccine strain⁶⁶ than serologically calculated r_1 -values. Both regions were identified by studying MAb-resistant mutant viruses.⁸⁵ For SAT2 viruses, amino acid

residues in the VP1 C-terminus (200–224), the VP2 β B– β C loop (70–82), and residue 178 in the VP1 β H– β I loop were found to be good predictors of cross-reactivity.⁶⁶ However, the aim in endemic settings is to identify potentially new vaccine viruses when existing vaccine strains are found to not cross-react to the outbreak virus sufficiently. The linear mixed-effect approach was found useful to assess the likely cross-reaction of new vaccine strains against a group of field viruses.⁶⁶ Taken together, this approach will assist in the future in the selection of potential new vaccine strains for sub-Saharan Africa.

Simple antibody recognition measures do not always correctly predict the ability of a vaccine to protect against an outbreak virus.¹⁰⁶ The antibody isotype, the avidity of the antibody to the virus in question, and the type of immune response elicited are also important factors to consider.^{107–109} In a recent study comparing the accuracy of traditional and novel serological assays to predict cross-protection, it was found that the use of VNT titers and r_1 -values are inaccurate indicators of protection.¹¹⁰ However, when the VNT titers were combined with the IgG1 titer, a more accurate estimate of FMD vaccine protection against the heterologous virus for serotype A was achieved. To date, the correlation of in-parallel serological data, like VNT and IgG1, IgG1/IgG2, or antibody avidity in cross-protection in the case of SAT viruses is unknown.

Control of FMD by vaccination

The existing vaccines against FMD consist of complete, chemically inactivated virions combined with an adjuvant.⁷ The adjuvant used in the vaccine formulation has undeniably a huge effect on the efficacy and potency of the vaccine and has been reviewed elsewhere.^{7,111} Despite successful application in the developed world, the effective administration and optimal induction of protective immunity are hampered by several factors in developing countries. In addition to the vaccine-matching constraints that have been discussed in the previous section, some viruses are very difficult to adapt to cell culture, slowing the introduction of new vaccine strains, reducing vaccine yield, and potentiating through prolonged passage the selection of undesirable antigenic changes.^{112,113} Vaccination does not induce sterile immunity, and animals may still be able to infect non-vaccinated animals and may also become persistently infected.^{7,114–117} The presence of contaminating non-structural proteins in some vaccine formulations makes it problematic to distinguish between vaccinated and convalescent animals, impacting on the ability to export from FMD-controlled regions. In addition, the hot climate in many African regions calls for

vaccines with improved stability and which are less reliant on a cold-chain. During production, the manufacturer also has to compensate for this instability by increasing the quantity of antigen per vaccine dose, which is expensive and reduces vaccine yield. Based on the findings of Doel and Bacarini,¹¹⁸ it is believed that unstable vaccines are less immunogenic due to degradation before and after inoculation. Therefore, FMD vaccines require frequent booster vaccinations in order to be effective. Lastly, the current vaccines are relatively expensive, especially for the small and subsistence farmer.

Vaccines used in the control of FMD in endemic regions are mostly used for mass prophylactic application. Such vaccines are multivalent to provide protection against multiple serotypes, and should have a potency of at least 3 PD₅₀ per dose.¹¹⁹ Generally, prophylactic vaccines incorporate 146S particles combined with saponin-alhydrogel or oil-adjuvant.¹¹⁹ Oil-adjuvanted vaccines have been used successfully in FMD-eradication campaigns in South America.^{5,120,121} A study evaluating different adjuvants for SAT vaccines has shown that a double water-in-oil-in-water adjuvant, ISA206, elicited protective antibody responses against SAT2 serotype in cattle.¹²² Inactivated vaccines induce short-lived immunity, and it is recommended that naïve animals receive two initial vaccinations (a primary and secondary dose) 3–4 weeks apart, followed by re-vaccination every 4–6 months^{101,122} to prevent spread of disease within populations. However, in the African environment, this may differ for different manufacturers, depending on the potency of the vaccine, and some manufacturers recommend five vaccinations per annum. There is a definite need to assess whether different adjuvants may enhance the duration of immunity against SAT antigens. For these reasons vaccination campaigns should be performed regularly, based on 1) the epidemiological circumstances and risk of disease spread, 2) the value and life expectancy of species, and 3) the economic status of the country. The interval between vaccinations is critical to prevent a “window of susceptibility” and where the continuous or sporadic presence of virus in carrier animals is present.

The PCP is the strategy proposed by OIE and FAO to control and ultimately eradicate FMD from endemic countries. Different regions in sub-Saharan Africa are at different developmental stages of control and are thus facing unique challenges and priorities in terms of FMD control (Figure 4). In many African endemic countries, there are various knowledge gaps, such as disease occurrence and mechanisms of virus maintenance and transmission, and therefore no routine vaccination campaigns are implemented (PCP Stage 1 countries; Figure 4). In other African endemic countries, even

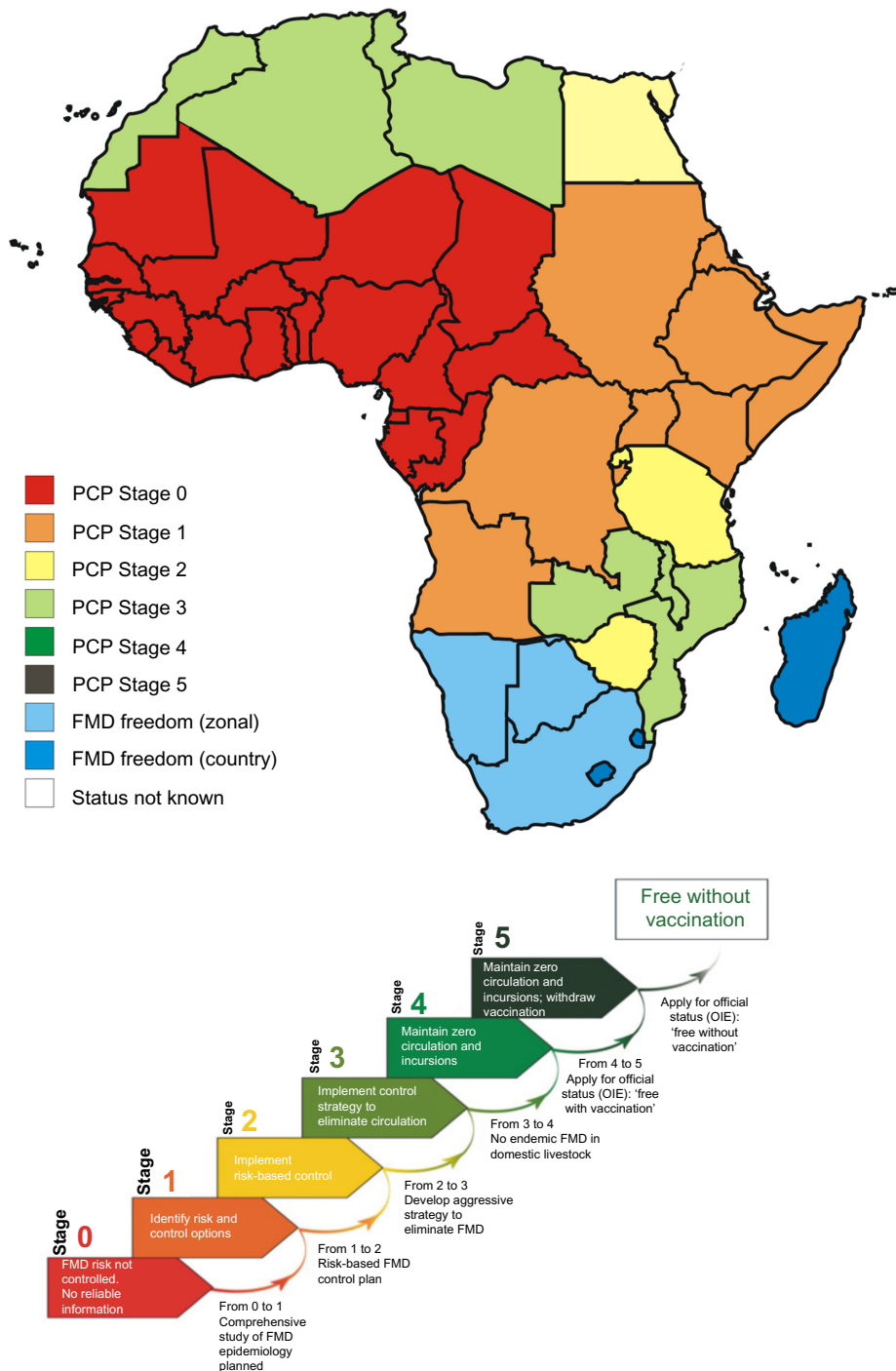


Figure 4 Map indicating the different PCP stages of countries in southern and eastern Africa.

Notes: The results are based on self-assessment and aspirations from the member states, with the goal to make progress on the PCP for FMD.^{208–210} The FMD PCP consists of six stages ranging from zero (0), when there is continuous FMD virus circulation with no reporting or control actions, to five (5), where a country is ready to be officially recognized by the OIE as free without vaccination. Currently, the OIE recognizes only three categories for countries with regard to FMD: 1) countries not free from FMD (PCP stages 0–3), 2) FMD-free countries or zones practicing vaccination (PCP stage 4), and 3) FMD-free countries or zones where vaccination is not practiced (PCP stage 5). This figure was adapted with permission from the Food and Agriculture Organization of the United Nations. © FAO 2011. Report of the 39th Session of EuFMD, Rome 2011. 1–31. <http://www.fao.org/ag/againfo/commissions/eufmd/commissions/eufmd-home/reports/general-sessions/2011-39th-session/en/>. Accessed July 25, 2014.²¹¹

Abbreviations: FMD, foot-and-mouth disease; OIE, World Organisation for Animal Health; PCP, Progressive Control Pathway.

where surveillance is conducted to provide knowledge about high-risk populations, often implementation of effective, scheduled vaccination campaigns still does not take place (PCP Stage 2 countries; Figure 4). There are various reasons

why governments do not subsidize FMD vaccines, leading to individuals needing to carry the cost and implement their own vaccine schedules. Additionally, individuals would need to source vaccines without knowledge of the current

circulating strains in their region, leading to a poor vaccine match. This often leads to no or ineffective control in endemic African regions. The development of new vaccines against FMD in endemic countries in Africa should therefore take into account the ecosystem-based synchronization as FMD control strategies employed in these regions.³⁰

Design of improved inactivated vaccines

Some of the abovementioned limitations of current vaccines are being addressed by the development of reverse genetic approaches. Additionally, new alternative vaccines that do not require infectious virus as well as efforts to understand the role of innate immunity and cytokines to induce protection and boost the immune response offer tremendous potential for the control of FMD in endemic regions.

Genome-length viral RNA (vRNA) derived from complementary DNA (cDNA) clones of FMDV is infectious when transfected into suitable mammalian cells.^{123–126} These cDNA clones are readily amenable to genetic engineering to introduce changes to the virus genome that allows for the replacement of the external capsid-coding region or structural, surface-exposed antigenic loops with the corresponding regions of an emerging virus. The outcome of such a chimeric virus is the transfer of the spectrum of neutralizing epitopes from the etiological agent to the recombinant virus^{123–127} and the ability to antigenically simulate the outbreak virus and induce protective immunity in host animals.^{123,128,129} Furthermore, it has been shown that inter-serotype chimeric vaccines with the capsid proteins of SAT1 within a SAT2 background¹²⁸ and O serotype within an A serotype background^{123,130} confer protective immunity.

However, capsid swapping may transfer other undesirable traits such as capsid instability and poor cell culture adaptation, which are limitations that can be overcome by site-directed mutagenesis of the amino acid(s) associated with improved performance as vaccine candidates.¹²⁹

The development of new vaccine FMDV strains relies strongly on virus growth and high antigen yields of the new strain in the production cell line.^{7,131,132} We have demonstrated that SAT-type viruses, previously impossible to adapt to cell culture, can be structurally modified by introducing an adaptation phenotype which is able to interact with sulfated glycosaminoglycans, enabling improved vaccine production.¹²⁹ Several glycosaminoglycan-binding sites have been identified for the various serotypes that can be used for this purpose.^{112,129,133–138} Furthermore, alternative cell entry pathways exist and can be applied in future to improve cell

adaptation.^{134,135,139} Continued reverse genetic approaches to rapidly adapt African FMDV to BHK-21 cell culture will greatly enhance the ability to produce region-specific vaccines tailored to current circulating strains.

The stability of vaccines is of crucial importance in Africa, where the logistical process to get the vaccine from the manufacturer to the animal may take months and in many remote regions is in the absence of a cold-chain. Vaccines with improved stability and less reliant on a cold-chain are needed and could improve the longevity of immune responses elicited in animals.¹¹⁸ FMD is known to be unstable, especially for O and SAT2 serotypes,¹¹⁸ in mildly acidic pH conditions or at elevated temperatures, leading to dissociation of the capsid (146S particle) and loss of immunogenicity. The residues at the capsid inter-pentamer interfaces, and their interactions, are important for the infectivity and stability of the virion,^{140,141} and mutations adjacent to these interfaces have an effect on the conformational stability of FMDV.^{142–145} However, experimental studies on the relative importance of residues and molecular interactions in viral capsid assembly, disassembly, and/or stability are still very limited. Recent research has compared more thermostable serotype A viruses with unstable O and SAT2 viruses, together with crystallography structures, sequence data, and in silico calculations of stability, to predict residue substitutions that could increase stability at inter-pentameric interfaces. With reverse genetic approaches, stabilizing mutations have been introduced into infectious copy clones. SAT2 and O viruses with improved stability have been developed, and their antigens are currently being tested in animal models for potential use as vaccines (unpublished observation).

Alternative vaccine strategies: subunit and live viral-vector vaccines

Due to the limitations of inactivated vaccines, alternative strategies for vaccine development have focused initially on the use of VP1-proteins and peptides either isolated from FMDV or produced by recombinant DNA;^{146,147} VP1-derived peptides¹⁴⁸ or chemically synthesized VP1 peptides;^{149–152} vectors expressing VP1 fusion proteins;^{153–155} inoculation with DNA expressing VP1 epitopes alone¹⁵⁶ or with DNA encoding IL-2;¹⁵⁷ and transgenic plants or recombinant tobacco mosaic virus expressing VP1.^{158,159} However, they rarely achieved protection against virus challenge in livestock,^{151,160,161} or as a result of a limited subset of epitopes, selected for antigenic variants that escaped from protection.¹⁶² The reduced level of protection may be due to a lack of T-cell epitopes.¹⁶³

Subviral particles or virus-like particles which are assembled as empty particles lack RNA (noninfectious), but are immunogenic, as their antigenic surface is indistinguishable from the complete virus.¹⁶⁴ Previous attempts to express the P1, leader, and 3C regions in recombinant baculovirus systems has yielded limited amounts of empty capsid due to toxicity in insect cells¹⁶⁵ or in *Escherichia coli* resulted in inefficient capsid assembly.¹⁶⁴ Major advances have been made, firstly in developing methods to efficiently express empty capsids in vaccinia virus or baculovirus expression systems by lowering viral protease activity, which is toxic to cells, and secondly by enhancing capsid stability.¹⁶⁶ Cattle vaccinated with such an A-serotype construct have shown sustained VNT titers and protection from challenge 34 weeks post-vaccination.¹⁶⁶ This approach has several advantages over conventional vaccines, by reducing production costs, eliminating risk of infectivity, and enhancing stability.¹⁶⁶ However, producing vaccines using insect cells requires highly skilled staff, and scaling up of the manufacturing process is difficult and expensive to optimize, which poses disadvantages for the African market. More studies are needed to assess whether this strategy would be effective in endemic regions of Africa for SAT serotypes and when applied to the field and to find out whether the production process would be economically viable.

Alternative strategies which incorporate sections of the FMDV genome inserted in a live viral vector have been investigated with bovine rhinotracheitis virus,¹⁵⁴ and the G–H loop region has been incorporated in a chimeric poliovirus;¹⁵⁵ capsid and/or non-structural proteins in vaccinia virus,^{167,168} fowlpox virus,¹⁶⁹ or pseudorabies virus^{170,171} provides partial protection in pigs and guinea pigs. A chimeric bamboo mosaic virus containing 1D epitopes induces humoral and cell-mediated responses and protection in pigs.¹⁷² The experimental vaccines so developed have several disadvantages, such as requirement for multiple doses, low-level antigen expression, and uncertain safety. Additionally, repeated vaccination with a viral-vector vaccine, as is necessary in endemic regions, leads to immunity against the vector and reduced protection. The most successful advances have arisen from incorporating FMDV genes into replication-defective human adenovirus (Ad) vectors.^{173,174} The resulting vaccine has several advantages over conventional vaccines: 1) it can induce protection within 7 days and within 1 day if combined with interferon- α expression, particularly useful for emergency use; 2) it is fully compatible with test systems that allow to differentiate infected from vaccine animals;

3) incomplete inactivation problems are not a concern; and 4) it has excellent immunogenicity, attributed to its natural adjuvant properties, high transduction efficiency of targeted cells, and ability to induce antiviral cytotoxic T-cells.¹⁷⁴ However, whether animals vaccinated with Ad-FMD vaccines would be protected from developing a carrier state is unclear, as is the significance of pre-existing immunity in the field. Finally, it is of major importance to determine the protective immunity and breadth of antigenic coverage against evolving variants in the field.

To be optimal for use in Africa, new vaccines should be easily delivered to the animal, proven to be more effective and induce longer lasting immunity than conventional inactivated vaccines, safe in production, easily made, and inexpensive. This is especially important for African producers that need to design vaccines tailored to African continental control, focusing on circulating serotypes and subtypes in endemic regions where eradication is difficult to attain due to the presence of maintenance hosts and continuous wildlife–livestock interfaces.

Current and novel diagnostic tests for field application in endemic regions

The accurate diagnosis of FMDV infection is of utmost importance for the control and eradication of the disease in endemic regions. The initial diagnosis of FMD is normally based on clinical signs, but this can easily be confused with other vesicular diseases.¹⁷⁵ Hence, it is vital that the recognition of signs of the disease by the farmer is promptly conveyed to the relevant veterinary authorities to verify clinical symptoms, and suspect samples should then be sent to the reference laboratory for confirmation. Rapid and precise data generated by laboratories provides vital support to FMD control and vaccination programs. However, in many African countries, samples received by the laboratory can be of poor quality due to an ineffective cold-chain and long transport periods. These factors make laboratory diagnosis challenging, and it is evident that sub-Saharan Africa requires diagnostic tools that are fit for purpose in these settings to allow for rapid diagnosis and the appropriate measures taken for control.

Existing diagnostic techniques for the detection of FMD are mainly based on the following principles:

- The identification of the infectious agent by virus isolation involving propagation on susceptible cell cultures¹⁷⁶
- The detection of viral antigen by ELISA systems using FMDV-specific antibody or capturing reagents^{177–179}

- Molecular detection of viral nucleic acid by reverse-transcription polymerase chain reaction (RT-PCR) and the genetic analysis of the nucleotide sequence, mostly of the VP1-coding region¹⁸⁰
- Detection of FMDV-specific antibody in animals previously exposed to the virus. The VNT is usually used as a confirmatory test for sera found positive by ELISA.⁸⁸

These techniques are primarily suited for well-equipped laboratories which are usually either national or regional reference laboratories.³⁰ The virus cell culture system, for example, requires careful handling of specimens to prevent environmental and cross-contamination, trained personnel, and a BSL3 (biosecurity level 3) laboratory. The success of virus isolation is dependent on the sample quality and requires special transport conditions from the sampling point to the laboratory.¹⁷⁶ Both the solid-phase competition ELISA and the liquid-phase blocking ELISA for serological detection of FMDV-specific antibodies against structural proteins are relatively simple procedures and easily implementable in diagnostic laboratories in endemic regions.^{181–183} The VNT on the other hand, requires technical skill to be performed accurately and is dependent on cell culture facilities,^{184,185} which may not be conducive for laboratories in endemic regions.

Molecular techniques, like RT-PCR and real-time RT-PCR have the advantage that a wide range of samples (eg, esophageal/pharyngeal scrapings, tissue, and serum) can be tested rapidly^{186,187} and are now widely considered to be front-line diagnostic tests for the detection of FMD.¹⁸⁸ Furthermore, RT-PCR is the first step to determine the nucleotide sequences and contributes to molecular epidemiology studies and provides a tool to support regional and country-wide FMD control programs in an outbreak situation. To various degrees, these technologies are being implemented and applied in many diagnostic laboratories across the African continent, although these approaches are sometimes still too expensive to be implemented as routine across the whole of the African environment.

One of the limitations in the early diagnosis of FMD in an endemic situation is that outbreaks often occur in remote areas where the nearest veterinarian and national laboratory can be many kilometers away.³⁰ This can delay the laboratory results for a month or longer. Lateral flow devices (LFDs) are immuno-chromatographic tests that allow the diagnosis of FMDV at the site of a suspected outbreak. Different LFDs have been developed and evaluated and are either serotype specific¹⁸⁹ or can detect all seven FMDV serotypes.^{190,191} The LFD can utilize vesicular fluid or vesicular epithelial suspensions but not nasal swabs or sera.^{189,191} The method makes use of capture and detection MAbs or specific polyclonal

antisera on a strip test, and studies thus far have shown the test to be as sensitive and specific as the ELISA; however, the sensitivity of the strip test may differ for the various FMDV strains.¹⁹² The use of LFDs will allow for immediate determination of an FMDV-positive or -negative result and thus will facilitate decisions to be undertaken immediately for FMDV control in case of a positive result. Furthermore, only those samples that test positive on the field devices can be sent to the laboratory for further confirmatory tests, subtyping, and genetic characterization.

Portable real-time PCR platforms offer many advantages in endemic point-of-disease situations.^{193–195} These platforms, which can be utilized by non-specialists, are designed to perform all the steps of an RT-PCR test (eg, capable of nucleic acid extraction and performing RT-PCR). The Enigma FL field laboratory platform (Enigma Diagnostics, Wiltshire, UK) is one such system capable of nucleic acid extraction, PCR thermocycling, and analysis of data without the requirement for user intervention and has been tested for FMD diagnosis.^{193,196} The question remains whether field-based assays can ensure the detection of new viruses as they continue to evolve in sub-Saharan Africa.

An alternative molecular detection technique is loop-mediated isothermal amplification (LAMP) assay, which has been widely used for the detection of RNA and DNA viruses that infect livestock.^{197,198} This approach is an autocycling, strand-displacement DNA-synthesis method performed by the large fragment of Bst DNA polymerase at a single temperature. To facilitate the LAMP assay in the field and because the RT-LAMP products are generated in abundance, the results can be visualized by the naked eye, either in the form of visual turbidity or visual fluorescence. The diagnostic performance of real-time RT-PCR and RT-LAMP are governed by the nucleotide variability of the genome signatures between the different serotypes and genetic lineages of the virus. Since the primer recognition sites used for RT-LAMP have a larger footprint than RT-PCR assays, designing assays that can accommodate the large range of sequence variability of FMDV can be challenging, particularly for SAT strains. There have been many studies that have shown the high specificity and high amplification efficiency of RT-LAMP.^{198–200} Furthermore, Yamazaki et al²⁰¹ developed the first application of a multiplex RT-LAMP approach to accommodate the high sequence variability encountered in RNA virus genomes and found the analytical sensitivity to be comparable to the singleplex RT-LAMP assays.¹⁹⁸ The combination of a simple method to prepare template RNA and RT-LAMP can prove useful for sub-Saharan Africa in

the field or laboratory where expensive equipment may not be readily available.

Additional novel diagnostic assays such as biosensors,²⁰² microarrays,²⁰³ gold nanoparticle improved immuno-PCR,²⁰⁴ and nucleic acid sequence-based amplification^{205,206} have been shown to enable rapid and reliable diagnosis, surveillance screening, and strain typing for FMDV. Although these assays have promising capabilities for sub-Saharan Africa and can improve many of the current problems faced, there are also many limiting factors that prevent the routine use of certain assays. For example, many of the novel assays are still to be optimized for the FMDV SAT serotypes where a high degree of sequence variability exists, the costs involved per test will determine how widely these assays will be used, the field-based novel assays will require training of personnel, and some laboratory-based tests require specialized equipment which is not readily available and personnel capable of correctly interpreting and analyzing the datasets produced.

Thus, open communication between national and international reference labs becomes important as a support system to endemic regions. Also, the possibility of diagnostic banks where diagnostic kits become readily available in outbreak situations can alleviate many of the problems faced by endemic regions.

Conclusion

Most countries in Africa are ill equipped to control transboundary animal diseases such as FMD because of the lack of infrastructure and financial resources, ineffective animal health authorities, civil unrest, and even military conflict. Furthermore, most governments ascribe low priority to the control of animal diseases in the face of many other pressing problems like human health and education. Even in countries that controlled FMD successfully in the past, like South Africa, Namibia, Botswana, and Zimbabwe, an increase in the amount of FMD outbreaks has been observed over the last two decades. However, for other countries, this may reflect the situation since the 1960s. In many others, the current situations regarding their FMD status is unknown, and the majority of outbreaks remain unrecorded. There are several reasons for this: 1) countries may not be involved in intercontinental trade in animals and animal products and therefore have little incentive to report FMD outbreaks; 2) in many regions where pastoral systems predominate, surveillance systems are inadequate or nonexistent; 3) transporting suitable material from the field to a suitably equipped laboratory to confirm and type the occurrence of FMDV is logistically complicated and expensive; and 4) very few laboratories in Africa have the means to diagnose FMD adequately.

Nevertheless, there have been successes in parts of Africa – for example, Botswana, Namibia, and South Africa have FMD-free zones certified by the OIE and protected by a vaccination zone surrounding high-risk areas. Furthermore, Morocco, Algeria, and Tunisia have OIE-endorsed control plans. These successes indicate that FMD control is possible in Africa and can be extended.

More effective control of FMD in Africa can be achieved if primary endemic areas and factors that influence disease dissemination are known to assist in the design of appropriate control strategies. This will assist the design of targeted, area-wide or ecosystem-based disease control strategies, which may include more effective movement control, improve decisions on appropriate vaccine strains, and improved vaccines and point-of-disease diagnostic assays. Various new and exciting technologies to improve vaccinology, vaccine matching, and diagnostic tests have seen the light. However, for these technologies to make an impact in the control of FMD in Africa, they should be tailored to the unique environment and needs of Africa.

Many countries are embarking on the stepwise PCP approach to improve their FMD control capacity in a sustainable manner. Different regions in sub-Saharan Africa are at different developmental stages of control and thus face unique challenges and priorities in terms of FMD control. Progressive control of FMD may be achieved if founded on sound epidemiological understanding of the disease that is ecosystem specific.

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Disclosure

The authors report no conflicts of interest in this work.

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