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Contribution à la connaissance de l'épidémiologie de la fièvre aphteuse au Niger

Contribution to the knowledge of the epidemiology of Foot and Mouth Disease in Niger

Bachir SOULEY KOUATO

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The pictures of the cover page were taken during FMD outbreaks in 2014

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Abstract

FMD is a severe, highly contagious viral disease affecting domestic and wild ruminants and pigs. FMD is endemic in Niger with potential impact on the national economy because of its negative effect on animal production. However, there is evidence that FMD is poorly investigated in Niger as the prevalence as well as the associated risk factors of the disease and serotypes circulating are not well known. These informations are of key importance to implement appropriate and efficient prevention and control measures against FMD. Therefore, the research presented in this thesis aimed to contribute to a better understanding of the epidemiology of FMD in Niger.

Firstly, two prerequisites systematic review studies were performed on FMD risk factors modelling and molecular epidemiology of FMD in Africa respectively. The findings of the first systematic review showed that the most reported factors related to FMD were the uncontrolled animal movement and the mixing of animals around water and grazing points. Depending on the model used, the included articles in this review presented some limitations. The lack of reliable data especially from endemic settings to perform these epidemiological modelling studies was also highlighted. On the other hand, the second systematic review showed an increasing interest from African countries to conduct research on molecular epidemiology of FMD. The identification and molecular characterization studies of African FMDV strains showed the complexity of the genetic relationships between circulating strains as reflected by the diversity and transboundary mobility of FMDV in the continent.

Further, an original study to get insight in the economic impact and the spatiotemporal pattern of transmission of FMD outbreaks in Niger was performed based on the retrospective analysis of 9-year (2007-2015) outbreak data. This study revealed that FMD outbreaks occurred in all regions but affecting more the districts bordering neighbouring countries. The animal density was the important predictor variable of outbreaks occurrence. The seasonal trend of FMD outbreak occurrence was confirmed by this study with most outbreaks occurring during the cold and dry season and starting at the end of the rainy season. This study revealed that at outbreak level, the mean stochastic estimates were respectively 52.33 cattle affected by the disease and 4.33 cattle assumed to die from FMD. In this analysis, the cost of FMD consists of the cost due to the morbidity assumed to be the loss of milk production and the cost of mortality of young animals. Thereby, the average total cost of FMD at herd level was estimated at 732.72

euros. The cost of mortality of young bulls was the largest portion of the total cost, contributing to 41.55%, while costs related to heifer mortality and reduced milk production were respectively 35.36% and 23.09% of the total cost of FMD at outbreak level. To estimate the cost of vaccination at FMD outbreak level, one scenario was considered consisting in vaccinating each animal with 2 doses of vaccine allowing to estimate a cost of vaccination at FMD outbreak level at 315.27 euros on average at herd level. Consequently, the average ratio total costs of FMD/ cost of vaccination at outbreak level was estimated at 2.31.

The performed field outbreak study in southwestern Niger indicated that 70% (158/227) of the sera were positive for the presence of antibodies against FMDV through NSP ELISA. Multivariate logistic regression analysis revealed that only the herd composition (presence of both cattle and small ruminants) was significantly associated with FMDV seropositivity (P-value = 0.006). Among the NSP ELISA positive sera tested by LPBE, 86% (136/158) were positive for one or more serotypes (A, O, SAT 1 and SAT 2). Additionally, either as single or as multiple serological reactions, there was a clear dominance of serotype O followed by serotypes A and SAT1. Moreover, FMDV serotype O was isolated and characterised within the O/WEST AFRICA topotype. One of the FMDV isolates from Niger (O/NGR/4/2015) showed a close antigenic match with three FMDV serotype O reference vaccine strains. The phylogenetic results showed a strong relation amongst and between collected samples from Niger and the result revealed that these isolates are closely related to strains previously isolated in some West African countries including Benin, Togo and Ghana.

In conclusion, the results of the field outbreak study together with the spatiotemporal distribution of FMD outbreaks confirm the endemic nature of the disease in Niger. Furthermore, the molecular characterization highlights the complex transboundary nature of FMD in Africa through uncontrolled animal movement, cross bordering transhumance and live animal trade. The key messages for decision makers resulting from this thesis are the need for further countrywide comprehensive epidemiological research on the epidemiology of FMD and the launching of a strategic plan for disease control in Niger. Moreover, the major implication of this study is the requirement for integrated and regional FMD control strategies with the aim to more effectively prevent or control FMD in Africa.

Résumé

La fièvre aphteuse est une grave maladie virale et hautement contagieuse qui affecte les ruminants domestiques et sauvages et les porcs. La fièvre aphteuse est endémique au Niger avec potentiellement un impact sur l'économie nationale en raison de ses effets néfastes sur les productions animales. Cependant, il est évident que la fièvre aphteuse est très peu étudiée au Niger car la prévalence ainsi que les facteurs de risque associés à la maladie d'une part et d'autre part les sérotypes circulants ne sont pas bien connus. Or, ces informations sont d'une importance capitale pour mettre en œuvre des mesures de prévention et de lutte adaptées et efficaces contre la fièvre aphteuse. Par conséquent, les recherches présentées dans cette thèse visent à contribuer à une meilleure compréhension de l'épidémiologie de la fièvre aphteuse au Niger.

D'emblée, le besoin d'effectuer deux revues systématiques s'est fait sentir et ce, respectivement sur la modélisation des facteurs de risque de la fièvre aphteuse et l'épidémiologie moléculaire de la fièvre aphteuse en Afrique. Les résultats de la première revue systématique ont montré que les facteurs les plus rapportés qui sont liés à la fièvre aphteuse sont le mouvement non contrôlé des animaux et le mélange des troupeaux autour des points d'eau et des pâturages. Il a été rapporté certaines limites selon le modèle utilisé et décrit dans les articles inclus dans cette revue. Il a également été mis en évidence le manque de données fiables pour effectuer ces études de modélisation épidémiologique et particulièrement dans le contexte des pays endémiques. Par ailleurs, la deuxième revue systématique a montré un intérêt croissant des pays africains à mener des recherches sur l'épidémiologie moléculaire de la fièvre aphteuse. L'identification et les études de caractérisation moléculaire des souches africaines du virus de la fièvre aphteuse ont mis en évidence la complexité des relations génétiques entre les souches virales circulantes, se traduisant par la diversité et la mobilité transfrontière du virus de la fièvre aphteuse au sein du continent.

Une étude a ensuite été conduite et est basée sur une analyse rétrospective de neuf années (2007-2015) de données sur des foyers de fièvre aphteuse. L'objectif de cette étude était d'avoir un aperçu de l'impact économique mais aussi de connaitre les caractéristiques spatiotemporelles de transmission de la maladie au Niger. Ainsi, cette étude a révélé l'apparition des foyers de fièvre aphteuse dans toutes les régions, avec les départements frontaliers avec les pays voisins étant les plus affectés. Il s'est également avéré que la densité animale était la principale variable prédictive de l'apparition de ces foyers. En outre, la tendance

saisonnière de l'apparition des foyers de fièvre aphteuse a été confirmée par cette étude, la plupart des épidémies se produisant pendant la saison sèche et froide et débutant à la fin de la saison des pluies. Cette étude a révélé qu'à l'échelle du foyer, les estimations stochastiques moyennes étaient respectivement de 52,33 bovins affectés par la maladie et de 4,33 bovins supposés mourir de cette maladie. Pour cette analyse, le coût estimé de la fièvre aphteuse est composé du coût dû à la morbidité qui est ici représenté par la perte de production laitière et le coût de la mortalité des jeunes animaux. Ainsi, le coût total moyen de la fièvre aphteuse au niveau du troupeau était estimé à 732,72 euros. Le coût de la mortalité des jeunes taureaux contribuant à 41,55% du coût total de la maladie représentait la plus grande part de ce coût estimé de la fièvre aphteuse, tandis que les coûts liés à la mortalité des génisses et à la réduction de la production laitière étaient respectivement de 35,36% et 23,09% du coût total de la maladie. Pour estimer le coût de la vaccination au niveau d'un foyer de fièvre aphteuse, un seul scénario consistant à vacciner chaque animal avec 2 doses de vaccin, a été pris en compte. Ainsi, le coût de la vaccination à l'échelle d'un foyer a été estimé en moyenne à 315,27 euros. Par conséquent, le ratio moyen du coût total de la maladie /coût de la vaccination pour un foyer est estimé à 2.31.

Une étude sur des foyers de fièvre aphteuse survenus dans le Sud-Ouest du Niger a révélé que 70% (158/227) des sérums étaient positifs pour la présence d'anticorps viraux par la méthode NSP ELISA. L'analyse de régression logistique multivariée a révélé que seule la composition du troupeau (présence de bovins et de petits ruminants) était significativement associée à la séropositivité (valeur P = 0,006). Parmi les sérums positifs à la NSP ELISA et testés par LPBE, 86% (136/158) étaient positifs pour un ou plusieurs sérotypes (A, O, SAT 1 et SAT 2). En outre, pour les réactions sérologiques spécifiques (à un seul sérotype) ou multiples (plusieurs sérotypes), il y avait une nette prédominance du sérotype O suivi des sérotypes A et SAT 1. Par ailleurs, le sérotype O du virus de la fièvre aphteuse a été le seul isolé et dont les résultats de la caractérisation moléculaire indique qu'il appartient au topotype ouest Africain (WA : West Africa). Un des isolats de ce virus du sérotype O (O/NGR/4/2015) a montré une étroite relation antigénique avec trois souches de vaccin de référence du même sérotype. Les résultats phylogénétiques ont montré une forte relation génétique entre les souches virales isolées au Niger et par ailleurs, ces souces virales sont étroitement liées à des souches isolées précédemment dans certains pays ouest africains à savoir le Bénin, le Togo et le Ghana.

En conclusion, les résultats sérologiques obtenus sur le terrain ainsi que la distribution spatiotemporelle des foyers de fièvre aphteuse confirment la nature endémique de la maladie au Niger. En outre, les résultats de la caractérisation moléculaire mettent en évidence le caractère transfrontalier et complexe de la fièvre aphteuse en Afrique à travers le mouvement non contrôlé des animaux et la transhumance transfrontalière ainsi que le commerce du bétail sur pied. Il résulte de cette thèse, un vibrant appel adressé aux décideurs politiques concernant le besoin urgent de mener plus de recherches épidémiologiques de la fièvre aphteuse sur tout le territoire et le besoin d'élaboration et la mise en œuvre d'un plan stratégique de lutte contre la maladie au Niger. En outre, l'implication majeure de cette étude est la nécessité absolue de mettre en place une stratégie intégrée et régionale de lutte contre la fièvre aphteuse visant à prévenir ou à combattre plus efficacement la maladie en Afrique.

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Thank you all!

“It always seems impossible until it's done” Nelson Mandela

“Everything must be made as simple as possible. But not simpler.” Albert Einstein

List of Abbreviations

°C: Degree Celsius
BHK: Baby hamster kidney
BVI: Botswana Vaccine Institute
CART: Classification and regression tree analysis
CBPP: Contagious Bovine Pleuro Pneumonia
CFA: Communauté Financière d'Afrique
CFT: Complement fixation test
CPE: Cytopathic effect
EA: East Africa
ECOWAS: Economic Community of West African States
FAO: Food and Agriculture Organization
FAOSTAT: Food and Agriculture Organization Statistics Division
FMD: Foot-and-Mouth Disease
FMDV: Foot-and-Mouth Disease virus
g: gravitational force
GDP: Gross Domestic Product
GLM: Generalized linear models
IB-RS-2: Swine kidney epithelial cells
ICTV: International Committee on Taxonomy of Viruses
Ig : Immunoglobulin
INS : Institut National de la Statistique
Km^2: Square kilometre
KOH: Potassium hydroxide
LABOCEL : Laboratoire Central de l'Elevage
LFD: Lateral flow devices
LPBE: Liquid-phase blocking ELISA
MEL : Ministère de l'Elevage
ME-SA: Middle East-South Asia
Na OH: Sodium hydroxide
$Na_2Co_{3:}$ Sodium Carbonate
NGR: Niger
NSP ELISA: Non-Structural Protein Enzyme-Linked Immuno Sorbent Assay

nt: Nucleotide

OIE: World Organization for Animal Health, former Organisation Internationale des Epizooties

OPF: Oropharyngeal fluid

PCP: Progressive Control Pathway

PPR : Peste des Petits Ruminants

RNA: Ribonucleic acid

RT PCR: Reverse transcription polymerase chain reaction

s/n/y: substitutions per nucleotide site per year

SAT: Southern African Territories

SONERA : Société Nationale d'Exploitation des Ressources Animales du Niger

SP: Structural Proteins

SPCE: Solid-phase competition ELISA

SSA: Sub Saharan Africa

SVD: Swine vesicular disease

SVDV : Swine vesicular disease virus

$TCID_{50}$: Tissue Culture Infective Doses 50

UNDP: United Nations Development Programme

VNT: Virus Neutralisation Tests

VP: Viral Protein (major capsid protein)

WA: West Africa

WAAPP: West African Agricultural Productivity Program

WRLFMD: World Reference Laboratory for FMD

Content

GENERAL INTRODUCTION

General introduction

Niger is a Sahelian country in West Africa located between the longitude 0°16' and 16° East and the latitude 11°1' and 23°17' North. The country covers a surface area of 1,267,000 square kilometres (Km^2). The three fourths of the country are occupied by deserts. This makes it the world's twenty-second largest country and the largest country in West Africa. Niger borders Nigeria and Benin to the South, Burkina Faso and Mali to the West, Algeria and Libya to the North and Chad to the East **(Figure 1)**. The climate is characterized by a short rainy season from three to four months (from May-June to September), and a dry season from eight to nine months (from September-October to May-June). The country is usually divided into four ecological zones: the "Sahara" zone, the arid central zone (Sahelo-Saharan), the "Sahel" and the "Sudan" zone **(Figure 2)**.

Niger's economy is mainly based on agriculture and livestock **(Figure 3)**. Livestock in Niger is the main or the secondary activity of around 87% of the population, and it contributes to their financial resources as well as to their food security. Livestock production contributes up to 35% of the agricultural gross domestic product (GDP) and 12 % of the total GDP (INS, 2010). After uranium, livestock production is the second largest export product of the country, which is believed to have one of the largest livestock population in West Africa comprising approximately 10.3 million of cattle, 25.02 million of sheep and 27.88 million of goats (MEL, 2012). The livestock system in Niger could be classified into three systems: the pastoral system, including transhumance and nomadism; the agropastoral system; and the peri-urban system (Lhoste, 1984, Bernus and Boutrais, 1994). The peri-urban farming system mainly consists of dairy farms. Agropastoral farming is mostly practised by sedentary people, but transhumance is also common in this type of breeding. In the pastoral system, the main feature is animal mobility. Pastures generally correspond to areas unsuitable for crop production in the northern part of the country. Over the eras, pastoralists have developed some strategies adapted to the difficult climatic conditions of semi-arid environments including the scarcity of pastoral resources. One of the approaches is relative to the mobility of pastoralists with their herds (Benoit, 1998; Convers *et al.*, 2007; Leclerc and Sy, 2011). It takes three main forms: (1) transhumance, or cyclic seasonal mobility between an initial point or locality (within or outside the country so-called an "attachment point") (in the rainy season) and "host" terroirs during the dry season, this mobility is performed over long distances that can range from a dozen to several hundred kilometers; (2) nomadism, characterized by mobility without an attachment point for

the whole household; (3) migration, which involves the change of attachment land of the whole household, which may include short-term "test" movements (Turner, 1999). This pastoral livestock system has indeed some advantages. For instance, each transhumant herdsman has its own motivations that guide its choices for moving. The major reasons include the search for water, pastures or crop residues (after the rainy season). Another advantage of this mobility is the existence of markets for dairy products and opportunities for livestock trade (especially the small ruminants). There is also the mineral complementation of their animals on salted land. The example of "cure salée" of Ingall in the region of Agadez in Niger is an illustration of this practice at the end of the rainy season and which allow several herdsmen to naturally feed their animal with mineral salts found in ground deposit as well as water or grasses and plant. However, the pastoral system has also some disadvantages including the frequent and violent conflicts between herdsmen and crop-farmers, and the introduction and/or reintroduction of animal diseases in a given area or region through livestock movements (Abiola *et al.*, 2005).

Thereby, Nigerien[1] livestock production based on extensive grazing is continuously challenging with climatic vagaries, pastures scarcity, and sanitary constraints that set limits to its performance. The animal health constraints include, inter alia, the persistence and/or resurgence of transboundary diseases, including foot-and-mouth disease (FMD).

[1] Nigerien' is used here to mean 'pertaining to Niger' and should not be confused with 'Nigerian', i.e., 'pertaining to Nigeria'.

Figure 1: Administrative map of Niger (**Source:** http://www.nationsonline.org/oneworld/map/niger-political-map.htm)

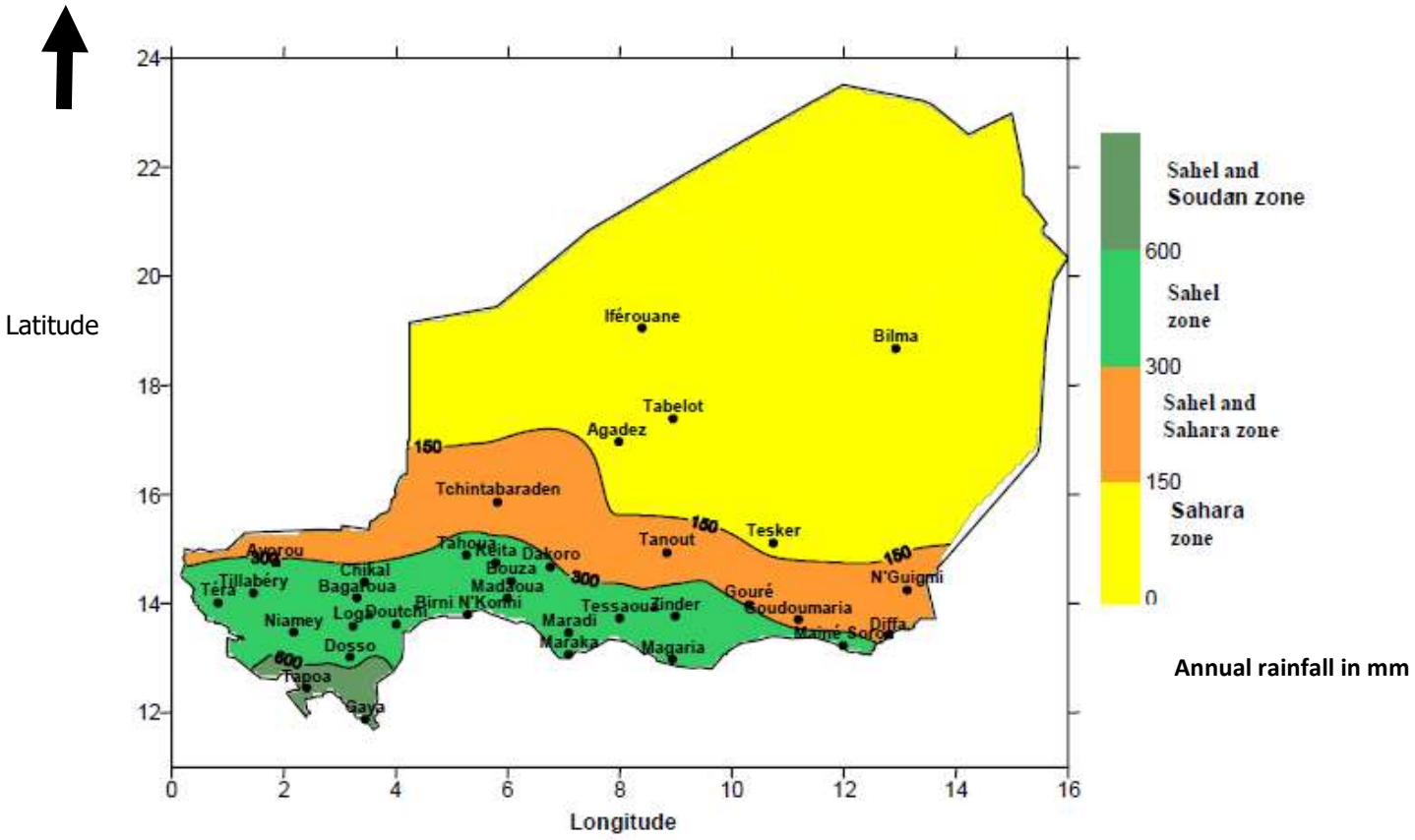

Figure 2: Climatic zones in Niger (Adapted from http://unfccc.int/resource/docs/napa/ner01e.pdf**)**

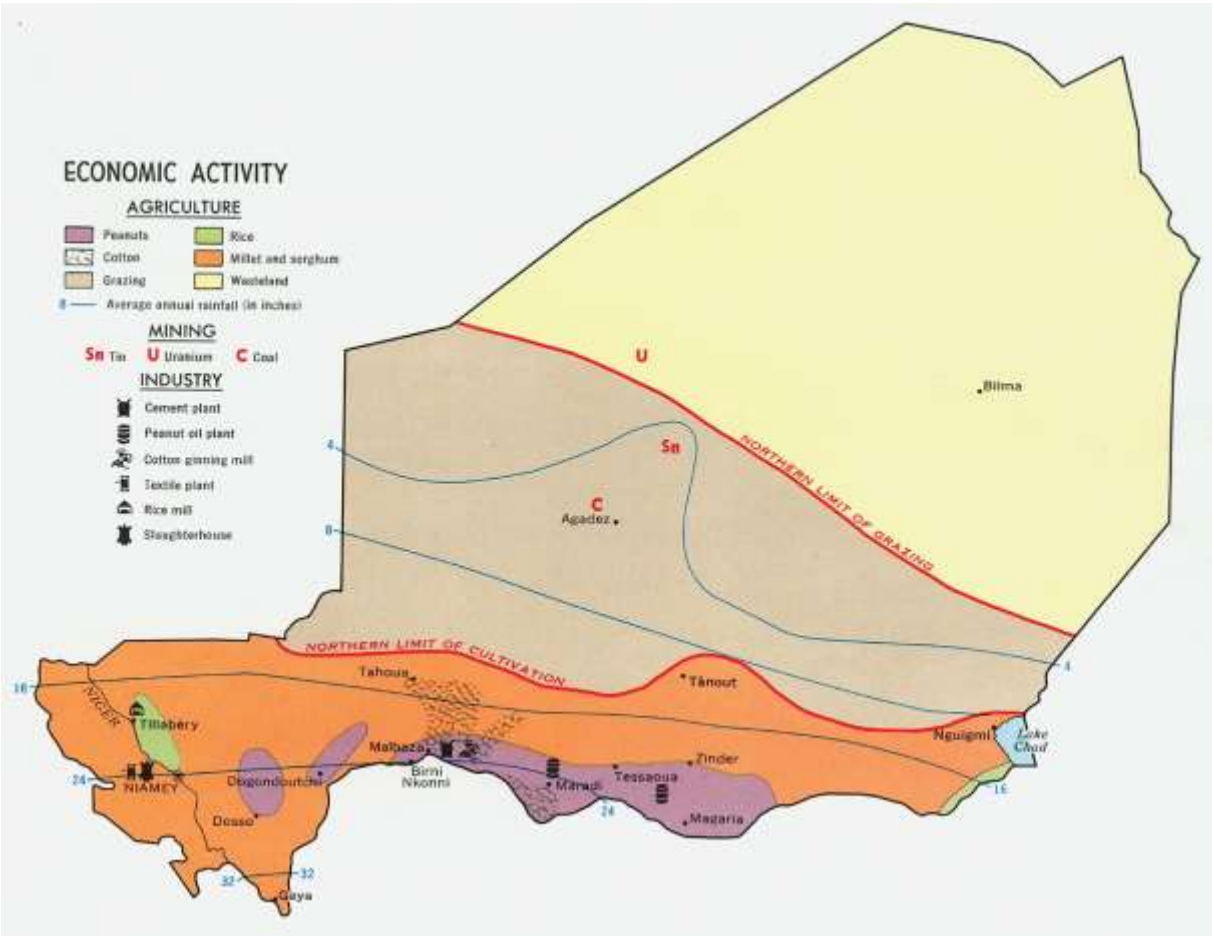

Figure 3: Spatial distribution of economic activity in Niger (Source: http://www.lib.utexas.edu/maps/niger.html)

FMD is a severe, highly contagious viral disease of livestock with significant economic impact (James & Rushton, 2002; Thompson *et al*., 2002). The disease affects domestic and wild ruminants and pigs. FMD is the most feared infectious animal disease owing to nearly 100% morbidity, rapid spread, severe decrease in livestock production, and mortality in young animals (Grubman & Baxt, 2004). Accordingly, FMD is a disease listed in the World Organization for Animal Health (OIE) Terrestrial Animal Health Code and the disease must be strictly reported to that organization. Moreover, FMD is the first disease for which the OIE established an official list of free countries and zones with or without vaccination. FMD remains widespread throughout the world, and is endemic particularly in Asia, the Middle East and Africa (OIE, 2016; WRLFMD, 2016). Indeed, FMD is endemic to most of sub-Saharan Africa (SSA), except in a few countries in southern Africa, where the disease is controlled by the separation of infected wildlife from susceptible livestock as well as by intensive vaccination. Historically, FMD has been reported in many West African countries. FMD virus (FMDV) was identified in Nigeria (1955), Burkina Faso (1964), Ghana (1958), Niger (1971), Côte d'Ivoire and Niger (1971), as well as in Senegal, Mauritania and Liberia (Habou, 1976). Due to the permeability of the borders and uncontrolled animal movements between countries, the existence of FMD in other West African countries cannot be excluded at that time. The identified FMDV serotypes were O, A, C, SAT1 and SAT2. However earlier in 1945, FMDV was already isolated in Niger and the virus which belonged to serotype C was typed by the Laboratoire Central de Recherches Vétérinaires of Maisons-Alfort in France (Pagot J, 1948). The occurrence of FMD outbreaks in Niger had important economic repercussions, notably in lucrative market access of live cattle but also in meat. About forty years ago, Niger was one of the largest meat exporters in West Africa through its company called "National Society of Export of Animal Resources" with French acronym "SONERA". The Niger exported meat to other African countries such as Ghana, Gabon, Benin, Togo, Libya, and even to the Caribbean. But since the multiple occurrence of FMD in Niger, the Libyan and Caribbean markets were closed to Niger, resulting in a significant slowdown of the company's activities (Habou, 1976).

Mainly due to the endemicity of the disease, and the fact that FMD does not normally cause high rate of mortality in adult animals as other animal epizootics do, FMD outbreaks were not perceived as important and consequently were not reported or further investigated to determine the causative serotypes. However, a number of countries within African continent realise at present that FMD is one of the transboundary diseases that should be controlled to

ensure economic stability and access to lucrative international export markets for animal and animal products. Furthermore, moving towards the global control of FMD has been considered as a priority for international donors. Therefore, interventions must fall within the framework of programmes developed by intercontinental organisations, such as the Food and Agriculture Organization of the United Nations (FAO) and the OIE, through the FAO/OIE Global Framework for the Progressive Control Pathway (PCP) of FMD and other transboundary animal diseases (Forman *et al*., 2009). Such interventions should specifically focus on thoroughly work including, epidemiological surveillance, communication, monitoring and evaluation, continuous strengthening of veterinary services and research activities.

Hence, an understanding of the epidemiology of the disease is critical for the implementation of efficient control programs and further eradication of the disease. For FMD, one of the important aspects of combating the disease is virus characterization, where the study of relationships between field isolates using reference and historical viruses is used to investigate the possible origins of the disease and to select suitable vaccine (Knowles & Samuel, 1998; Knowles & Samuel, 2003; Sahle *et al*., 2007). Unlike southern Africa and parts of central and East Africa, little is known about the FMD situation in West Africa. Although, recently, a few studies on FMD were conducted in that part of the continent (Ehizibolo *et al*., 2014; Fasina *et al*., 2013; Gorna *et al*., 2014; Sangare *et al*., 2001; Sangare *et al*., 2003; Sangare *et al*., 2004; Ularamu *et al*., 2016; WRLFMD, 2016). In Niger, despite the endemicity of FMD, the prevalence of the disease and serotypes circulating are not well known. Moreover, until at the time of this study, there are no scientific evidence on the spatiotemporal patterns of FMD occurrence as well as on the associated risk factors. Consequently, at present there is no possibility of preventing and controlling effectively the disease such as by vaccination. It is in this context that the West African Agricultural Productivity Program (WAAPP[2]) has selected FMD as one of the priority areas of research in Niger and has accordingly funded this thesis.

This thesis is structured in three main parts and is presented in seven chapters. It aims to improve the current knowledge on the epidemiological status of FMD in Niger. The introduction part includes two chapters. **Chapter 1** gives an overview on the disease, its etiological agent, clinical signs and pathology, epidemiology, and diagnosis. In addition,

[2] WAAPP is funded by World bank, globally, the aim of the program is to achieve agricultural growth and increased food production and availability in West Africa. Thirteen west African countries are included in this program.

prevention and control of FMD with focus on Sub Saharan Africa are presented in this chapter too. **Chapter 2** presents the objectives of the experimental part of the thesis whose research contributions are outlined in chapters 3, 4, 5 and 6. **Chapter 3** includes a review of risk models for FMD providing a synopsis of the strengths and weaknesses of these models and their relevance to FMD prevention policy, focusing on their use in African countries where the disease remains enzootic. In relation to the use of epidemiological modelling, a retrospective study was performed and reported in **chapter 4**. **Chapter 5** provides a systematic review of molecular epidemiology of FMD in Africa. It gives an overview of the distribution and diversity of FMDV, pointing out the need to develop more comprehensive surveillance and reporting systems for effective prevention and control of FMD in Africa with the respect of the PCP-FMD. In the respect of molecular epidemiology, an outbreak investigation and molecular characterization of FMD was conducted and described in **Chapter 6**. Finally, the last part includes **chapter 7** presenting a general discussion on the overall contribution of the thesis as well as the conclusion and recommendations that arise from this research work and the perspectives to be considered.

Part one: literature review

Chapter 1: Foot-and Mouth Disease: Etiological agent, clinical signs and pathology, Epidemiology, Diagnosis, Surveillance, prevention and control of FMD with focus on Africa

Part one: Literature review

Chapter 1: Foot-and Mouth Disease: Etiological agent, clinical signs and pathology, Epidemiology, Diagnosis, Surveillance, prevention and control of FMD with focus on Africa

1.1 Etiological agent of FMD

1.1.1 Brief History of foot-and-mouth disease virus

The earliest description of what was probably Foot-and-mouth disease (FMD) was proposed by Hieronymi Fracastorii in 1546. He described the disease, which occurred in Northern Italy in 1514, as being unusual and affecting only cattle. In 1780, in Southern Africa, Le Vaillant described in 1795 a disease in cattle which "*attacked the feet of oxen causing them to swell prodigiously and after producing suppuration, sometimes the hooves dropped off*". In 1897, Loeffler and Frosch proved that a filterable agent caused FMD (Brown, 2003). This was the first demonstration that animal disease was caused by a filterable agent and marked the beginning of the era of animal virology. This happened after Ivanovski had shown in 1892 that the agent of tobacco mosaic disease would pass through a bacteria-proof filter candle but before Beijerinck developed the concept of a filterable virus that he called *contagium vivum fluidum* (Bos, 2000; Mahy, 2004). For many years after its discovery, research on FMD virus (FMDV) was inhibited by the lack of a suitable experimental animal model to study the disease. Subsequently, Waldmann and Pape discovered in 1920 the sensitivity of guinea pigs to FMD. In 1922, a new progress was made when Vallée and Carrée demonstrated that there were different antigenic FMDV types (serotypes) suggesting the possibility of the same animal to be infected successively. They discovered 2 serotypes named O and A based on their origin, namely in a department in the North of France and in Germany respectively. In 1926, Waldmann and Trautwein discovered the third antigenic type which they called C. Thus, the three first serotypes became known, named by international agreement, Vallée O, Vallée A and Waldmann C and later simply O, A and C. Many atypical virus strains were later described, mainly from Africa, until in 1948 a sample submitted to the world reference laboratory for FMD (WRLFMD) from Bechuanaland (current Botswana) yielded a virus which in cross-protection tests in cattle and guinea pigs was found to be distinct from O, A and C. Subsequently a virus isolate from northern Rhodesia (equivalent in territorial terms to current Zambia) was identified as yet another distinct type. Retrospective testing of viruses isolated between 1931 and 1937

revealed isolates from southern Rhodesia which were close to the 1948 isolates from Bechuanaland (isolates from 1937) and northern Rhodesia (isolates from 1931) (Brooksby, 1958). An additional virus isolated in Southern Rhodesia in 1934 was found to be a third new type. These new types were called SAT (Southern African Territories) types 1, 2 and 3. The seventh serotype, designated Asia1, was first identified in the early 1950's when viruses were isolated from India in 1951 and 1952 (Dhanda *et al*., 1957) and Pakistan in 1954 (Brooksby & Rogers, 1957). Hence, at present 7 immunologically distinct serotypes of FMDV are known since there is no cross protection between these serotypes (Brooksby, 1982). Additionally, within each serotype several genetic and antigenic subtypes with different degrees of virulence exist (Fontaine *et al*., 1968; Kitching *et al*., 1989; Pereira, 1975; Rweyemamu, 1984; Vallée & Carrée, 1922; Toma, 2003).

The development of *in vitro* techniques for the growth of the virus have been crucial for the large-scale production of vaccines and for the accurate assay of virus infectivity. However, early work was already undertaken by Hecke and the Maitlands in the early 1930s, and was followed by the crucial demonstration by Frenkel in 1947 that large amounts of a virus could be produced in live tongue epithelium. This formed the basis for the vaccination programmes initiated in Europe in the 1950s (Brown, 2003).

1.1.2 Economic importance

FMD is on the earlier list A of infectious diseases of animals of the Office International des Epizooties (OIE), the disease has considerable economic consequences. This impact can be divided into two components: (1) direct losses due to reduced production, loss of draught power; growth retardation, abortion and (2) indirect losses caused by costs of FMD control, poor access to markets and limited use of improved production technologies. However, FMD consequences are not the same throughout the world (Knight-Jones & Rushton, 2013). In recent past, in many FMD endemic countries, especially in East, Central and West Africa, the importance of FMD was not considered with much attention by livestock owners and by the veterinary services since the acute phase of the infection last only a short time and mortality is low in adult animal but relatively high in young animals (James & Rushton, 2002; Perry *et al*., 2003; Perry & Rich, 2007). Additionally, production losses due directly to FMD include reduced milk production (Bayissa *et al*., 2011) affecting both the humans and calves that depend

on it. Hence, FMD production losses have a big impact on the world's poorest including Africa where more people are directly dependent on livestock and affect negatively food security (Barasa *et al*., 2008; Rufael *et al*., 2008). However, at the beginning of the last century the full economic importance of the disease received proper consideration in some part of the world. The negative impact of FMD can be properly illustrated by the example of the outbreak of serotype O (the PanAsian) strain in the United Kingdom (UK), a country which had been free for FMD since 1981. This devastating epidemic in 2001 spread to Ireland, France and the Netherlands where the UK alone were forced to slaughter about 4 million infected and contact animals. The cost of this epidemic in the UK was estimated to be more than US $29 billion (Knowles *et al*., 2001; Samuel & Knowles, 2001; Knight-Jones & Rushton, 2013). Although, in many Sub Saharan African countries, it is difficult to assess losses caused by FMD, especially the indirect losses, due to the complexity of the production systems (Domenech, 2011).

1.1.3 Taxonomy, Genome organization, Genetic and Antigenic variation of FMDV

1.1.3.1 Taxonomy of Picornaviruses

The Foot-and-mouth disease virus (FMDV) belongs to the picornavirus family, a diverse group of non-enveloped, positive sense, single stranded RNA (ssRNA) viruses. A picornavirus is a virus belonging to the family *Picornaviridae* within the order of *Picornavirales*. The family name *Picornaviridae*, is derived from '*pico*' referring to their small size and '*rna*' referring to their RNA genomes. Based on genome size and organization, virus replication strategy and sequence homologies, the family is currently divided into 31 genera (ICTV, 2016) **(Table 1)**. Viruses within this family cause diseases of medical (e.g. poliovirus, common cold virus, human hepatitis A virus) and agricultural importance, including FMDV which is the prototype of the *Aphthovirus* genus comprising beside FMDV, 3 other viruses namely Bovine Rhinitis A virus, Bovine Rhinitis B virus and Equine Rhinitis A virus. The genus name is derived from the Greek word *aphtha* meaning 'vesicles in the mouth' and refers to the vesicular lesions that they produce in cloven-hoofed animals (Melnick, 1983; Brooksby, 1982).

Table 1: Genus composition of the family *Picornaviridae* (Adapted from ICTV, 2016)

Genus	*Total number of species per genus*
Aphthovirus	4
Aquamavirus	1
Avihepatovirus	1
Avisivirus	1
Cardiovirus	3
Cosavirus	1
Dicipivirus	1
Enterovirus	12
Erbovirus	1
Gallivirus	1
Hepatovirus	1
Hunnivirus	1
Kobuvirus	3
Kunsagivirus	1
Limnipivirus	3
Megrivirus	1
Mischivirus	1
Mosavirus	1
Oscivirus	1
Parechovirus	2
Pasivirus	1
Passerivirus	1
Potamipivirus	1
Rosavirus	1
Sakobuvirus	1
Salivirus	1
Sapelovirus	3
Senecavirus	1
Sicinivirus	1
Teschovirus	1
Tremovirus	1

1.1.3.2 Morphology and Physicochemical properties of FMDV

In common with other picornaviruses, FMDV is non-enveloped and has a roughly spherical capsid, exhibiting icosahedral symmetry. The virion has a diameter of 22 -25 nm and it consists of approximately 70 per cent protein and 30 per cent RNA (Cooper *et al*., 1978; Melnick *et al*., 1974). It has a molecular mass of about 8.5×10^6 D with a sedimentation constant of 146S (Rueckert,1996). This characteristic sedimentation rate in sucrose gradients is widely

used in vaccine production to determine the mass of intact virions present in culture harvests because disintegration of virus particles results in loss of immunogenicity. The capsid consists of 60 capsomers each consisting of four protein (VP1, VP2, VP3 and VP4) (**Figure 4**). VP1 is the most antigenic protein, is involved in cell attachment and carries an immunological important G-H loop which is one of the most important neutralizing sites of the virus (Logan *et al*., 1993).

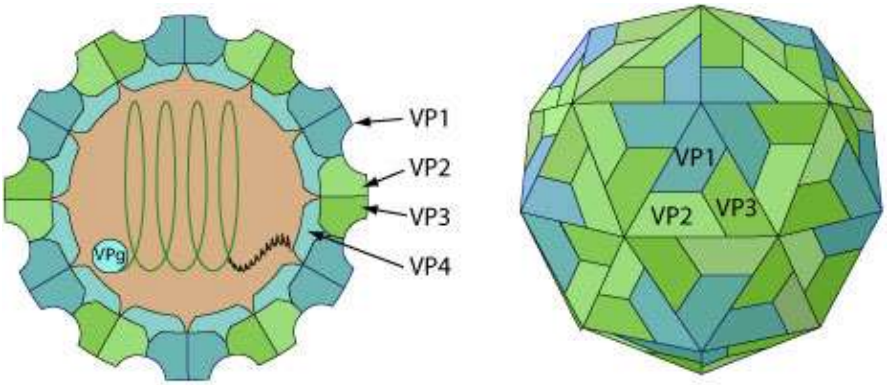

Figure 4: Diagram of the typical picornavirus icosahedral capsid (Adapted from Arias et al., 2010)

Source: Viral Zone, 2008 (Swiss Institute of Bioinformatics) Available at http://viralzone.expasy.org/all_by_species/33.html

Legend: Non-enveloped, spherical, about 30 nm in diameter, an icosahedral capsid surrounding the naked RNA genome. The capsid consists of a densely-packed icosahedral arrangement of 60 protomers, each consisting of 4 polypeptides, VP1, VP2, VP3 and VP4. VP4 is located on the internal side of the capsid.

FMDV exhibits a remarkable resistance to such bactericidal agents as the narcotic solvents (alcohol, ether, chloroform), or such antiseptics as phenol or cresol (Harada *et al*., 2015; Hong *et al*., 2015), although, two percent solutions of NaOH or KOH and 4% Na_2Co_3 are effective disinfectants for FMD contaminated objects (Harada *et al*., 2015; Hong *et al*., 2015). On the other hand, in acidic conditions the FMDV particles are disrupted into pentameric subunits composed of five copies each of the virus structural capsid proteins (VP1-3) with the liberation of the internal capsid protein (VP4) and the RNA (Hong *et al*., 2015; Newman *et al*., 1973). The most important difference between the physicochemical properties of viruses within the *Picornaviridae* family is their pH stability (Pereira, 1981). FMDV is stable between pH 7

and 9 at 4°C and -20°C. However, in milk and milk products, the virion is protected, and can survive at 70° C for 15 seconds and pH 4.6. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes (Mckercher & Callis, 1983). The size of droplet aerosol also plays a role in the survival or drying out of the virus. Indeed, a droplet aerosol size of 0.5 – 0.7 µm is optimal for longer survival of the virus in the air while smaller aerosols dry out. Moreover, in dry conditions the virus also survives longer in proteins, for example in epithelial fragments (Donaldson, 1987; Donaldson *et al*., 1987; Sellers *et al*., 1983).

1.1.3.3 Genome organisation

FMDV genome consists of a positive sense single stranded RNA molecule, of approximately 8500 nucleotides in length, and comprises a 5' non-coding region (NCR), a single large open reading frame (ORF) and a short 3' NCR (Belsham, 1993). The 5' NCR is exceptionally long (about 1300 nt) and has a virus encoded protein, 3B, called virus protein genome (VPg) attached to the 5' end (**Figure 5a**). The first portion of the 5' NCR is termed the S fragment and is approximately 400 nt long. This is followed by the poly C tract, a homopolymeric tract of predominantly cytidyl residues which is 150-250 nt long and which only occurs in cardioviruses and aphthoviruses within the *Picornaviridae* family (Rueckert & Wimmer, 1984). The last region of approximately 720 nt contains inverted repeats which are predicted to form pseudo-knots (Clarke *et al*., 1987). The internal ribosome entry site (IRES) which is immediately upstream of the first AUG initiation codon and is approximately 435 nt in length also occurs within this region (Belsham & Brangwyn, 1990; Ohlmann & Jackson, 1999). The main portion of the virus genome is a single very large open reading frame of 6996 nucleotides encoding a polyprotein of 2332 amino acids (for serotype O) (Forss *et al*., 1984). Four polyproteins (L1, P1, P2 and P3) are translated and processed into the different structural and non-structural proteins by viral encoded proteases (L^{pro}, 2A, oligopeptide and $3C^{pro}$) (Rueckert, 1996) (**Figure 5b**). The L protein represents the leader protein, where two initiations sites (AUG codons) have been identified in FMDV, namely Lab and Lb (Burroughs *et al*., 1984; Sangar *et al*., 1988). The P1 gene product is the precursor of the capsid proteins 1A, 1B, 1C and 1D (also known as VP4, VP2, VP3 and VP1 respectively) (**Figure 5b**). Firstly, the intermediate P1 precursor is processed with the help of viral proteinase $3C^{pro}$ to produce VP0, VP1 and VP3 where the products combine to form empty capsid particles. The mature virion is produced after the encapsidation of the virion RNA which is accompanied by the cleavage of VP0 to VP2 and VP4. VP1, VP2 and VP3 are exposed on the capsid surface (Acharya *et al*.,

1989). The P2 (2A, 2B, 2C) and P3 (3A, 3B, 3C, 3D) regions encode for non-structural proteins that are involved in viral RNA replication and protein processing (Belsham, 1993).

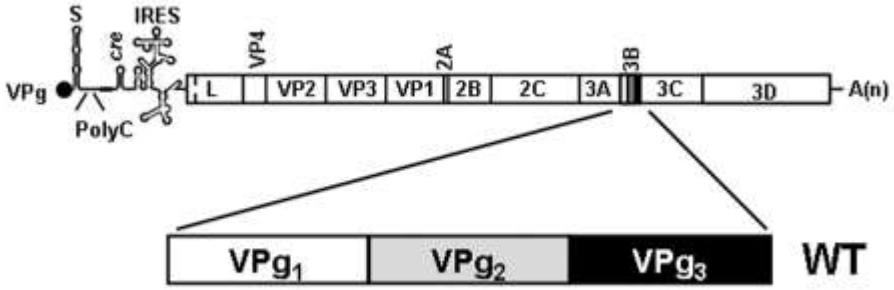

Figure 5a: Schematic representation of the FMDV genome (Adapted from Arias et al., 2010)

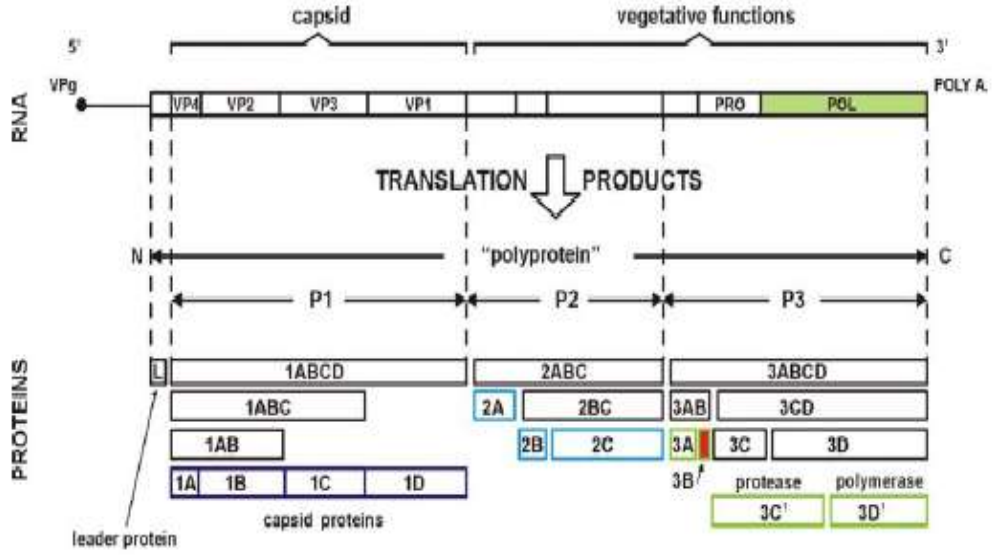

Figure 5b: Diagram of general structure of picornavirus with cleavage sites of the polyprotein (Adapted from Rueckert & Wimmer, 1984)

1.1.3.4 Genetic variation

1.1.3.4.1 Mutations

As mentioned above, FMDV exists in seven distinct serotypes which can be further subdivided into a great number of subtypes. This diversity is expressed mainly in the structural genes leading to more than 30% amino acid exchanges in the capsid proteins between serotypes, whereas the non-structural proteins differ by 2–7% (Domingo *et al*., 2003). The viruses are subjected to a high genetic drift with a mutation rate of up to 3% base exchanges per year in the structural genes (Beck & Strohmaier, 1987; Beck, 1988). Due to the absence of proofreading-repair activity by the viral replicase (lack of replication error checking mechanisms), FMDV RNA genome replication is highly error-prone (Holland *et al*., 1982). The high mutation rates result in populations that consist of genetically related but non-identical viruses known as quasispecies. Studies revealed that the rates of mutations of the European serotype FMDV RNA genome can reach 10^{-2} substitutions per nucleotide site per year (s/n/y) (Gebauer *et al*., 1988). Similar studies conducted on SAT 1 and SAT 2 FMDV have estimated nucleotide changes of 1.64 % and 1.54 %, respectively per year for the VP1 gene (Vosloo *et al*., 1996). Moreover, it was estimated that a mutation rate of up to $10^{-8} – 10^{-9}$ nucleotide substitution per year during an epizootiological cycle of FMDV can occur (Domingo *et al*., 1990). Several *in vivo* experiments report the generation of highly variable FMD viruses from single animals during infection studies. These observations may have been influenced by molecular host factors and/or selective pressures indirectly incurred from laboratories methodologies (Carrillo *et al*., 1998; Martinez *et al*., 1988). Recently, a study conducted during the UK 2001 epidemic demonstrated that nucleotide changes occur throughout the genome at a rate of 2.26×10^{-5} nucleotide substitutions per site per day. Hence, data obtained from outbreaks like the 2001 epidemic support the experimental observations, demonstrating the role of host-related selective pressures on the variability and evolution of FMDV (Cottam *et al*., 2006). Comparative genomics studies using full-length sequences representative of all seven serotypes have identified highly conserved genomic regions, indicating functional constraints for variability as well as undefined motifs with likely biological significance (Carrillo *et al*., 2005). At least 64% of all nt sites within the FMDV genome are susceptible to substitution, including compensatory substitutions. It is important to clarify that most of the "variant" or substitutable residues within the FMDV genome mutate in response to detrimental effects produced by mutations elsewhere in the genome (Carrillo, 2012). Therefore, new variants of FMDV are

continuously arising after each replication cycle. The generation of new variants is considered as one of the major problems in the control of FMD by vaccination.

1.1.3.4.2 Recombination

Recombination is another important process driving viral biology and evolution. In RNA viruses, recombination involves the exchange of genetic material between two non-segmented RNA genomes resulting from polymerase 'jumping' during RNA synthesis. Consequently, the generation of new antigenic variants may escape immune pressure (King *et al*., 1982). Mutations through recombination were first reported in picornaviruses following the replication of a mixture of mutants in the same cell monolayer (Domingo *et al*., 2012; Hirst, 1962). Since then, it has been shown that genetic recombination occurs between viruses of the same serotype (King *et al*., 1985; Pringle, 1965) as well as between serotypes (Chitray *et al*., 2014; Haydon *et al*., 2001). For example, recombination has been demonstrated between serotypes O and C (Krebs & Marquardt, 1992), and relatively recent reports document the occurrence of inter-serotypic recombination between serotypes A and Asia 1, resulting in altered antigenic characteristics (Jamal *et al*., 2011). Intratypic recombination occurs more frequently than intertypic recombination and it appears that recombination events in FMDV occur more readily in the 3' half of the genome, than in the capsid genes of FMDV (Domingo *et al*., 1995; King *et al*., 1985). It was also shown that recombination can involve single or multiple crossover events when two viruses of the same serotype co-infect cell cultures (King *et al*., 1982). Although recombination is not frequent in most RNA viruses, for FMDV, this phenomenon poses a real threat when attenuated vaccines are used, as reversion to virulence following natural infection of a vaccinated individual is likely given the high recombination frequency in FMDV.

1.1.3.5 Antigenic variation

The concept of antigenic variation derived from the observation of Vallée & Carré in 1922 that an animal that has recovered from FMDV infection can be re-infected and develops clinical signs. The observed genetic variation in the FMD viral genome is the result of a viral evolution process including the replication of viral RNA that is error-prone due to the absence of proofreading in the 3D-encoded RNA-dependent RNA polymerase (Domingo *et al*., 1990). Hence, antigenic variation can be caused by nucleotide mutations or recombination in the RNA

viral genome. One of the consequences of genetic variation through mutation and recombination is that new antigenic variants are constantly being generated as mentioned above. Apart from the non-existence of cross-protection between the 7 FMDV serotypes (Brooksby, 1982) one of the worrying implications of antigenic variation is the fact that vaccination with one antigenic variant of a serotype does not necessarily protect an animal when challenged with a different virus of the same serotype (Cartwright *et al*., 1982). Among the capsid proteins, VP1 is the most antigenic one and carries the domain mainly responsible for antigenic heterogeneity and cell-virus interaction. The contribution of capsid proteins other than VP1 to the antigenicity of FMDV was demonstrated by many researchers (Barnett *et al*., 1989; Baxt *et al*., 1989; Meyer *et al*., 1997; Meyer *et al*., 1994; Parry *et al*., 1989). These independent antigenic sites were identified on the VP2 and VP3 genes. For example, the B-C loop (VP2) was found in serotype A, O and Asia1 (Marquardt *et al*., 2000; Saiz *et al*., 1991). However, serological studies and observation in the degree of virulence of the virus in recovered animals have shown that there are significant differences between strains within each serotype (subtypes) (Brooksby, 1982; Grubman & Mason, 2002).

Progress made in the understanding of the genetic differences underlying observed antigenic variation, has played a major role in the epidemiology of FMD. Nowadays, nucleotide sequencing is routinely used to identify the genetic relationships between different isolates and historical strains. However, co-circulation of different types of FMDV is a reality in most parts of the endemic regions which represents a serious complication in the epidemiology of FMDV (Ayelet *et al*., 2009; Balinda *et al*., 2010; Ludi *et al*., 2016; Vosloo *et al*., 2002a; Wekesa *et al*., 2015a). Therefore, considering the continual antigenic drift in enzootic situation, vaccine strains selection should be implemented with considerable attention.

1.1.4 Pathogenesis

The pathogenesis of FMD is complex and there is at present many gaps in the level of understanding of this phenomenon (Arzt *et al*., 2011a; Arzt *et al*., 2011b). The main route of infection of FMDV in cloven-hoofed animals including ruminants is through the inhalation of droplets, but ingestion of infected feed, inoculation with contaminated vaccines, insemination with contaminated semen, and contact with contaminating clothing, veterinary instruments, etc. can produce FMDV infection (Arzt *et al*., 2011a; Arzt *et al*., 2011b; Arzt *et al*., 2014). However,

recent experimental studies have confirmed some aspects of conventional wisdom by demonstrating that pigs are more susceptible to FMDV infection via exposure of the upper gastrointestinal tract (oropharynx) than through inhalation of virus (Stenfeldt *et al*., 2016a). Three basic phases of FMD pathogenesis *in vivo* are distinguished: (i) pre-viraemia characterized by infection and replication at the primary replication site(s), (ii) sustained viraemia with generalization and vesiculation at secondary infection sites and (iii) post-viremia/convalescence including resolution of clinical disease that may result in long-term persistent infection.

In cattle, the tissues most consistently infected during the pre-viraemic phase of the disease are the epithelia of the naso-pharynx and larynx (Arzt *et al*., 2011b). It is therefore likely that this is the primary replication site in ruminants. There is a complex relationship between the tissues of the naso-pharynx and FMDV because not only does initial infection of ruminants take place there but the naso-pharynx is also the site of viral persistence in chronically infected animals (so-called carriers) (Stenfeldt *et al*., 2016b; Parthiban *et al*., 2015; Pacheco *et al*., 2015). Indeed, more than 50% of ruminants that recover from illness and those that are vaccinated and have been exposed to virus can carry virus particles in the naso-pharyngeal region up to 3.5 years in cattle, 9 months in sheep, and more than 5 years in African buffalo (Thomson, 1996).

Vesicle formation, cell lysis and significant inflammation occur at secondary replication sites (oral mucosa, skin of the horn-hoof junction & skin of the teats) but not in the epithelium of the primary replication site. The cells which support viral replication are located in the basal layer of naso-pharyngeal epithelium. However, the mechanism by which viral replication occurs in the naso-pharyngeal epithelium without causing cell lysis is unknown; nor is there an explanation as to why virus can be readily cultured from pharyngeal scrapings (obtained using probing cups) that, in recently infected animals, may contain high levels of antibody (mainly IgA) directed against the infecting virus (Arzt *et al*., 2011b; Stenfeldt *et al*., 2015). In pigs, delayed clearance of viral RNA from pharyngeal and lymphoid tissues has been observed but that has not been shown for infectious virus (Arzt *et al*., 2011a). It is currently concluded that persistent infection of pigs does not occur or at least is not epidemiologically important (Sutmoller & Casas, 2002).

One or two days before the onset of clinical signs, cattle and pigs develop viraemia which may endure for up to 3 days. In summary, at the viraemia stage, FMDV is distributed throughout the body, to reach the best sites of multiplication sites such as the epithelium of oro-

pharynx, oral cavity, feet, the udder and heart (Burrows *et al*., 1981; Zhang & Alexandersen, 2004; Arzt *et al*., 2010). Virus may also accumulate in the spleen, liver, adrenals, myocardium, pancreas, thyroid and mammary glands. In mammary tissue and myocardium, however, viral replication occurs in secretory epithelial cells of the alveoli and myocytes respectively, resulting in clear microscopic lesions. Development of characteristic vesicular lesions in FMD is dependent on persistent local irritation or friction. In transplantation studies in guinea pigs it was shown that epithelium from predilection sites grafted to other body areas lost that predilection and vice versa *(Platt, 1960).* This explains why the mouth, feet and teats are predilection sites for the development of lesions and why pigs often develop lesions on the dorsum of the snout, because of "snuffling".

Viral excretion starts about 24 hours prior to the onset of clinical disease and continues for several days. The acute phase of the disease lasts about one week and viraemia usually declines gradually coinciding with the appearance of strong humoral responses (Murphy *et al*., 1999). Recovered cattle produce neutralizing antibodies and can resist to re-infection by the same subtype of virus for up to one year. In various parts of the world including South America, East Africa and India/Pakistan, a heat-intolerance syndrome (sometimes referred to as 'hairy panters') has been associated with previous infection or 'chronic FMD', with a putative endocrine-related pathogenesis. Although, there is still limited information available on this syndrome, Arzt *et al*., (2011a) have indicated in their review that the extent of the syndrome's association with FMD remains speculative.

1.2 Clinical signs and pathology

1.2.1 Clinical signs

The incubation period of an infectious disease is defined as the time interval between exposure to an infective dose and first appearance of clinical signs (OIE, 2016). When susceptible animals are in contact with clinically infected animals, clinical signs usually develop in 3 to 5 day (Kitching & Hughes, 2002; Kitching, 2002). However, the incubation period of FMD is variable and depends on the host (age, breed, species and degree of immunity), environment, route of exposure, exposure dose, husbandry conditions and virus strain. Hence, it was estimated that after infection with FMDV, the average incubation period for sheep and goats is 3 to 8 days, at least 2 or more days for pigs, and 2 to 14 days in cattle

(Gailiunas & Cottral, 1966; Grubman & Baxt, 2004; Hugh-Jones & Tinline, 1976). The incubation period can be as short as 18 hours for host-adapted strains in pigs, especially under intense direct contact (Kitching & Alexandersen, 2002). The signs can range from a mild or unapparent disease in sheep or goats to a severe one occurring in cattle or pigs (OIE, 2016).

In cattle, following an initial pyrexia around 40°C, lasting one or two days, a variable number of vesicles develop on the tongue, hard palate, dental pad, lips, gums, muzzle, coronary band and interdigital space (Brooksby, 1982; Kitching, 2002; Woodbury, 1995). However, mouth lesions are less common and less pronounced in other species such as sheep and pigs. Vesicles may also be seen on the teats, particularly of lactating cows. Young calves may die before the appearance of vesicles because of the predilection of the virus to invade and destroy cells of the developing heart muscle (Kitching, 2002). Once infection is established within cattle herds, morbidity can approach 100% (Salt *et al*., 1996; Woodbury, 1995). A chronic panting syndrome characterized by dyspnoea, anaemia, hair overgrowth, and lack of heat tolerance has been reported as a sequela in cattle (Kitching, 2002). Additionally, it has been shown that in cattle, pregnant cows may abort (Radostits *et al*., 2006).

In sheep and goats, if the clinical signs occur, it tends to be very mild, and may include dullness, fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Commonly in sheep and other small ruminant lesions occur where (usually on the dental pad) they may be difficult to detect (Coetzer *et al*., 1994; Geering, 1967). Mild lameness may be the only sign. In lame animals, there may be vesicles or erosion on the coronary band or in the interdigital space. Infected nursing lambs may die without showing any clinical sign (Kitching & Hughes, 2002). Abortion may result from infection with FMDV and is thought to occur more frequently in sheep than other species (Arzt *et al*., 2011a).

Infected pigs initially show mild signs of lameness, blanching of the skin around the coronary bands and may develop a fever of up to 42°C but most often, this is in the range of 39°C to 40°C (Kitching & Alexandersen, 2002). The fever is most often associated with anorexia, reluctance to move, and squeal when forced to move. These signs are followed by vesicles on the coronary band, vesicles on the heals, vesicles in the interdigital space (foot involvement is usually severe), and vesicles on the snout. Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle and tend to be a "dry"-

type lesion; there is no drooling; sows may abort; and piglets may die without showing any clinical sign (Coetzer *et al*., 1994; Kitching & Alexandersen, 2002; Radostits *et al*., 2006).

1.2.2 Pathology

FMDV replicates at the site of entry, either in mucosa and lymphoid tissue of the upper respiratory tract or in the dermal and subdermal tissue of a skin abrasion (Kitching, 1992). The virus enters the blood circulation as free virus or associated with mononuclear cells and is distributed around the body to glandular tissue and predilection sites in the stratum spinosum, where secondary replication occurs. The cells of the stratum spinosum undergo ballooning degeneration and as the cells rupture and oedema fluid accumulates, vesicles develop which coalesce to form the aphthae and bullae that characterise FMD (Kitching, 1992). The lesions on the dental pad and tongue appear as reddened areas and progress within a few hours into vesicles. The vesicles are easily ruptured within 24 hours leaving a raw surface and healing occurs within one to two weeks of rupture. Lesions at interdigital areas occur and animals can lose their hooves in severe cases (Donaldson *et al*., 1984; Geering, 1967). There has also been supportive evidence that FMD virus replicates in the bovine mammary gland and mastitis may occur due to secondary bacterial infection. Moreover, histological studies have revealed the presence of clumps of necrotic secretory epithelial cells in the mammary gland alveolar tissue. A week after the onset of the disease in cattle, an increase in the number of alveoli containing necrotic cells, and luminal exocytosis of all alveoli occurs with concomitant increase in non-secretory areas (Blackwell *et al*., 1983; Kitching, 1992). In young animals, the virus invades the cells of the myocardium and macroscopic grey areas may be observed, particularly in the wall of the left ventricle, which appears striped (tiger heart). Cells of the skeletal muscle may also undergo hyaline degeneration (Blackwell *et al*., 1983).

1.3 Epidemiology

Considering the following definition of epidemiology as “study of the frequency and distribution of diseases over time and space, and the role of factors that determine this frequency and distribution within a population at risk” (adapted from Toma *et al*., 1996), in this section devoted to the epidemiology of FMD, an overview will be given of susceptible hosts, source of infection and mode of transmission, global distribution, serotype diversity and their distribution in Africa. In addition, two important questions related to FMDV transmission will be tentatively

clarified. These questions are: (i) what are carriers and how do they contribute to FMDV transmission? and (ii) what is the role of wildlife in FMDV? Lastly, in this section, an overview of epidemiological modelling and statistics used in the thesis, and molecular epidemiology will be briefly presented.

1.3.1 Susceptible hosts

FMDV has a wide host range and can affects over 70 species of both domestic and wild cloven-hoofed animals. Although, not all FMDV have the same host range (Saiz *et al*., 2002) the most sensitive species belong to the mammalian order of *Artiodactyls.* Of the domesticated species, cattle, pigs, sheep, goats and water buffalo are susceptible to FMD. The Bactrian camel (two-humped camel) is susceptible to FMD and develops severe lesions, while the dromedary camel (one-humped camel) is apparently resistant to infection. Lamas and alpacas have a high natural resistance to infection. Some will develop mild clinical signs following direct contact with infected cattle, but will not transmit FMD to other camelids under field conditions. Horses are not cloven hoofed and are therefore resistant. Similarly, many species of wildlife, such as African buffalo (*Syncerus caffer*), bison (*Bison spp*.), moose (*Alces alces*), chamois (*Rupicapra rupicapra*), giraffe (*Giraffa camelopardalis*), wildebeest (*Connochaetes gnou*), blackbuck (*Antilopa cervicapra*), warthogs (*Phacochoerus aethiopicus*), kudu (*Tragelaphus strepsicornis*), impala (*Aepyceros melampus*), and several species of deer, antelopes and gazelles may become infected with FMDV. Several clinical cases have been reported in captive Asian elephants (*Elephas maximus*), but there are few reports of FMDV in African elephants (*Loxodonta africana*), and the latter species is not considered susceptible under natural conditions in southern Africa (Anderson *et al*., 1993; Ayebazibwe *et al*., 2010; Bronsvoort *et al*., 2008; Bruckner *et al*., 2002; Thomson, 1995; Thomson *et al*., 2003; Thomson *et al*., 2013; Vosloo *et al*., 1996; Vosloo *et al*., 2002; Ward *et al*., 2007; Weaver *et al*., 2013). The receptivity of hippopotamus (*Hippopotamus amphibious*) to FMDV has not yet been reported through seroprevalence in wildlife species (Di Nardo *et al.*, 2015; Thomson *et al.*, 2003).

FMD is not a zoonosis, and only a few possible cases of infection of humans have been described (Bauer, 1997; Berrios, 2007; Capella, 2001; Simmons & Feldman, 2001) and where infection of humans with FMDV does occur the results have only mild and transient consequences (Bauer, 1997). Therefore, human infection does not appear to have any

significant role in the natural epidemiology of FMD. However, people often play a significant role in passive transfer of the virus from infected animals or contaminated surfaces to susceptible animals, and may even passively carry the virus in the respiratory tract for a day or more (Sellers *et al*., 1970), and this is important to take into consideration in control programmes and in biosecurity measures (Alexandersen & Mowat, 2005).

Experimentally, other species, including mice, rats, guinea pigs, rabbits, embryonating chicken eggs, and chickens, may be infected, but this often requires artificial transmission of the virus, and infection of these species has not been implicated in significant spread of FMD (Mahy, 2004).

The susceptibility of cloven-hoofed animals varies with animal species and strain of the virus. The disease is considerably less obvious or sub-clinical in sheep and goats indigenous to Africa and Asia, where FMD is endemic while cattle appeared to be more susceptible followed by pigs (Alexandersen *et al*., 2002b; Kitching & Hughes, 2002; Kitching, 2002; Kitching & Alexandersen, 2002). Among wildlife, the disease can be severe or subclinical in impala making this animal a possible transmission route of FMD virus from buffalo to cattle (Bastos *et al*., 2000). Experimental infection of warthog *(Phacochoerus aethiopicus)* and bush pig *(Patomachoerus porcus)* with SAT 2 viruses showed severe clinical signs of infection and transmission to in-contact animals (Thomson *et al*., 2003). However, these animals do not excrete virus to the levels of domestic pigs, and are not believed to play an important role in the epidemiology of FMD in Africa.

1.3.2 Source of infection and mode of transmission

Foot and mouth disease is very contagious because a small dose of the virus is infectious and several routes of FMD virus infection and excretion have been reported. The most common method of spread of FMDV is by contact between an infected and a susceptible animal (Kitching *et al*., 2005a). In densely populated areas the disease may spread extremely rapidly because of the high level of challenges from infected animals (Boender *et al*., 2007). Conversely, disease spread in extensive grazing areas in hotter climates can be more insidious. The movement of infected animals (including transhumance or nomadic systems) is considered to be the most important factor in the spread of FMDV (Bronsvoort *et al*., 2003; Bronsvoort *et*

al., 2004; Di Nardo *et al*., 2011) particularly with animals showing discrete or no clinical signs of disease (Barnett *et al*., 1989; Charleston *et al*., 2011; Mansley *et al*., 2003).

FMDV can also be transmitted indirectly by a variety of inanimate objects including animal food staff, beddings, farm equipment, livestock holding areas, transport vehicles that have been contaminated with acutely infected animal excretions and secretions such as saliva, milk, faeces and urine (Brooksby, 1982; Grubman & Baxt, 2004; Woodbury, 1995). Evidence was provided that the movement of infective raw milk can play an important part in the spread of FMD during outbreaks. Of considerable epidemiological importance is the fact that cattle, and probably other milking animals, such as goats and sheep, can excrete the virus in their milk for several days before the clinical signs of disease become apparent (Donaldson, 1997). The released viruses can also survive in dry blood and defragmented epithelium in the environment for varying periods of time depending on the weather condition. Immediate freezing of carcasses after dressing enhances preservation of live infectious virus and outbreaks across international borders have been ascribed to this manner through meat trading. Indeed, the source of FMD outbreaks occurred in 1967/68 in UK was attributed to infected sheep meat imported from Argentina (Leforban & Gerbier, 2002). Transmission of FMDV can also occur via viruses escaping from research and vaccine production centres (Cottam *et al*., 2008) and the semen of infected bull can be a source of infection by artificial insemination (Radostits *et al*., 2006). Personnel handling infected animals can be contaminated on hands, clothes or in nasal passages with live FMD virus and mechanically carry virus to susceptible animals by direct contact (Kitching *et al*., 2007). A person in contact with infected animals can serve as a source of infection for 24 h post infection (Kitching *et al*., 2007). It has been shown that similarly to man, pets such as dogs, cats and birds can transmit the disease mechanically (Radostits *et al*., 2006; Woodbury, 1995).

On the other hand, an important mode of transmission of FMDV is via respiratory aerosols since the virus can replicate mainly in the respiratory tract of animals and a large amount of the virus particles are excreted from this area although the virus may occur in all the secretions and excretions of infected animals during the acute phase of infection (Geering *et al*., 1995; Kitching *et al*., 2007; Woodbury, 1995). Indeed, transmission of FMDV by aerosol spread can occur over considerable distances, especially in the temperate regions (Garner & Cannon, 1995). Cattle and sheep infected with FMDV serotype O can excrete up to $10^{4.3}$ infectious virus units/day as an aerosol, while pigs can generate large amounts of aerosolized virus estimated at

$10^{6.1}$ infectious units/day. However, pigs are considerably less susceptible to aerosol infection, possibly requiring as much as 6000 Tissue Culture Infective Doses 50 ($TCID_{50}$) (Alexandersen & Donaldson, 2002; Alexandersen *et al*., 2002a) whilst cattle and sheep are particularly susceptible to infection by the aerosol route, requiring as little as 10 $TCID_{50}$ (Donaldson *et al*., 1983; Kitching *et al*., 2005a).

During the FMD outbreak that occurred in France and then in the UK in 1981, virus spread from France to the UK over 250 km (Sorensen *et al*., 2000). Moreover, FMDV spread over distances of 60 km over land, and some 250 km over sea, are also believed to have occurred (Garner & Cannon, 1995). At present, there are number of computer models which can predict the most likely wind-borne spread of the virus from infected herds and allow the examination of a variety of control strategies (Backer *et al*., 2012; Doran & Laffan, 2005; Halasa & Boklund, 2014; Highfield *et al*., 2008; Howey *et al*., 2012; Keeling *et al*., 2001; Kitching *et al*., 2005b; Lawson *et al*., 2011; Rautureau *et al*., 2012). However, aerosol transmission is less effective in hot, dry environmental conditions, particularly in Sub-Saharan Africa (Alexandersen *et al*., 2002a; Hutber & Kitching, 2000).

Additionally, sexual transmission could be a significant route of spread for the SAT type viruses in African buffalo populations (Bastos *et al*., 1999).

1.3.3 Role of carriers in the epidemiology of the disease

FMDV carrier animals are defined as those from which the virus can be isolated in oropharyngeal fluid (OPF) samples more than 28 days after infection (Moonen & Schrijver, 2000; Salt *et al*., 1996; Sutmoller *et al*., 1968; Sutmoller & McVicar, 1972; Sutmoller & Casas, 2002). Persistent infection can occur either after a clinical or a subclinical FMD infection, and occurs in vaccinated animals as well as in non-vaccinated animals (Doel *et al*., 1994; Moonen & Schrijver, 2000). The mechanism of persistence depends on the characteristics of the virus, such as type of replication, type of genome, and its targeted cell (Belsham, 1993; Brooksby, 1982), but is also influenced by the characteristics of the host (Samina *et al*., 1998). Indeed, the duration of the persistence of the virus varies with the species. Most cattle carry FMDV for six months or less, but some animals can remain persistently infected for up to 3.5 years (Alexandersen *et al*., 2002b). The virus or its nucleic acids have been found for up to 12 months in sheep (although most seem to be carriers for only 1 to 5 months), up to 4 months in goats,

for a year in water buffalo (Salt, 1993). Individual African buffalo can be FMDV SAT types carriers for at least five years, and the virus persisted in one herd of African buffalo for at least 5 years (Condy *et al*., 1985; Vosloo *et al*., 1996). Camelids do not seem to become carriers (Wernery & Kaaden, 2004). Pigs are not thought to become carriers because the infection is cleared and virus disappeared less than 3 weeks after infection (Alexandersen *et al*., 2002b; Stenfeldt *et al*., 2016a). However, there have been a few reports documenting the presence of viral nucleic acids after 28 days in pigs (Grubman & Baxt, 2004; Stenfeldt *et al*., 2016a).

The epidemiological significance of livestock FMDV carriers is uncertain and controversial (Bronsvoort *et al*., 2016), although, it has been demonstrated that carrier animals may transmit FMDV (Bengis *et al*., 1986; Dawe *et al*., 1994; Hedger & Condy, 1985; Vosloo *et al*., 1996). Indeed, the only successful experimental FMDV infection were those that involved African buffalo carrying SAT viruses, which transmitted the virus to other buffalo and sporadically to cattle (Bastos *et al*., 1999; Bastos *et al*., 2000; Vosloo *et al*., 1996; Vosloo *et al*., 2001; Vosloo *et al*., 2002).

1.3.4 The role of wildlife in FMD transmission

As mentioned above, FMDV can infect several wildlife species and it has been reported that these animals play an important role in the epidemiology of the disease (Di *et al*., 2015a; Tekleghiorghis *et al*., 2016). Indeed, the transmission dynamic of FMD in sub-Saharan Africa is mainly determined by two epidemiological cycles: one in which the virus circulates between wildlife hosts and domestic animals (Ayebazibwe *et al*., 2010; Bastos *et al*., 1999; Bengis *et al*., 1986; Thomson, 1996; Thomson *et al*., 2003; Vosloo *et al*., 2002) and another in which the virus spreads among domestic animals, without the involvement of wildlife. A specific characteristic of FMD epidemiology in Africa is the presence of the three South African Territories (SAT) serotypes FMDV, which are maintained within the African buffalo (*Syncerus caffer*) population (Brito *et al*., 2016; Jori *et al*., 2016). In southern Africa, the involvement of African buffaloes (*Syncerus caffer*) in the epidemiology of FMD has been extensively studied. Consequently, in this region, it has been shown that contacts between African buffaloes and cattle are mainly responsible for most of the FMD outbreaks in cattle (Brito *et al*., 2016; Hargreaves *et al*., 2004; Jori *et al*., 2009; Jori *et al*., 2016; Phologane *et al*., 2008). Conversely, in other parts of Africa, particularly in East Africa and especially in Central and West Africa the role which the wildlife populations play in the transmission dynamics of FMD is not well

investigated. However, many studies have recently been conducted in these areas and these studies have reported the involvement of wildlife such as African buffalo in the transmission of the FMDV to domestic animals (Ayebazibwe *et al*., 2010; Bronsvoort *et al*., 2008; Dhikusooka *et al*., 2015; Dhikusooka *et al*., 2016; Di Nardo *et al*., 2015b; Wekesa *et al*., 2015). For instance, it has been demonstrated that multiple FMDV serotypes (O, SAT1 and SAT2) circulate in wild ruminants populating both West and Central Africa rangelands and in particular in African buffalo (Di Nardo *et al*., 2015c). In addition, the role of wild animal species other than African buffaloes has also been demonstrated in the epidemiology of FMD (Anderson *et al*., 1993). The Impala (*Aepyceros melampus*) is frequently infected and acts as intermediary in disease transmission between cattle and African buffalo. The Impala (*Aepyceros melampus*) is frequently infected and number of studies have confirmed its potential role in spreading FMDV (Vosloo *et al*., 2009; Brahmbhatt *et al*., 2012; Jori *et al*., 2009b; Ocaido *et al*., 2009; Hargreaves *et al*., 2004b; Hedger *et al*., 1980). Outside Africa, the role of wildlife including deer and boar in the transmission of the FMDV has been studied, but in terms of prediction or modelling simulation (Highfield *et al*., 2008; Highfield *et al*., 2009; Ward *et al*., 2007).

1.3.5 Spatiotemporal distribution of FMDV

FMDV has a wide distribution around the world. By December 2016, there are 180 countries member of OIE. Out of them, 97 countries have no official status, 67 are recognized as FMD free country where vaccination is not practised, and Uruguay is being the only country which has FMD free status where vaccination is practised. A total of 15 other countries have a FMD free zone where vaccination is carried out or not (OIE, 2016). The countries recognized as free of FMD without vaccination include almost all European countries, west of the Russian Federation plus the Balkan countries of Bosnia-Herzegovina, Macedonia and Serbia-Montenegro (including the territory of Kosovo administered by the United Nations) (Rweyemamu *et al*., 2008b).

In recent years, many authors delivered comprehensive reviews of the geographical distribution of FMDV recorded during a length of period (Brito *et al*., 2015; Di Nardo *et al*., 2011; Rweyemamu *et al*., 2008b; Tekleghiorghis *et al*., 2016). The distribution of the 7 FMDV serotypes varies in space and time. Accordingly, the OIE/FAO, as well as the world reference laboratory for FMD (WRLFMD), provide regularly reports on the occurrence of the disease worldwide. Moreover, FMDV *pools* have been defined by OIE/FAO and these pools **(Figure**

6) are often the result of ecological similarities, common livestock exchange and cultural traditions (Brito *et al*., 2015). Each of these *pools* contains at least two serotypes of virus, and as 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 vaccines (Paton *et al*., 2009).

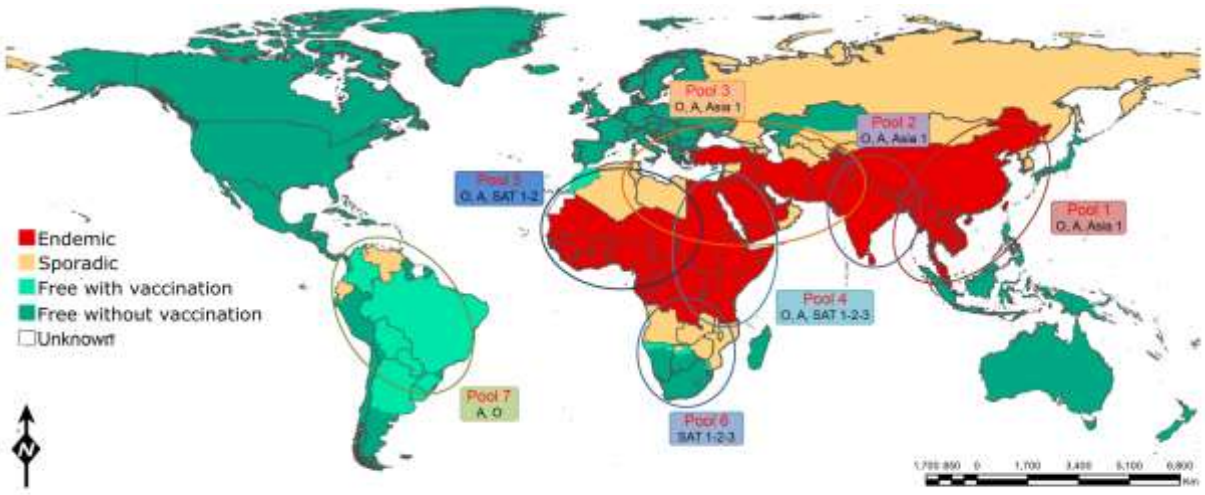

Figure 6: Global distribution of the FMDV serotypes

Source: WRLFMD, 2016 (Available from http://www.foot-and-mouth.org/sites/foot/files/quick_media/WRLFMD_status.png)

Historically, FMDV serotypes A, O, C and Asia 1 were originally confined to Eurasia where they were closely associated with domestic livestock, cattle and pigs particularly. In South America, FMD is presumed to be restricted to specific areas in the region and the viruses belong to one single pool, referred to as FMDV pool 7, where serotype A topotype Euro-SA and serotype O topotype Euro-SA circulate. In North America, FMD has not been reported for more than 60 years. The last US outbreak occurred in 1929, while Canada and Mexico are FMD-free since 1952-1953 (Carpenter, 2013; Sutmoller *et al*., 2003). An eighth pool of FMD infection, in western Europe, was present until the 1980s, but has been eradicated through a combination of preventive vaccination and zoo-sanitary measures (Paton *et al*., 2009; Valarcher *et al*., 2008). Western Europe was affected by some recent outbreaks between 2001 and 2007 (Cottam *et al*., 2006a; Cottam *et al*., 2008; Jamal & Belsham, 2013; Knowles *et al*., 2005; Valarcher *et al*., 2008; Valdazo-Gonzalez *et al*., 2012b) but these outbreaks have been

contained and eradicated rapidly (Kitching *et al*., 2007; Leforban & Gerbier, 2002; Paton *et al*., 2009).

Although, FMD can occur sporadically in typically free areas, the disease is still endemic in several parts of Asia, most of Africa and the Middle East. In Latin America, many countries applied zoning and are recognized free of FMD with or without vaccination, and the disease remains endemic in only a few countries (OIE, 2016). Until 2004-2005, the cumulative incidence of FMD serotypes showed that six of the seven serotypes of FMD (O, A, C, SAT 1, SAT 2, SAT 3) occurred in Africa, while Asia contended with four serotypes (O, A, C, Asia-1), and South America with only three ones (O, A, C) (Rweyemamu *et al*., 2008). Hence, FMDV serotypes A and O have the widest distribution occurring in Africa, Asia and South America. Serotype O is the most prevalent FMDV in the world and within this serotype there are some strains with transcontinental spread. This was the case of the PanAsia strain (within the O/ME-SA topotype) that spread from 1990 to 2003 to Asia, Europe and South Africa (Knowles *et al*., 2005; Mason *et al*., 2003; Sangare *et al*., 2001). In addition, FMDV serotype O has a particular lineage (Ind-2001d within the topotype ME-SA: Middle East-South Asia) which is normally endemic in the Indian subcontinent but has recently caused outbreaks in the Middle East and in North Africa (Bachanek-Bankowska *et al*., 2016; Knowles *et al*., 2016; Valdazo-Gonzalez *et al*., 2014).

At present, FMDV serotype C appears to be extinct (WRLFMD, 2016), The last reported outbreaks of FMD due to serotype C occurred in Amazonia, Brazil (Sumption *et al*., 2007) and in Kenya in 2004 (Sangula *et al*., 2011; WRLFMD, 2016). The serotype Asia1 is nowadays generally confined to Asia. However, two incursions of this serotype have occurred into Greece, one in 1984 and a second in 2000 (Jamal & Belsham, 2013). Moreover, periodically spreads of Asia1 serotype were reported to the west into the Middle East, and to the North and the East into former soviet republics (such as Kyrgyzstan, Tajikistan, Uzbekistan) and China (Valarcher *et al*., 2009). The three SAT serotypes are normally restricted to sub-Saharan Africa. However, there have been some outbreaks due to SAT1 viruses in Greece in 1962 for example (WRLFMD, 2016). Additionally, there have been reports of outbreaks due to serotype SAT2 in the Middle East and recently in northern African countries, namely Egypt and Libya (Ahmed *et al*., 2012; EL-Shehawy *et al*., 2014; Elhaig & Elsheery, 2014; Valdazo-Gonzalez *et al*., 2012; WRLFMD, 2016).

1.3.6 Overview of epidemiological concepts, methodologies and statistics used in the thesis

13.6.1 Systematic review and meta-analysis

A systematic review is a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyse data from the studies that are included in the review (Gopalakrishnan & Ganeshkumar, 2013; Moher *et al*., 2015). Often, systematic reviews include a meta-analysis[3] component which involves using statistical techniques to synthesize the data from several studies into a single quantitative estimate or summary effect size (Petticrew & Roberts, 2008; Uman, 2011). The term meta-analysis has been used to denote the full range of quantitative methods for research reviews (Garg *et al*., 2008). Systematic reviews adhere to a strict scientific design based on explicit, pre-specified, and reproducible methods. Accordingly, when carried out well, systematic review provides reliable and good quality data or information for decision-making support. Additionally, systematic reviews can also demonstrate where knowledge is lacking in a specific area of research. Systematic reviews and meta-analyses have become increasingly important notably in health care as well as in animal disease (Haidich, 2010; Brainard *et al*., 2016; Bian *et al*., 2015; Allan *et al*., 2015; Coral-Almeida *et al*., 2015). However, application of recommended guidelines is requisite to ensure good quality of both systematic reviews and meta-analysis (Moher *et al*., 2015).

1.3.6.2 Generalized linear models

A range of statistical methods is available to analyse data from epidemiological studies according to the objectives. These statistical methods include the generalized linear models (GLM) promoted by McCullagh and Nelder (1989). The GLM are a broad class of models that includes linear regression, Poisson regression, log-linear models, negative binomial regression, etc. Regression modelling is one of the most important statistical techniques used in analytical epidemiology. By means of regression models, the effect of one or several explanatory variables (e.g., exposures, risk factors) on a response variable such as mortality or disease occurrence can be investigated (Bender, 2009). Depending on the nature of the data, three statistical methods

[3] The meta-analysis was not used in this thesis.

were mainly used in this thesis: logistic regression, Poisson regression and negative binomial regression.

Logistic regression is the appropriate regression analysis to conduct when the dependent variable is dichotomous (binary). Like all regression analyses, the logistic regression is a predictive analysis. Logistic regression is used to describe data and to explain the relationship between one dependent binary variable and one or more nominal, ordinal, interval or ratio-level independent variables (Lewis & Ward, 2013).

Poisson regression model is a statistical method used to analyse count data as a function of a set of predictor variables. However, these models have many applications, not only to the analysis of counts of events, but also in the context of models for contingency tables and the analysis of survival data (Viel, 1994). Poisson regression assumes the response variable Y has a Poisson distribution and has a logarithmic link function. Indeed, the Poisson regression assumes the logarithm of its expected value can be modelled by a linear combination of unknown parameters. A Poisson regression model is sometimes known as a log-linear model, especially when used to model contingency tables.

Negative binomial regression is a popular generalization of Poisson regression because it releases the highly restrictive assumption that the variance is equal to the mean made by the Poisson model. Indeed, the negative binomial model provides an alternative approach for the analysis of discrete data where over dispersion is a problem, if the model is correctly specified and adequately fits the data (Bennett, 1981; Byers *et al*., 2003).

1.3.6.3 Classification and regression tree (CART) analysis

CART is a classification method which uses data to construct so-called decision trees. Decision trees are then used to classify new data (Song & Lu, 2015). CART methodology was developed in 80s by Breiman *et al.,* 1984 (1984). A CART analysis is a non-linear and non-parametric model that is fitted by binary recursive partitioning of multidimensional covariate space (Breiman *et al*., 1984; Crichton *et al*., 1997). CART can statistically demonstrate which factors are particularly important in a model or relationship in terms of explanatory power and variance. This process is mathematically identical to certain familiar regression techniques, but presents the data in a way that is easily interpreted by those not well versed in statistical analysis (Lemon *et al*., 2003).

1.3.6 Molecular epidemiology

Molecular epidemiology is the study of distribution and determinants of health and disease using molecular biology methods (Riley, 2004; Zadoks & Schukken, 2006). The combined use of molecular and descriptive epidemiology is strongly required to establish the temporal and geographical evolution of FDMV (Thiry *et al*., 2001). In the last 30 years, there was an increasing progress in the understanding of FMD epidemiology. This was widely thanks to the application of the molecular biological techniques of PCR amplification, nucleotide sequencing and phylogenetic analysis (Knowles & Samuel, 2003). In practice, FMDV isolates are characterized by the nucleotide sequence mostly of the gene encoding VP1. These isolates are therefore compared based on the percentage of nucleotide differences in this restricted part of the genome. Phylogenetic analysis of the VP1 region of FMDV was used in an extensive way to investigate the molecular epidemiology of the disease worldwide. These techniques allowed to determine the genetic relationships between different FMDV isolates, the geographical distribution of lineages and genotypes, the establishment of genetically and geographically linked topotypes and to trace the source of virus during outbreaks (Abdul-Hamid *et al*., 2011; Bastos *et al*., 2003b; Bastos *et al*., 2003a; Cottam *et al*., 2006b; Klein *et al*., 2006; Knowles *et al*., 2007; Sahle *et al*., 2004; Sangare *et al*., 2001; Sangare *et al*., 2004a; Vosloo *et al*., 2001). Nucleotide sequence differences of 30% to 55% of the VP1 gene were obtained between seven serotypes of FMD while different subgroups (genotypes, topotypes) were defined based on differences of 15% to 20% (Knowles & Samuel, 2003). However, even if within FMDV serotypes, topotypes remain constant over time (Sahle *et al*., 2004), it has been shown that viruses belonging to several topotypes may be present in a particular region. Hence, the topotype concept should be considered cautiously (Thiry *et al*., 2001). Although the concept of topotype relates to a great genetic relationship between isolates, it does not exclude that viruses of the same topotype can circulate in several different regions (Bachanek-Bankowska *et al*., 2016; Knowles *et al*., 2005; Knowles *et al*., 2016; Valarcher *et al*., 2009; Wekesa *et al*., 2015a).

1.4 Diagnosis

Diagnosis of FMD can be distinguished into two categories. First the field diagnosis based on clinical signs and lesions and study of the epidemiological situation; and secondly the laboratory diagnosis which is essential for disease confirmation. These two categories of

diagnosis will be further presented in this section. However, regarding the laboratory diagnosis mainly referencing herein to the OIE manual of diagnostic tests and vaccines for terrestrial animals for FMD, chapter 2.1.8 (OIE, 2016), the presentation will be limited to the purpose, principles and summaries of the methodology of the applied tests. A more detailed description of these tests is available in the referenced OIE manual.

1.4.1 Field diagnosis

Commonly named clinical diagnosis, this diagnosis is difficult, due to the absence of pathognomonic signs of the disease although in endemic zones even the farmers know in some respects the disease (Chisembele, 2005; Morgan *et al*., 2014). However, FMD should be suspected when salivation and lameness occur simultaneously in susceptible animals and when a vesicular lesion is seen or suspected. Usually vesicles appear on the feet and lesion around the oral cavity and on the mammary glands (Barnett & Cox, 1999). Vesicles can also occur in other sites such as nostrils and pressure points on the limbs especially in pigs. Fever often precedes other clinical signs. Therefore, febrile animals should be carefully examined. In addition to these clinical signs, the disease shows a high morbidity that can reach 100% and in dairy farms, milk production may be reduced drastically (Barasa *et al*., 2008; Lyons *et al*., 2015). In some extreme cases, death may occur. Mortality from multifocal myocarditis is most commonly seen in young animals (Alexandersen & Mowat, 2005; Arzt *et al*., 2011b; Aslani *et al*., 2013).

However, the severity of clinical signs of FMD varies with virus serotype and strain, host species, age and breed of the animal, and its degree of immunity (Grubman & Baxt, 2004). Nevertheless, clinical signs alone are not sufficient to make a sound diagnosis since other vesicular diseases *inter alia* swine vesicular virus disease or bluetongue disease, may produce similar signs. A definitive and accurate diagnosis can only be established after further laboratory tests.

1.4.2 Laboratory diagnosis

Laboratory diagnosis being the only reliable method to detect the FMDV, it is therefore a prerequisite for any control and prevention planning. On one hand, there are techniques enabling the identification of the agent including virus isolation, immunological methods and

nucleic acid recognition methods, and on the other hand, serological tests enabling to detect structural antibodies as well as antibodies against non-structural protein as indicators of infection irrespective of vaccination status. Several laboratory techniques have been developed to detect and confirm FMD and as mentioned above these are described in the OIE manual of diagnostic tests and vaccines for terrestrial animals for FMD, chapter 2.1.8 **(Figure 7)**. Due to the highly contagious nature and economic importance of FMD, the laboratory diagnosis should be done in a virus-secure laboratory specifically that meets the requirements for Containment Group 4 pathogens as outlined in Chapter 1.1.4 Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities (OIE, 2016). Additionally, for an effective diagnosis, the biological specimen should be collected appropriately.

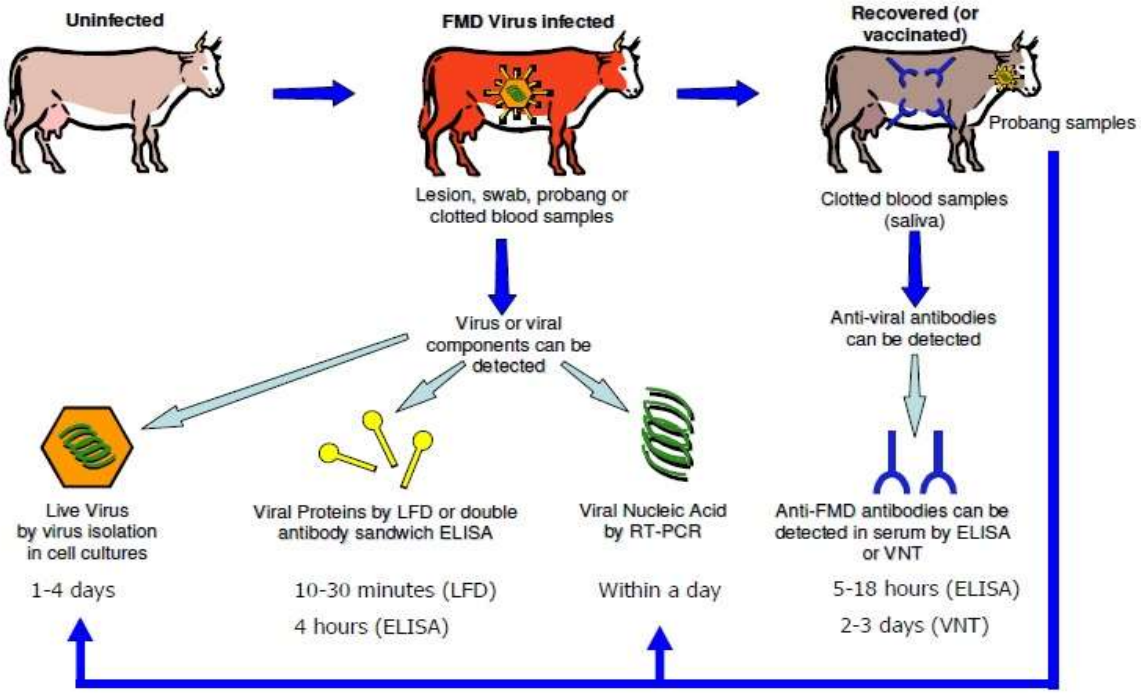

Figure 7: Principals FMD diagnosis

Source :http://www.fao.org/ag/againfo/commissions/docs/training/material/Diagnostic_sampling_procedures/Diagnostic_sampling_procedures.pdf

Legend: ELISA: Enzyme-Linked Immuno Sorbent Assay, LFD: Lateral Flow Device, VNT: Virus Neutralisation Test

1.4.2.1 Specimen collection

For laboratory diagnosis, the tissue of choice is the epithelium from early vesicles and from recently ruptured vesicles. Ideally at least 1g of epithelial tissue should be collected from un-ruptured or recently ruptured vesicles. Epithelium should be collected and placed in a transport medium composed of equal amounts of glycerol and 0.04M phosphate buffer pH (7.2-7.6) and preferably with some antibiotics. Samples should be kept refrigerated on ice until received by the laboratory. When epithelium tissue is not available from ruminant animals e.g. in advance or convalescent cases and infection is suspected in the absence of clinical sign, samples of oesophageal-pharyngeal fluids (OPF) is collected by means of a probang and used for virus isolation. In addition, other samples such as bovine milk, blood with anticoagulant, serum, and some post-mortem samples such as lymph nodes, thyroid gland, adrenal gland, kidney, and heart are also useful to confirm the disease. Countries lacking access to a specialised national or regional FMD diagnosis laboratory should send specimens to an OIE FMD Reference Laboratory. In this case, the samples should be carefully packaged, labelled, and transmitted to the laboratory by the fastest practicable mean, with the appropriate temperature control (OIE, 2016; World Health Organization, 1997).

1.4.2.2 FMDV identification

1.4.2.2.1 Virus Isolation

Virus isolation is an ultimate method of confirming the presence of live virus. In practice, clarified suspensions of field samples suspected to contain FMDV are inoculated onto cell cultures or into unweaned mice. Sensitive cell culture systems include primary bovine (calf) thyroid cells and primary pig, calf or lamb kidney cells. Established cell lines, such as BHK-21 (baby hamster kidney) and IB-RS-2 cells, may also be used but are generally less sensitive than primary cells for detecting low amounts of infectivity. The use of IB-RS-2 cells aids to differentiate FMDV from swine vesicular disease (SVD) (as SVD virus will only grow in this cell type). The cell cultures should be examined for cytopathic effect (CPE) for 48 hours. If no CPE is detected, the cells should be frozen and thawed, used to inoculate fresh cultures and examined for CPEs for another 48 hours. If no CPE was not observed after 3 passages, this could presumably indicate the absence of FMDV in the samples. Virus isolation is a very sensitive method, but laborious and expensive and there is a risk of disseminating the virus into

the environment (Kitching *et al*., 1989). It should be noted that virus isolation may not be of use in identifying the involved FMDV serotypes.

1.4.2.2.2 Antigen detection by indirect sandwich ELISA

The preferred procedure for the detection of FMD viral antigen and identification of viral serotype is the ELISA method (Ferris & Donaldson, 1992). This is an indirect sandwich test in which different rows in multi-well plates are coated with rabbit antisera to each of the seven serotypes of FMDV. When the test sample is added, the antigen (if present) is trapped by the immobilized antibodies. Specific guinea pig anti-FMDV detecting antibodies are subsequently added which in turn react with the trapped antigen. The bound guinea pig antibodies are detected by adding anti-guinea pig Ig conjugated to horse radish peroxidase. Next, with the addition of a substrate/chromogen solution, a coloured product develops indicating a positive reaction. But results can also be read spectrophotometrically at an appropriate wavelength. In this case, an absorbance reading greater than 0.1 above background indicates a positive reaction; and the serotype of FMDV can also be identified. Depending on the affected species and the geographical origin of the samples, it may be appropriate to simultaneously test for swine vesicular disease virus (SVDV) or vesicular stomatitis virus (VSV). Ideally, a complete differential diagnosis should be undertaken in all vesicular conditions. There are also other immunological methods, including lateral flow devices (LFD) (Ferris *et al*., 2009) and complement fixation test (CFT), to demonstrate the presence of the virus in samples but the antigen detection by indirect sandwich ELISA remains the most sensitive and most used of the available tests.

1.4.2.2.3 Nucleic acid detection

The nucleic acid of FMDV can be detected using Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) assays. RT-PCR can be used to amplify genome fragments of FMDV in diagnostic materials including epithelium tissue, milk, serum and probang samples. Reverse- Transcriptase (RT) combined with real-time PCR has a sensitivity comparable to that of virus isolation and automated procedures enhance sample throughput (Reid *et al*., 2002; Reid *et al*., 2009; Reid *et al*., 2010). Significant advances have been made to improve the performance of this molecular test. Indeed, specific primers have been designed to distinguish the seven serotypes (Vangrysperre & De Clercq K., 1996). In Sub-Saharan Africa countries,

few national laboratories use molecular diagnosis in routine (Namatovu *et al*., 2013). Simplified RT-PCR systems for potential field-use are under development (Abd El *et al*., 2013; Callens & De Clercq K., 1997; Yamazaki *et al*., 2013). The application of the molecular biological techniques such as PCR amplification and nucleotide sequencing, contributed greatly to a better understanding of FMD epidemiology. These techniques allowed comparisons and geographical tracing of FMDV strains. Accordingly, in epidemiological studies of FMDV, nucleotide sequencing of the VP1 gene has been used extensively to determine the relationships between the field isolates.

1.4.2.3 Serological tests

According to the OIE, FMD serological tests are used for four main purposes: 1) to certify individual animals prior to import or export (i.e. for trade); 2) to confirm suspected cases of FMD; 3) to substantiate absence of infection (for which different approaches are required) according to whether the population has been vaccinated or not and if vaccination has been used, whether this has been applied as an emergency application or as part of an ongoing programme of vaccination; 4) to demonstrate the efficacy of vaccination. Serological tests for FMD are of two types; those that detect antibodies to viral structural proteins (SP) and those that detect antibodies to viral non-structural proteins (NSPs) (OIE, 2016).

An ELISA that detects antibodies to non-structural proteins of the FMDV (NSP-ELISA) can be used to discriminate infected and non-infected animals regardless of their vaccination status, and thereby help countries to substantiate absence of infection. However, there is experimental evidence that some cattle, vaccinated and subsequently challenged with live virus and confirmed persistently infected, may not be detected in some anti-NSP tests, causing false-negative results (Brocchi *et al*., 2006). On the other hand, the lack of vaccine purity may affect diagnostic specificity as the presence of NSPs in some vaccine preparations may result in misclassification in animals that have been repeatedly vaccinated. Thus, attempts to improve these NSP ELISA tests have been carried out and have led to the development of different methods and techniques such as methods of detecting antibodies against 3AB or 3ABC polyproteins. The detection of antibodies to the NSP 3ABC of FMDV has been shown to be a sensitive and specific method to differentiate between infection and vaccination (Clavijo *et al*., 2004). Indeed, these tests measure antibody to NSPs using antigens produced by recombinant techniques in a variety of *in-vitro* expression systems. Subsequently, antibodies to the

polyproteins 3AB or 3ABC are generally considered to be the most reliable indicators of FMD infection.

The SP tests are serotype-specific and detect antibodies produced by vaccinated or infected animal. The SP tests include among others the virus neutralisation test (VNT) (Golding *et al*., 1976), the solid-phase competition ELISA (SPCE) (Goris & De Clercq K., 2005; Mackay *et al*., 2001; Paiba *et al*., 2004; Chenard *et al*., 2003), and the liquid-phase blocking ELISA (LPBE) (Hamblin *et al*., 1986; Hamblin *et al*., 1987). These tests are more frequently used and are highly sensitive. The VNT requires cell culture facilities, the use of live virus and takes 2–3 days to provide results. The ELISAs are blocking or competition based assays that use serotype-specific polyclonal antibodies (PAbs) or MAbs. They are quicker to perform and are not dependent on tissue culture systems and the use of live viruses (OIE, 2016). . The solid-phase competitive ELISA is more specific but as sensitive as the liquid-phase blocking ELISA (Mackay *et al*., 2001). An approach combining screening by ELISA and confirming the positives by the VNT minimises the occurrence of false-positive results. Reference sera to standardise FMD SP serological tests for some serotypes and subtypes are available from the Reference Laboratory at Pirbright. SP tests can be prescribed for trade and are appropriate for confirming previous or ongoing infection in non-vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field. However, serological tests, despite their intensive use in epidemiological surveillance as screening method for FMD diagnosis, may not be of use to identify the viral strains.

1.5 Prevention and control of FMD with focus on Africa

Considering the economic importance and the extreme speed in which the virus can spread FMD, prevention and control activities need to be rigorously toned down to drastically reduce the negative impacts of the disease. The means of control in sub Saharan Africa must be based on specific epidemiological cycles of FMD. Indeed, FMD in sub-Saharan Africa has two distinct but overlapping situations to deal with. The first is cattle to cattle transmission involving all the types of FMDV prevalent in Africa. The second is disease associated with wildlife, especially African buffalo, caused by the three SAT virus types (Thomson & Bastos, 1994). In general, for each animal disease including FMD, the control and/or eradication programme is based on three main principles (i) the prevention of the pathogen agent from entering the area, (ii) early detection and diagnosis and (iii) rapid implementation of control measures and

management of occurred outbreaks (OIE, 2016; Saegerman *et al*., 2007). However, the choice of control policy adopted by a given country depends on its FMD status, the risks of incursion of the disease and its economy (Ahl *et al*., 1990). There are two main approaches to FMD control frequently used: slaughter (or stamping out) and vaccination. In this section, the FMD prevention and control strategies with focus on those adapted to the context of sub-Saharan Africa will be presented, although within this vast region there is a diversity of ecosystems but herein an emphasis will be put on the pastoral system.

1.5.1 Overview of successful FMD control strategies in Europe and other free FMD areas

In 1892, Britain was the first country with a substantial program for FMD control. The decision was made to eradicate every outbreak by stamping out. This implied the killing and destruction of all infected animals and their immediate susceptible contacts, followed by thorough cleaning and disinfection of the affected premises (Sutmoller *et al*., 2003). This slaughter policy associated with strict movement controls achieved success, but the scale of slaughter at times overwhelmed the financial or organizational capacity and unfortunately fresh introductions occurred regularly. The USA also successfully applied stamping-out. The last outbreak occurred in 1929. Canada also controlled the 1951-1952 outbreaks by this method and was declared FMD free in 1953. Most European countries opted for quarantine policies until mass preventive vaccination became possible from the mid-1950s.

Preventive vaccination, coupled with stamping out of cases, was adopted by most European countries in the 1950s until 1990, when freedom from FMD allowed vaccination to stop in Europe, except for Turkey and parts of the Russian Federation at that time. Policies based on vaccination, mostly involving quarantine rather than slaughter of cases, have been applied in other regions, such as South America and southern Africa (Paton *et al*., 2009). In summary, countries that have achieved FMD-free status, have applied strict zoo-sanitary measures involving import controls on animals and their products from affected countries, early detection and culling of cases, tracing to identify undisclosed sources of infection and onward spread, controls on movements of animals and contaminated materials and intensive surveillance until freedom is re-established. Moreover, in southern Africa, where there is evidence of involvement of wildlife in the maintenance and transmission of the FMDV, in

particular the SAT serotypes, fencing, for controlling the movement of wild and domestic animals, has been one of the supplementary measures of FMD control but has engendered much acrimonious debate with regard to its efficacy and the deleterious effects it has on wildlife (Thomson & Bastos, 1994).

1.5.2 Stamping out

Stamping out is a recognized and proven strategy for rapid elimination of an introduced exotic disease or other emerging livestock disease (Geering *et al*., 2013). However, to achieve success there are crucial elements for stamping out policy application. These elements include among others, the following:

- designation of infected zones;
- intensive disease surveillance to identify infected premises and dangerous-contact premises or villages within these zones;
- imposition of quarantine and livestock movement restrictions;
- immediate slaughter of all susceptible animals either on the infected and dangerous-contact premises or in the whole infected area;
- safe disposal of their carcasses and other potentially infected materials;
- disinfection and cleaning of infected premises;
- maintaining these premises depopulated of susceptible animals for a suitable period.

Slaughter or stamping out may be used on its own, as in the UK in 2001 and 2007 (Leforban, 2002), or in combination with vaccination. Most of the European countries have agreed to a policy of non-vaccination and in the case of an outbreak, infected as well as in contact animals are slaughtered (Kahn *et al*., 2002). The strategy used to combat the outbreak of FMD that occurred in the UK in 2001 has stimulated a larger debate on the policy of disease control by stamping out (Crispin *et al*., 2002; Sutmoller & Casas, 2002; Thompson *et al*., 2002). Additionally, the possibility of a major increase in cost must be considered when a country or region decides to stop vaccination and instigate a policy of stamping out. This will require the establishment of a contingency fund so that in the event of an outbreak the affected farmers will be fully and speedily compensated, otherwise the policy will not be sustained. Accordingly, in developing countries including most African countries, control by stamping out appeared to be very costly and in some respect not realistic, hence, in these countries FMD control is mainly

through regular vaccination in conjunction with the control of animal movement to prevent the virus spread.

1.5.3 Vaccination

The finding by Mowat and Chapman in 1962, that FMD virus could multiply efficiently in a baby hamster kidney (BHK) cell line opened new areas in vaccine production resulting in better control of the disease (Barteling & Vreeswijk, 1991; Lubroth *et al*., 2007). For FMD an inactivated vaccine is used. Depending on the type of adjuvant, the vaccines can be in aqueous or in oil form. The aqueous vaccines are commonly used in cattle, sheep, goats and buffalo's but are not effective in pigs while oil vaccines are used in all species. The recommended vaccination schedule includes a two-dose primary course to achieve six months of protection (FAO/OIE, 2016). Several other types of vaccines (based on proteins, peptides, DNA) were also developed but only the conventional vaccine (inactivated vaccine) has proven to be effective in the field (Paton & Taylor, 2011). However, vaccine strains are required to be antigenically similar to those involved in the outbreak. In addition, the vaccine must contain all the serotypes that are circulating in the field and should induce protective immunity against each vaccine component. Hence, it is fundamental to briefly remind the basis of immunity of FMDV to have an overview on how immunity of susceptible animal response to FMDV when challenged with infection.

1.5.3.1 Immune response

FMDV infection elicits a rapid humoral response in either infected or vaccinated animals (Grubman & Baxt, 2004), this is accompanied by clearance of virus–antibody complexes through phagocytic cells (McCullough *et al*., 1988). Protection against FMDV correlates with the induction of high levels of neutralising antibody in serum, first detectable as early as 3–4 days following infection (Doel, 2005). However, cattle which have recovered from infection with one of the seven serotypes of FMDV are not immune to the other serotypes but remain protected against the first serotype for a considerable period (Callis *et al*., 1968; Cox & Barnett, 2009; Doel, 1996; Doel, 2005). Additionally, within the FMDV serotypes there are subtypes against which vaccines of the same serotype will fail to fully protect (Paton *et al*., 2005). The response is directed to epitopes on the viral capsid protein, VP1, and good protective immunity is apparent between 7 and 14 days after either infection or vaccination. In cattle, the

immunoglobulin G1 (IgG1) response predominates over IgG2 (Capozzo *et al*., 1997; Mulcahy *et al*., 1990; Salt *et al*., 1996a), and antibodies, including IgA, can be detected in upper respiratory secretions early in infection (Pega *et al*., 2013; Salt, 1993; Salt *et al*., 1996a). The neutralization of virus within the host may occur by mechanisms like those occurring in *in vitro* neutralization; however, there is a suggestion that macrophages may play a role in clearing the virus from the infected animal by phagocytosis of opsonized virus (McCullough *et al*., 1988; McCullough *et al*., 1992; Rigden *et al*., 2003).

On the other hand, the role of cellular immunity in the protection of infected animals is still well established (Grubman & Baxt, 2004). Although specific T-cell antiviral responses, involving $CD4^+$ and $CD8^+$ cells, have been observed in cattle and swine following either infection or vaccination (Bautista *et al*., 2003; Childerstone *et al*., 1999; Collen & Doel, 1990; Saiz *et al*., 1992), it has been suggested that cell-mediated immunity is involved in clearance of virus from persistently infected animals. In addition, other components of innate immune system may be involved in the immune response of the host. Indeed, several studies have shown that IFN-α, -β, and -γ may be involved in the host defence against FMDV infection (Brown *et al*., 2000; Diaz-San *et al*., 2010; Diaz-San *et al*., 2016; Oh *et al*., 2012; Parida *et al*., 2006; Ramirez-Carvajal *et al*., 2016; Toka *et al*., 2009). In addition to the IFNs, other cytokines may also play a role in the host response.

The age of the animals has also been shown to influence the antibody response against FMD virus (Doel, 2005; Samina *et al*., 1998). In the absence of maternally derived antibody, it has been shown that cattle respond well to vaccination as early as one week of age, in terms of both antibody and protection (Nicholls *et al*., 1984). Furthermore, the response of animals ranging in age from one week to eighteen months were broadly equivalent (Nicholls *et al*., 1985). Other factors such as animal breed, animal husbandry system, etc., may also be involved in the antibody response to FMDV **(Table 3)**.

Table 3: Different variables which influence the immune response to foot and mouth disease virus and vaccine

Adapted from (Doel, 1996)

Stimulus variables	Responses variables
Host Species, breed, age, health status (concomitant infections), physiological state (pregnancy), FMD immune status (maternal antibody), other stress factors (climate, husbandry, etc.	**Antibodies** Specificity, affinity, isotype, half-lives, synergy or competition between different antibodies, titres and distribution
Virus Dose, route of infection, serotype, strain, etc.	**Cells (including memory)** Density and number, distribution/tropism, type (B-cells, T-cells, phagocytes), specificity, relative proportions of different cells, half-lives

1.5.3.2 Vaccination in endemic situation with special reference to Sub Saharan Africa

Vaccination is one of the main tools proven to better manage or eliminate the disease when properly applied and with desirable quality and composition of vaccine.

However, Ringa and Bauch (2014) have demonstrated that there are significant differences between FMD-free settings and FMD-endemic settings in such vaccination. Indeed, in endemic situation, the efficacy of vaccination can vary widely depending on factors such as the duration of natural and vaccine immunity (usually 6 months) and the rate of disease re-introductions. In endemic settings, the main objective of FMD vaccination is to reduce the overall incidence of the disease (Hunter, 1998; OIE/FAO, 2016). Nevertheless, controlling FMD cannot rely exclusively on vaccination. Vaccination should be implemented as a part of a control program that includes other zoo-sanitary measures (Nicholls *et al*., 1983). In Africa, a successful strict application of zoo-sanitary measures in support of a vaccination program is best illustrated by the FMD control programs in southern Africa including Botswana. In the later country, the

control program is based on the division of the country in risk zones and implementation of appropriate disease surveillance, livestock identification and movement restriction and control in the different risk zones. Vaccination is carried out in the designated vaccination zones (Falconer, 1972; Letshwenyo *et al*., 2004). The situation of southern Africa is very different from that of other regions, particularly that of West Africa. The pastoral farming system in the Sudan/Sahel region, which is characterized by long-distance movements of livestock due to either transhumance or trade, has been suggested to contribute to FMD outbreaks (Bronsvoort *et al*., 2004b; Bronsvoort *et al*., 2004a; Rweyemamu *et al*., 2008b; Ularamu *et al*., 2016). Hence, the nomadic system is a major epidemiological consideration for FMD control in these regions. Indeed, it is the custom for farmers in the Sahel, to move hundreds of thousands of cattle within a very short period. It would be impractical to establish quarantine stations capable of handling such large numbers of animals within the same area (Sangare *et al*., 2004b). In most developing countries where FMD is mostly endemic, other challenges encountered the effectiveness of vaccination in the control of FMD. The constraints could be summarised as following: restricted financial and infrastructure, inadequate policies, lack of public awareness and lack of commitment (Knight-Jones & Rushton, 2013; Paton *et al*., 2009; Sinkala *et al*., 2014).

Two key issues should be considered in SSA, firstly repeated FMD outbreaks occurrence and secondly transboundary FMDV transmission pattern via uncontrolled animal movement including nomadic pastoralism and animal trade. These two elements make the regional and integrated approach an urgent need for effective prevention and control of animal diseases in general and FMD in particular (Leforban & Gerbier, 2002; Rweyemamu *et al*., 2008a; Sutmoller & Casas, 2002; Sutmoller *et al*., 2003). Additionally, it is recognised that, to be effective, FMD vaccine strains should be closely related antigenically to those strains which are circulating in the field (Sutmoller *et al*., 2003; Balinda *et al*., 2010; Jamal & Belsham, 2013; Freimanis *et al*., 2016; Lubroth *et al*., 2007). Hence, control activities in endemic area should include passive and active surveillance to monitor FMDV serotypes circulating in domestic animals as well as in wildlife.

Part one

Chapter 2: Objectives of the thesis

Chapter 2: Objectives of the thesis

As mentioned in the introductory part of this thesis, livestock production in Niger, despite its role as an important support of the national economy, is challenged by enormous constraints. Due to multiple causes, the lack of competitiveness of this Niger's economic activity is observed both at local level as well as at the level of regional markets such as Economic Community of West African States (ECOWAS), the African Union or even on an intercontinental scale. It was also mentioned in the introduction that among the constraints of livestock production in Niger, they are those related to animal diseases. Although, some deadly epizootics of livestock such as rinderpest have been eradicated, other important animal diseases including FMD, remain endemic and could negatively impact the development of animal resources in Niger. Additionally, despite its known negative economic impact and the ability of the virus to spread rapidly, FMD was not considered as a priority disease and therefore remained neglected, underreported and uncontrolled in Niger as in many west African countries.

On the other hand, the dynamics of regional and international demand for animal products, including livestock, are becoming increasingly requiring high quality products. Export is accordingly complying with international standards and product traceability. The response to those requirements is undeniably the improvement of the livestock health status through, *inter alia*, strengthening the national epidemiological surveillance system for improved reporting of diseases, and preventing and controlling transboundary animal diseases, including FMD. However, given that the balance of FMD impacts are not the same throughout the world, in Niger, as in most of developping countries, international trade of livestock and animal products would not be a realistic priority for subsistence animal husbandry. Indeed, according to FAO and OIE, much of the global FMD burden of production losses falls on the world's poorest communities, and those which are most dependent upon the health of their livestock. In addition, the presence of FMD in these countries has an impact on the overall herd fertility, modifying the herd structure and affecting the selection of breeds. Overall the direct losses in developing countries, limit livestock productivity creating a food security issue and contributing to malnutrition. In Niger, there is evidence that FMD is scarcely investigated as it emerges from the few data on this disease in the databases of international animal health organizations such as OIE, FAO and at the world reference laboratory for FMD. Otherwise, it is well accepted that a better knowledge of the epidemiology of diseases such as FMD is crucial

for the implementation of efficient control measures. Therefore, this study aims to bridge this knowledge gap by providing relevant information.

The overall objective of the research carried out for this PhD thesis is to improve the knowledge on the epidemiology of FMD in Niger allowing the implementation of future strategic control planning (**Figure 8**). For this purpose, prerequisites review studies have been performed firstly on FMD risk factors modelling and secondly on molecular epidemiology of FMD in Africa (**Chapters 3 and 5** respectively).

The specific objectives were to:

- ☞ Determine the incidence, geographical and temporal distribution of FMD outbreaks
- ☞ Assess at outbreak level clinical and economic impact of FMD outbreaks;
- ☞ Estimate the costs and benefits of potential control options by vaccination in Niger

It implies the understanding of FMD epidemiology and gaining knowledge on FMD occurrence, its clinical incidence, the disease transmission pathways, the spatiotemporal analysis and quantitative assessment of FMD economics, and its control to support decision making. (**Chapter 4**).

- ☞ Determine the seroprevalence of FMD and to assess potential risk factors associated with seropositive FMDV
- ☞ Isolate, identify and molecularly characterize FMDV involved in recent FMD outbreaks that occurred in Niger and further to determine their relationship with reference vaccine strains.

Undeniably, implementing effective control strategies require thorough understanding of the seroprevalence and the molecular epidemiology of the disease and this can be done by serological tests followed by virus isolation and identification and by further molecular characterization through PCR and nucleotide sequencing of the viruses. Additionally, given that the available commercially vaccines do not necessary confer protection against all antigenic

FMDV strains and consequently for a successful vaccination, vaccines should antigenically be matched to the epidemic virus. (**Chapter 6**).

- ☞ Provide to decision-makers some recommendations for FMD prevention and control as well as some perspectives to be considered for further investigations. (**Chapter 7**).

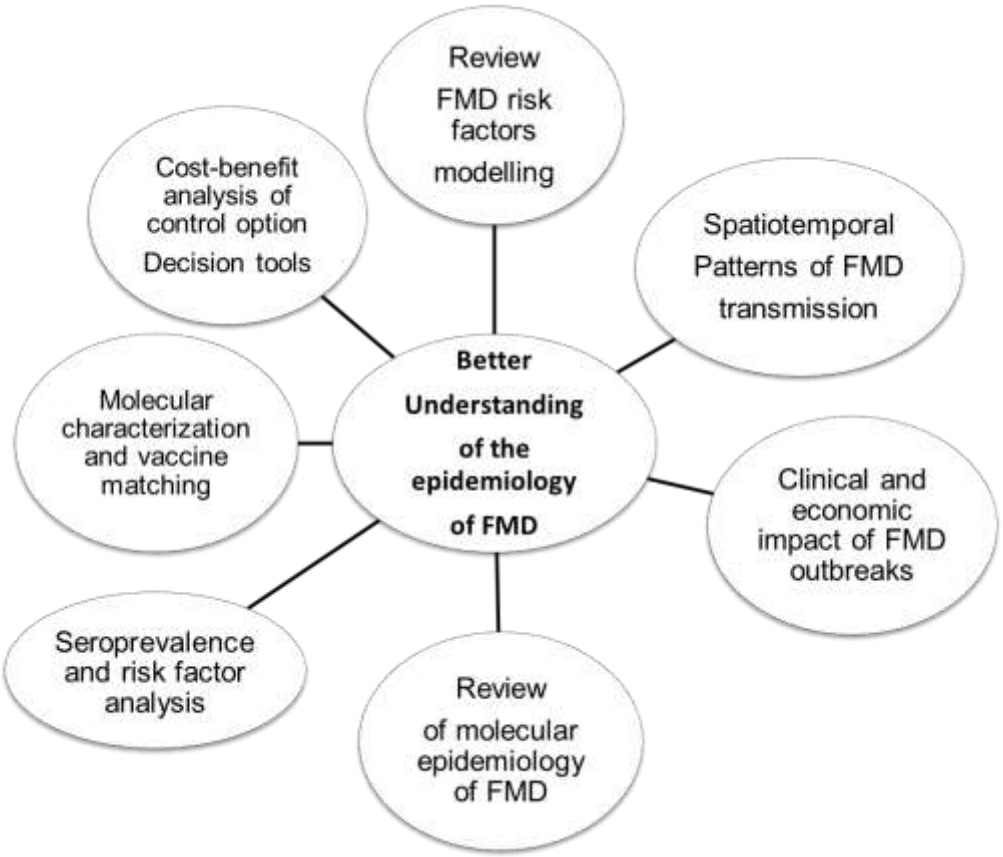

Figure 8: Summary of the mains objectives of the thesis

Part two

Chapter 3: Review of epidemiological risk modelling of foot-and-mouth disease: implications for prevention strategies and perspectives with focus on Africa

Part two: Experimental section

Chapter 3: Review of epidemiological risk modelling of foot-and-mouth disease: implications for prevention strategies and perspectives with focus on Africa

(Under review in PLOS ONE)

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Abstract

Foot-and-mouth disease (FMD) is a highly infectious and transboundary disease that affects domestic and wild cloven-hoofed animal species. The characteristics of FMD have been widely modelled to estimate disease control options. The aim of this review was to identify and characterise risk models for FMD that are well-supported by scientific evidence from the literature. This study attempted to provide a synopsis of the strengths and weaknesses of these models and their relevance to FMD prevention policy with a focus on their use in African countries where the disease remains enzootic. A literature search was conducted to identify relevant data on quantitative and qualitative risk assessments for FMD. This search included studies reporting FMD risk factor modelling and spatiotemporal analysis. A description of retrieved papers and a critical assessment of the modelling methods, main findings and their limitations were performed. Different types of models have been used depending on the purpose of the study and the nature of available data. The most reported factors related to FMD were the movement (especially uncontrolled animal movement) and the mixing of animals around water and grazing points. Based on the included qualitative and quantitative risk assessment studies, the critical pathway analysis showed that the risk of FMDV entering a given involved country is overall low. In some cases, this risk can be elevated, especially when illegal importation of meat and the movement of terrestrial livestock are involved. Depending on the approach used, the selected published studies presented some shortcomings associated with the type of model and the lack of reliable data from endemic settings. The application of modelling in endemic countries including Africa should be encouraged.

Introduction

Foot-and-mouth disease (FMD) is a highly infectious and transboundary disease that affects domestic and wild cloven-hoofed animal species. The disease has direct and indirect tremendous economic consequences resulting mainly from constraints in international trade in animals and animal products originating from infected countries [1,2]. The etiological agent of FMD is a small, non-enveloped, positive-sense, single stranded RNA (8.4 kb in length) virus belonging to the genus *Aphthovirus* of the family *Picornaviridae* called foot-and-mouth disease virus (FMDV). The primary mode of transmission of FMDV is via direct contact between infected and susceptible animals [3]. The virus can also spread mechanically by contaminated organic debris, fomites or personnel and materials from infected farms that may carry the virus to susceptible animals in another farm [4-6]. FMDV transmission can also be airborne, a mechanism by which virus exhaled into the air by infected animals can be spread over long distances depending on the wind speed and direction [7,8]. The rapid spread of FMDV highlights the need for a rapid and effective prevention and/or control of the disease. Development of an efficient FMD surveillance and relevant control policies for different scenarios requires deep understanding of FMD epidemiology through for example accurate epidemiological models [9].

An epidemiological model is usually defined as 'a mathematical and/or logical representation of the epidemiology of disease transmission and its associated processes' [10]. These models provide a representation of the transmission dynamics of diseases among animals, and/or among groups of animals in time and/or space [11,12]. Although, there is no agreed classification system for models, several authors have focused on different aspects of models which may distinguish them from each other. For instance, according to the treatment of variability, probability and uncertainty, models can be stochastic or deterministic. Models which assign averages or most likely values to all parameters and model the average or most likely outcome of probability events are named 'deterministic' models. They produce a single output or result for each set of input values or scenario [13]. For example, deterministic models were used by Ferguson et al. [14,15] for the FMDV epidemic in the UK in 2001. Models which included variability and the effect of probability are termed 'stochastic'. As parameter values within the model can vary and the occurrence of chance events is randomized, stochastic models must be run repeatedly to produce a range of outcomes from the same input scenario. Such models were used by Keeling *et al*., [16] also in the 2001 FMD epidemic in the UK. There are

several overviews, reviews and critiques of FMD models in the literature [17-20], but often with a strong focus on the 2001 UK epidemic. Furthermore, most of the models related to FMD transmission were intended for epidemic settings, where control measures are designed to contain a single epidemic. However, in endemic settings, long-term factors such as waning of natural immunity or vaccine-induced immunity, and frequent disease re-introduction should be considered for FMD control [21]. Consequently, it is difficult or even wrong to extrapolate the experience in one country to another one as farming practices, farm density, farm size, and contact patterns may differ [22].

In contrast to developed regions where mostly FMD has been eradicated, the disease is still endemic in some parts of the world, especially in Asia, parts of South America and Africa [23]. Currently**,** in some endemic countries, as it is the case in West Africa, there is no efficient control plan as FMD risk factors are poorly understood. Consequently, for FMD free countries, these endemic areas constitute a real and permanent threat through numerous transmission pathways. Considering the need to mitigate this potential event of FMDV entry from endemic to non-endemic FMD countries, the implementation of FMD risk assessment in endemic areas such as Africa is warranted. However, at present, most of the parameters required for the models may be unknown. Accordingly, one of the most relevant issues is whether suited models for endemic countries exist? Therefore, the aim of this paper is to systematically collect information on studies related to risk models for FMD that are well-supported by scientific evidence from the literature. This review will specifically focus on (i) analytical models which seek to establish associations between occurrence of disease and risk factors and (ii) risk models which describe qualitatively and/or quantitatively the risk of introduction of disease into a population through particular routes (risk pathways). Based on the data extracted from the included studies, recommendations will be presented on critical disease prevention and control options with focus on Sub Saharan African (SSA) conditions.

Materials and methods

Systematic review

Literature search process

Relevant published articles were searched based on PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) method [24]. The search was conducted through online search engines, particularly in PubMed () and Scopus (http://www.scopus.com) using

combinations of seven keywords. These keywords were: (a) "Foot-and-Mouth Disease", (b) "Modelling", (c) "Risk assessment", (d) "Risk factors", (e) "Spatiotemporal", (f) "Transmission" and (g) "Spread". The search was restricted to articles written in English or in French, with an available abstract and published between January 1997 and December 2016. Two screening steps were applied based on defined inclusion and exclusion criteria **(Table 1)**. The first step was applied to the titles and abstracts to select potential relevant papers while the second screening was applied on the full text. Additionally, some other documents were identified from the references of included articles and were added to the present review.

Table 1: Inclusion and exclusion criteria

Exclusion criteria	Inclusion criteria
• Studies related to another pathogenic agent (such as enterovirus) instead of FMDV	• Studies should be original articles published in a peer-reviewed journal during the last 20 years (from 1997 to 2016)
• Studies reporting the use of biological models rather than statistical or mathematical models	• Studies should focus on different spatial and spatiotemporal models to estimate the risk of occurrence or transmission of FMD
• Articles describing models of the transmission dynamics of FMDV spread through population or compartmental models	• Studies describing quantitative and/or qualitative risk modelling of FMD
• Modelling studies reporting the exploration of either different strategies or resource requirements in hypothetical outbreaks (simulation models)	• Studies reporting patterns of different epidemiological outbreaks in terms of FMDV spatiotemporal distribution
• Articles describing only the modelling of economic impact of FMD	• Retrospective analysis of historical outbreaks data with the purpose to highlight FMD risk factors
• Studies carried out for assessing laboratory tests or surveillance system performance (sensitivity and specificity)	
• Experimental studies related to factors associated with secretion and excretion of FMDV	
• Modelling studies that did not explicitly discuss FMDV transmission and risk factors for its spread	

Data collection and analysis

To be included in the analysis of this review, the following had to be available for the retrieved papers: (1) the country of interest, (2) the type and features of the model, (3) the mode of transmission discussed in the study, (4) the assessment process, (5) the main transmission risk factors identified, (6) and if any the practical applications. The extracted data were compiled in an excel datasheet and subsequently a descriptive analysis was performed to provide state of the art - knowledge on FMD epidemic models and risk analysis.

Results

The literature search yielded a total of 3,716 records through the two databases (PubMed and Scopus). After removing duplicates, 1,302 unique publications were identified as potentially relevant references and were screened using titles, abstracts and keywords. Out of these screened articles, 128 full texts were assessed for eligibility. A total of 108 references were selected and presented in this review, including 60 additional articles retrieved after screening the reference lists of the eligible papers giving that the 48 retrieved published papers met at least one of the inclusion criteria. The flow diagram in **Fig 1** shows the search process. The PRISMA check list, the search strategies, and the results for the consulted databases are provided in **S1 Table and S2 Table** respectively.

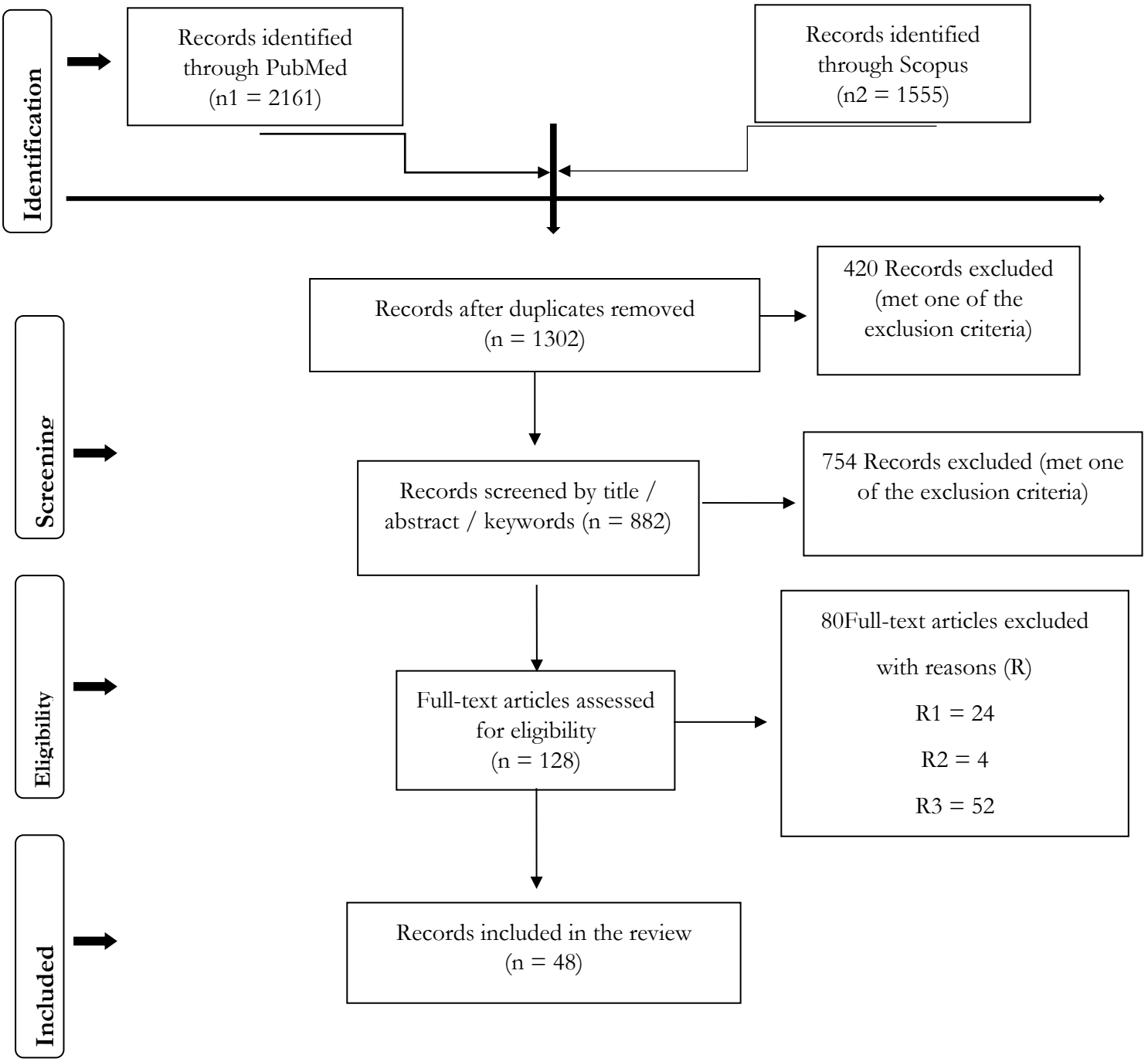

Fig 1. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Diagram

Legend: R (reason) 1: UK FMD 2001 epidemic models; R2: Japan 2010 FMD epidemic models; R3: Other simulated epidemic models.

General description of the included studies

To simplify the analysis, the selected articles were categorized into two types: (1) modelling FMD risk factors and spatiotemporal analysis, (2) FMD risk assessment models, subdivided into two components (quantitative and qualitative). Hence, out of the 48 included articles, 14 described quantitative risk models, 7 were related to qualitative risk assessments while 27 reported results of spatiotemporal or risk factors analysis.

The chronology of publication of the included articles showed that the concern for risk modelling is relatively recent. Although the use of a type of mathematical or statistical model depends on the purpose of the study and the nature of the data, logistic regression and stochastic models were the most frequently used in the modelling studies included in this review (**Fig 2**). Regarding the geographical origin of articles related to risk modelling, it is not surprising that many studies were implemented in developed countries, which are free of the disease. However, a significant number of spatiotemporal and risk factor analysis studies were performed in endemic countries or regions such as Sub-Saharan Africa.

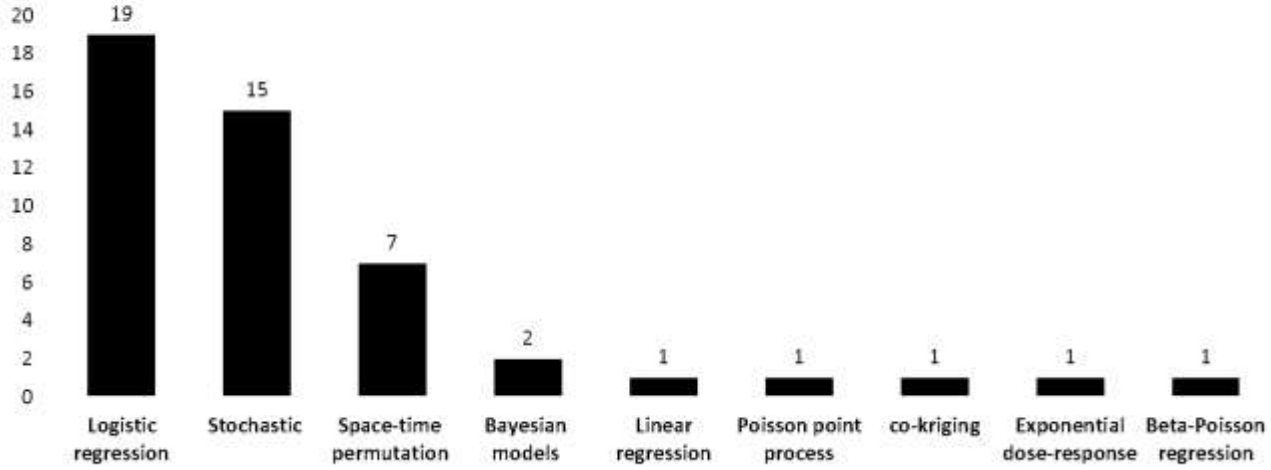

Fig 2: Frequency of type of models among the studies included in this review

Modelling FMD risk factors and spatiotemporal analysis

Out of the 27 studies reporting spatiotemporal and risk factor modelling of FMD (**Table 1**), 19 were designed as retrospective studies using mostly historical data and were often associated with survey results based on questionnaires [25-43]. Among this type of selected studies, 4 were designed as case-control [44-47], and 4 others were conducted as cross-sectional or seroprevalence studies [48-51].

It should be noted that the identification of risk factors in these published articles (**Table 2**), has not only been based on model results but also by considering their implications. Accordingly, despite the geographical diversity of the studies, there were indeed some common risk factors. In almost all articles, the most frequently reported factor was the animal movement *sensu lato*. The uncontrolled animal movement leads to other risk factors such as mixing of animals around water and grazing points, a risk factor that is widely identified in Africa, undoubtedly linked to the farming and transhumance practices. However, there are some specific risk factors like the contact between wild animals and domestic animals which are more relevant in Africa [26,51,52], and animal density which is predominant in Europe [28,29,33]. The other identified risk factors such as the seasonal pattern of occurrence of FMD outbreaks [25,27,51] or the factor of susceptibility related to the age of animals [31,45,49] were less frequently reported in the selected studies.

Table 2: Main risk factors identified through selected modelling studies and presented in this review

Identified main risk factors	Country of interest	Reference
Animal movement	Tanzania, Uganda, Cameroon, Togo/West Africa, Turkey, Zambia, Ethiopia, Japan, Pakistan,	[26,27,32,34,36,40,47,48,50,52,76,79]
Animal trade	Cameroon, Togo/West Africa, Iran, Ethiopia, Pakistan, Zambia, Scotland	[34,37,48,49,52,76,79]
High animal density	Turkey, Japan, Ethiopia, Iran, United Kingdom (UK), Turkey	[28,29,33,34,40,89,106]

Mixing herds around water points and on pastures	Uganda, Cameroon, Bhutan, Nigeria, Zambia, Ethiopia	[27,32,46,48,50,107]
Contacts between domestic animals and wildlife	Tanzania, Nepal, Zambia, South Africa	[26,36,48,51,52,79]
Human activities and / or lack of compliance with biosecurity measures	Tanzania, Nepal, England, Japan	[26,30,38,44,47]
Seasonal pattern of occurrence of FMD outbreaks	Middle East, Uganda, South Africa	[25,27,51]
Young animals identified as being most susceptible to infection	Israel, Iran, Bolivia	[31,45,49]
Lack of early screening/detection	UK	[35,37]
Shorter distances to the nearest infectious source	UK	[29,108]

FMD risk analysis models

There were two main approaches to risk analysis: the qualitative and the quantitative. In a qualitative risk analysis, the risk level is appreciated in qualitative terms; like, for example, "the risk of introduction is "negligible". In a quantitative analysis, the risk is appreciated in quantitative terms e.g. by risk rates, usually as a probability. Additionally, there was broad agreement concerning the definition of risk analysis defined as *"A process consisting of three components: risk assessment, risk management and risk communication"* and in other words as *"A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization"* [53].

Quantitative risk assessment model

In this review, 14 articles presenting a quantitative analysis of risk were selected. In quantitative risk analysis, Monte Carlo simulation is usually used to assimilate the probability components of the import scenario. Several software programmes have been developed within a spreadsheet environment for Monte Carlo simulation. The uncertainty associated with an input and its known variability were modelled as a probability distribution. Although the electronic search

yielded only few articles, published in recent years, risk analysis has been earlier applied in the field of animal health, particularly in food safety (microbiological risk assessment) and import risk analysis (IRA), also including number of studies on FMD risk assessment. Indeed, most of the studies reported risks related to the importation of potentially contaminated animal products (milk or meat) [54-56] or live animals [57-59]. Some studies were related to the risks associated with movement of either people or animal products possibly infected with FMDV [60,61]. Most reviewed IRAs originated from FMD free countries, mainly in Europe and USA [55,57,58]. Only one included published study on quantitative risk assessment was performed in a FMD endemic country namely Zimbabwe [62]. Through these quantitative risk assessment studies, the critical pathways analysis showed that the risk of FMDV entering a country is overall low [6,54,57,60-64]. However, depending on the research question and model assumptions, some risks could be considered as relatively high depending on their nature, i.e. the illegal importation of meat and the terrestrial movement of livestock [55,56,65,66] (listed in **Table 3**). The reviews performed by Garland & De Clercq [67] and by Potier [68] related to the risk assessment approach were not included in the analysis of this review, based on the exclusion criteria. However, important insight has been provided by these reviews, for instance, Garland & De Clercq [67] reported a comprehensive review of risk assessment related to vaccinated animal import. It was demonstrated through this review that the risk from products derived from vaccinated animals is very low when risk mitigation measures are correctly applied.

Table 3: Estimated risk of introduction and/or exposure of FMDV through quantitative risk assessment

Country	Nature of risk	Estimated Risk/ probability	Risk level	Reference
United Kingdom	Annual frequency with which the illegal importation of meat will result in infection with FMD in the UK livestock population	• Total amount of illegal meat entering UK each year is estimated on average to be 11,875 tonnes	High	[55]
	Risk to the UK livestock population of FMD, CSF, ASF and SVD from the	• Mean flow of the quantity of illegally imported meat that is	High	[56]

	illegal importation of any meat product from any region in the world.	contaminated with FMDV per year into UK = 214.2 Kg • Mean Frequency of infection per year for infection with FMDV because of the illegal importation of meat and meat products into UK = 0.015		
	Frequency with which meat waste from ships or aircraft might expose British livestock to infection with FMD	• Total weight of FMD contaminated waste estimated to be 26 kg per year • Mean value of Frequency of livestock infection in UK = 0·0007 per year (1,429 years between outbreaks of FMD due to ship and aircraft waste)	Low	[60]
	Risk of new outbreaks occurring as a result of the six burning pyres during FMD epidemic in 2001 in UK	• The probability of a cow or sheep being infected were estimated, with 95 per cent certainty, to be less than 0.003 and 0. 0004 respectively	Low	[64]
United States	Potential spread of FMD if infected livestock had been exhibited at the 2005 California State Fair	• The mean probability that at least 1 animal that became infected with FMD would subsequently leave the state ranged from 28% to 96% as the number of index cases increased from 1 to 10, respectively	High	[65]
	Probability of an outbreak of FMD occurring in the USA as a result of the importation of livestock and to understand the sensitivity of the results to the various risk parameters used in	• Total probability of introduction into the USA of FMD from imported livestock is estimated to be 0.415% per year, which is equivalent to one introduction every 241 years	Very low	[58]

	estimating the probability.			
	Probability of introduction of FMDV into the USA via the importation of cloned bovine embryos	• Mean Probability of introducing FMDV via cloned embryos was estimated to be 3.1×10^{-7}	Very low	[63]
Malaysia	Likelihood of an introduction of FMD through terrestrial movement of livestock.	• Mean probability of an animal accepted for import having FMD was 2.9%, and the risk was as high as 11%.	High	[59]
Peru	Risk for potential FMD re-introduction into Peru and to quantify the FMD spread and economic impact associated with hypothetic FMD epidemics.	• Mean (95% probability interval) number of outbreaks, infected animals, epidemic duration, and direct costs were 37 (1, 1164), 2152 (1, 13, 250), 63 days (0, 442), and US$ 1.2 million (1,072, 9.5 million), respectively	High	[66]
The Netherlands	Probability of infecting dairy cows that were drinking FMDV contaminated surface water due to illegal discharges of contaminated milk.	• The probability of infection of a herd of 53 cows in the case of a dilution factor of 44 is 8.5×10^{-5}	Low	[6]
	Risk of exporting FMD-infected pig carcasses from a vaccinated area	• The probability that a processed carcass was derived from an FMD-infected pig was on average 2.0×10^{-5} directly after final screening, and 1.7×10^{-5} after a six-month waiting period	Very low	[54]
Spain	Probability of FMD epidemic occurring in Spain because of the introduction of live	• Mean probability of FMDV introduction into Spain via import of live animals per year	Low	[57]

	animals into the country from another European Union member country.	was estimated as 2.36×10^{-2}, with a 95% • Probability Interval of (7.37×10^{-6}, 1.61×10^{-1}), which corresponds to approximately one outbreak every 40 years		
Zimbabwe	Effectiveness of the containment of FMD in buffaloes within the conservancies	• Greatest annual risk (2×10^{-4}) for cattle would be from antelope jumping over the outer perimeter fence of the conservancy and infecting cattle on the outside	Low	[62]
Taiwan	FMD entrance caused by passengers who illegally carry meat products of cloven-hoofed animals through international airports into a country.	• The probability of FMD virus risk caused by the passenger event from area A (3.11×10^{-10}) was four times lower than the corresponding probability from area B (2.00×10^{-7})	Very low	[61]

Legend: ACF: African swine fever, CSF: classical swine fever, FMD: Foot-and-Mouth Disease, SVD: swine vesicular disease, UK: United Kingdom

Qualitative risk assessment model

Based on the method of data extraction used in this review, the key findings of the included articles related to FMD qualitative risk assessment (n = 7) were summarized in a narrative description of each study. Taking into account the design of these studies, an exception was made to include some published reviews with respect to the defined time frame of publication which is between 1997 and 2016. In general, FMD qualitative risk assessment was based on the OIE assessment framework, using available data from published sources and various unpublished sources [69-71]. As mentioned above, the main application of risk analysis in the animal health field has been directed to import risk analysis, which is the assessment of disease risks associated with international trade in animals and their products. This is illustrated by the research question of some included articles which served as basis for the qualitative assessment of risk [70-73]. However, for both quantitative and qualitative risk analysis, the fields of application of these assessment methods were extensive and diverse [20,69,74]. Notwithstanding, these studies revealed some risks that ranged from negligible to moderate (**Table 4**). Based on these qualitative assessments the authors proposed useful or important recommendations for the prevention and control of FMD.

Table 4: Highest risk level reported in selected articles related to FMD qualitative risk assessment

Risk assessed	Overall risk	Reference
Risk of FMDV release outside Kruger National Park (South Africa) and subsequent spread in the buffer zone with vaccination	Moderate	[69]
Risk of introducing FMD into Russia and Europe from Georgia, Armenia and Azerbaijan: Probability of occurrence of FMD	Moderate	[72]
Risk associated with International trade in deboned beef	Low	[70]
Introduction of FMD virus into New Zealand in legally imported animals and animal products	Low	[73]
Risk posed by cattle slaughtered during the carrier stage for the international beef trade	Negligible	[71]

Legend: * Two articles [20] and [74] related to qualitative risk assessment were not included in this table. In the first paper [20], the authors have highlighted the importance of the risk analysis based on which policy changes has been implemented to control the epidemic that occurred in UK in 2001. In the second article [74], the authors described a risk assessment conducted with local expert's opinions. They concluded that FMDV entry risk pathways in Mongolia was estimated high in relation with livestock movements.

Discussion

FMD risk factors and spatiotemporal distribution modelling

The risk factor concept in the epidemiology of animal disease including FMD is based on the findings of statistically significant associations between incidence or prevalence of the disease and levels of the relevant variables in question. This review demonstrated that in the field of FMD epidemiology, several studies have been performed with the aim to show that a given risk factor contributes to the occurrence and/or transmission of the disease. However, it is likely that some identified risk factors are not causative and merely reflect increased risk via correlation with other risk factors. Therefore, the logistic regression model (mainly multivariate) is a theoretically acceptable method of analysis of the risk dependence of several variables. One of the advantages of such approach is that specific risk factors can be identified and their impact quantified, and therefore abled to be managed or controlled [75]. On the other hand, this review showed the importance of using spatiotemporal models like the space-time scan statistic permutation model [25,30,38,52]. Indeed, assessing the spatiotemporal clustering of FMD prevalence or incidence appears to be a useful method for identifying geographical regions and

periods of time in which the disease is more likely to occur. Hence, in the identified significant clusters, further FMD investigation should be implemented to identify predictors for outbreaks and epidemics to improve the effectiveness of preventive plans in reducing the occurrence of disease outbreaks [76]. In our point of view, this is greatly needed, specifically in the context of endemic countries in SSA with a broad common pastoral space but mostly with limited financial and logistical resources.

The collected published papers highlighted several factors that contribute significantly to the occurrence of FMD outbreaks. As mentioned above, even though these studies were carried out in different geographical areas, the predominant risk factor of FMD remains the uncontrolled animal movements. Other risk factors, such as mixing animals around water points, on pastures and in livestock markets were also elucidated. Nevertheless, it should be noted that the magnitude of these risk factors, most likely related to the farming system, do not have a similar impact on the prevalence or incidence of the disease as well as on the control measures to be implemented. For example, during the UK FMD epizootic in 2001, in addition to the policy of slaughtering animals on infected farms, further control measures were initiated, including a ban on all animal movements, the closure of markets, and the restricted public use of footpaths across agricultural land [15]. In contrast, in endemic countries with a huge epidemiological complexity and considering the livestock production system such as the transhumance or nomadism, the application of the preventive and control options mentioned above would be nowadays unrealistic. Indeed, the context is so far different from that which prevails in several SSA countries where the animal husbandry system includes a seasonal cyclical movement, and where large herds must migrate on long distances in search of grass and water, within the country of origin or by crossing over the border to neighbouring countries (transboundary transhumance). This favours the contact between infected and healthy animals and between potentially infected wildlife and domestic animals and as result induces a significant risk of disease spread, FMD included [27,48,77-79]. Although there are specific risk factors for certain regions such as the presence of wildlife which plays an important role in the maintenance of FMDV of SAT serotypes in Africa [80-83], some other identified risk factors including international livestock trade [76,79] and transboundary movements of animals, stress the absolute necessity for an integrated control at country, regional or continental level [84-86]. This could be based, for example, on coordinated vaccination programs against FMDV serotypes circulating within a region.

Despite the proven significance of these modelling studies of risk factors and spatiotemporal distribution of FMD, there are some limitations in their implementation, and in the accuracy and reproducibility of their methods and results. Although the technical development is identical, the application of models can and should vary based on the purpose of the research. Also, some of the limitations of the risk factors analysis and of the spatiotemporal distribution could be related to the applied model type [87,88]. For example, in the logistic regression analysis, large sample sizes are required to provide sufficient number of positive cases for proper estimation [45]. Moreover, for this type of model widely used in risk factor modelling, no assumption is made concerning the distributions of the explanatory variables. In fact, in logistic regression, the explanatory variable should not be highly correlated with another variable because this could induce problems of estimation [75,88].

The permutation model was also extensively used by some authors [25,52,89]. Nevertheless, it has a disadvantage due to the shape of the clusters constrained by the cylindrical shape (with a circular base) of the window used to scan the studied area. This could lead to a serious constraint when the geographical extension of the detected clusters is large [90].

Another example of limitation due to the applied model is given by Perez *et al.*, [76]. Indeed, these authors have used the co-kigring model to estimate the spatial risk of FMD in Pakistan. The co-kriging model uses information on covariates that are assumed to be associated with the outcome and to be known throughout the study area. Consequently, the findings of this type of study are formulated from a model that is based on a probability interpolation method which does not consider the variability.

The limitations of models in relation to the used data will be further discussed in the next section devoted to qualitative and quantitative risk FMD modelling. However, the limitations due to the use of questionnaires should be mentioned. Indeed, some authors presented a possible reporting bias when using data recorded by questionnaire rather than by using a prospective collection of objective data [34,46,47]. Using questionnaires may also lead to some variables of the questionnaire to be subject to confusion with others [32,48]. Likewise, the analysis of risk factors based on seroprevalence studies can present limitations related to the low sensitivity and specificity of the applied serological test [49-51].

FMD risk assessment models

Despite the relatively few articles reporting risk assessment models (n = 21) collected for this review, it is observed that, especially in developed FMD free countries, FMD risk assessment modelling was performed, with the aim to estimate the risk of introduction of FMDV via several pathways including import of animals or animal products [91-94]. Irrespective of the differences between the two approaches (quantitative *versus* qualitative), the decision-makers gained a thorough understanding of the FMD risk through risk assessment which resulted in sensible and realistic recommendations. If implemented, these recommendations can lead to a sustainable strengthening of capacities to prevent, control and even to eradicate FMD [20,74,95].

Given the risks estimated by the two assessment methods reported in the included articles, the risk of introduction ranged overall from low to high. The interpretation of these results must be made cautiously. Indeed, the low level of an estimated risk is very different from the absence of the risk. Some authors explicitly reported the low level of risk in relation to the deficiency of available data to make their models more useful [6,57,58,61,62], although in some models, some values of parameters were either assumed [55,58,60,63,64] or determined from experimental studies [54]. According to some authors, livestock does not represent a risk because the importation of susceptible live animals into FMD-free countries from countries that are not FMD-free is prohibited [72,73].

Depending on the used approach, the selected studies have also some shortcomings that can be ascribed to the risk assessment methodology. As noticed above, qualitative risk assessments express risks in relative qualitative terms and often involve the aggregation of expert opinions. A comprehensive collection of data combined with expert opinion, was first undertaken by the European Commission for the Control of Foot and Mouth Disease (EuFMD), but thereafter extended and reviewed by the working group on FMD risk coordinated by the European Food Safety Authority (EFSA). This was done to assess the risk of FMDV entering through a pathway that could lead to its eventual release in the European Union from FMD risk regions such as Africa, Asia and South America [95]. In this case, the methodology for qualitative risk assessment must be rigorous to ensure that the true risk, and not the false risk perception, is assessed as most likely, any decision can lead to a major animal health and economic impact [96]. In addition, from a methodological point of view, qualitative risk analysis has usually a lack of reproducibility and accuracy, compared to quantitative risk models. Furthermore,

quantitative risk assessment allows to model uncertainty and accordingly to undertake sensitivity analysis to determine the relative importance of variation in different inputs on the output(s) [54,55,57,60,61,63,64,66]. However, quantitative risk analysis may be too complex to carry out as they require more time, resources and accurate data. Indeed, a major and common problem for modelling is the lack of strong reliability and accuracy of recorded data [25-27,34,37,45,49-52,89]. Similarly, it should be emphasized that several FMD endemic countries with substantial animal populations provide no information on FMD outbreaks or provide data that are considered to reflect a significant under-reporting of the true situation [95,97]. The best example that illustrates the importance of data in modelling is given by the well-developed database of the 2001 UK FMD outbreak which allowed the expansion of detailed epidemiological models that are more accurate than those generally generated for other epidemics [14-17,98]. In a recently published review, Pomeroy *et al.,* [99] elegantly demonstrated the crucial importance of data availability and accessibility for model implementation. Moreover, whatever the modelling approach (quantitative or qualitative), the uncertainty of each step of the model should be clearly underlined and reported to decision-makers.

Apart from the limitations related to the types of models and the quality of data used, some weaknesses of this review should also be noted. The limitations could essentially be related to the methodology applied. The time criteria as well as the Boolean operators used may have caused to inadvertently miss pertinent research articles. For example, the use of the term “model” instead of “prediction” or “simulation” could probably result to miss certain published articles which do not include in their titles, abstracts and/or keywords one of these keywords. But, the Boolean operators “Foot-and-Mouth Disease" AND "Epidemiology" were used to avoid this and typically this could encompass all epidemiological studies of FMD. Moreover, it excluded the epidemics (real or simulated) models, especially those based on UK FMD 2001 models and similar models. The heterogeneity of the selected studies, mainly in relation to the used assumption and parameters, was a major constraint for data extraction and accordingly it precluded a more extensive quantitative comparison between studies. Additionally, not all the included studies presented detailed models, especially those related to risk factors analysis through seroprevalence studies that could be criticized for their sensitivity and specificity. Consequently, this fact has unfortunately not enabled to rank the identified risk and the associated contributing factors.

One of the strengths of this review is to have highlighted some FMD risk factors that subsequently may allow the proposition of some basic recommendations for preventive measures of FMD. First, it should be noted that the control measures depend largely on the epidemiological status of a given country or region, the livestock production system, but notably also on the available financial resources. For example, in developed countries, in case of an FMD outbreak, one of the recommended policy is to strictly implement stamping out (or pre-emptive culling when the risk of transmission or spread is present). Although the economic impact is very important, the application of these measures is possible and effectively allows to control the epidemic. On the contrary, in developing countries, with most of them being FMD endemic, this option cannot reasonably be considered for many reasons including the financial issue. Hence, the following control or preventive measures are formulated with emphasis to endemic countries. For the principal risk factor (animal movement) and other factors resulting from the movement (as mixing herds around water points and on pastures), the recommended control measure is the prohibition or restriction of movements during FMD outbreaks as much as possible. Considering the transhumance or nomadism system, dominant in some African regions like SSA, vaccination of animals before going on transhumance could effectively reduce the incidence of the disease. However, for implementing this measure, there is an ultimate need of an updated knowledge of FMDV serotypes circulating in the region. For animal trade at local or national level, the application of quarantine measures should be strictly applied. In case of FMD clusters with a well-known seasonal pattern of occurrence of the disease, selective vaccination campaigns, surveillance activities and control of movements before and during the season at higher risk could be appropriate. Some studies reported that in detected FMD clusters young animals are the most susceptible to FMD infection. Therefore, increasing the frequency of vaccination among herds followed by the intensification of surveillance activities (where young calves are abundant, surveillance targeted to this specific animal group) would be highly interesting to recommend. In addition, the implementation of risk based surveillance, would certainly improve the efficiency of the use of resources.

In areas where wildlife constitutes a threat for FMDV transmission, building fences at the fringes of game reserves to avoid contact between wild and domesticated animals has been adopted in some regions as a FMD prevention method. Also, given the fact that human activities through several pathways could be an important risk factor, the enhancement of compliance of biosecurity measures and the awareness of all stakeholders (e.g. farmers and veterinarians) should be taken into consideration in planning control options.

In some FMD endemic countries, the World Organisation for Animal Health (OIE) has recognized some zones within the country (i.e. Botswana) allowed to export livestock on the international market. For these areas, it is highly desirable to understand and model the risks of FMD importation in FMD free zones. This assessment could thereby assist decision-makers during further outbreaks by implementing appropriate measures in due time. Consequently, the application of modelling including epidemic models, could be interesting even in an endemic setting. A valuable modelling study, recently carried out in an endemic country is illustrative and strongly encouraging for the application of models especially in areas where the threat of disease is persistent. Indeed, by catalytic and reverse catalytic models applied to serological data to estimate the force of infection and the rate of waning immunity and to detect periods of sustained transmission, Pomeroy *et al.,* [100] were able to reconstruct the historical burden of FMDV in Cameroon and to quantify control efforts necessary to stop the transmission. Additionally, in recent years, relevant studies demonstrated the feasibility of implementing epidemiological modelling based on simulations in SSA endemic areas [101] as well as in countries where exist FMD free zones, such as in southern Africa [102-105].

Conclusions

Our understanding of FMD epidemiology is continuously improving. The growing knowledge can be further enhanced by the use of epidemiological modelling in order to improve disease data interpretation and control actions. This review highlighted the unavoidable prerequisites of good-quality data to perform modelling. Hence, FMD could be effectively controlled, if certain conditions are met. The recommended measures to be adopted include a regional approach to disease control, setting up a global or regional surveillance partnership. In addition, especially in developing countries where mostly FMD is endemic, political and administrative authorities should consent more efforts on strengthening the veterinary services and the veterinary laboratory capacities. When these steps are achieved, improving the data collection and the disease reporting system could be expected.

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The authors declare no conflict of interest.

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Supplementary materials

S1 Table. PRISMA Check list

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3 - 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4 - 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4 - 5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	NA
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	NA
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5 Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5 - 8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8 – 11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11 – 14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14– 15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

Adapted from: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal. pmed1000097. For more information, visit: www.prisma-statement.org

S2 Table. Search strategies and results for PubMed & Scopus databases

Last date of search	Database consulted	Search algorithms applied	Results
15-12-16	Pubmed	Foot-and-Mouth Disease AND Epidemiology	1168
	Scopus		790
Subtotal 1			1958
15-12-16	Pubmed	Foot-and-Mouth Disease AND Epidemiology AND Risk assessment*	56
	Scopus		37
Subtotal 2			93
15-12-16	Pubmed	Foot-and-Mouth Disease AND Model*	466
	Scopus		223
Subtotal 3			689
15-12-16	Pubmed	Foot-and-Mouth Disease AND Risk factor AND Model*	95
	Scopus		84
Subtotal 4			179
15-12-16	Pubmed	Foot-and-Mouth Disease AND Quantitative AND Risk AND Assessment	20
	Scopus		36
Subtotal 5			56
15-12-16	Pubmed	Foot-and-Mouth Disease AND Qualitative AND Risk Assessment	8
	Scopus		10
Subtotal 6			18
15-12-16	Pubmed	Foot-and-Mouth Disease AND Spread AND Model*	154
	Scopus		171
Subtotal 7			325
15-12-16	Pubmed	Foot-and-Mouth Disease AND Transmission AND Model*	194
	Scopus		204
Subtotal 8			398
Total of records			**3716**

Part two

Chapter 4: Spatiotemporal patterns of FMD transmission in cattle between 2007 and 2015 and quantitative assessment of the economic impact of the disease in Niger

Chapter 4: Spatiotemporal patterns of FMD transmission in cattle between 2007 and 2015 and quantitative assessment of the economic impact of the disease in Niger

(Under review in Transboundary and Emerging Diseases)

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ABSTRACT

Foot-and-mouth disease (FMD) is endemic in Niger, with outbreaks occurring every year. Recently, there was an increasing interest from veterinary authorities to implement preventive and control measures against FMD. However, for an efficient control, improving the current knowledge on the disease dynamics and factors related to FMD occurrence is a prerequisite. The objective of this study was therefore to obtain insights into the incidence and the spatio-temporal patterns of transmission of FMD outbreaks in Niger based on the retrospective analysis of 9-year outbreak-data. Negative binomial regression was used to explore the relationship between FMD occurrence and possible associated variables including the period (year and month), the location (region) and the animal-contact density. In addition, a regression tree analysis model was used to identify statistically significant predictors associated with FMD incidence. This study provided also a first report on economic losses associated with FMD. From 2007 to 2015, 791 clinical FMD outbreaks were reported from the 8 regions of Niger; the number of outbreaks per region ranging from 5 to 309. The statistical analysis revealed that 3 regions (Dosso, Tillabery and Zinder), the months (September to December and January to February; i.e. end of the rainy season and during the dry and cold season), the year (2007 and 2015) and the density of contact were the main predictors of FMD occurrence. The quantitative assessment of the economic impacts showed that the average total cost of FMD at herd level was 733 euros while the average price for FMD vaccination of one outbreak was estimated to be more than 315 euros. Despite some limitations of the clinical data used, this study will guide further research into the epidemiology of FMD in Niger and will promote a better understanding of the disease as well as an efficient control and prevention of FMD.

Keywords: Foot-and-Mouth Disease; Outbreak; Clinical; Retrospective study; CART; Spatio-temporal distribution; Negative Binomial regression; Economic impacts; Costs; Niger.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious transboundary disease that affects all cloven-hoofed animals. The causative agent is a member of the *Picornaviridae* family, belonging to the genus *Aphthovirus* (Belsham & Sonenberg, 1996). There are seven FMD virus (FMDV) serotypes namely O, A, C, South African Territories (SAT1, SAT2 and SAT3) and Asia1, with limited cross-protection between them (Paton *et al*., 2009). FMDV serotype C was last detected in Kenya and Brazil in 2004 (Sangula *et al*., 2011; WRLFMD, 2016). Serotypes O, A, and the SAT FMDVs are endemic in Africa; serotype O is the most widely distributed in eastern and western Africa, whereas SAT FMDVs are mostly found in sub-Saharan Africa (SSA) (Brito *et al*., 2015; Tekleghiorghis *et al*., 2016).

In Niger, FMD is endemic and causes several outbreaks every year due to continuous infection of FMDV in the absence of prevention and control measures. Referring to the data recorded monthly in the frame of the official passive (clinical) surveillance, FMD is the second most widely distributed disease in Niger after pasteurellosis. Recently in 2014, the country confirmed outbreaks of FMDV serotype O (WRLFMD, 2016). In contrast, to the best of our knowledge, there are no FMD control measures in Niger such as vaccination since the circulating antigenic types of FMDV are not well known. Factors associated with FMD outbreaks are not clearly understood and the spatio-temporal distribution of FMDV has not been studied obviously. On the other hand, the economic impact of FMD in Niger, particularly the reduction in milk production and the depreciation in value of meat, has been overlooked or is not well understood by livestock-owners. These factors, combined with the low mortality rate in adult animals, may explain the relative lack of attention to FMD infections in livestock. However, in recent years the situation has changed with the increasing interest from veterinary authorities to implement FMD prevention and control. However, to effectively prevent or control the threats posed by FMD or by other diseases, there is a need to understand clearly the epidemiology of the animal disease in question (Grubman & Baxt, 2004; Knight-Jones & Rushton, 2013). Nevertheless, in general, few studies were performed on FMD in West African countries, fact that makes that those countries represent a potential risk for other regions such as North Africa and the Middle East through i.a. the trade of live animal from the Sahel (e.g. Niger and Mali) to North African countries like Libya and Algeria (Di Nardo *et al*., 2011; Rweyemamu *et al*., 2008). More specifically, no recorded studies in Niger have been carried out to determine the prevalence of FMD as well as to investigate the disease distribution, the risk factors and the economic costs. For a developing country with such a large area as Niger, a deep understanding of FMD

epidemiology is strongly recommended to understand when and where resources should be optimally directed to prevent or to reduce the incidence of the disease directly related to the dynamic of FMD. In addition, to determine epidemiological evidence for the need to invest resources to control FMD in such a country, it would be appropriate to better understand the economic impact of the disease. The objective of this study was therefore to obtain insights into the incidence and related economic costs of the disease as well as to determine the spatio-temporal patterns of transmission and the predictors of FMD outbreaks in Niger based on a retrospective analysis of 9 years (from 2007 to 2015) outbreak data.

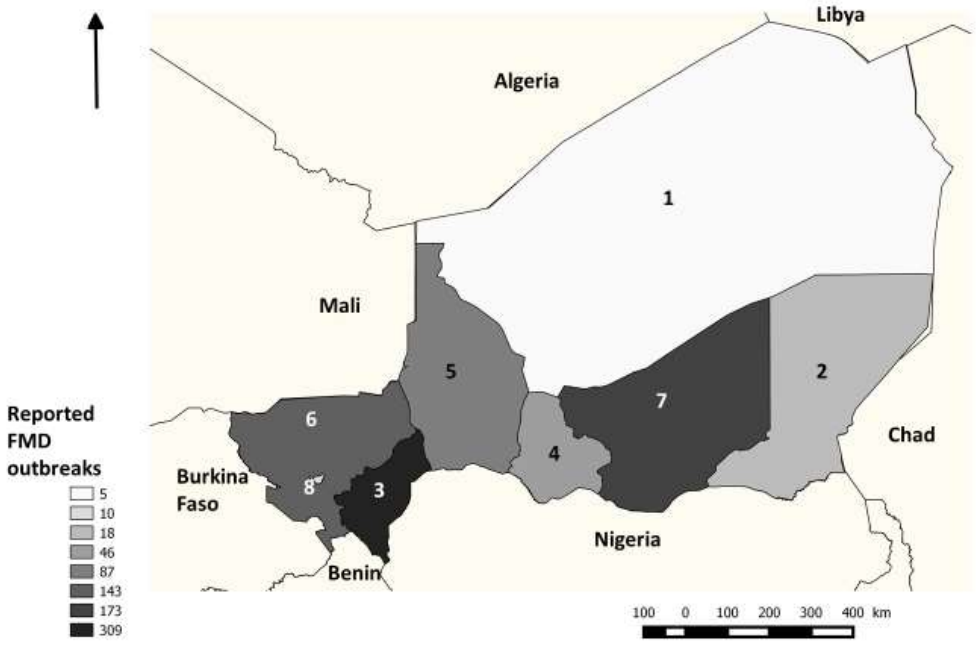
MATERIALS AND METHODS

Study area

The Republic of Niger covers 1,267,000 square kilometres (490,000 square miles). It is a landlocked country bordered by seven other countries namely Algeria and Libya to the north, Chad to the east, Nigeria and Benin to the south, Burkina Faso to the southwest, and Mali to the west **(Fig. 1)**. Niger is in the heart of the Sahel, the transitional zone between the tropical West African coast and the Sahara Desert. Since 2002 and until 2012, Niger is administratively divided into 8 regions, 37 departments and 265 municipalities. In this paper, the regions are considered as the epidemiological units of interest. Niger has an arid sub-tropical climate characterized by a short rainy season (RS) from May-June to September, and a long dry season lasting from 8 to 9 months. The dry season is composed of 2 periods namely the dry and cold season (DCS) from October to January; and the dry and hot season (DHS) from February to May.

Crop and livestock production are greatly important to the national economy, contributing around 40% to its gross domestic product (GDP). Agricultural and pastoral activities are carried out in four distinct major agro-ecological zones namely: (i) the semi-desert area in the north, with a rainfall of 0 to 50 mm per year, (ii) the sub-Saharan pastoral zone in the longitudinal East-West centre core of the country with a yearly rainfall of 50 to 200 mm, (iii) the Sahelian agro-pastoral zone extending in the central to southern part of the country with 200 to 500 mm of yearly rainfall , and (iv) the Sudano-Sahelian zone covering the southern part of the country, receiving 600 to 800 mm of rain per year, and being the most suitable for agriculture. The well-known informal cross-border movement of animals or animal products and feed is a traditional practice among the countries in the Sahel region including Niger. In addition, livestock production is highly limited by multiple constraints including disease occurrence (e.g. FMD).

FMD is in general clinically and economically more important in cattle and pigs (Grubman & Baxt, 2004; Kitching, 2002). However, in Niger the pig population was estimated in 2013 to be only 42,500 heads, hence negligible from an economic point of view. Based on the latest livestock census, 10.3 million of cattle, 25.02 million of sheep and 27.88 million of goats are estimated to be distributed across the country (MEL, 2012). Although the great economic importance of small ruminants, FMD in cattle seems to be more impacting than in another domestic animals. Accordingly, cattle which constitutes the main livestock sector in Niger, will be the only species considered in this study.



1: Agadez, 2: Diffa, 3: Dosso, 4: Maradi, 5: Tahoua, 6: Tillabery, 7: Zinder, 8: Niamey

Fig. 1. Map of Niger showing the regions where FMD outbreaks were notified from 2007 to 2015 plotted as graduated gray rectangles (see legend)

Nature and source of data

A database with the total number of cattle FMD outbreaks in Niger from 1st January 2007 to 31 December 2015 was provided by the Statistical Unit of the Ministry of Livestock. For this study, a FMD outbreak was defined as the occurrence of one or more cases of the disease in a district as clinically diagnosed by district veterinary officials. A continuous sequence of cases within a district was considered as one outbreak unless successive cases were separated by a time gap of at least one month. Usually, animals seen by the veterinary officer are sick animals presented by farmers. The signs and/or lesions are typically sufficient for veterinary officers to make a provisional diagnosis of the endemic diseases such as FMD in Niger. The livestock district services send monthly passive surveillance reports to the regional level office, which in turn send them to the Statistical Unit of the Ministry of Livestock. The collected data include the number of cattle with FMD signs (morbidity data), the number of dead cattle (mortality data) as well as the monthly climate data for each district (rainfall, temperature and humidity) and the cattle, sheep and goat population for each district. In addition, data related to water points, livestock markets and pastoral enclaves, were also included in the statistical analyses. The pastoral enclaves are defined as "traditionally, areas reserved for pastures in agricultural zones". Population and contacts (water points, livestock markets and pastoral enclaves) data were standardized using its density by area of surveillance.

Descriptive analysis

The recorded data were first transferred to a spreadsheet program (Excel 2016, Microsoft). A descriptive statistical analysis was conducted to determine (i) the reported outbreaks per year and per month, (ii) the seasonality trends of the FMD outbreak occurrence, and (iii) the most affected areas in relationship with the time of onset of the disease. For the seasonality analysis, each year was divided into the three seasons (see study area): rainy season (RS), dry and cold season (DCS) and dry hot season (DHS). The seasonal distribution was assessed by summing the frequency of cases (cumulated incidence) into these three seasons. The database was cleaned and merged to the list of all districts in Niger obtained from the Pastoral Unit of the Ministry of Livestock. All geographical data were projected to UTM Zone 31N coordinate system (datum WGS84 EPSG:32631) and represented using QGIS 2.12.0.

Statistical analysis

Regression analysis

One of the main research question addressed in this article is whether the distribution of the occurrence of FMD outbreaks (count data) is influenced by the recorded temporal data such as the year and months, and the spatial data including the region, the animal density (cattle, sheep and goats), the water crossing points, the livestock markets and the pastoral enclaves. The latter three were merged as they are related to the animal contact frequency. In a first step, Poisson regression analysis was used. The response variable is the number of outbreaks recorded at each time-space unit (region-month) during the period between 2007 and 2015. The aggregation by region was necessary because administrative subdivisions at the district level do not reflect the distinction between agro-ecological zoning within the regions. For example, all the 7 regions except Agadez (located in the far north in the desert area), include at least two agro-ecological zones, hence some data such as the climate data were only available at district level. Due to extra-binomial variability, univariate negative binomial regression was used. Multivariable negative binomial regression model was further used to evaluate the relationship between FMD outbreaks occurrence and variables found to be significant in the univariate analysis (with *P* value ≤ 0.20). The regression analyses were performed using STATA/SE Acad. 14 (Stata Corp., College Station, Texas). The level of significance for the tests performed was defined at P value ≤ 0.05.

Classification and regression tree (CART) analysis

In addition, all variables from the univariate analysis were also entered into a regression tree model with FMD occurrence at time-region level as response variable. The Regression Tree model was used to identify predictors and their interactions which influence FMD occurrence at region level (Speybroeck, 2012). Specifically, in this study, a regression tree analysis was conducted. The response variable was the FMD occurrence for a specific region and year (time-space unit).

A CART analysis is a non-linear and non-parametric model that is fitted by binary recursive partitioning of multidimensional covariate space (Breiman *et al*., 1984; Crichton *et al*., 1997). Using Salford Predictive Modeller software (Salford Systems, San Diego, CA, USA), the analysis successively splits the dataset into increasingly homogeneous subsets until it is stratified to meet specified criteria. The Gini index was used as the splitting criteria, and 10-fold cross-validation was used to test the predictive ability of the obtained trees. CART

performs cross validation by growing maximal trees on subsets of data then calculating error rates based on unused portions of the data set. To accomplish this, CART divides the data set into 10 randomly selected and roughly equal parts, with each "part" containing a similar distribution of data from the populations of interest (i.e., FMD outbreaks). CART then uses the first 9 parts of the data, constructs the largest possible tree, and uses the remaining 1/10 of the data to obtain initial estimates of the error rate of the selected sub-tree. The process is repeated using different combinations of the remaining 9 sub-sets of data and a different 1/10 data sub-set to test the resulting tree. This process is repeated until each 1/10 sub-set of the data has been used as to test a tree that was grown using a 9/10 data sub set. The results of the 10 mini-tests are then combined to calculate error rates for trees of each possible size; these error rates are applied to prune the tree grown using the entire data set. The consequence of this process is a set of fairly reliable estimates of the independent predictive accuracy of the tree, even when some of the data for independent variables are incomplete and/or comparatively small. For each node in a CART generated tree, the "primary splitter" is the variable that best splits the node, maximizing the purity of the resulting nodes. Further details about CART are presented in previously published articles e.g., (Chaber & Saegerman, 2016; Saegerman *et al*., 2011; Saegerman *et al*., 2015; Saegerman *et al*., 2016).

Stochastic estimate of the economic FMD impacts

A framework of economic impact of animal disease including FMD has been outlined by Rushton (2009) (**Fig. 2**). The visible losses include milk production loss, draft power loss, weight loss, and death loss. The invisible losses include fertility problems that lead to a change in herd structure and a delay in sale of animals and/or livestock products. On the other hand, the additional costs are related to control, diagnostic and surveillance costs while the revenue foregone are essentially related to denied access of market and the use of less productive but disease resistant breeds (Rushton, 2016). However, in this study, two components of the visible losses, namely the milk production losses and losses due to animal deaths (specifically of young animals) were considered for the direct impact. The indirect impact considered in the study is related to the costs associated with FMD vaccination.

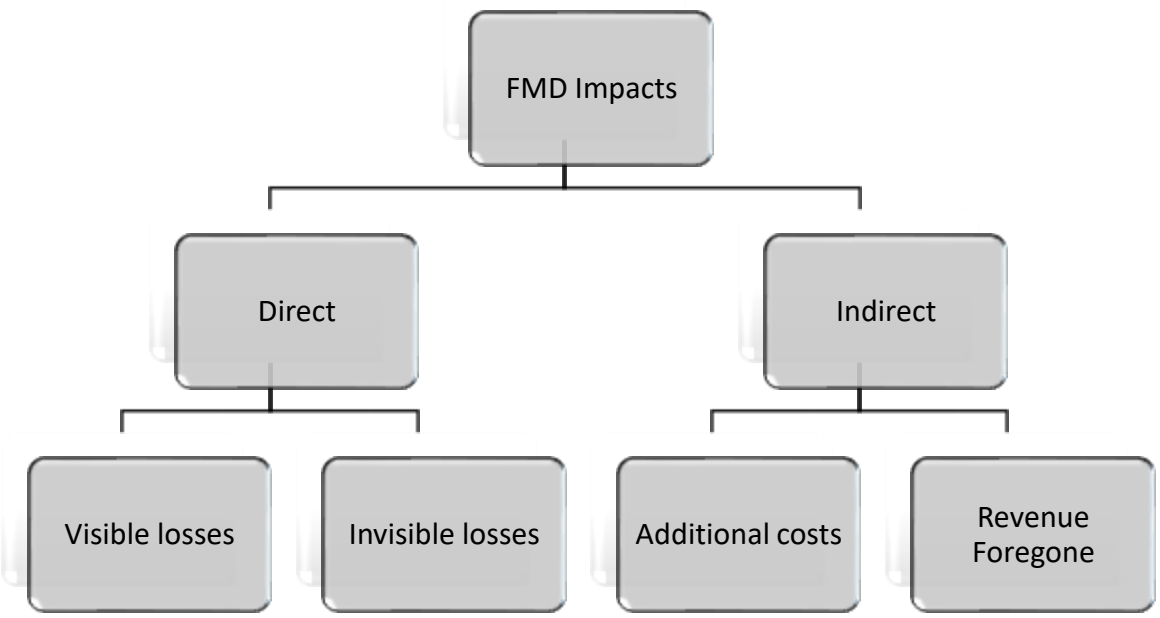


Fig. 2: Framework of economic impact of FMD (adapted from Rushton, 2009)

Model inputs

Model input variables used to estimate the economic impacts of FMD are in **Table 1**. Data used to create input variables are based on the following information: the structure of the population in a FMD outbreak, the clinical impact of FMD at outbreak level and the costs of FMD (morbidity, mortality and costs of FMD vaccination).

Table 1: Model inputs and output* to estimate the economic impacts of FMD in cattle and the costs of the vaccination

Inputs and outputs	Value	Unit	@Risk function	Description and/or source
Structure of the population in a FMD outbreak				
Number of bovines per outbreak (1)	82.17	Heads	=Risk Pert[1] (23;55;250)	Inputs (1) to (5) Derived from FMD outbreak
Proportion of cows in the outbreak (2)	0.25	Heads	Fixed	investigation study (Souley Kouato *et al.*, 2017)
Proportion of heifers in the outbreak (3)	0.34	Heads	Fixed	
Proportion of bulls in the outbreak (4)	0.17	Heads	Fixed	
Proportion of young bulls in the outbreak (5)	0.24	heads	Fixed	
Clinical impact of FMD at outbreak level				
Morbidity per outbreak (6)	52.33	heads	=Risk Pert (4;15;250)	This study
Mortality per outbreak (7)	4.33	heads	=Risk Triang[2] (1;1;11)	This study
Costs of FMD				
a) Morbidity (only milk losses were considered)				
Number of cows (8)	20.54	heads	= (1) * (2)	Calculation
Number of liters of milk per day (9)	2.22	liter	=Risk Uniform[3] (2;2,44)	Vias et al., 2003
Duration of illness (10)	10.50	days	=Risk Uniform (7;14)	OIE, 2012
Price per liter (11)	0.35	euros	=Risk Uniform (0,34;0,36)	Boukary et al., 2007
Cost of milk losses (12)	-	euros	=Risk Output (12)+ (8) * (9) * (10) * (11)	Calculation
b) Mortality (only young animals were considered)				
Number of young bulls affected (13)	1.04	heads	= [(7) * (5)]	Calculation
Number of heifers affected (14)	1.47	heads	= [(7) *(3)]	Calculation
Price by young bulls (15)	207.00	euros	=Risk Pert (152;210;250)	CountrySTAT (FAO) Niger, 2017
Price by heifer (16)	176.33	euros	=Risk Triang (152;152;225)	CountrySTAT (FAO) Niger, 2017
Costs of young bulls died (17)	-	euros	=Risk Output (17) + (13) *(15)	Calculation
Costs of heifers died (18)	-	euros	=Risk Output (18) + (14) * (16)	Calculation
Total costs of FMD at herd level (C_{FMD})	-	euros	=Risk Output (C_{FMD}) + (12) + (17) + (18)	Calculation

Costs of vaccination (inactivated vaccine with 3 serotypes)				
Price per doses of FMD vaccine (19)	1.60	euros	Fixed	Anonymous (BVI)
Cost of vaccine delivery, distribution and cold storage (based on experience for CBPP vaccination) (20)	0.33	euros	= Risk Pert (0.07;0.12;1.42)	Anonymous (MAG/EL, 2017)
Costs for the FMD vaccination of one outbreak (2 doses/animals) (C_{VACC})	-	euros	=Risk Output (C_{VACC}) + [(1) *2 * (19)] + [(1) *2*(20)]	Calculation
Ratio Costs of FMD / Costs of vaccination at outbreak level (R)	-		=Risk Output (R) + (C_{FMD})/ (C_{VACC})	Calculation

Legend: [1]Pert distribution includes minimum, most likely, and maximum parameters. Values around the most likely are more likely to occur. It can generally be considered as superior to the Triangular distribution when the parameters result in a skewed distribution. [2]Triangular distribution includes minimum, most likely, and maximum parameters. [3]Uniform distribution in which all values have an equal chance of occurring, it includes the minimum and maximum parameters. * Output was indicated as Risk Output in the column of @Risk function. [#] 2 doses per animal (inactivated vaccine).

Structure of the cattle population

The structure of the cattle population in an outbreak of FMD (number of cattle per outbreak, proportions of cows, heifers, bulls and young bulls in the outbreak) were extracted from a study on FMD outbreaks which occurred in 2014 in south-western of Niger (Souley Kouato *et al.*, 2017).

Clinical impacts and associated costs of FMD at outbreak level

The number of sick animals as well as the number of dead animals recorded during each FMD outbreak were included in the overall data used for this study. However, because of the fact that these variables are included in the case definition, they were not considered in the regression analysis. Nevertheless, they were used to analyse the clinical impacts and associated costs of FMD in Niger at herd level. Indeed, in this study, the costs of FMD include the cost due to the morbidity (i.e. the loss of milk production) and the cost due to the mortality of young animals. In this analysis, heifers and young bulls were considered as young cattle susceptible to die from acute FMD. Indeed, data on prices of heifers and young bulls were available in the FAO databases used (http://www.countrystat.org/home.aspx?c=NER). The prices per litre of milk and the average daily milk production per cow (in the rainy season, the dry and cold season and the dry and hot season) were extracted from studies carried out in Niger respectively by Boukary et al., (2007) and Vias *et al.*, (2003). The duration of acute FMD illness was considered to be between 7 and 14 days (OIE, 2012).

Costs of vaccination (scenario using an inactivated vaccine with 3 serotypes)

In Niger, vaccination against CBPP is annual and mandatory for all cattle over 6 months of age. Other vaccinations of cattle as against pasteurellosis, anthrax, and blackleg disease are optional. FMD vaccination strategy considered as preventive mass vaccination strategy (PMVS) would be similar to that of Contagious Bovine Pleuropneumonia (CBPP) with some differences. For the PMVS, it is assumed that all cattle above 4 months of age are vaccinated. An initial double vaccination with a 4–6 weeks interval is considered, followed by an annual vaccination until the incidence of the disease becomes less than 5% after which the strategy would be re-adopted to maintain the incidence at this level. A trivalent vaccine (with serotypes A, O and SAT2) supposed to match with the circulating field strains, was assumed to be used in the country. The data of the cost of the vaccine was provided by the Botswana Vaccine Institute laboratory which manufactures and provides this vaccine to some west African countries neighbouring Niger. The vaccine cost is 159.60 euros per 100 doses, so 1.596 euros per dose.

The vaccine delivery costs per animal, distribution and cold storage based on the experience of the CBPP vaccination campaign, were also included in the assessment of the total costs of vaccination. At the time of this study, there was no official FMD vaccination program in Niger. FMD infected cattle are either treated with antibiotics or by traditional means or not treated at all. Data on the costs of vaccination against CBPP were provided by the Ministry of Agriculture and Livestock.

Table 2 reports the estimated costs of vaccination campaign implementation in each region of Niger based on the CBPP vaccination experience. Indeed, for the 2016-2017 vaccination campaign, Niger imported CBPP vaccines from Ethiopia (Anonymous, MAG/EL, 2017). To determine the part of the cost of the vaccine per animal in the total budget allocated for each region, estimates are made taking into account the respective cattle population. The cattle population for each region in 2016 was estimated based on the results of the last general census of agriculture and livestock in 2007. Hence, an annual growth rate of 1.06 has been applied for each year since 2007. For CBPP vaccination, an objective of 80% of the cattle population was considered to be vaccinated. Total required number of vaccine doses was estimated as the sum of 80% of the cattle population and 5% of this latter number (considering the possible losses of vaccine in the field during the vaccination process).

Table 2: Estimation of vaccination campaign implementation costs (based on current CBPP vaccination program 2016-2017)

Region	Cattle population (estimates for 2016) **(a)** (head)	Number of cattle to be vaccinated **(b)** (head)	Vaccine doses required **(c)**	Vaccine cost **(d)** (FCFA)	Overall budget **(e)** (FCFA)	Part of the vaccine cost in the overall budget **(f)** (%)	Cost of vaccine distribution, delivery and cold storage **(g)** (euros)	Vaccine cost by animal (euros) **(h)**
Agadez	99.383	79.506	83.481	4.257.531	78.051.005	5.45	112.497	1.41
Diffa	1.425.179	1.140.144	1.197.151	61.054.701	149.559.400	40.82	134.925	0.12
Dosso	1.336.658	1.069.327	1.122.793	57.262.443	153.015.302	37.42	145.974	0.14
Maradi	1.914.002	1.531.202	1.607.762	81.995.862	152.141.425	53.89	106.936	0.07
Tahoua	2.428.403	1.942.722	2.039.858	104.032.758	224.130.512	46.42	183.088	0.09
Tillaberi	2.618.909	2.095.127	2.199.883	112.194.033	312.213.249	35.94	304.927	0.15
Zinder	2.741.712	2.193.369	2.303.037	117.454.887	212.795.965	55.20	145.347	0.07
Niamey	58.297	46.637	48.969	2.497.419	16.202.000	15.41	20.892	0.45
National	12.622.543	10.098.035	10.602.937	540.749.787	1.298.108.858	41.66	1.154.586	0.11

Legend:
(b) = 80%* (a); (c) = (b*1.05); (d) = (45+6) * (c). The vaccine was purchased at 45 FCFA per dose plus 6 FCFA for the dilution solution; (f) = (d) *100 / (e); (g) = ((e) – (d)) /655.957); one euro corresponds to 655.957 FCFA (XOF - CFA Franc); (h) = (g) / (b).

Model Development

The spreadsheet with economic model was constructed in Microsoft Excel (Microsoft® Office 2007, Redmond, WA). The model was run for 10,000 iterations (Monte Carlo sampling) in @Risk version 7.5 (© Palisade Corporation, Ithaca, NY). This allowed the convergence of all the output probability distributions using a 1.5% convergence tolerance with 95% confidence level. The sensitivity analysis was performed by means of the sensitivity analysis tool in @Risk version 7.5. Hence, probability density and tornado graphs were produced using the same software.

Sensitivity Analysis

To identify those inputs which were more influential on the final outputs, a sensitivity analysis was carried out using the rank order correlation method, which is based on the Spearman rank correlation coefficient calculations. With this analysis, the rank correlation coefficient is calculated between the selected output variable and the sampled values from each of the input distributions.

RESULTS

Spatiotemporal distribution of outbreaks

From 2007 to 2015, 791 FMD outbreaks were reported from the 8 regions of Niger, with the number of outbreaks per region ranging from 5 to 309 **(Fig. 1)**. The regions where outbreaks were less recorded were the regions of Agadez in the north and Diffa in the far south of the country. The most affected regions are those of Dosso, Zinder and Tillabery. Although, the geographical distribution of outbreaks varies according to the year, FMD-affected districts were mainly located at the borders of neighbouring countries, especially districts in the southwest bordering Benin and in the south-centre of the country bordering with Nigeria. The geographical distribution of outbreaks according to the year is provided in **Appendix 1 (summarize the findings from those plots here)**.

Although each year there were more than 50 FMD outbreaks, the number of reported outbreaks varied over the study period. During 2007 and 2015, the number of outbreaks were high (126 and 161, respectively) compared to the rest of the years **(Fig. 3)**. This number decreased from 2007 to 2009 after which it remained relatively stable up to 2013 with a small peak in 2011. The incidence of reported outbreaks then increased steeply from 2013 to 2015.

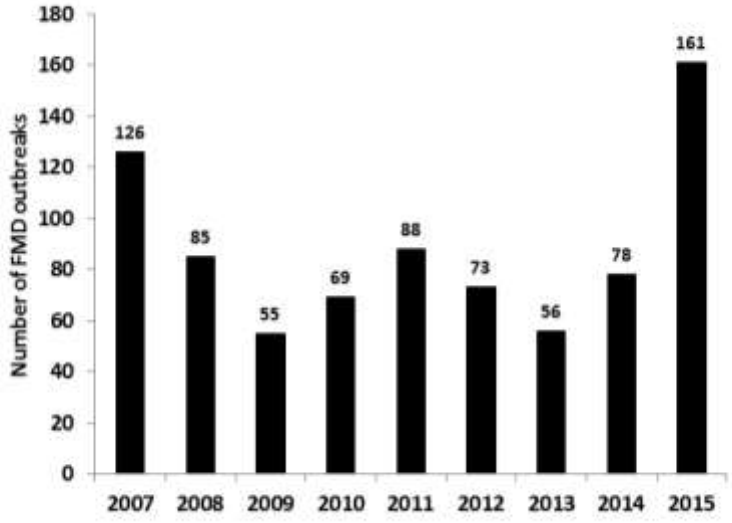


Fig. 3: Annual distribution of reported clinical FMD outbreaks in Niger during the period 2007-2015

There is an important monthly variation in the occurrence of FMD outbreaks. Indeed, a high number of outbreaks were recorded in January and February. The number of FMD episodes was low from March to August with a modest peak in May. From September to December, the number of outbreaks increased significantly **(Fig. 4)**. This monthly trend was confirmed by the multivariate regression model, which revealed that the months at risk were January and February and from September to December. In Niger, this period corresponds with the end of the rainy season (September) and with the cold dry season (October to January or February).

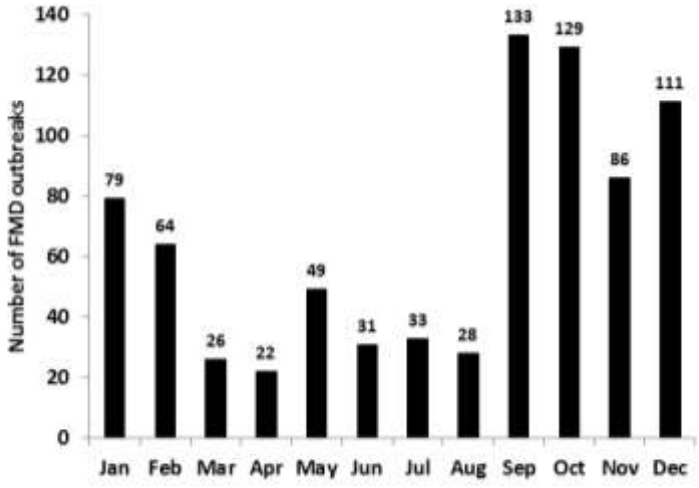


Fig. 4: Monthly trend of clinical FMD outbreaks from 2007 to 2015

The regression tree analysis revealed that 3 regions (Dosso, Tillabery and Zinder), the months (September to December and January), the years (2007 and 2015) and in addition the density of animal contacts were the main predictors of FMD occurrence in Niger **(Fig. 5 and Table 3)**.

Table 3: Relative importance of the different FMD predictors obtained after regression tree analysis (maximum relative importance = 100)

Predictor	Variable importance
Region	100
Density of contacts	75.86
Density of sheep	65.12
Density of goats	55.24
Year	48.15
Density of cattle	28.33
Month	20.01

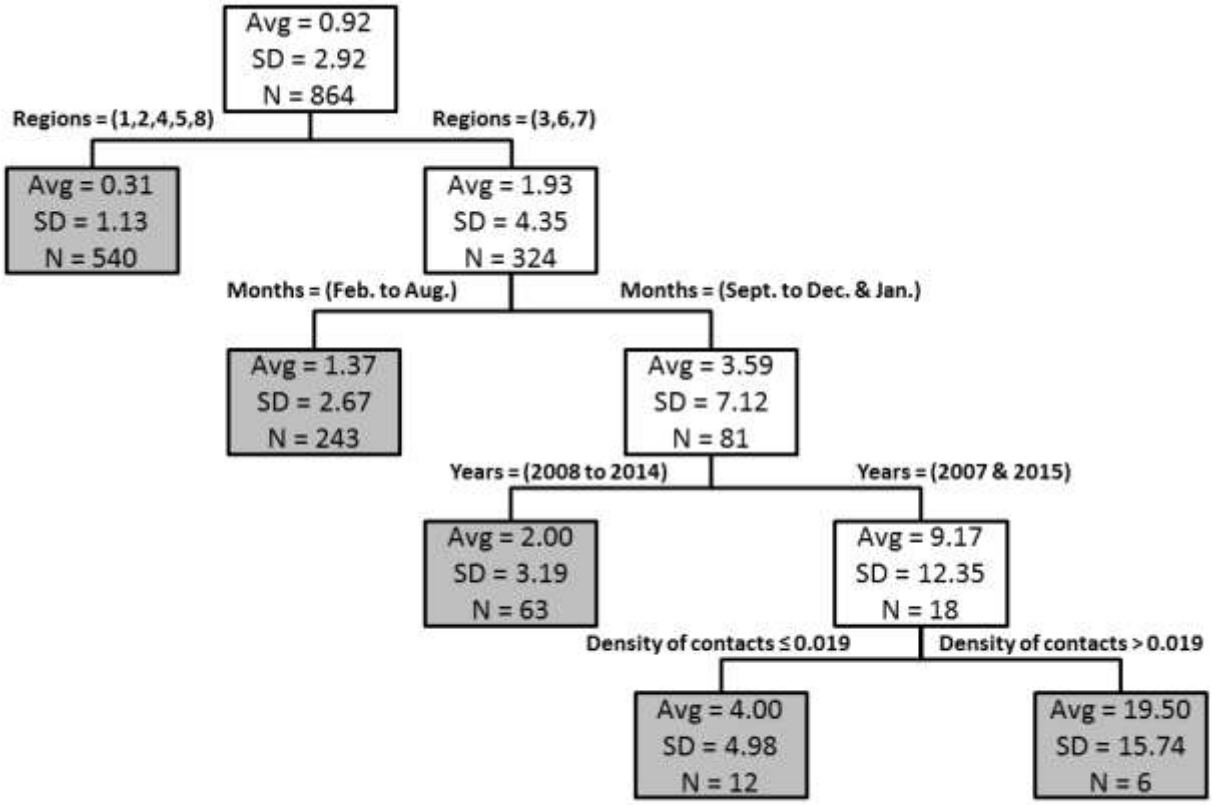


Fig. 5: Regression tree analysis for the main significant variables and their interactions on the incidence of reported FMD outbreaks

Legend: Avg: Average of FMD cases; SD: Standard deviation; N: Number of observations; Region 1: Agadez, Region 2: Diffa, Region 3: Dosso, Region 4: Maradi, Region 5: Tahoua, Region 6: Tillabery, Region 7: Zinder, Region 8: Niamey; Month: Jan, Feb, Aug and Sept for January, February, August and September, respectively.

Stochastic estimation of the economic impact of FMD

Clinical impacts and estimated production losses due to FMD

During the 791 FMD outbreaks recorded during the study period, 8,804 cattle were clinically affected and among these 247 animals died from the disease. **Fig. 6** shows the yearly variation in the number of sick animals with peaks in 2008, 2012, 2013 and especially in 2015. The mortality rate appeared to be stable during the study period, although the number of dead animals was relatively high in 2007 (n=36) and in 2015 (n=51). However, at outbreak level, the mean stochastic estimates were respectively 52.33 cattle affected by the disease and 4.33 cattle assumed to die from FMD **(Table 1)**.

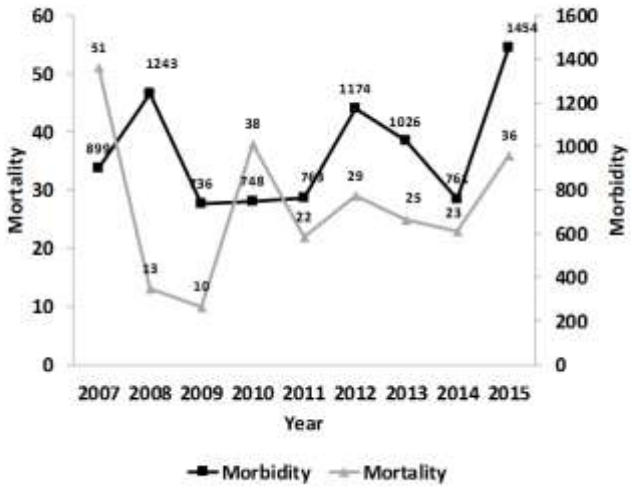


Fig. 6: Trends of FMD morbidity and mortality between 2007 and 2015

Table 4 summarizes the results of the Monte Carlo simulations estimating the economic impacts of FMD at outbreak level. The average total costs of FMD at herd level (C_{FMD}) were estimated at 732.72 euros (S.D. 322.01 euros). The cost of mortality of young bulls was the largest portion of the total costs, contributing for 41.55% (average: 304.45 euros; S.D.: 169.16 euros), while costs related to heifer mortality and reduced milk production were respectively 35.36% (average: 259.06 euros; S.D.: 144.25 euros) and 23.09% (average: 169.20 euros; S.D.: 85.93 euros) of the total costs of FMD at outbreak level.

FMD vaccination costs

The average cost of implementing vaccination in the field was estimated at 0.30 euro per vaccinated animal (median of 0.12). Although an important variation of this cost was observed from one region to another, the highest costs were observed for the regions of Agadez (in the north of the country) and Niamey (capital city) with 1.41 and 0.45 euros per vaccinated cattle, respectively **(Table 2)**.

To estimate the cost of vaccination at FMD outbreak level (C_{VACC}), one scenario was considered. It consists in vaccinating each animal with 2 doses of the vaccine (one primary dose and a second one after 4 to 6 weeks of interval). Moreover, in this simulation, it was assumed that FMD vaccination have been carried out during a campaign devoted exclusively to vaccination against FMD rather than being part of a vaccination program against other livestock diseases such as CBPP. Thus, the costs of vaccination at FMD outbreak level (C_{VACC}) was estimated at 315.27 euros on average at herd level. Consequently, the average ratio total costs of FMD/ costs of vaccination at outbreak level (R) (C_{FMD} / C_{VACC}) was estimated at 2.31 **(Table 4)**.

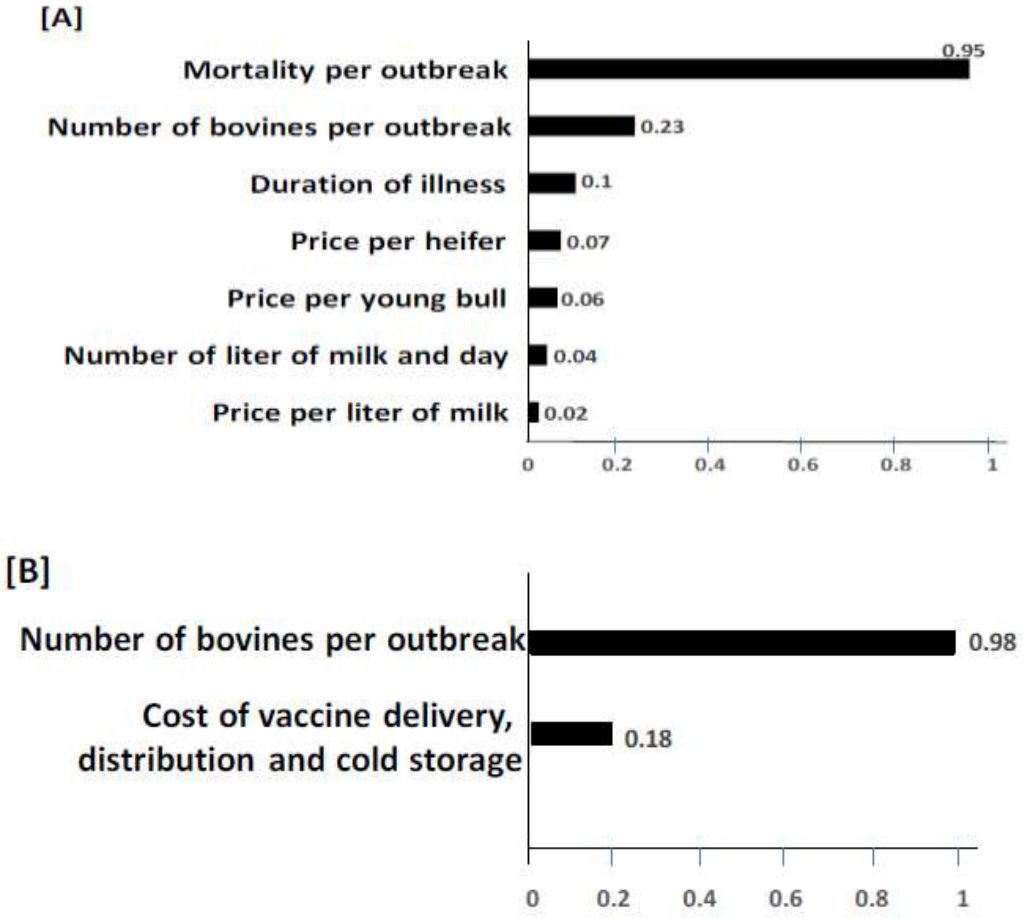
Table 4: Results of Monte Carlo simulations estimating the economic impacts of FMD at outbreak level (expressed in euros)

Outputs	**Minimum**	**Maximum**	**Mean**	**Standard Deviation**	**Median**
Costs for milk losses	32.16	662.24	169.20	85.93	151.67
Costs for young bulls died	60.30	879.72	304.45	169.16	272.79
Costs for heifers died	51.97	787.42	259.06	144.25	232.32
Total costs of FMD at herd level (C_{FMD})	171.82	1821.66	732.72	322.01	681.24
Costs of FMD vaccination of one outbreak (2 doses/animal) / Value (C_{VACC})	80.95	1139.96	315.27	148.55	289.34
Ratio Costs of FMD / Costs of vaccination at outbreak level / Value (R)*	0.49	15.87	2.31	1.80	2.29

Sensitivity analysis

Fig. 7 A, B and C show tornado graphs with the inputs that accounted for the greatest variation in the outputs of the model. The most influential input parameter (i.e. with the highest rank order correlation coefficients) on the total costs of FMD (C_{FMD}) at herd level, was the mortality per outbreak which had a correlation coefficient greater than 0.9. The number of affected cattle per outbreak also showed a relative high correlation with C_{FMD} and the stage of FMD infection

in relation to the duration of illness **(Fig 7 A)**. Likewise, the mortality per outbreak and the number of affected cattle per outbreak, were the two input variables to which the Ratio C_{FMD} / C_{VACC} was most sensitive, based upon Spearman rank correlation coefficients. Indeed, the number of affected cattle per outbreak significantly influenced the cost of vaccination per FMD outbreak (C_{VACC}) with a correlation coefficient greater than 0.9 **(Fig 7 B**) accordingly with increase in the number of affected cattle, the ratio would change significantly **(Fig. 7 C)**.



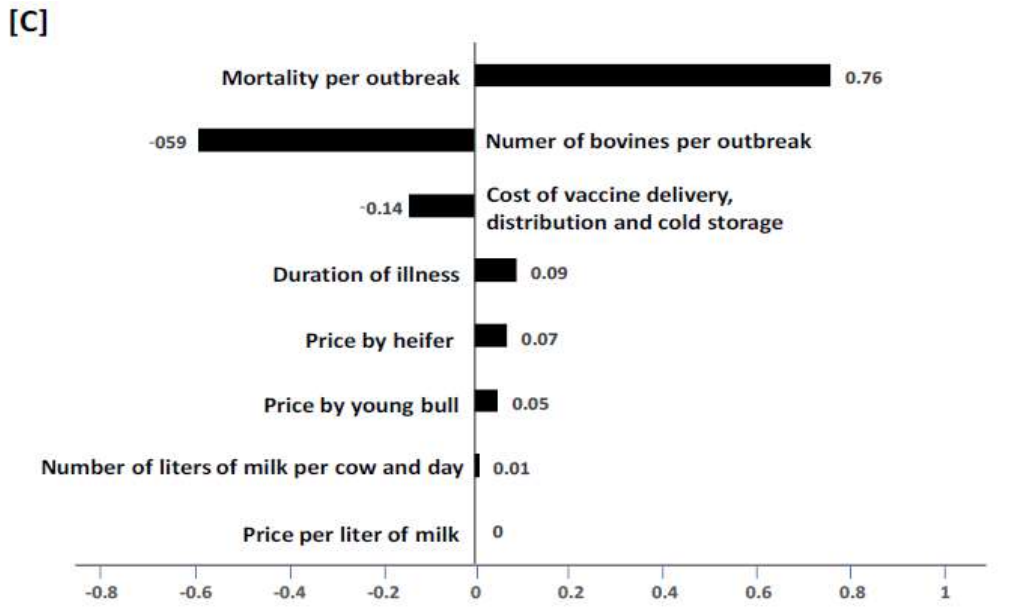


Fig. 7: Tornado graphs showing correlation coefficients between model input variables and the total costs of FMD [A], costs of FMD vaccination of one outbreak [B], and Ratio Costs of FMD / Costs of vaccination at outbreak level [C]

DISCUSSION

This study was performed with an overall objective of generating epidemiological information and economic estimates of FMD in Niger to support decision making in a future control plan. Initially, a spatio-temporal analysis of reported clinical FMD was conducted. Several FMD outbreaks were recorded in Niger for about a decade. This study obviously illustrated that the occurrence of FMD is more frequent and more widespread through regions than generally accepted. Indeed, only the semi-desert areas including Agadez and Diffa were less affected by FMD, although the farmers or the veterinary officers must consider this cautiously because of the fact that in Niger the notification of the disease is not always performed. From the results, certain areas were more prone to FMD outbreaks. Accordingly, the results of the regression analysis showed that regions with a high risk of occurrence of FMD were the regions of Dosso, Tillabery and Zinder. These 3 regions account for more than half (53%) of the country's livestock population when considering the projections made for the livestock population in 2015. It was therefore expected that the animal density would be an important predictor variable of outbreaks occurrence as it is indicated by the regression tree analysis. In accordance with the transboundary nature of the disease (Balinda *et al*., 2010; Knowles *et al*., 2016; Ludi *et al*., 2016), FMD has been mostly recorded in districts bordering with neighbouring countries, in particular with Benin and Burkina Faso in the south-west; Mali in the west; and with Nigeria

in the south of the country **(Fig. 1)**. This would be related to one of the livestock systems prevailing in Niger, characterized by the practice of both internal and cross border transhumance consisting in long distance animal movements in search of better feeding conditions in neighbouring countries. This study is with some respect in agreement with that of Couacy-Hymann *et al.,* (2006), which identified among others the regions of Niger bordering with Nigeria, Chad and Mali and the park W area (which is at the junction between Benin, Burkina Faso and Niger) as primary sources of infection of FMD in West Africa.

This retrospective study showed also that in Niger, FMD occurs almost everywhere but also at any time period of the year indicating that the disease is endemic all over the country. However, from the study of the monthly occurrence of the outbreaks it appeared that most outbreaks occurred during the cold and dry season (from October to January) and started at the end of the rainy season (September). The seasonality of FMD in Africa and elsewhere has been reported by several studies (Molla & Delil, 2015; Genchwere & Kasanga, 2014; Bayissa *et al*., 2011; Dukpa *et al*., 2011; Rufael *et al*., 2008; Bronsvoort *et al*., 2003) even though the eco-climatic conditions differ from one region to another. However, in the case of Niger this is undeniably related to the livestock system. Indeed, transhumance in the Sahel region in general is practiced based on a classical pattern rarely modified and consistent with seasonal cycles. Overall, from November to July (corresponding to the dry season until the beginning of rainy season) herdsmen keep their animals locally to exploit the available pastures. From July to October (corresponding to the rainy season until the beginning of cold and dry season), transhumant herdsmen move with their animals towards the north of the country (pastoral zone) or the neighbouring countries. Consequently, during the dry season there is a high concentration of animals in the south of the country where pastures are more abundant and where the animal can often benefit from agricultural products. Moreover, this high animal density could explain the large number of FMD outbreaks in this period (Allepuz *et al*., 2015; Sumption *et al*., 2008; Shiilegdamba *et al*., 2008).

Initially, the potential risk factors which were investigated included several factors such as rainfall, temperature and humidity, factors promoting contacts between animals including pastoral enclaves, cattle market and herds water crossing points. These factors were initially used because they are predictively related to FMD onset. However, the aim of the use of these factors was not to investigate whether they were risk factors of FMD because, for instance, cattle population is already known to be a risk factor for FMD occurrence (Elnekave *et al*., 2015; Emami *et al*., 2015).

As mentioned above, one of the main purpose of this study was to assess the economic impact of FMD. The epidemiological information presented in this paper is essential to such assessment. However, based on Rushton's (2009) economic impact framework for FMD, most of the required data to achieve these economic analyses, are currently lacking for Niger and consequently only some aspects of the production losses (milk production losses and animal mortality) and the vaccination costs such as indirect impact input variables, were considered in this analysis. Furthermore, in the context of Niger in particular, the influence of these input variables related to livestock production and access to international markets could not be attributed solely to FMD. However, with the available data mostly based on already performed studies, economic assessment was possible using a stochastic modelling approach which allowed to generate a range of model outputs that give insights in the impacts of FMD in the country.

This study revealed a high herd level morbidity of about 50 cattle per outbreak affected by FMD and resulting in a mortality of more than 4 animals per outbreak. However, to get an idea of the percentage of clinical affected cattle (morbidity) and dead animals (mortality), the cattle population structure of the herds investigated during FMD outbreaks that occurred in 2014 (Souley Kouato *et al.*, 2017) has been considered. Therefore, based on 74.43 cattle per herd on average, approximately 67% were sick and about 5% died. Moreover, although high FMD mortality rates are often reported (Grubman, 2004), this mortality rate of 5% could be explained not by solely by FMD but also by other factors such as the possible malnutrition or other infectious or parasitic diseases prevailing in Niger.

The direct consequence of these clinical impacts is the drastic economic losses with an average total cost of 733 euros per outbreak. Although these estimates on FMD costs could be considered as minimum because some variables were not considered (e.g. the draft power losses), this study revealed that FMD infection resulted in important economic losses for a poor country like Niger.

The mean cost of milk losses was estimated at 169 euros per outbreak in Niger. Lyons (2015) and Barasa, (2008) showed also that milk yield decreased due to FMD. In Niger, livestock breeding and particularly milk production play a major role in poverty alleviation and economic growth (Boukary *et al*., 2007). Indeed, in peri-urban dairy farms, the daily milk production consists of two parts, namely a sold fraction of 62% of the daily milk production and 38% for self-consumption (Vias *et al*., 2003). Hence, these estimates highlighted the considerable

impacts of FMD on rural communities due to the reduced income of households from dairy sale as well as the negative effects on human nutrition.

Despite these adverse consequences of FMD in Niger, there is no control and prevention plan yet for FMD. Although, FMD eradication seems not to be realistic at short time, especially in the context of Sahel countries including Niger, it will be economically beneficial to protect livestock by vaccination (James & Rushton, 2002; Orsel & Bouma, 2009). Results of the economic assessment from this paper revealed that the mean price for FMD vaccination of one outbreak was more than 315 euros. However, it would be beneficial to vaccinate because the costs related to the losses due to the disease (733 euros) is more than 2 times higher than that of the costs of the vaccine. The costs of vaccination were variable from region to region, probably influenced by different factors. For instance, the estimated vaccine costs per animal (**Table 2**) were much higher for the region of Agadez (in semi desert area) and for Niamey. The region of Niamey, likely because of its position as capital of the country, has a relatively smaller cattle population than the other regions and consequently the allocated budget for the vaccination is lower than that of the rest of the regions. On the other hand, for the region of Agadez, the overall relatively more expensive vaccination costs could be explained by the existence of more long distances between two vaccination centres within the region. However, the overall vaccine cost per animal (0.11 euros) estimated in this study was in some respect in accordance with that of Jemberu *et al*., (2016) in Ethiopia (0.08 euros). Although for Niger the estimated cost of the vaccine was provided by the Botswana Vaccine Institute, the same laboratory where Ethiopia purchased their FMD vaccine, in contrast to the cost calculation of Jemberu *et al*., (2016) the estimations from our study were based on empirical data rather than on expert opinion. Moreover, the empirical data in this study at regional level and the use of a stochastic modelling approach, most likely considered the uncertainty and variability of the input parameters in the analysis (Briggs *et al*., 2012).

On the other hand, it should also be noted that the costs of the vaccine are probably high because it is a multivalent vaccine composed of 3 serotypes (A, O and SAT2). Likely, this vaccination costs could possibly be lower for a monovalent vaccine which has a single serotype prevalent in the field as it was the case during the last FMD outbreak in the southwestern part of Niger where only FMD serotype O was isolated (Souley Kouato, personal communication). Furthermore, in a case where the FMD vaccination would be integrated in the present national vaccination framework, this study demonstrated that this option would allow positive economic returns on the costs of FMD vaccination. Indeed, with this strategy of FMD vaccination

simultaneously applied with that against other transboundary disease such as CBPP, the cost-benefit ratio would be better and therefore economically more profitable. Since these estimates were carried out only for the bovine species, it would be interesting to vaccinate as well other sensitive species, such as small ruminants and pigs.

This study has some limitations that are worth mentioning. One of the shortcomings is that no records on laboratory confirmation of FMD outbreaks could be found in the statistics of the Ministry of Livestock. The only laboratory findings confirming FMD outbreaks are those of the world reference laboratory for FMD (WRLFMD) and recently that of one study performed in Niger (Souley Kouato *et al.,* 2017). However, Morvan *et al.,* (2014) stressed that in Cameroon (another endemic country) estimates reported by herdsmen (clinical surveillance) were comparable to those obtained from serologic testing indicating the high level of awareness about FMD among herdsmen. On the other hand, the constraints to this study are perceived to be related to the disease reporting system. In fact, over the 9-year period of this study, the levels and the reliability of reporting of FMD outbreaks varied from one region to another. For some reports, the only information available was that outbreaks occurred in a specific district. No indication was given regarding the exact location and the number of exposed animals (GPS coordinates). Furthermore, in addition to missed diagnosis, there was underreporting of animal disease in general and especially of FMD. It is therefore likely that some FMD outbreaks could have been missed and were never recorded or reported. This could result in inaccurate estimations of the disease impact. The abovementioned discrepancies resulted in values of predictors that are not always necessarily reflecting actual spatio-temporal patterns of FMD outbreaks. Therefore, the effect of these shortcomings is that the estimates of the associations between the predictors and the outcome may be biased. In addition, in Niger, major issues to account for the continuing occurrence of transboundary animal diseases such as FMD include inadequate monitoring, surveillance and disease reporting, lack of herdsmen awareness, and lack of any controls over animal movements.

However, despite some limitations, this study explored useful epidemiological information to support national decision making related to FMD control. For the first-time, the location and season of all the recorded FMD outbreaks in the country were documented. Additionally, the clinical incidence was statistically estimated at herd level through FMD mortality and morbidity. This study is also the first estimation of the economic impacts of FMD and evaluation of the economic benefits of vaccinating against FMD in Niger. Indeed, the quantitative assessment of this study provides an overview of the significant economic impacts

of the disease when considering the total losses due to animal mortality and reduced milk production. On the other hand, this study reported the temporal and spatial distribution of FMD outbreaks in Niger and highlighted which areas are more susceptible to experience an outbreak. The statistical analysis also showed that higher animal densities were mostly apparent in the dry season and thus increasing the probability of FMD outbreaks. Accordingly, intensive FMD control should be more focused in these high-risk areas, specifically in districts bordering neighbouring countries. Future vaccination programs must also consider the transhumance schedules. The transhumant animals should be vaccinated before and after transhumance. Additionally, the high-risk period, which is the dry and cold season, coincides in Niger with the vaccination of cattle against CBPP. It would be therefore technically appropriate and as mentioned above economically profitable to associate this annual vaccination campaign with that against FMD.

However, given the limitations of the study as discussed above, the suggested approaches may not be conclusive enough and further studies are needed to evaluate the effectiveness of these options. Moreover, for an effective FMD control using vaccination, a thorough understanding of the specific frequency, distribution of FMDV serotypes and subtypes causing the outbreaks is required, highlighting the need of more extensive molecular epidemiology studies. In conclusion, this study will certainly guide further research into the epidemiology of FMD in Niger and will promote a better understanding of the disease. This will accordingly help to set up FMD risk-based surveillance as well as better preparedness for the disease prevention and control. Additionally, for FMD to be efficiently controlled especially in West Africa it is strongly recommended to implement a regional strategy which considers the true epidemiological situation as well as the existing livestock system including transhumance, nomadism and live-animal trade.

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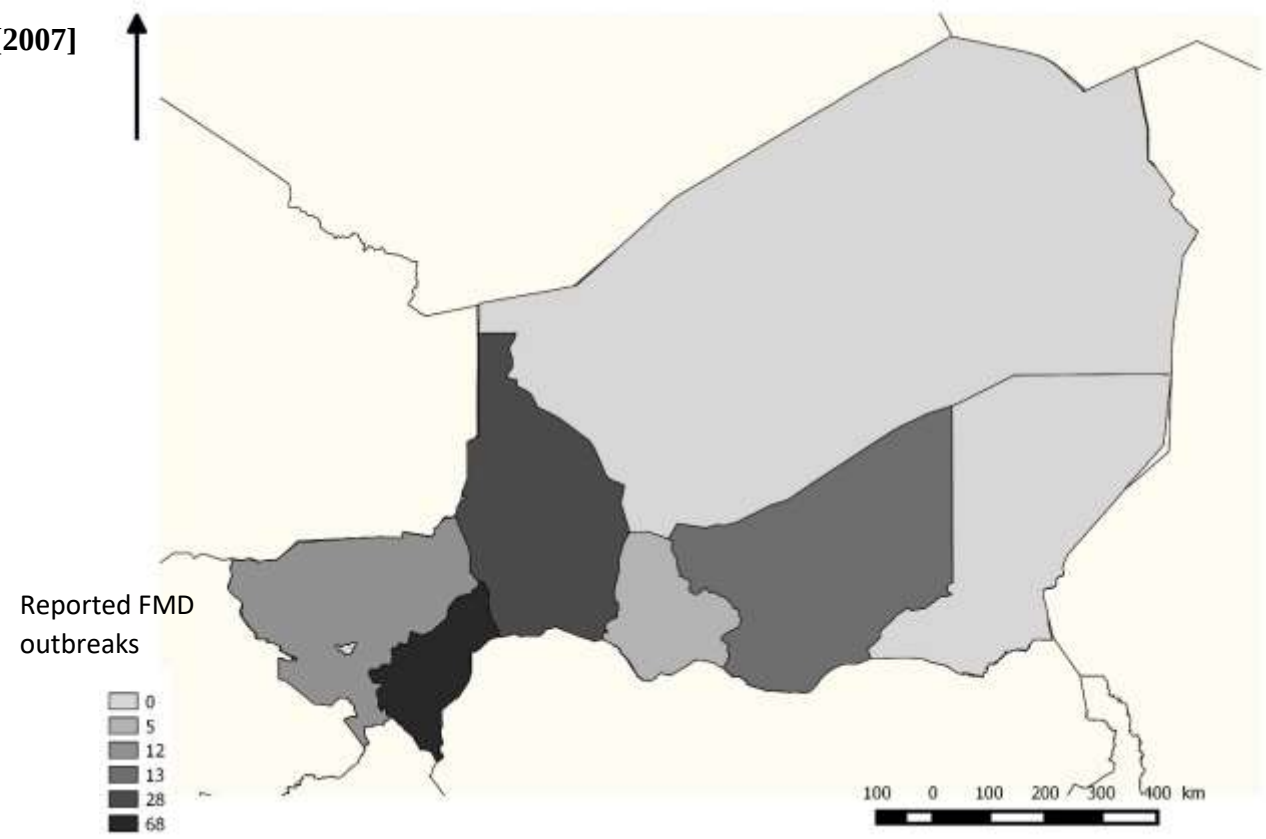
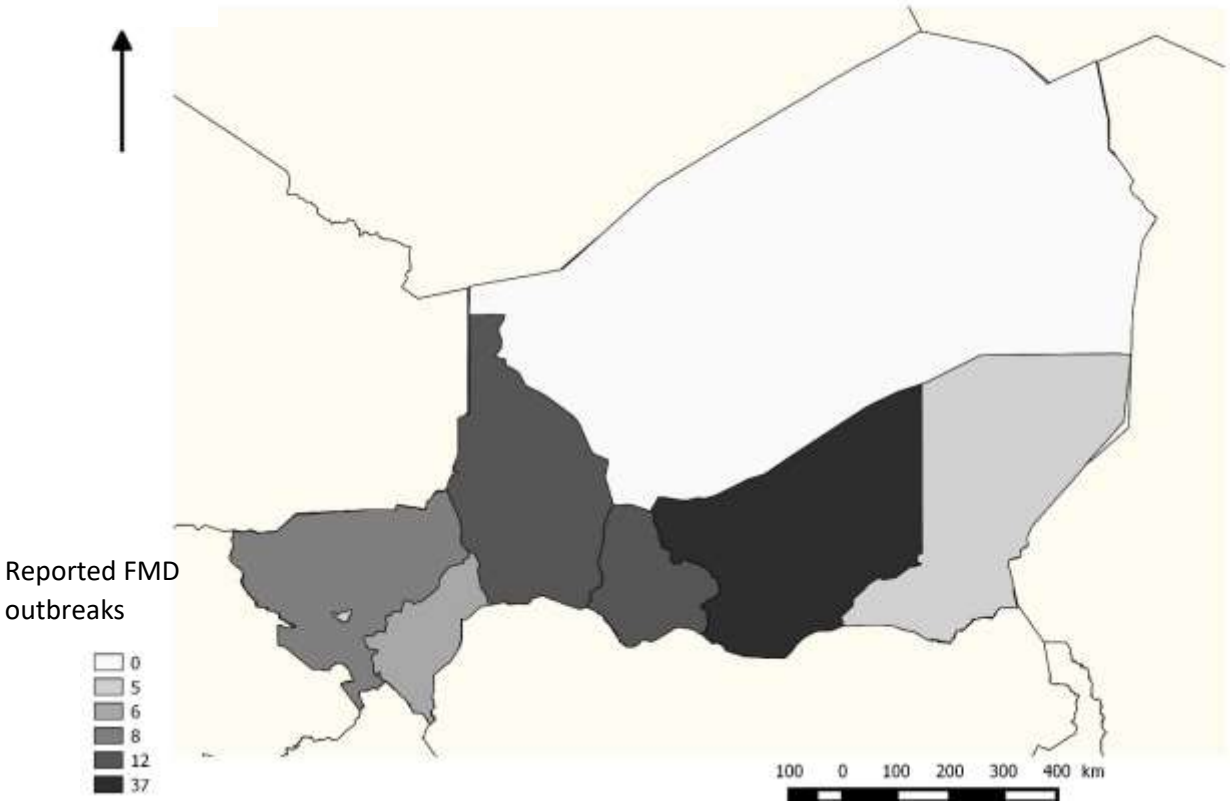
Appendix

Annual spatial distribution of suspected outbreaks of FMD in Niger from 2007 until 2015

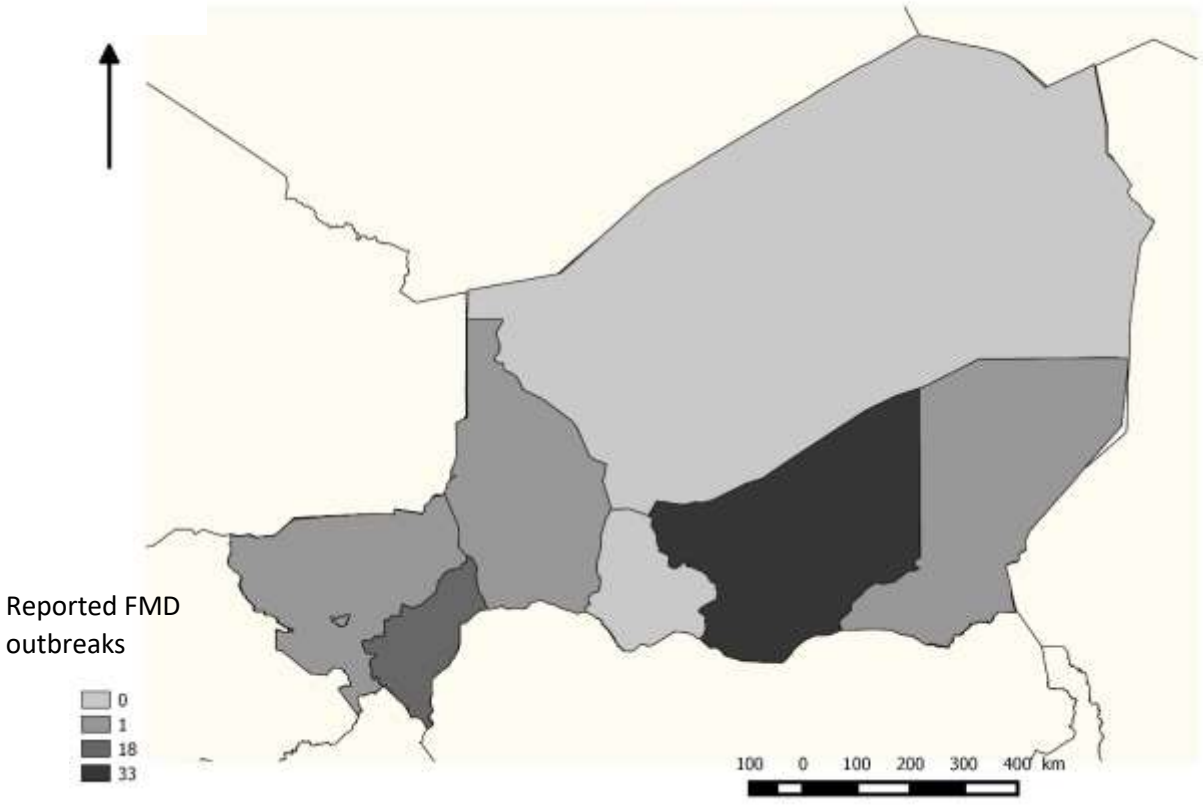
[2007]



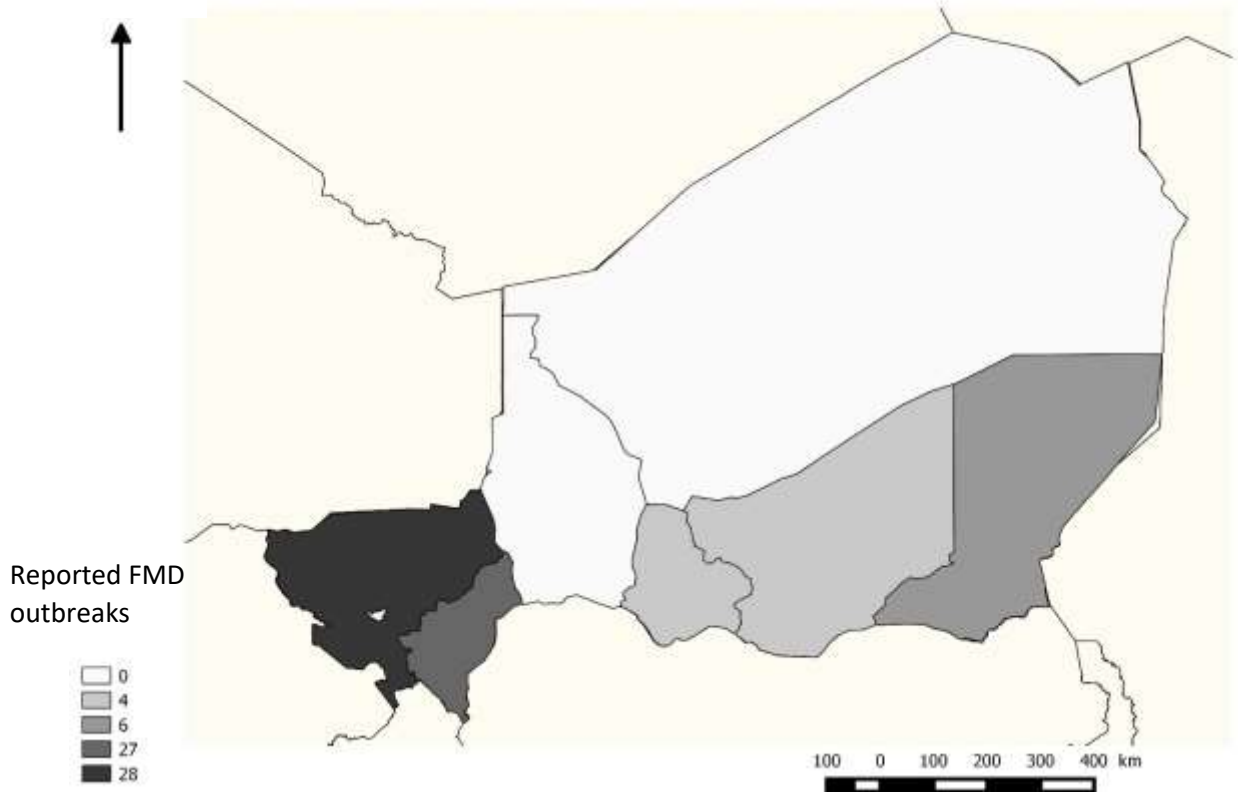
[2008]


Reported FMD outbreaks
0
5
6
8
12
37
100 0 100 200 300 400 km

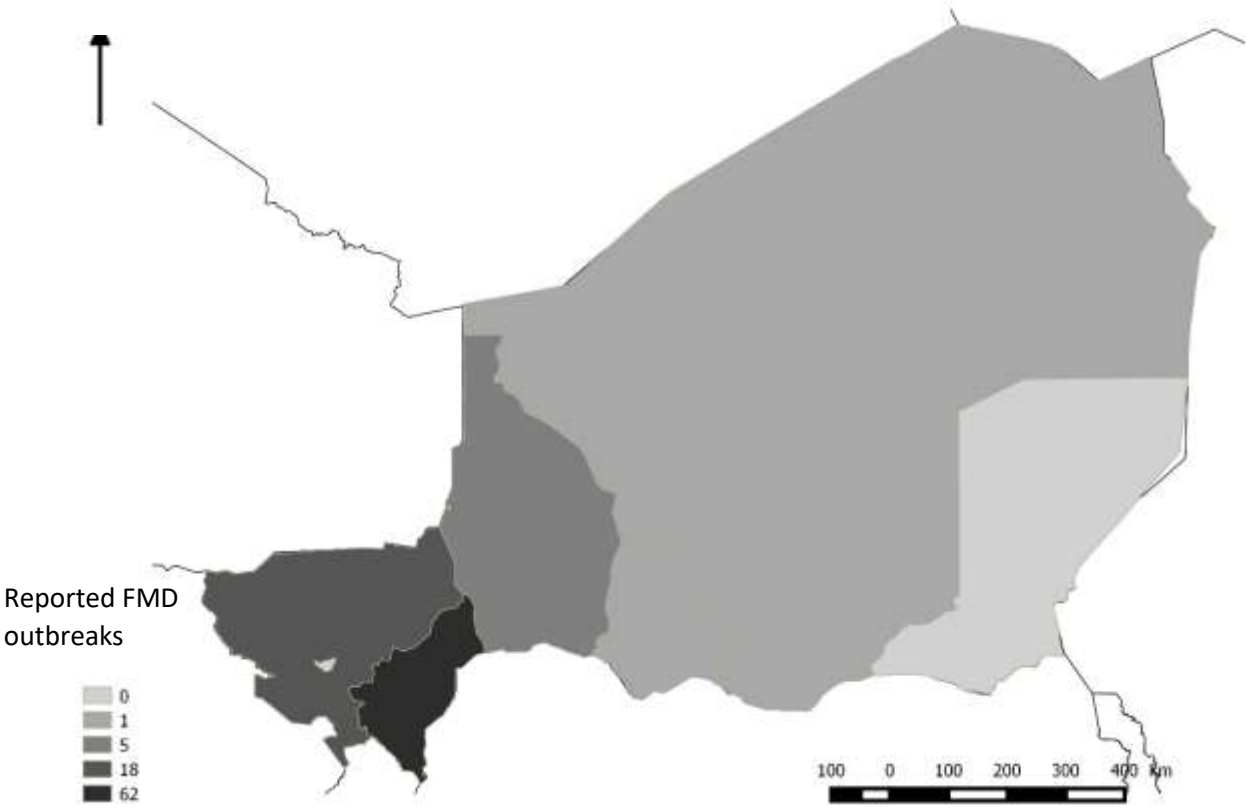
[2009]


Reported FMD outbreaks
0
1
18
33
100 0 100 200 300 400 km

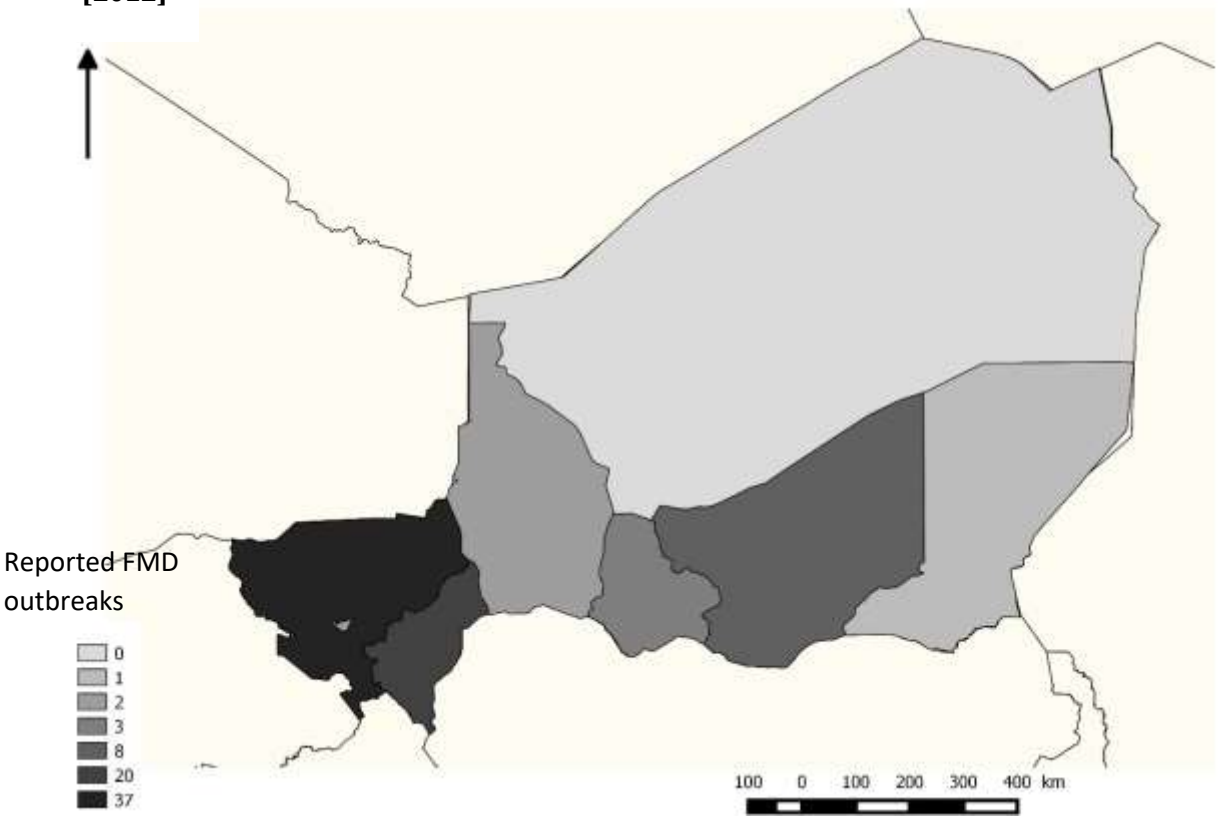
[2010]


Reported FMD outbreaks
0
4
6
27
28
100 0 100 200 300 400 km

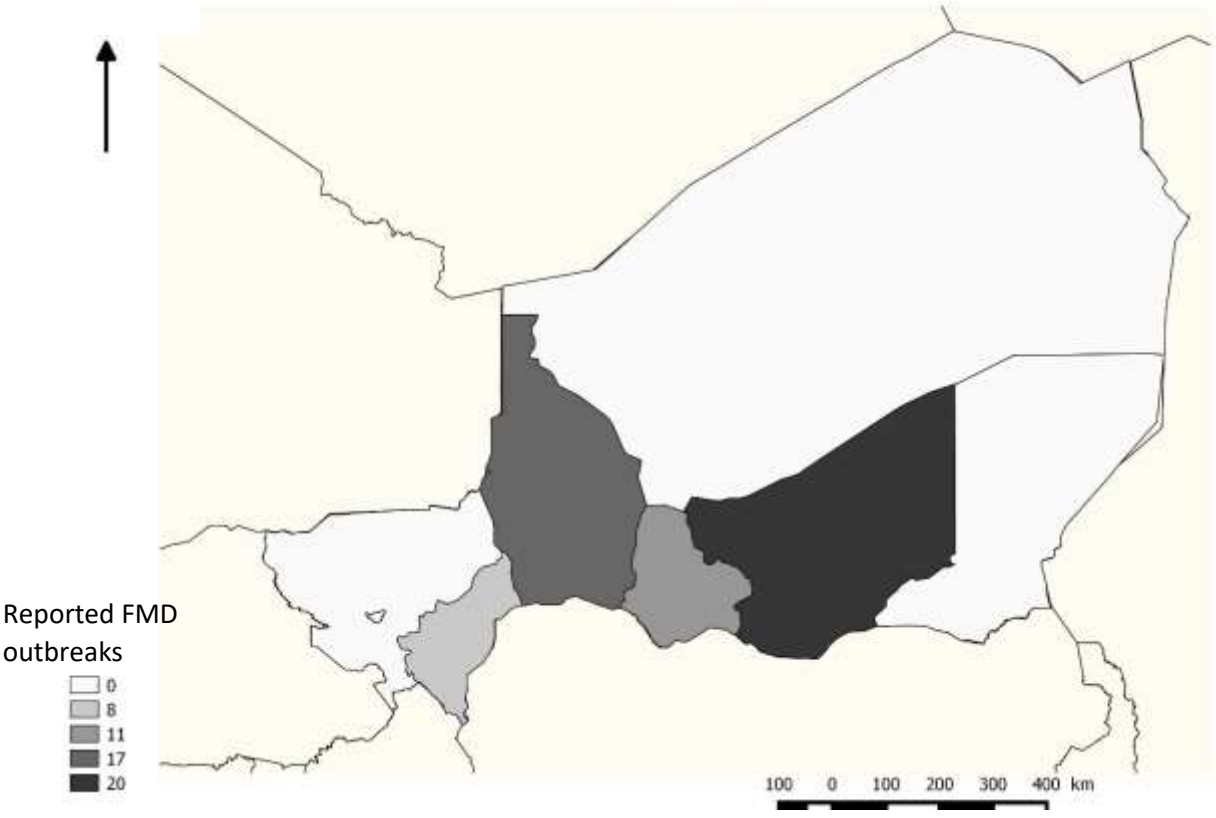
[2011]


Reported FMD outbreaks
0
1
5
18
62
100 0 100 200 300 400 km

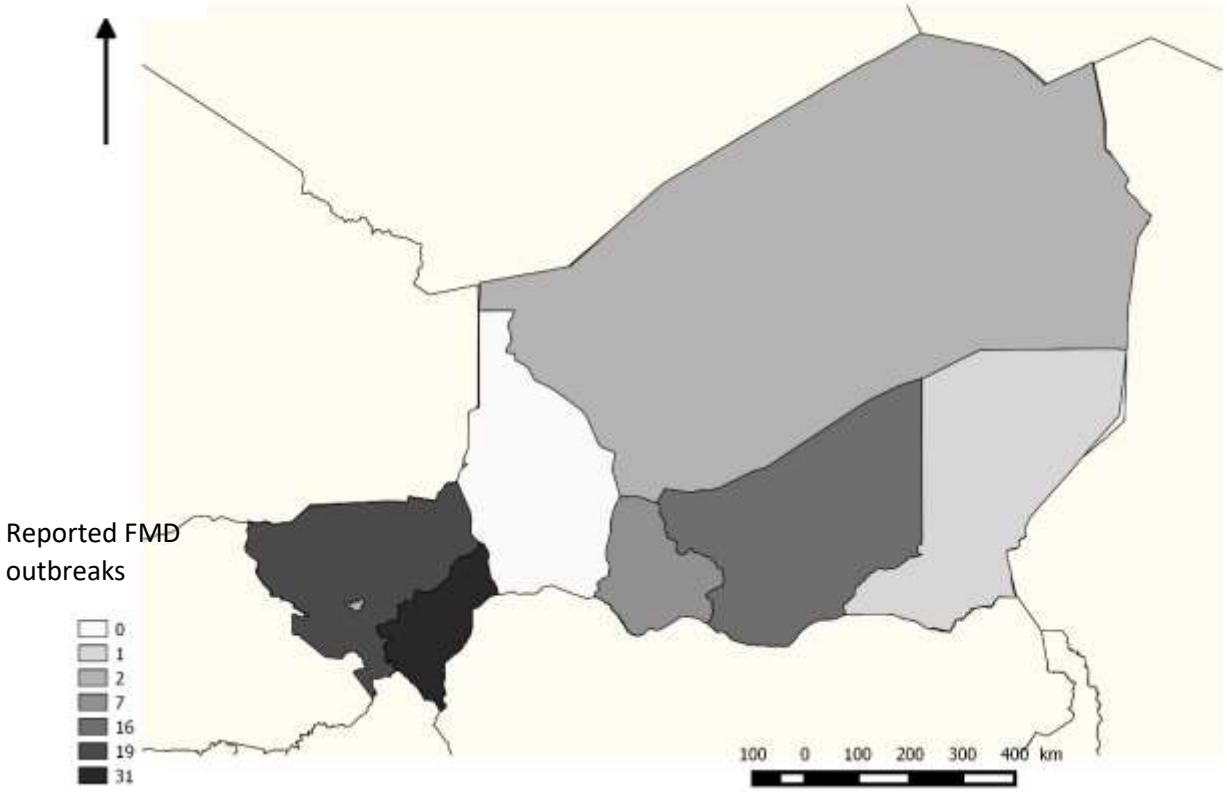
[2012]


Reported FMD outbreaks
0
1
2
3
8
20
37
100 0 100 200 300 400 km

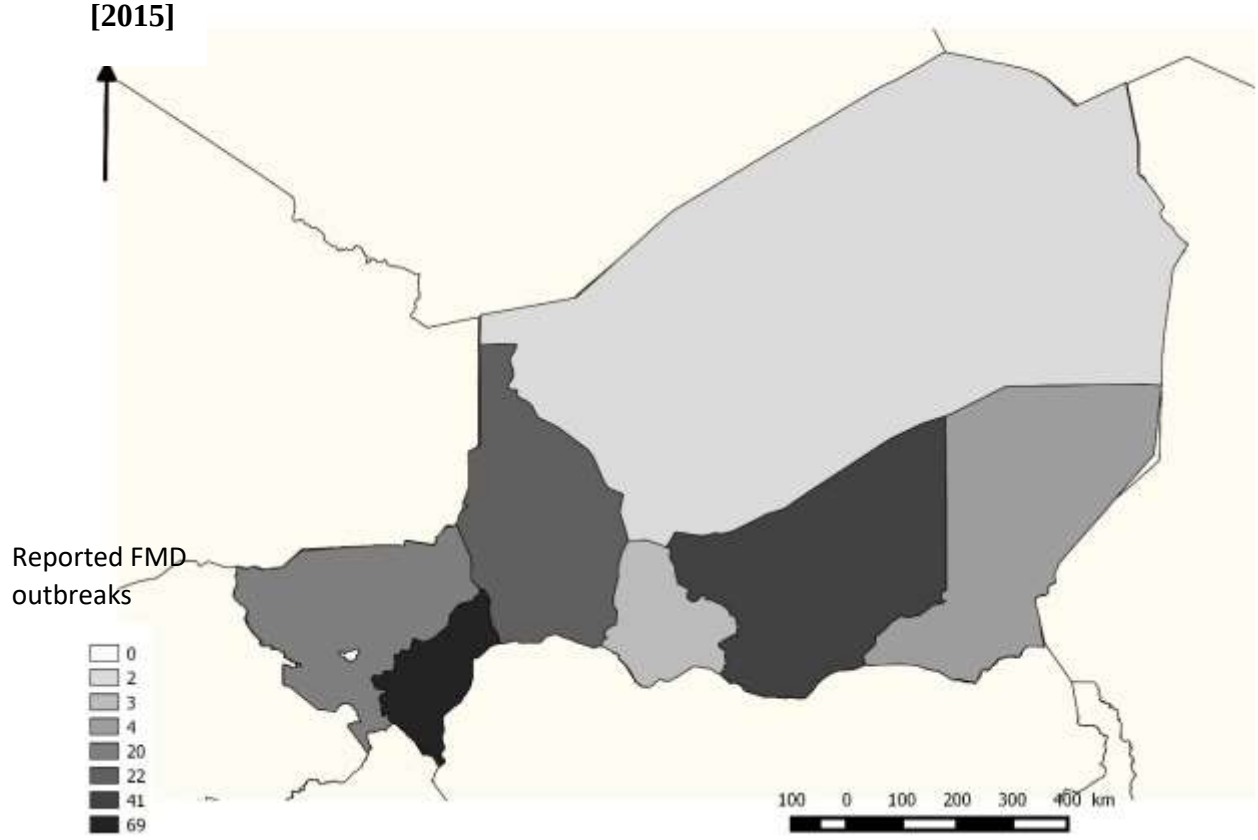
[2013]


Reported FMD outbreaks
0
8
11
17
20
100 0 100 200 300 400 km

[2014]


Reported FMD outbreaks
0
1
2
7
16
19
31
100 0 100 200 300 400 km

[2015]


Reported FMD outbreaks
0
2
3
4
20
22
41
69
100 0 100 200 300 400 km

Part two

Chapter 5: Systematic review of molecular epidemiology of foot-and-mouth disease in Africa: implications for more integrated control and regional strategies

Chapter 5: Systematic review of molecular epidemiology of foot-and-mouth disease in Africa: implications for more integrated control and regional strategies

(Manuscript in preparation for PLOS ONE)

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Abstract

Background: Foot-and-mouth disease virus (FMDV) causes a highly contagious viral disease of cloven-hoofed animals and is one of the most important economic diseases for livestock. There are seven recognized serotypes of FMD which differ in distribution across the world. In the last 20 years, there have been significant advances in the understanding of FMD epidemiology with molecular tools. The purpose of this review is to give an overview of the current knowledge in molecular epidemiology of FMD and some perspectives of integrated control and regional strategies in African countries through a systematic search. **Methodology and principal findings:** The systematic search was conducted following the PRISMA guidelines, mainly using electronic databases but also including additional records obtained from other sources. Based on defined criteria, the identified publications were analysed to select available relevant articles related to molecular epidemiology of FMDV. A total of 124 references were selected and presented in this review, including 57 additional articles from other primarily sources than electronic databases. **Conclusions/Significance:** It was observed that research articles related to molecular epidemiology of FMD in Africa have significantly increased in recent years, especially in the 7 last years (from 2010 to 2017). Most of these studies are based on comparison of VP1 gene sequence. The identification and molecular characterization studies of African FMDV strains have highlighted the complexity of the genetic relationships between strains circulating and/or co-circulating in the African continent. The results of these studies also pointed out the high intricacy of the epidemiology of FMDV in Africa as well as the diversity and transboundary mobility of FMDV. Therefore, there is an urgent need for integrated and regional FMD control strategies with the ultimate target to more effectively prevent or control disease in Africa.

Keywords: Foot-and-Mouth Disease Virus; Identification, Characterization; Molecular epidemiology; Prevention; Control; Transboundary; Strategy; Africa.

Introduction

Foot-and-mouth disease (FMD) is the most contagious disease of cloven-hoofed domestic and wild animals. Although mortality caused by FMD in infected animals is low, outbreaks result in significant economic consequences due to direct losses, such as low milk and meat production, treatment costs, reduced draught power, as well as indirect losses including losses due to animal and animal products trade limitations (James & Rushton, 2002; Jemberu *et al*., 2016; Knight-Jones *et al*., 2016; Perry & Rich, 2007). The agent of the disease, the FMD virus (FMDV) is a single-stranded, positive-sense RNA virus in the genus *aphthovirus*, family *Picornaviridae*. FMDV has high antigenic and phenotypic variability which is reflected in the existence of seven serotypes: O, A, C, Asia 1, South African Territories (SAT) types 1 to 3 and further numerous variants and lineages, described as topotypes (Knowles & Samuel, 2003). In Sub Saharan Africa (SSA), several factors make the epidemiology of FMD particularly complex. In this region two cycles of FMD exist, one in which the virus circulates between wildlife and domestic animals and another related to virus spread within the domestic animals without the involvement of wildlife (Arzt *et al*., 2011; Ayebazibwe *et al*., 2010; Bastos *et al*., 2000; Vosloo *et al*., 2002b; Weaver *et al*., 2013). The complexity of FMD in SSA needs to be taken into consideration when developing control and prevention strategies in endemic settings. It is basically important to consider the distribution and diversity of circulating serotypes in different ecological systems. One of the purposes of better understanding the epidemiology of transboundary animal diseases in general, would certainly be the implementation of integrated control approach based on regional strategies. For the specific case of FMD, a Progressive Control Pathway (PCP-FMD) was developed in 2012 by the FAO to assist and facilitate FMD endemic countries to progressively reduce the impact of the disease. One of the principles of PCP-FMD is an active monitoring of FMDV circulation and understanding of the epidemiology of FMD. Indeed, molecular characterization of the FMDV should be carried out following each FMD outbreak.

More than 20 years ago, molecular epidemiology has significantly increase our understanding of the factors that shape the spatial and temporal distribution of pathogens and diseases (Muellner *et al*., 2011; Zadoks & Schukken, 2006). Consequently, many articles related to molecular epidemiology of FMD have been published worldwide. Indeed, FMD molecular epidemiological patterns have been reviewed by several authors notably by Vosloo et *al*,

(2002a), Knowles & Samuel (2003), Rweyemamu et al. (2008), Klein (2009), Di Nardo *et al*. (2011) and more recently by Brito *et al*. (2015) and Freimanis et al. (2016). Specifically, Tekleghiorghis et al. (2016) provided a comprehensive overview of FMDV occurrences reported until 2013 in Africa. Other recent reviews were also presented by some authors (Casey *et al*., 2013; Maree *et al*., 2014) emphasizing the limiting factors of FMD control in Africa, including the presence of wildlife, the diversity of FMDV strains as well as their distribution in the continent. In this review, although we do not pretend to be exhaustive, our aim is to provide a state of knowledge on the molecular epidemiology of African FMDV over the last 20 years and based on PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) method (Moher *et al*., 2015). Hence, by collecting and summarizing currently available data on the continent of Africa, the purpose of this systematic review is to describe the distribution and diversity of FMDV; and to highlight the need to develop more comprehensive surveillance and reporting systems for effective prevention and control of FMD in Africa with the respect of the PCP-FMD.

Materials and methods

Systematic Review

A systematic literature search on FMDV molecular epidemiology from African countries was conducted on indexed literature published during the period from 01/01/1997 to 31/03/2017. The search was performed based on the reporting guidelines of PRISMA **(S1 Table** and **S2 Table)**.

Source of data

The literature search was conducted online using PubMED (www.ncbi.nlm.nih.gov/pubmed) and Scopus (www.scopus.com). Six keywords were identified: (a) "Foot-and-Mouth Disease", (b) "Foot-and-Mouth Disease Virus", (c) "Epidemiology", "Molecular epidemiology", (d) "Serotype", and (e) "Topotype". From these keywords, four search algorithms were applied on title/abstract/keywords: (1) "Foot-and-Mouth Disease" OR "Foot and mouth disease" OR "FMD" AND "Epidemiology"; (2) "Foot-and-Mouth Disease" AND "Foot-and-Mouth Disease Virus" AND "Molecular epidemiology"; (3) "Foot-and-Mouth Disease Virus" AND "Serotype"; (4) " Foot-and-Mouth Disease" AND "Foot-and-Mouth Disease Virus" AND "serotype" AND "Topotype". Further data available from conference papers, reports of international organisations and databases (e.g. the website of the World Reference Laboratory

for FMD (WRLFMD), the Office International des Epizooties (OIE), the Global Foot-and-Mouth Disease Research Alliance, and the National Centre for Biotechnology Information (NCBI) database for nucleotide sequences, were manually searched and included in this review. The search on NCBI (PubMed / nucleotide) was done using the following algorithm: "FMDV" AND "SEROTYPE" OR "TYPE" (A, O, SAT1, SAT2 and SAT3 successively) AND "Country" (all African countries except Madagascar). Serotype C is not included in the search query considering the known apparent decline of this virus since 2004. However, recorded articles reporting molecular characterization of this serotype (alone or associated with other serotypes) are not excluded from the present review and could be therefore included.

Eligibility criteria and search strategy

To have comparative data, the electronic search focused on FMD molecular epidemiology articles in which data were obtained using the following techniques: virus isolation and serotype identification by Enzyme Linked Immunosorbent Assay detecting circulating FMDV antigens (Ag-ELISA), at least one of a Polymerase Chain Reaction (PCR) techniques used for molecular diagnosis of FMD (namely real time and conventional PCR), sequencing and phylogenetic analysis. The online literature search was applied for title/keywords/abstract and it was restricted to English and French languages. To refine the search results, other criteria were also applied, including: article type (Journal Article/review), text availability (abstract is available), species (Other Animals instead of Human), subject (Veterinary Science), and journal category (MEDLINE for PubMed). The articles were selected following three steps. In the first step, the search strategy was tested and fine-tuned in Scopus. Subsequently, two databases were created in Reference Manager (Thomson Reuters Professional Edition version 12): "PubMed" and "Scopus" **(S2 Table)**. For each database, records were imported into Reference Manager and duplicates[4] were removed. Thereafter, a single database was created by merging the two initial databases. The same process of removing duplicates was implemented. However, the remaining duplicates were identified by progressive decrease of the degree of similarity between titles, but without the publication date as a criterion. These duplicates were manually removed and titles and abstracts screened for eligibility. As expected the number of eligible studies was huge. Selection criteria were refined to better meet the aim of the review. Consequently, the second step consisted in the exclusion of articles from the title and abstract review as for the following exclusion criteria: (1) Studies on other Picornaviruses (as Enteroviruses) or other pathogens

[4] Duplicates are defined as records with similarity in titles below than 87% (default parameters in Reference Manager) and same publication date.

instead of FMDV, (2) Studies carried out for assessing laboratory tests performance (improving specificity or sensitivity), (3) Studies describing only molecular characterization of FMDV strains with exclusively the purpose to select vaccine strains (it is assumed that preliminary genetic characterization has been done to isolate the FMDV), (4) Articles describing experimental studies on a given FMDV strain, (5) Articles describing a specific prevention/control measure of FMD such as vaccination or stamping out, (6) Studies describing the use of models in the epidemiology of FMD (such as spatial and spatiotemporal models to estimate the risk of occurrence or transmission of FMD), Studies in which only the serological tests (as a diagnostic technique) were performed and (7) Full-text articles written in another language than English and French, and when its summary does not give accurate information on the objectives, methodology and results of the study. The third step was applied when full texts were read and consisted in the study selection based on the following inclusion criteria: (1) Molecular study/analysis focused on different serotypes of FMDV and their distribution, (2) Comparative study/analysis of FMDV genetic differences through molecular tools, (3) Studies related to molecular epidemiological patterns of FMD outbreaks, (4) Molecular investigation of the origin and spread of FMD outbreak. However, some other documents were identified from the references of included articles and were subsequently added to the systematic review.

Data Collection process and analysis

Of every selected article the following items were collected and introduced in a Excel database: (1) the aim of the study, (2) year of samples collection, (3) nature of samples, (4) origin (country) of sample, (5) tests performed, (6) serotypes detected (the serotype involved in the described FMD outbreak), (7) topotypes identified, (8) relationship with other isolates from neighbouring countries, (9) the main findings and their implications and (10) references of the study (i.e. authors and year of publication). All countries where FMDV were isolated and characterized were visualized using QGIS software (version2.8).

Definition of frequently used words

Isolate and strain: when FMDV is obtained from, for example, an epithelium tissue, and when a cell culture is used for storage or further study, this would be referred to as FMDV isolate. FMDV strain is an isolate or group of isolates exhibiting characteristics that set it apart from other FMDV isolates (adapted from Zadoks & Schukken, 2006).

Lineage and genotypes: for FMDV, there are no uniform criteria or nomenclature for these taxonomic classifications that enable a clear definition of these terms. However, while it is likely justified to use "lineage" as a general term without referring to a kind of taxonomic level, FMDV genotype is defined as any phylogenetically unique RNA sequence. FMDV genotypes are used to be studied by VP1 sequence and comparison between related genotypes is used to infer evolutionary relationships (by phylogenetic analysis) (Haas, 1997). The following examples are given to illustrate how difficult it is to define with consistent criteria these terms: (1) FMDV of serotypes O, A, C and Asia-1 have been further classified into genotypes based on differences in VP1 coding sequences of up to 15% (Jamal *et al*., 2011; Knowles & Samuel, 2003); (2) Serotypes O and A FMDVs have been classified into lineages but serotype Asia-1 FMDVs have been classified into groups (Jamal *et al*., 2011; Valarcher *et al*., 2009); (3) The seven serotypes of the FMDV cluster were classified into distinct genetic lineages with approximately 30-50% difference in the VP1 gene (Knowles & Samuel, 2003).

Molecular characterisation: in genetic terms, characterization refers to the detection of variation because of differences in either DNA sequences or specific genes or modifying factors. Thereby, molecular characterization can be simply defined as the use of molecular data to improve or even to allow the elucidation of phylogeny, and provide the basic knowledge for understanding taxonomy and evolution of FMDV strain (adapted from King *et al*., 2012; Riley, 2004)

Molecular investigation: is a study using molecular tools (for example Polymerase Chain Reaction methods) to enhance case definition, increasing specificity and reducing misclassification of outbreak cases. Furthermore, outbreak investigations are used to systematically identify causes (risk factors) of disease outbreaks or to identify patterns of disease occurrence (adapted from Riley, 2004).

Outbreak: an outbreak is defined as the occurrence of one or more clinical cases of FMD reported in animal population at risk. An outbreak is considered as confirmed when FMDV was identified from tissue samples taken from one or more clinical cases (adapted from Cleland *et al*., 1995)

Serotypes: FMDV serotypes can be simply defined as serologically distinct types (A, O, C, SAT1, SAT2, SAT3 and Asia1). Moreover, FMDV serotypes are characterized by the lack of cross-protection between viruses (WRLFMD, 2016).

Topotype: can be defined as genetically and geographically FMDV distinct evolutionary lineages (adapted from Knowles & Samuel, 1998). Based on the VP1 coding sequence, topotypes are defined as geographically clustered viruses that form a single genetic lineage

generally sharing >85% (O, A, C, and Asia 1) or >80% (SAT 1, SAT 2, and SAT 3) nucleotide identity (Ayebazibwe *et al*., 2010; Ayelet *et al*., 2009; Samuel & Knowles, 2001a).

Results

The PRISMA diagram on the process of screening and selecting records is shown in **Fig 1**. Among 2433 studies returned from the searches and after removing duplicates across molecular epidemiological studies, a total of 1049 published articles were screened for suitability. During the first screening applied to title, abstract and keywords, 797 articles were selected. Out of these 797 articles, 154 full texts were assessed for eligibility. A total of 124 references are selected and presented in this review, including 57 additional articles retrieved after screening the references list of the eligible papers suggesting that 67 retrieved published papers were related to molecular epidemiology of FMD in Africa **(Table 1)**. The PRISMA checklist and the search strategy are given in **S1 Table** and **S2 Table** respectively. Five other tables are also provided in appendixes (**S3 Table**, **S4 Table**, **S5 Table**, **S6 Table** and **S7 Table**). Regarding the 4 supplementary tables (**S3, S4, S5 and S6**), each constitutes a list of FMDV serotype (and topotype) isolated from each part of the African continent during the period between January 1997 and March 2017. It should be noted that during one specific year there may be several identified strains of FMDV belonging to the same serotype and topotype isolated in a country. For this review, this is mentioned only once a year and per country because the number of isolates has not been considered. However, the period is repeated if another serotype or topotype is identified in the same country. These data included in the supplementary tables were mainly based on WRLFMD genotyping reports supplemented by those from published articles and NCBI (PubMed / nucleotide). The references of published articles were primary mentioned, but in default those of WRLFMD, NCBI and other sources are cited. Among the references of NCBI, the choice was made to include one of the citing RefSeq (https://www.ncbi.nlm.nih.gov/refseq/publications/) such as "O'Leary *et al*., 2016". The **S7 Table** summarizes descriptions of relevant molecular epidemiology studies.

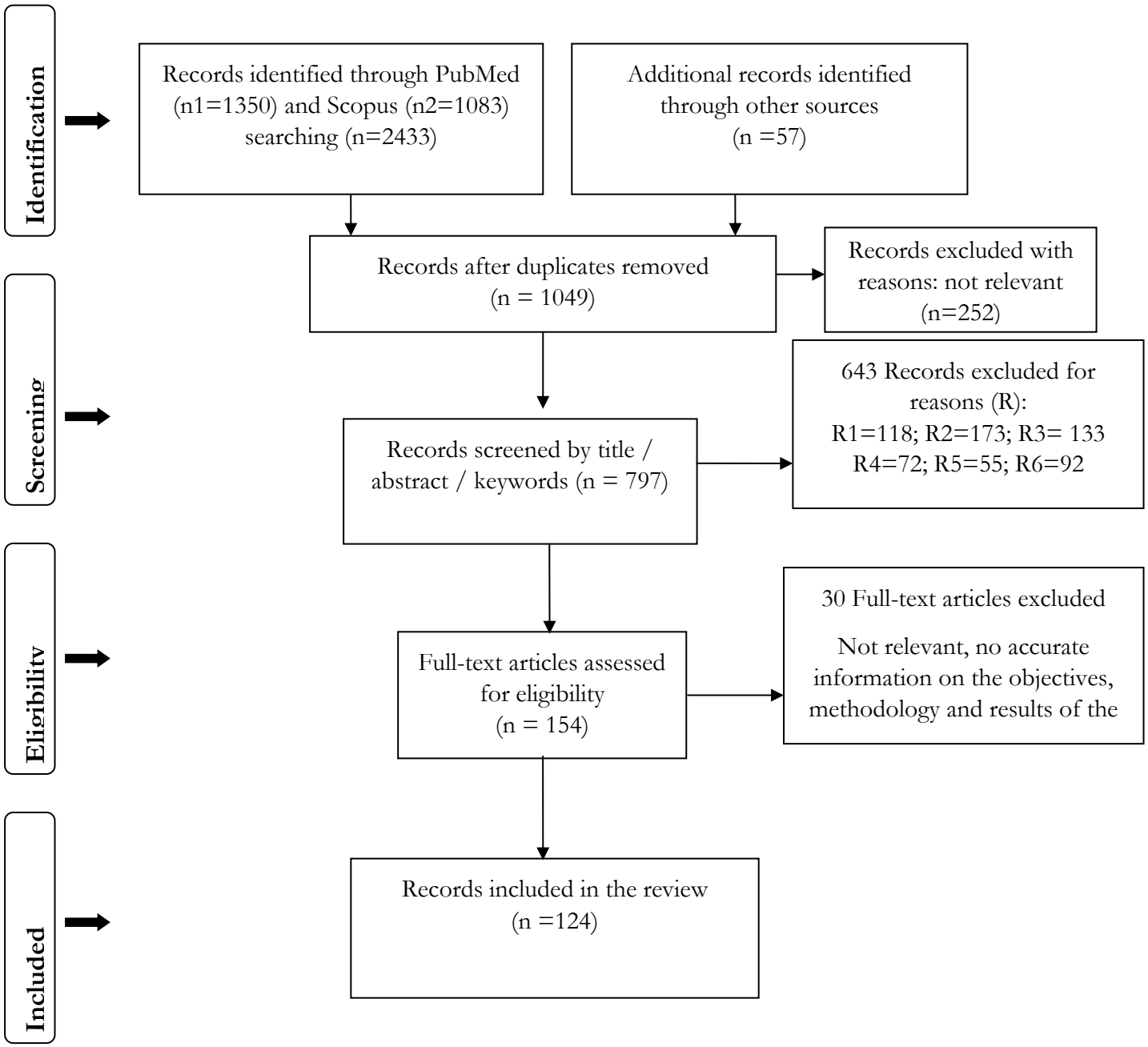


Figure 1: PRISMA Flow diagram of the review *(Adapted from* Moher *et al., 2009*)

Legend: R1: Other virus and pathogen than FMDV; R2: Focus on modelling; R3: Molecular epidemiology study not performed for African FMDV; R4: Articles related on African FMDV but not focus on molecular epidemiology; R5: Not relevant (matches some of the exclusion criteria); R6: Duplicates

Overview on molecular epidemiology of African FMDV

The epidemiology of FMD in Africa is exceptional in the sense that six of the seven serotypes of FMD viruses (Southern African Territories [SAT] 1, SAT2, SAT3, A, O, and C), except for Asia-1, have occurred in the last 2 decades (Maree *et al.*, 2014; Vosloo *et al*., 2002a). Serotype O is the most prevalent of the seven FMDV serotypes and occurs in many parts of the world. For illustration, based on the network labs of FMD world reference reports, out of the 1269 characterised FMDV isolates from FMD endemic countries, 46% (n=586) belonged to serotype O (WRLFMD, 2015). However, there is no exact genetically explanation for the higher prevalence of this FMDV (Mason *et al*., 2003). Based on data from the World Reference Laboratory for FMD, there have been no reports of serotype C since the 2004 outbreak in Kenya (KEN/1/2004 belonging to topotype AFRICA) (WRLFMD, 2016). However, at present, the discussion on this serotype within the OIE (World Organization for Animal Health), FAO (Food and Agriculture Organization of the United Nations) and the scientific community is whether serotype C has extinct or whether it remains undetected due to sub-clinical infection and limited spread within small populations of indigenous livestock breeds or wildlife in remote areas. The SAT (1, 2, and 3) serotypes are usually confined to SSA and they differ from each other regarding geographic distribution, infection rate and wildlife involvement in the FMD outbreaks in livestock (Vosloo *et al*., 2002a). The 3 SAT serotypes are maintained effectively in their wildlife reservoir, the African buffalo, and individuals may harbour multiple SAT-serotypes in the pharyngeal region for extended periods (Maree *et al*., 2016). Although serotype O is the most prevalent of FMDV in the world, serotype SAT 2 (n = 29, serotype taken individually) is the most frequently reported in molecular epidemiology studies included in this review. The number of reports on SAT 2 is followed by that on serotype O (n=28), SAT 1 (n=17), serotype A (n=11) and serotype SAT 3 (n=4). FMDV serotype C was reported in a single article related to previous isolates involved in the historical FMD outbreaks in Kenya (**Fig 2 F)**. The extracted data from included articles (origin, sampling year, the FMDV serotypes detected or studied, the topotypes of each serotype and references) are presented in **Table 1**. Based on genetic characterization 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 (Egypt), with predominance of serotypes A, O, SAT 1, and SAT 2; pool 5 restricted to West and Central Africa, with serotypes O, A, SAT 1, and SAT2; and pool 6 restricted to southern Africa, with SAT 1, SAT 2, and SAT 3 serotypes (Maree *et al*., 2014). Hence, from a spatial point of view, the results of the bibliographic search are consistent with some respects

to the FMDV pool subdivision. Indeed, the serotype A was reported in published articles describing FMDV A strains mainly from pools 4 and 5 whereas reports on serotype O are related to viruses from North, East, Central and West Africa corresponding to pools 4 and 5. Molecular characterization of FMDV SAT 1 is regularly described in papers from east and southern Africa, the SAT 2 is reported in articles from all three FMD African pools (4,5 and 6), while the SAT 3 is mainly described in Southern Africa **(Table 1).** However, due to overlapping between FMDV pools within the African continent, the results of this systematic review will be presented based on the cardinal points such as North, West associated with the Centre, East and South Africa.

Table 1: Selected Molecular epidemiological studies of FMD with emphasis on Africa published between January 1997 andMarch 2017

Region/Country	Sampling year	FMDV serotypes detected/studied	Topotypes (Genotype)	References
Egypt	2012	SAT2	VII	Ahmed *et al*., 2012
	2012	SAT2	VII	EL-Shehawy *et al*., 2014
	2012	O		El Rahman *et al*., 2015
	2012	SAT2	VII	Elhaig & Elsheery, 2014
	2012	SAT2	VII	Kandeil *et al*., 2013
	2006	A	AFRICA (G-VII KEN^{-05})	Knowles *et al*., 2007
	2012	SAT2	VII	Valdazo-Gonzalez *et al*., 2012
Libya	2013	O	ME-SA (Ind-2001)	Knowles *et al*., 2016
	2013	O	ME-SA (Ind-2001)	Valdazo-Gonzalez *et al*., 2014
Morocco	2015	O	ME-SA (Ind-2001)	Bachanek-Bankowska *et al*., 2016
North Africa	1987 - 1994	O		Samuel *et al*., 1999
Benin	2010	O	WA	Gorna *et al*., 2014
	2010	A	AFRICA (G-VI)	
Cameroon	2000	A	AFRICA	Bronsvoort *et al*., 2004
	2000	O	WA	
	2000	SAT2	VII	
	2010	O	EA-3	Ludi *et al*., 2016
	2012	O	WA	
	2012	SAT2	VII	
Niger	2015	O	WA	Souley Kouato *et al*., 2017
Nigeria	2009	A	AFRICA	Ehizibolo *et al*., 2014
	2015	A	AFRICA	Ehizibolo *et al*., 2017a
	2014	O	EA-3	
	2013	O	WA	
	2013	SAT2	VII	

	2015	SAT1	X	Ehizibolo *et al*., 2017b
	2007-2009	O	EA-3	Fasina *et al*., 2013
	2011	A	AFRICA	Olabode *et al*., 2014
	2011	SAT2	VII	
	2007-2011	O	EA-3	Ularamu *et al*., 2016 *
	2011-2014	O	WA	
	2009-2013	A	AFRICA (G-IV)	
	2007-2014	SAT2	VII	
West Africa	1974-1991	SAT2		Sangare *et al*., 2004*
SSA including West Africa (Burkina Faso, Niger and Ghana)	1946-2000	O		Sangare *et al*., 2001*
Southern and West Africa (Niger and Nigeria)	1975-1976	SAT1		Sangare *et al*., 2003*
Ethiopia	1977-2007	O	EA-3	Ayelet *et al*., 2009*
	2005	O	EA-4	
	1981-2007	A	AFRICA	
	1971-1983	C	AFRICA	
	2007	SAT1	IX	
	1990	SAT2	IV	
	2007	SAT2	XIII	
	1991	SAT2	XIV	
	2011	O	EA-3	Kassaw *et al*., 2013
	2007	SAT1		Legesse *et al*., 2013
	2008-2009	A	AFRICA (G-VII)	Negusssie *et al*., 2011
	2008-2009	O	EA-3	
	1979-2001	O	I (EA)	Sahle *et al*., 2004
Kenya	1967-2004	C	AFRICA	Sangula *et al*., 2011
	2011-2012	SAT1	I	Wekesa *et al*., 2015b

	2004-2012	SAT2	IV	
	2010-2011	O	EA-2	Wekesa *et al*., 2015a
Sudan	2004-2008	O	EA-3	Habiela *et al*., 2010
	2004-2008	A	AFRICA (G-IV)	
	2004-2008	SAT2	VII and XIII	
Tanzania	1967-2009	A	AFRICA (G-I)	Kasanga *et al*., 2015
	1985-2008	O	EA-2	
	1971-1999	SAT1	I	
	1975-2009	SAT2	IV	
	2008-2013	O	EA-2	Sallu *et al*., 2014
	2008-2013	A	AFRICA (G-I)	
	2008-2013	SAT1	I	
	2008-2013	SAT2		
Uganda	2007	SAT2		Ayebazibwe *et al*., 2010
	2004	SAT2		Balinda *et al*., 2010a
	2013	SAT3	V	Dhikusooka *et al*., 2015
	2013	SAT1	IV	Dhikusooka *et al*., 2016
	2008 - 2009	O	EA-2	Kasambula *et al*., 2012
	2006	O		Mwiine *et al*., 2010
	2013	A	AFRICA (G-I)	Namatovu *et al*., 2015b
	2013	SAT2	I	
	2011	O	EA-2	Namatovu *et al*., 2015a
East Africa	1978-2008	O	EA-1, EA-2, EA-3, EA-4	Balinda *et al*., 2010b
East Africa (Kenya and Uganda)	1992-2005	O	EA-2, EA-1	Balinda *et al*., 2010c
SSA including East Africa	1971-2000	SAT1	I-VI	Sahle *et al*., 2007a*
	1975-2000	SAT2	I-III	Sahle *et al*., 2007b*
	1948-2007	SAT1		Sangula *et al*., 2010a*
	1948-2007	SAT2		Sangula *et al*., 2010b*

Southern and East Africa	2010	SAT1, 2, 3		Kasanga *et al*., 2014a
Botswana	2002	SAT2		Baipoledi *et al*., 2004
South Africa (KNP)	1974-1991	SAT2		Bastos *et al*., 2000*
	SAT1	1981-2003		Vosloo *et al*., 2007
South Africa	2000	O	ME-SA(PanAsia)	Knowles *et al*., 2005
	2001	SAT2		Phologane et al, 2008
	1998	SAT3	I	Vosloo *et al*., 2001
	2000	SAT1		Vosloo *et al*., 2002
	2001	SAT2		
Namibia	2010	SAT1		Van *et al*., 2016
Zambia	2010	O		
	2009	SAT2		
	2011-2012	SAT2		Sikombe *et al*., 2015
	2011-2012	SAT1		
	2012	SAT1		Banda *et al*., 2014
		SAT2		
Southern Africa	1977-1999	SAT1		Bastos *et al*., 2001*
	1948-2000	SAT2		Bastos *et al*., 2003b*
	1983-2011	SAT2		Brito *et al*., 2016
	2010	SAT2	I	Jori *et al*., 2016
	2010	SAT3	I	
		SAT1		Kasanga *et al*., 2014b
	1948-1998	SAT1		Vosloo *et al*., 2006*
SSA including Southern Africa	1965-1999	SAT3		Bastos *et al*., 2003a*
Zimbabwe	1997	SAT2	I	Hargreaves *et al*., 2004

Legend: EA (East Africa), KNP (Kruger National Park), ME-SA (Middle East-South Asia), SSA (Sub Saharan Africa), WA (West Africa)

* Retrospective studies that used a large database of FMDV isolates **(cf. Supplementary materials S7)**

FMDV in northern Africa

Geographically northern Africa (Maghreb) is close to western Europe. The region is located between the Mediterranean Sea, the Libyan desert, the Sahara and the Atlantic Ocean. The results of the electronic search (for the period from 1997 to 2017) yielded 11 published articles in relation to molecular epidemiological studies on FMDV from North Africa (**Table 1)**. Out of these, 5 studies were related to molecular studies on FMDV serotype O (Bachanek-Bankowska *et al*., 2016; El Rahman *et al*., 2015; Knowles *et al*., 2016; Samuel *et al*., 1999; Valdazo-Gonzalez *et al*., 2014) while 5 other published papers focused on outbreaks due to serotype SAT 2 in Egypt during 2012 (Ahmed *et al*., 2012; EL-Shehawy *et al*., 2014; Elhaig & Elsheery, 2014; Kandeil *et al*., 2013; Valdazo-Gonzalez *et al*., 2012). The bibliographic search yielded only one published paper reporting molecular epidemiology of serotype A from northern Africa (Knowles *et al*., 2007).

Based on data from published articles supplemented by those from WRLFMD and PubMed (nucleotide) **(S3 Table)**, FMDV serotypes O and SAT 2 were the most recorded in recent years (2009-2016) in North Africa. From 2006 onwards, FMD outbreaks due to serotype O have been recorded in all the Maghreb countries. Egypt has the highest number of recorded outbreaks with a continuous occurrence for a decade (2006 to 2016). From 2009, FMDV serotype O was isolated in Algeria (2009-2014) and Libya (2009 to 2013). Tunisia and Morocco were the last countries in north Africa where the FMDV O virus was isolated in 2014 and 2015 respectively (Bachanek-Bankowska *et al*., 2016). In North Africa, two topotypes of FMDV serotype O virus were found: topotype ME-SA was recorded in all countries of this region **(Fig 2 A)** and topotype EA-3 (East Africa-3) recorded for the first time in only two countries namely Egypt (2012 - 2016) and Libya (2012), this virus O/EA-3 is usually recovered in East Africa. Indeed, the Libyan O/EA-3 was closely related to viruses isolated in 2011 from Eritrea and northern Ethiopia. In contrast, Libyan FMDV O/ME-SA/PanAsia 2ANT-10 sublineage, was closely related to those found in Pakistan and Iran in 2011, suggesting co-circulation of two different lineages of FMDV O (O/ME-SA/PanAsia 2ANT-10 and O/ME-SA/Ind-2001). The re-emergence of FMDV serotype O in Tunisia and Morocco in 2014 and 2015 occurred since 1999 (Samuel *et al*., 1999). Phylogenetic analysis revealed relationships of the Moroccan isolates to other viruses pertaining to the Middle East-South Asia (ME-SA) topotype, the Ind-2001d lineage (O/ME-SA/Ind-2001d) (Bachanek-Bankowska *et al*., 2016). However, these viruses belonged to a FMDV lineage that was originally isolated in the Indian subcontinent, but their emergence in the Middle East (United Arab Emirates and Saudi Arabia) and North Africa

(Libya) was reported in 2013 (Knowles *et al*., 2016; Valdazo-Gonzalez *et al*., 2014), with further spread to Algeria (WRLFMD, 2016).

FMDV SAT 2 typically confined to SSA, was isolated in 2012 in Egypt (Ahmed *et al*., 2012; Elhaig & Elsheery, 2014; EL-Shehawy *et al*., 2014; Kandeil *et al*., 2013) and in Libya (WRLFMD, 2016). The Egyptian viruses belonged to two distinct lineages (designated as SAT 2/VII/Ghb-12 and SAT 2/VII/Alx-12). Molecular analysis of samples showed that these SAT 2 isolates were genetically related to Sudan and Nigeria isolates from 2007 (Ahmed *et al*., 2012). The occurrence of this FMDV SAT 2 in Egypt was the first reappearance since 1950 (Ahmed *et al*., 2012; WRLFMD, 2016). This was suspected to have occurred through movements of people and animals into the region from further south during the "Arab spring". Although Libyan FMDV SAT2 belonged to the same topotype, these viruses were different from those isolated in Egypt in the same year (SAT 2/VII/Lib-12) suggesting independent introductions of the virus. However, unlike Egypt, Libya has experienced FMD outbreak due to serotype SAT 2 in the more recent past, i.e. in 2003. This virus belonging to topotype VII **(Fig 2 B)** and was genetically related to the virus isolated in Cameroon in 2000 (Bronsvoort *et al*., 2004b; Ludi *et al*., 2016), Saudi Arabia in 2000 and Eritrea in 1998 (Ahmed *et al*., 2012).

Based on data recorded from the World Reference Laboratory, serotype A has been isolated in two countries of northern Africa such as Libya in 2009 (A/ASIA/ Iran-05^{BAR-08}) and Egypt (2006-2016) **(Fig 2 C)**. In Egypt where several outbreaks due to FMDV serotype A occurred, presumably there is a fluctuation of occurrence of 2 topotypes: Asia (2010-2011; 2013-2014) and Africa (2006, 2009, 2012 and 2015-2016). FMDV A/Asia/Iran-05^{BAR-08} detected in Libya in 2009 was subsequently isolated in Egypt in 2010. However, phylogenetic analysis of VP1 nucleotide sequences of Egyptian isolates from 2006, demonstrated a close relationship to recent FMD virus isolates from East Africa, rather than to viruses currently circulating in the Middle East (Knowles *et al*., 2007). Recently in March 2015, serotype A belonging to topotype AFRICA (genotype IV) was isolated in Algeria 40 years after the last outbreak of foot-and-mouth disease due to this serotype.

FMDV in West and Central Africa

In this part of Africa, the number of publications contrasts with the occurrence of the disease. Indeed, despite the endemicity of this region to FMD, very few studies have been published concerning the molecular epidemiology of FMD. Overall, for the period between January 1997 and March 2017, the literature search has identified ten published articles about FMDV from

West and Central Africa **(Table 1)**. Although four serotypes (i.e. A, O, SAT 1 and SAT 2) are suspected to be found in this area, three serotypes (A, O and SAT 2) were prevalent during the last two decades **(S4 Table)**.

FMDV serotype A belonging to topotype AFRICA is often isolated in West and Central Africa, the most recent cases are those from Cameroon in 2013, Nigeria in 2011 (Olabode *et al*., 2014; Ularamu *et al*., 2016), Congo DR in 2011 (WRLFMD, 2016) and Benin in 2010 (Gorna *et al*., 2014). Earlier FMDV serotype A have been isolated in Cameroon from 2000 to 2005 (Bronsvoort *et al*., 2004b), Mali in 2004 and Togo in 2005 (WRLFMD, 2016). Within the AFRICA topotype, the most recovered genotype was the genotype G-IV **(Fig 2 C)**. However, the isolated FMDV serotype A from Congo DR in 2011 belonged to genotype G-I rather than G-IV. Phylogenetic analyses have mostly revealed, a close similarity to FMDV serotype A isolated in each country with those previously isolated in the same country and/or with isolates from countries of the sub region (Fasina *et al*., 2013; Gorna *et al*., 2014; Ularamu *et al*., 2016). Nevertheless, sequences analysis on the 1D coding region of FMD viruses topotype AFRICA (G-IV genotype) from Togo in 2005, Cameroon in 2005 and Nigeria in 2009 indicated that these isolates have a close relationship with the serotype A viruses from Eritrea in 1998 and Sudan from 2006 to 2011 in East Africa (WRLFMD, 2016).

Within the serotype O, the widely distributed topotype in West and Central Africa, is the topotype WA (West Africa). This virus belonging to topotype WA has been found in more than ten West and Central African countries from 1999 to 2015 (Bronsvoort *et al*., 2004b; Gorna *et al*., 2014; Ludi *et al*., 2016; Souley Kouato *et al*., 2017; Ularamu *et al*., 2016; WRLFMD, 2016). Nonetheless, incursions of topotypes historically found in East Africa (EA-3) occurred also in West Africa (Nigeria in 2007, 2009 and 2011) (Fasina *et al*., 2013) and Central Africa (Cameroun in 2010) (Ludi *et al*., 2016). In DRC (adjacent with East African countries), normally included in the FMD pool 4 (of east African countries), only the topotype EA-2 has been recorded in 2006 and 2010 **(Fig 2 A)**.

FMDV serotype SAT 1 is one of the suspected serotypes in this region. Indeed, Serological studies have shown evidence of the existence of antibodies against the serotype SAT 1 in Chad between 2007 and 2011 (Ouagal *et al*., 2010), Nigeria in 2008 (Ehizibolo *et al*., 2014) and Cameroon in 2010 (Ludi *et al*., 2016). More recently in 2015, FMDV serotype SAT 1 was isolated, identified and characterized from an FMD outbreak in cattle in Nigeria, 35 years after the last report of FMDV SAT1 in West Africa (Ehizibolo *et al*., 2017b).

FMDV serotype SAT 2 from West and Central Africa was molecular characterized during the last two decades. FMD SAT 2 viruses were more recently isolated in Mauritania in 2014, Cameroon from 2012 and 2013 (Ludi et al., 2016; WRLFMD, 2016), Nigeria in 2007, 2008, 2011 and 2012 (Fasina et al., 2013; Olabode et al., 2014; Ularamu et al., 2016) and Senegal in 2009. These viruses belonged to topotype VII (Fig 2 B). Within this topotype, the SAT 2 isolates from Cameroon in 2013, appeared to belong to a distinct lineage similarly to the Libyan lineage denoted as SAT 2/VII/Lib-12 (WRLFMD, 2016).

FMDV in eastern Africa

East Africa is a highly endemic area of FMD, of the seven FMDV serotypes, five serotypes (A, O, SAT1, SAT 2 and very little SAT 3) have been identified in this region **(S5 Table)**. Likewise, compared to other African regions, East Africa has the largest number of published molecular investigations of FMD outbreaks during the last two decades. Twenty-five recent publications were found through electronic search for the period January 1997 to March 2017. All serotypes suspected to be present in this region have been reported in these published articles **(Table 1)**.

East African FMD serotype A viruses belonged to topotype Africa and within this topotype, there is a diversity of genotypes (G-I, G-IV and G-VII). FMDV serotype A of G-I genotype were recovered primarily in Kenya, Tanzania (Kasanga *et al*., 2015; Sallu *et al*., 2014) and Uganda (Namatovu *et al*., 2015b). Viruses belonging to genotype G-IV were isolated in Eritrea, Somalia and Sudan (Habiela *et al*., 2010b). From the 2000s, FMDV serotype A of G-VII genotype were recorded in Ethiopia (Ayelet *et al*., 2009; Negusssie *et al*., 2011), but also in Kenya where two genotypes of FMDV serotype A co-circulated in 2005 (G-I and G-VII) **(Fig 2 C)**. The diversity and complexity of genetic relationships among these FMDV strains are illustrated by the following examples: (i) the virus isolated in 2007 in Ethiopia (A/Africa/G-VII genotype) was more closely related to the virus isolated from Kenya in 2005 than to that isolated from in the same country (Ethiopia) in 2000–2002 (Ayelet *et al*., 2009); (ii) FMDV serotype A FMDVs isolated in Uganda in 2013 belonged to a different sub-lineage from those recently found in neighbouring country such as Kenya (2012-2013) (Namatovu *et al*., 2015b). Additionally, a recent study has shown that within the Africa topotype, new lineage has apparently emerged from genotype G-I; while genotypes G-III and G-VIII previously isolated in 1964 in Kenya, were thought to be extinct. The genotype G-VII was last recorded in 2005, while G-I (including the apparently new lineage) is currently in widespread circulation (Wekesa

et al., 2014). Therefore, considering the high diversity of genetic and antigenic of FMDV belonging to serotype A, at present there is a discussion about the need for reformulation of FMDV A serotype commercial vaccines in this region as the currently used vaccines contain rather the Kenyan (A-KEN-05-1980) and Ethiopian (A-ETH-06-2000) antigens (Namatovu *et al*., 2015b; Negusssie *et al*., 2011; Wekesa *et al*., 2014; WRLFMD, 2016).

FMD virus serotype O has been responsible for most reported outbreaks of the disease in East Africa (Balinda *et al*., 2010b; Habiela *et al*., 2010a; Sahle *et al*., 2004; Wekesa *et al*., 2015a). Consequently, these viruses have been intensively molecular characterized in this area, especially between 2005 and 2013. Four topotypes (EA-1, EA-2, EA-3 and EA-4) within serotype O exist in eastern Africa region (Balinda *et al*., 2010b). Of these, topotypes EA-2 and EA-3 were by far the most dominant. On the other hand, topotypes EA-3 and EA-4 were mainly found in Ethiopia (Ayelet *et al*., 2009; Kassaw *et al*., 2013; Negusssie *et al*., 2011), Eritrea and Sudan (Habiela *et al*., 2010b), although both topotypes appeared to have previously co-circulated in Kenya in 2005 (Ayelet *et al*., 2009) and in 2013 (WRLFMD, 2016). Topotype EA-3 was isolated in Kenya in 1998 and 1999 (Wekesa *et al*., 2015a) while an incursion of EA-4 into Uganda occurred in the same period (Ayelet *et al*., 2009; Balinda *et al*., 2010b). Conversely, EA-2 topotype is most prevalent in Kenya (Balinda *et al*., 2010b; Balinda *et al*., 2010c; Wekesa *et al*., 2015a), Tanzania (Kasanga *et al*., 2015; Nsamba *et al*., 2015) and Uganda (Asfor *et al*., 2014; Ayelet *et al*., 2009; Balinda *et al*., 2010b; Balinda *et al*., 2010c; Kasambula *et al*., 2012; Namatovu *et al*., 2015a; Nsamba *et al*., 2015) **(Fig. 2 A)**. This topotypes distribution is consistent in some respects to the two geographical clusters described within this area (Di Nardo *et al*., 2011), namely the Horn of Africa and the area of the Great Lakes. The Horn of Africa includes Djibouti, Ethiopia, Eritrea, Sudan and Somalia while the Great Lakes comprises northern areas of Tanzania, Uganda, Kenya, Rwanda, Burundi and Zambia (included in pool 6). Although FMDV O/EA-2 on the one hand, EA-3 and EA-4 on the other hand, are the dominant viruses in the Horn of Africa and the Great Lakes respectively, O/EA-1 is traditionally used to formulate vaccines in eastern African countries including Uganda, resulting in low cross-protection with circulating viruses (Namatovu *et al*., 2015a).

In East Africa, FMDV serotype SAT 1 is responsible for occasional severe outbreaks in livestock and is known to be maintained within the buffalo populations (Sangula *et al*., 2010a). During the past two decades, many of East African countries were affected by this virus including Burundi, Ethiopia, Kenya, Tanzania and Uganda. Within East African FMD SAT 1 viruses, the distribution of topotypes vary across areas **(Fig 2 D)**. In Kenya, the topotype I

(NVZ) of FMDV SAT 1 was most prevalent among the virus isolated from 1998 to 2013 (Nsamba *et al*., 2015; Sahle *et al*., 2007a; Sangula *et al*., 2010a; Wekesa *et al*., 2015b) while in Ethiopia all FMDV SAT 1 belonged to topotype V and IX (Ayelet *et al*., 2009). Uganda experienced with FMD outbreaks due FMDV SAT 1 belonging to different topotypes. Those isolated in 1997-1999 (Sahle *et al*., 2007a), in 2007 from the African buffalo (Ayebazibwe *et al*., 2010) and recently in 2013, belonged to topotype IV (EA-1), although, the last isolate of SAT 1 FMDV was markedly different from the earlier buffalo isolates in 2007 (Dhikusooka *et al*., 2016).

Of the FMDV SAT types, SAT 2 is the serotype that is most often associated with outbreaks of FMD in livestock in SSA (Bastos *et al*., 2003b). Additionally, SAT 2 is the only SAT type to have been recorded outside the African continent in the last decade (Ahmed *et al*., 2012; Kandeil *et al*., 2013; Valdazo-Gonzalez *et al*., 2012). In Africa, especially in East Africa, SAT 2 is one of the most characterized FMDV and therefore many data are published concerning this virus (Balinda *et al*., 2010a; Habiela *et al*., 2010b; Hall *et al*., 2013; Namatovu *et al*., 2015b; Nsamba *et al*., 2015; Sangula *et al*., 2010b; Wekesa *et al*., 2015b). In Angola, for example, one of the East African countries where data are scarce on FMD, serological results indicated that FMD outbreak due to FMDV SAT 2 serotype occurred in 2009 (WRLFMD, 2016). On the other hand, FMDV SAT 2 serotype has a larger number of topotypes compared to other FMDV serotypes (I-XIV) and of these topotypes, eight have been detected in East Africa, suggesting a multitude of topotypes circulating in the same area. However, based on our data generated by electronic search over the last twenty years, it appears that topotypes IV and VII were the most prevalent in the region **(Fig 2 B)**. In addition, depending on each country, FMD outbreaks were mainly due to a specific topotype: VII and XIII in Ethiopia (Ayelet *et al*., 2009; Hall *et al*., 2013; WRLFMD, 2016), IV in Kenya (Sangula *et al*., 2010b; Wekesa *et al*., 2015b) and Tanzania (Kasanga *et al*., 2015), VIII in Rwanda (Bastos *et al*., 2003b; Hall *et al*., 2013; Nsamba *et al*., 2015) and VII in Sudan (Habiela *et al*., 2010b; Hall *et al*., 2013). It should be noted that the FMDV (SAT 2/ VII/Alx-12) identified in North Africa is most related to those isolated in Sudan (2012-2014) and in Ethiopia from 2014 to 2015 (Ularamu *et al*., 2016; Valdazo-Gonzalez *et al*., 2012; WRLFMD, 2016). Moreover, FMDV SAT 2 serotypes were isolated from African buffalo's in Uganda between 1998 and 2013 and various topotypes were identified (Ayebazibwe *et al*., 2010; Balinda *et al*., 2010a; Christensen *et al*., 2004; Nsamba *et al*., 2015; Sahle *et al*., 2007b).

In East Africa, FMDV serotype SAT 3 was only isolated in Uganda in 1997 and 16 years later in 2013, the virus belonging to topotype V (EA) **(Fig 2 E)**. The VP1 coding sequence of this later Uganda's FMDV SAT 3 was about 20% different from the most closely related virus strains within Uganda (1997) and up to 36% divergent from southern African SAT 3 viruses. This suggest the requirement of further epidemiological studies to elucidate the implication of infection by this SAT 3 virus (Dhikusooka *et al*., 2015).

FMDV in southern Africa

For the period between January 1997 and March 2017, 19 articles related to molecular epidemiology of FMD in southern Africa were recorded **(Table 1)**.

Although, FMDV SAT serotypes are the most commonly recovered in southern African countries, compared to Euro-Asian serotypes (A and O), FMDV serotypes A and O were isolated in some countries such as South Africa (serotype O in 2000), Malawi (serotype O in 1998) and Zambia (serotype O in 2010 and serotype A in 2015) **(S6 Table)**. Thereby, FMDV serotype O was mostly isolated in southern African countries bordering Central and East Africa. For example, FMDV serotype O has been isolated in 2010 at Mbala in the northern province of Zambia (Banda *et al*., 2014; Mweene *et al*., 1996). The phylogenetic analysis revealed that this virus belonged to topotype EA-2 **(Fig 2 A)** and that. it was most closely related to viruses from DR Congo (2006), Uganda (between 2004 and 2007), and Tanzania (2009). Earlier in 1998, FMDV serotype O was also isolated in Malawi, a country between two southern African countries (Mozambique and Zambia) and one East African (Tanzania). Additionally, FMDV serotype A was also isolated more recently in 2015 in northern Zambia. This virus belonging to topotype Africa and lineage G-I was most closely related to viruses from Kenya (2008) and Tanzania between 2009 and 2013 (WRLFMD, 2016). However, FMD serotype O was isolated 16 years ago in South Africa, country quite far from the east and Central Africa borders. In fact, Kwa Zulu Natal province of South Africa experienced in 2000 an FMD outbreak in pigs and cattle caused by serotype O topotype ME-SA PanAsia-1 virus, most likely introduced from Asia (Knowles *et al*., 2005; Mason *et al*., 2003; Sangare *et al*., 2001) **(Fig 2 A)**. Although this South African FMDV serotype O was genetically most-closely related to that that have caused several outbreaks in UK in 2001, there was no evidence of an epidemiological link, and it is most probable that these viruses had a common origin, rather than being directly related (Samuel & Knowles, 2001b). However, the so-called Euro-Asian FMDV serotypes (A and O)

are believed to be exotic to the southern African region, since unlike the SAT serotypes, antibodies to these classical serotypes do not occur in wildlife (Thomson *et al*., 2003).

Molecular investigations of FMD outbreaks have been more focused on the SAT serotypes. SAT 2 was the most recorded serotype followed by SAT 1 and SAT 3. Within the serotype SAT 1, the topotype I (NWZ) was relatively more frequent during the last two decades and it was mainly isolated in South Africa in 1998, 2000, 2002, 2003 and 2010 (Bastos *et al*., 2001; Vosloo *et al*., 2006) and Zambia from 2004 to 2009. In Botswana, the frequently recovered SAT 1 topotypes were topotype II (SEZ) (Bastos *et al*., 2001) and topotype III (WZ) in 2006 and from 2014 to 2015. The topotype II (SEZ) was also isolated in Namibia in 1998 and 2010 (Bastos *et al*., 2001; Nsamba *et al*., 2015) and in Swaziland in 2000 and more recently in 2015. Mozambique has the highest topotypes diversity of serotype SAT 1 as at least three topotypes have been identified such as topotype I (NWZ) in 2002, topotype III (WZ) in 2002 and 2010 and topotype IV (EA-1) in 2010 **(Fig 2 D)**.

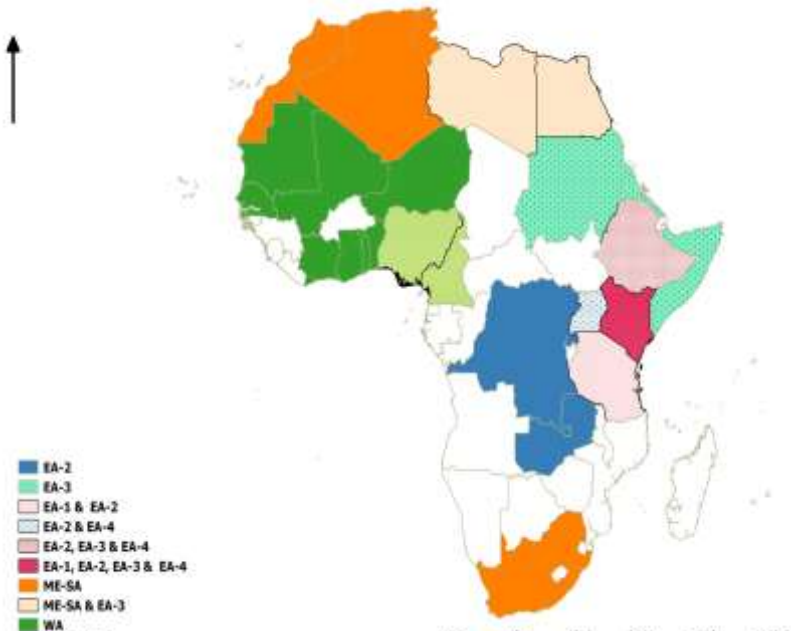
Over the past twenty years FMDV serotype SAT 2 caused several outbreaks in almost all southern African countries (Baipoledi *et al*., 2004; Banda *et al*., 2014; Bastos *et al*., 2003b; Brito *et al*., 2016; Kasanga *et al*., 2014a; Phologane *et al*., 2008; Sikombe *et al*., 2015), except in Swaziland and Lesotho. As illustrated by the **Fig 2 B**, Botswana, South Africa, Zimbabwe and Zambia were likely most affected by this virus. Similarly to FMDV SAT 1 serotype, the most prevalent topotype for FMD SAT 2 virus was the topotype I. This topotype was identified in Malawi from 2008 to 2015, in Mozambique in 2002, 2010, 2014 and 2015, in South Africa in 2001, 2007-2008, and 2010-2012 (Bastos, 1998; Bastos *et al*., 1999; Bastos *et al*., 2000; Brito *et al*., 2016; Jori *et al*., 2016; Phologane *et al*., 2008) and in Zimbabwe in 1997-1998, 2000-2003, 2010 and 2014 (Bastos *et al*., 2003b; Brito *et al*., 2016; Hargreaves *et al*., 2004). The topotype II of SAT 2 virus was mainly reported in Botswana in 1998 and 2006 (Brito *et al*., 2016), in Namibia in 2007-2008 and in Zimbabwe in 2010 and 2014-2015. Likewise, FMDV SAT 2 topotype III was most secondly reported in southern Africa **(Fig 2 B)**. It was isolated in Botswana during twelve of the last twenty years and in Namibia in 2007-2008 and 2015, in Zambia in 2007-2009 and in Zimbabwe in 2010 and 2014-2015.

FMDV Serotype SAT 3 is one of the least serotypes involved in FMD outbreaks in southern African region. However, it was isolated between 1997 and 2016 in several countries, like Botswana in 1998 and 2010 (Bastos *et al*., 2003a; WRLFMD, 2016), South Africa in 1997-1998, 2001, 2006 and 2010 (Bastos, 1998; Bastos *et al*., 1999; Bastos *et al*., 2003a; Jori *et al*.,

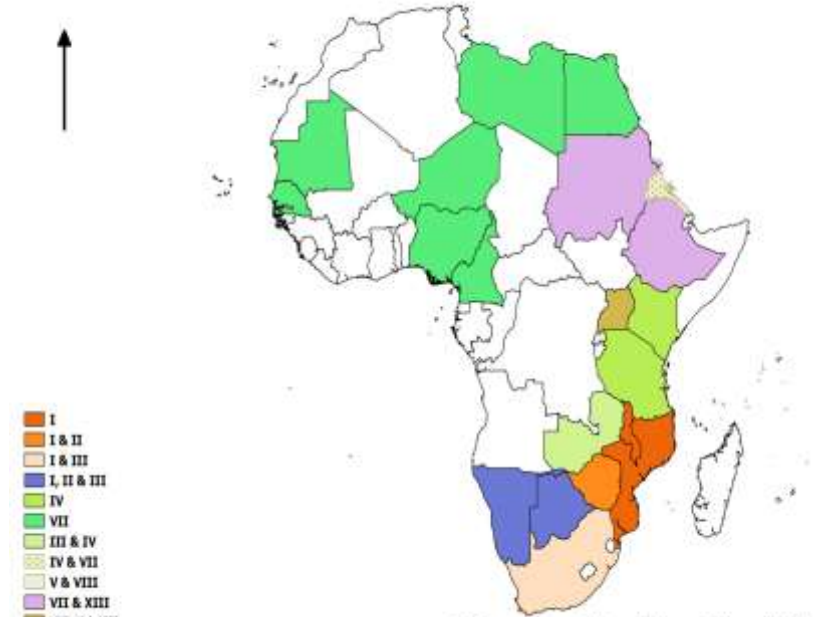
2016; Vosloo *et al*., 2001), in Zimbabwe in 1998 and 2010 (Bastos *et al*., 2003a; Jori *et al*., 2016) and recently in Zambia in 2015 **(Fig 2 E)**. This later Zambian FMDV SAT 3 which belonged to topotype II was most closely related to that isolated from the African Buffalo in Botswana in 1998.

Furthermore, it should be noted that it is mainly in the southern Africa area, that the role of wildlife in the maintenance of FMDV SAT serotypes, was the most investigated (Banda *et al*., 2014; Bastos *et al*., 2000; Bastos *et al*., 2003b; Brito *et al*., 2016; Hargreaves *et al*., 2004; Jori *et al*., 2016; Kasanga, 2014; Kasanga *et al*., 2014b; Phologane *et al*., 2008; Thomson *et al*., 2003; Vosloo *et al*., 2006; Vosloo *et al*., 2007). Molecular epidemiological studies showed that African buffaloes are indeed the most likely source of infection for susceptible cloven-hoofed animals living in close proximity (Bastos *et al*., 2000; Brito *et al*., 2016; Hargreaves *et al*., 2004; Jori *et al*., 2016; Kasanga *et al*., 2014a; Vosloo *et al*., 2001; Vosloo *et al*., 2002b; Vosloo *et al*., 2006), that interspecies transmission occurs between cattle and antelope and that trans-boundary transmission of virus remains a threat to disease control in southern African countries as well as in the rest of the African continent.

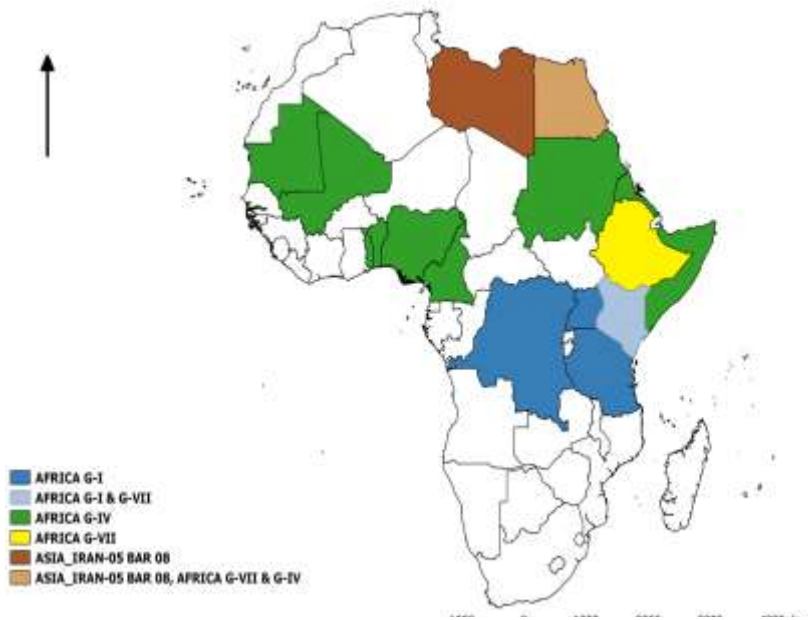
[A: Serotype O]



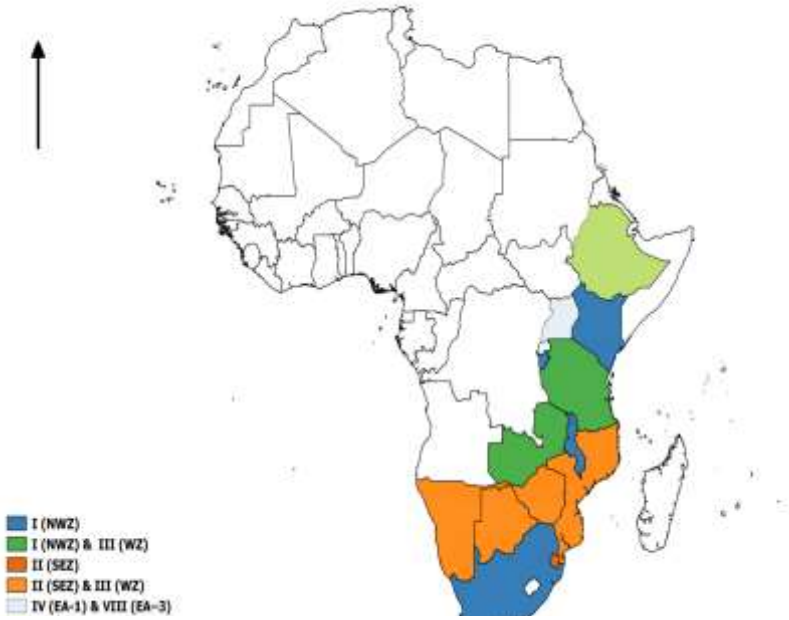
[B: Serotype SAT 2]



[C: Serotype A]



[D: Serotype SAT 1]



[E: Serotype SAT 3]

[F: Serotype C]

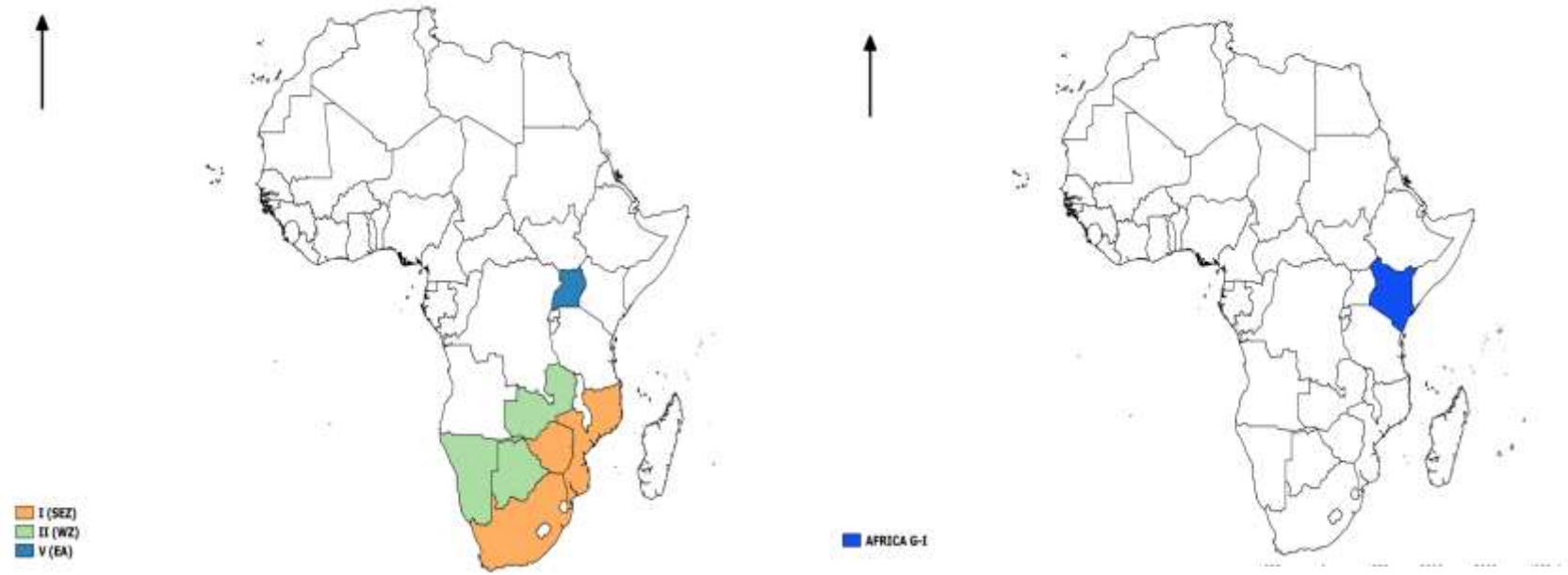


Fig 2. Map of Africa showing the topotypes distribution for each FMDV serotypes for the period between 1997 and 2016.

Legend: The topotypes are shown in different colours, countries with more than one topotype are also shown in different colours without considering individual topotype as well as the location of the isolate within the country. **[A]:** FMDV O topotypes distribution; **[B]:** FMDV SAT 2 topotypes distribution; **[C]:** FMDV A topotypes distribution; **[D]:** FMDV SAT1 topotypes distribution; **[E]:** FMDV SAT3 topotypes distribution; **[F]:** FMDV C topotypes distribution.

Discussion and conclusion

The present systematic review allowed to collect the published papers related to molecular epidemiology of FMD in Africa over the last twenty years. The main findings of these studies pointed out the complexity of the epidemiology of FMD in Africa, which is particularly reflected by the huge potential of African FMDV strains to spread over large areas within the continent. Due to the continuous spread of certain FMDV strains from one region to another, the delineation between African FMDV pools (4, 5 and 6) is highly unstable as some FMDV topotypes are restricted to one pool while others occur in multiple overlapping pools (Paton et al., 2009). Following the conclusions of the study conducted by Salhe et *al,* (2004), Knowles et *al,* (2004) demonstrated the existence of a ninth, and possibly a tenth topotype of FMDV serotype O. One of these new lineages, which has been named East Africa 2 (EA-2) was found in Tanzania in 1996 and 1998, in Malawi in 1998, in Zambia in 2000, in Kenya in 2002, in Uganda in 2002 and 2004, in Burundi in 2003 and in Rwanda in 2004 and possibly earlier in Uganda in 1972 while the second one, East Africa 3 (EA-3), was recovered in Ethiopia, Eritrea and Sudan. Additionally, in recent years, some authors confirmed the occurrence of this later topotype (EA-3) in the rest of the continent such as in the West African country Nigeria in 2007, 2009 and 2011 (Fasina et al., 2013; Ularamu *et al*., 2016), in Central African Cameroun in 2010 (Ludi et al., 2016) and in the northern African countries Egypt (from 2013 to 2016), and Libya in 2012 (WRLFMD, 2016). More recently, the FMDV SAT 2/Topotype VII isolated in Mauritania in 2014 was identified as genetically close with the same FMDV serotype SAT 2 and topotype VII previously isolated in Nigeria in 2011-2012 and in Cameroon in 2005 (WRLFMD, 2016). Recently from 2013 to 2016, FMD has re-emerged in several North African countries. Although North Africa applied intensive vaccination campaign to control the disease for many years, several FMD outbreaks occurred in the region since 2013, these outbreaks being due to an unusual FMDV O strain originating from India (O/ME-SA/Ind-2001). This strain spread first to Saudi Arabia and to Libya in the last quarter of 2013 (Knowles et *al.* 2016). It spread further to Algeria and Tunisia in 2014 and finally to Morocco in 2015 (Bachanek-Bankowska *et al*., 2016). Given this intensive and rapid spread of the virus associated with intensive movements between the Maghreb and the Mediterranean Europe, this FMDV strain is at present considered to be the most serious threat to Europe. Evidence for inter-continental transmission was earlier provided by the PanAsia FMDV O strain which was responsible for an explosive pandemic in Asia, spread to South Africa and further to Europe in the 2000s (Knowles *et al*., 2005; Sangare *et al*., 2001) (**Fig 3**).

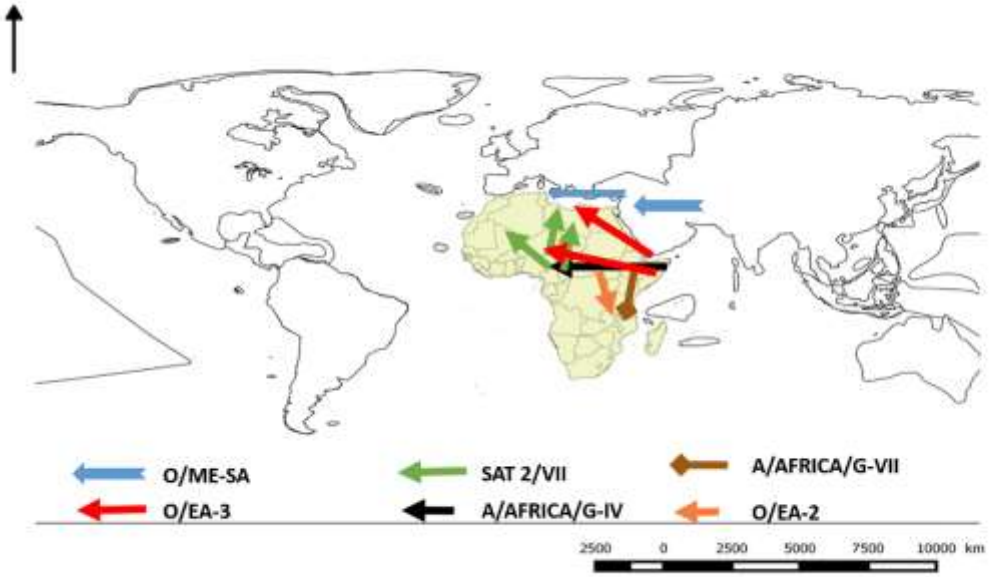


Fig 3. Likely trend of FMDV spread toward Africa and within the continent

Mostly, the findings of the published articles suggest that the transboundary and uncontrolled livestock mobility is the main source of FMDV introduction in a country or region. Likewise, in most studies, the identified and characterised FMDV have shown close relationship with FMDV strains previously isolated in neighbouring countries or regions. As an illustration of this, the Nigerian FMDV SAT 2 isolated in 2007-2009 was closely related to those found in neighbouring countries such as Republic of Niger in 2005, in Cameroon for the last ten years and in Sudan in 2007 (Fasina *et al*., 2013). This can be explained by extensive livestock trade as Nigeria has one of the biggest and most attractive West African cattle markets. Indeed, to meet the demand for animal products, the major production basins of the Sahel and Sahel Saharan belt developed since long cross-border trade with coastal countries (Mankor, 2013). On the other hand, during the transhumance, Nigerian herders move southwards with their herds into Cameroon while herders from Niger move into Nigeria during the dry season. This strongly indicates once more that cross border animal movement (transhumance or nomadism) as well as live animal trade to be hypothesized as the most plausible source of infection. To the best of our knowledge, to illustrate the great mobility of animals, the Sahel region in West Africa

appears to be a good example. With a population estimated at approximately more than 60 million of cattle and 160 million of small ruminants, around 70-90% of the cattle and 30-40% of the small ruminants are raised in a transhumant pastoral system (Kamuanga *et al*, 2008). Hence, the large majority of countries in West Africa are concerned with cross-border transhumance either as countries of departure or as receiver or transit countries. Depending on the season, the following transhumance axis have been identified: (a) a central axis composed by Benin, Burkina Faso, Ivory Coast, Ghana, Mali, Niger and Togo; (b) the west axis with Senegal, Gambia, Mauritania, Guinea and Mali; (c) the east axis with Benin, Nigeria and Niger; (d) another specific axis which involved the north of Niger and the northern Nigeria. Based on data of prevalence, serotype and topotype distribution, expert evaluation of animal movement patterns, and on the impact of wildlife and farming systems, some epidemiological clusters were proposed for Africa (Rweyemamu *et al*., 2008). In the Sudan/Sahel cluster which includes Burkina Faso, Chad, Mali, Mauritania, Niger, Northern Nigeria and Senegal, the farming system is predominantly pastoral. Rweyemamu et *al*, (2008) reported in a comprehensive FMD epidemiological review, that this cluster is an important disease corridor, linking the east African cluster with West Africa and probably West Africa with North Africa. In this cluster, transhumance is most often associated with the occurrence of FMD outbreaks and other transboundary animal diseases. Therefore, considering the animal movement features described above, the spread of FMD outbreaks due to FMDV SAT 2 from SSA to North Africa is easier to understand. Indeed, Egypt and Libya import considerable number of livestock from FMD endemic SSA countries. Additionally, number of published papers highlighted the impact of uncontrolled livestock movements such as transhumance in the transmission of FMDV in Africa (Bronsvoort *et al*., 2004b; Bronsvoort *et al*., 2004a; Macpherson, 1995).

Based on the number of publications recorded in this systematic search, it can be argued that the number of molecular epidemiological studies significantly increased in the African continent. Consistent with this observation, it could also be stated that interest and capabilities are growing in African national laboratories in implementing studies related to molecular epidemiology, although the role of many national laboratories is often limited to the collection and storage of samples before their shipment to some reference laboratories (WRLFMD, Pirbright Institute in the United Kingdom is the most requested for further analysis) (Namatovu *et al.*, 2013). However, some African FMD references laboratories such as the Agricultural Research Council/Onderstepoort Veterinary Institute (ARC/OVI) in South Africa and the Botswana Vaccine Institute (BVI) in Botswana have a high level of abilities in performing virus

isolation, identification and serotyping by Ag-ELISA, in molecular analysis by PCR methods and sequencing. Subsequently, the level of FMD control is much better in Southern Africa than in the rest of Africa (Perry *et al*., 2003; Scoones *et al*., 2010). Since May 2016, three southern African countries namely Botswana, Namibia and South Africa, are in the OIE list of FMD free zone where vaccination is not practised (OIE, 2016). Unfortunately, there is a continuing threat of infection of these zones from wildlife escaping from transfrontier conservation areas as well as from FMD endemic neighbouring countries (Jori *et al*., 2016). For instance, the Kruger National Park (KNP) is an endemic FMD area in South Africa, because the African buffalo's (*Syncerus caffer*) in the Park are considered as permanent carriers of the virus (Vosloo *et al*., 2001; Vosloo *et al*., 2002b; Vosloo *et al*., 2007). Consequently, frequent FMD outbreaks are diagnosed in wildlife in the Kruger National Park as well as in other southern African countries such as Botswana (Baipoledi *et al*., 2004) and Zimbabwe (Hargreaves *et al*., 2004).

However, in recent years, there were increasing contributions of certain laboratories from eastern African countries including Ethiopia, Kenya, Tanzania and Uganda mostly in relation to collaborative projects with FMD laboratories in Europe or in the United States of America. In contrast, relatively few studies have been conducted in North Africa and in central and western Africa. Although in some Maghreb countries (such as Algeria, Morocco and Tunisia) FMD occurs sporadically, the epidemiological situation is clearly opposite to that of West and Central Africa, which remains endemic to the disease. Among the reasons that may explain the lack of sufficient data from West and Central Africa, there is the underreporting of outbreaks and the fact that when the clinical cases are identified, they are mainly not confirmed by laboratory analysis (Ouagal *et al*., 2010). However, in the last two decades, samples were frequently send to the WRLFMD in Pirbright (UK) for serotyping and genotyping allowing numerous comparisons of VP1 gene sequences of viruses to be made.

Nonetheless, despite that FMD is an economically dramatic disease in most African countries, the disease was not ten years ago considered as a priority compared to some deadly animal diseases such as contagious bovine pleuropneumonia (CBPP) or Peste des Petits Ruminants (PPR). The lack of political awareness negatively affected the implementation of epidemiological studies and therefore in these countries there is very little known about the circulating strains and currently prevention and control measures such as effective vaccination are not performed. Another limitation to the better understanding of the epidemiology of FMD in Africa is the lack of sampling in wild animals as well as in small ruminants and pigs. Regarding to the articles dealing with the involvement of wildlife in the epidemiology of FMD,

the authors demonstrated the evidence of the important role of wildlife in the transmission of FMDV (Ayebazibwe *et al*., 2010; Jori *et al*., 2016; Vosloo *et al*., 2002b). Apart from the countries of southern Africa and recently from East Africa, data on wildlife were only provided by few epidemiological investigations. In many molecular investigations, cattle were most sampled than another animal species. The paucity of sampling small ruminants, pigs and wildlife animals could unfortunately overshadow the accurate epidemiological characteristics to be considered for implementing effective prevention and control measures.

However, it should be noted that the current systematic literature review has some weaknesses. One of the major challenges in attempting to synthesise such a broad selection of articles is the diversity of methodologies used by their authors. This could limit recording comparative data from the published papers. Nevertheless, it is generally assumed that an incursion with a specific strain of FMDV generally lasts to a period after which the animal population has become immune for this FMDV strain (Arzt *et al*., 2011). In endemic areas, such as SSA, this will be followed by an incursion with another FMDV strain for which the population has not yet immunity. Accordingly, the spatiotemporal distribution pattern of FMDV is therefore changing rapidly over time. In our point of view, this would be an alternative reason of the lack of comparative data from a range of studies conducted without a standardized study design in different area over a long period. Likewise, some exclusion criteria (notably the third and fourth exclusion criteria described above in materials and method section) are unlikely to have resulted in the exclusion of relevant papers or introduced bias. In addition, only two bibliographic databases were used (PubMed and Scopus), which could exclude articles not included in these databases especially some studies published not in English of French that can be relevant. Moreover, certain criteria as the time interval delimited by the chosen study period could also exclude some relevant papers previously published. Furthermore, regarding epidemiological events of FMD in Africa, the time criterion for study selection is very subtle because of the rapid change occurring in the continent. Indeed, there have been several FMD epidemiological events that have occurred, including the last outbreak in Algeria with the new serotype A in March 2017; two publications (in 2017) from studies in Nigeria: 1) the first containing new information on the virus strains in North-Nigeria (Ehizibolo *et al*., 2017a) and 2) the detection of SAT 1 in Nigeria 35 years after the last report (Ehizibolo *et al*., 2017b) and lastly, the identification and molecular characterization of FMDV serotype O in Niger in 2015 (Souley Kouato *et al.*, 2017).

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Despite these limitations, the comprehensive search and systematic methodology of this review is likely to have identified and selected a huge number of available relevant literature information. To the best of our knowledge, this is the first systematic review on molecular epidemiology of FMD in Africa based on a transparent and standardized procedure (PRISMA guidelines). It should be noted that some fifteen years ago significant review efforts have been made by Vosloo et *al*. (2002a) and Knowles & Samuel (2003) by providing interesting insights into the application of molecular tools of FMD epidemiology. Recently, some review articles were published on FMD epidemiology (Brito et *al*, 2015; Casey *et al.*, 2013; Maree *et al*., 2014; Tekleghiorghis et *al*, 2016). Notwithstanding the difference in the methodological approach between this systematic review with the earlier review papers, their findings generally agreed with the results of this review and also concluded that the main factor of FMD transmission is the uncontrolled cross-border animal. Although, the impact of this factor can vary from region to another because of farming system. One of the benefits of this systematic review is providing an updated knowledge on molecular epidemiology of FMD in Africa.

Globally, to achieve the goal of FAO/PCP-FMD in endemic area, especially in SSA, several studies need to be realized. In summary, these studies should include following objectives: (i) to implement the use of molecular tools for accurate and early diagnosis of FMD; (ii) to undertake studies on the dynamics of transmission of FMDV by using molecular biology tools and modelling; (iii) to carry out genetic, antigenic and evolutionary characteristics studies of FMDV; (iv) to investigate the transmission dynamics of FMDV both in domesticated livestock and wildlife; (v) to model FMD outbreaks for risk mapping by studying the spatiotemporal distribution of FMDV serotypes taking into account the impact of animal movements on to FMD spread. From a political and institutional point of view, some efforts must also be consented in strengthening veterinary laboratories capacities. This could be achieved either through training or technical assistance to resource constrained laboratories either by laboratory twinning at sub regional or regional level. The training and/or twinning programs of laboratories must essentially include among others the following objectives: use of standardized and rapid FMD diagnostic tests; implementation of secure communication and rapid reporting systems and setting of adequate biosafety and biosecurity measures. The strengthening of capacity of existing regional agencies devoted to animal disease control is also important. For example, the West and Central African veterinary laboratories networks for avian influenza and other transboundary diseases diagnostic (called RESOLAB), which already exists with the support of FAO, could be restructured and reactivated for this purpose. Through these veterinaries labs

networks, it would be possible to establish and to implement standardized protocols to ensure that outbreak investigation results in collecting and shipping of viable viral material for characterisation at reference laboratories recognised by FAO/OIE. It would be also interesting that the governments of these countries to improve the control of cross border livestock movement through more intensive surveillance in the high-risk areas such as the transhumance routes. Moreover, the implementation of vaccination should be based on the transhumance schedule. Additionally, where outbreak occurred, strict quarantines should be enforced to avoid the spread of the disease to new FMD free areas. While these additional efforts are welcome, the globalization of trade is a strong and legitimate argument for developed countries (free of FMD) to consider the urgent needs in endemic developing countries and to design regional and integrated FMD control strategies with the decisive purpose to more effectively prevent or control FMD worldwide.

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Supplementary materials

S1 Table. PRISMA Check list

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design PICOS.	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed e.g., Web address, and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics e.g., PICOS, length of follow-up and report characteristics e.g., years considered, language, publication status used as criteria for eligibility, giving rationale.	4- 6

Information sources	7	Describe all information sources e.g., databases with dates of coverage, contact with study authors to identify additional studies in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5 - 6
Study selection	9	State the process for selecting studies i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis.	5 - 6
Data collection process	10	Describe method of data extraction from reports e.g., piloted forms, independently, in duplicate and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought e.g., PICOS, funding sources and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies including specification of whether this was done at the study or outcome level, and how this information is to be used in any data synthesis.	NA
Summary measures	13	State the principal summary measures e.g., risk ratio, difference in means.	NA
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency e.g., I^2 for each meta-analysis.	NA
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence e.g., publication bias, selective reporting within studies.	NA
Additional analyses	16	Describe methods of additional analyses e.g., sensitivity or subgroup analyses, meta-regression, if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8-9

			Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted e.g., study size, PICOS, follow-up period and provide the citations.	9 - 19
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment see item 12.	NA
Results of individual studies	20	For all outcomes considered benefits or harms, present, for each study: a simple summary data for each intervention group b effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies see Item 15.	NA
Additional analysis	23	Give results of additional analyses, if done e.g., sensitivity or subgroup analyses, meta-regression [see Item 16].	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups e.g., healthcare providers, users, and policy makers.	19 – 23
Limitations	25	Discuss limitations at study and outcome level e.g., risk of bias, and at review-level e.g., incomplete retrieval of identified research, reporting bias.	23– 24
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	24 – 25
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support e.g., supply of data; role of funders for the systematic review.	25

Legend: Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group 2009. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 66: e1000097. doi:10.1371/journal. pmed1000097.

S2 Table. Search strategies and results for PubMed & Scopus databases

Last date of search	Database consulted	Search algorithms applied	Results
30-10-16	PubMed	Foot-and-Mouth Disease AND Epidemiology	707
	Scopus		579
Subtotal 1			**1286**
30-10-16	PubMed	Foot-and-Mouth Disease AND Foot-and-Mouth Disease Virus AND Molecular epidemiology	153
	Scopus		104
subtotal 2			**257**
30-10-16	PubMed	Foot-and-Mouth Disease Virus AND Serotype	443
	Scopus		341
Subtotal 3			**784**
30-10-16	PubMed	Foot-and-Mouth Disease AND Foot-and-Mouth Disease Virus AND serotype AND Topotype	47
	Scopus		59
subtotal 4			106
Total of records			**2433**

S3 Table. FMDV isolated from North Africa for the period between 1997 and 2016

Country	Year	Serotype	Topotype	Genotype/Strain	References
Algeria	2009	O	-	-	O'Leary *et al*., 2016
Algeria	2014	O	ME-SA	Ind-2001d	WRLFMD, 2016
Algeria	2017	A	Algeria	G-IV	WRLFMD, 2016
Egypt	2006	A	AFRICA	G-VII^{KEN-05}	Knowles *et al*., 2007; WRLFMD, 2016
Egypt	2006	A	AFRICA	Sharqia-72	WRLFMD, 2016
Egypt	2009	A	AFRICA	G-VII^{KEN-05}	
Egypt	2010	A	ASIA	Iran-05^{BAR-08}	
Egypt	2011	A	ASIA	Iran-05^{BAR-08}	
Egypt	2012	A	AFRICA	G-IV^{ISM-12}	
Egypt	2013	A	ASIA	Iran-05^{BAR-08}	
Egypt	2014	A	ASIA	Iran-05^{BAR-08}	
Egypt	2015	A	AFRICA	G-IV	
Egypt	2016	A	AFRICA	G-IV	
Egypt	2006	O	ME-SA	Sharquia-72	
Egypt	2007	O	ME-SA	PanAsia-2	
Egypt	2008	O	ME-SA	Sharquia-72	
Egypt	2009	O	ME-SA	Sharquia-72	
Egypt	2010	O	-	-	O'Leary *et al*., 2016
Egypt	2011	O	ME-SA	PanAsia-2	WRLFMD, 2016
Egypt	2011	O	ME-SA	Sharquia-72	
Egypt	2012	O	EA-3	-	
Egypt	2012	O	-	-	El Rahman *et al*., 2015
Egypt	2013	O	EA-3	-	WRLFMD, 2016
Egypt	2014	O	EA-3	-	
Egypt	2015	O	EA-3	-	

Egypt	2016	O	EA-3	-	
Egypt	2012	SAT2	VII	Alx-12	Ahmed *et al*., 2012; EL-Shehawy *et al*., 2014; Elhaig & Elsheery, 2014; WRLFMD, 2016
Egypt	2012	SAT2	VII	Ghb-12	
Egypt	2013	SAT2	-	-	O'Leary *et al*., 2016
Egypt	2014	SAT2	VII	Alx-12	WRLFMD, 2016
Egypt	2015	SAT2	VII	Alx-12	
Libya	2009	A	ASIA	Iran-05^{BAR-08}	
Libya	2010	O	ME-SA	PanAsia-2^{ANT-10}	
Libya	2011	O	ME-SA	PanAsia-2^{ANT-10}	
Libya	2012	O	ME-SA	PanAsia-2^{ANT-10}	
Libya	2012	O	EA-3	-	
Libya	2013	O	ME-SA	Ind-2001^{KAR-13}	Knowles *et al*., 2016; Valdazo-Gonzalez *et al*., 2014
Libya	2009	O	ASIA	Iran-05^{BAR-08}	WRLFMD, 2016
Libya	2003	SAT2	-	-	O'Leary *et al*., 2016
Libya	2012	SAT2	VII	-	WRLFMD, 2016
Morocco	1999	O	-	-	O'Leary *et al*., 2016
Morocco	2015	O	ME-SA	Ind-2001d	Bachanek-Bankowska *et al*., 2016
Tunisia	1999	O	-	-	WRLFMD, 2016
Tunisia	2014	O	ME-SA	Ind-2001d	

S4 Table. FMDV isolated from west and central Africa for the period between 1997 and 2016

Country	Year	Serotype	Topotype	Genotype/Strain	References
Benin	2010	A	AFRICA	G-IV	Gorna *et al*., 2014
Benin	2010	O	WA	NK	
Burkina Faso	2002	O	WA	NM	Ularamu *et al*., 2016
Cameroon	2000	A	AFRICA	G-IV	Bronsvoort *et al*., 2004; Ularamu *et al*., 2016
Cameroon	2005	A	AFRICA	G-IV	Ularamu *et al*., 2016
Cameroon	2012	A	AFRICA	G-IV	
Cameroon	2013	A	AFRICA	G-IV	WRLFMD, 2016
Gambia	1999	A	AFRICA	-	Knowles *et al*., 2007
Cameroon	2000	O	WA	-	Bronsvoort *et al*., 2004
Cameroon	2005	O	WA	-	WRLFMD, 2016
Cameroon	2000	O	-	-	Bronsvoort *et al*., 2004; Ludi *et al*., 2016
Cameroon	2010	O	-	-	O'Leary *et al*., 2016
Cameroon	2012	O	-	-	
Cameroon	2010	O	EA-3	-	Ludi et *al*, 2016
Cameroon	2000	SAT2	VII	-	Bronsvoort et al, 2004 ; Ludi et al, 2016 ; Ularamu et al, 2016
Cameroon	2005	SAT2	VII	-	Ludi et *al*, 2016 ; Ularamu et al, 2016
Cameroon	2012	SAT2	VII	-	Ludi et al, 2016; WRLFMD, 2016
Cameroon	2013	SAT2	VII	Lib-12	WRLFMD, 2016
Cote d'Ivoire	1999	O	WA	-	Ularamu et *al*, 2016
Congo DR	2011	A	AFRICA	G-I	WRLFMD, 2016
Congo DR	2006	O	EA-2	-	
Congo DR	2010	O	EA-2	-	
Ghana	1993	O	WA	-	Ularamu et *al*, 2016
Ghana	2012	O	WA	-	

Ghana	1991	SAT2	-	-	O'Leary *et al*., 2016
Mali	2004	A	AFRICA	G-IV	Ularamu et *al*, 2016
Mali	2006	A	AFRICA	G-IV	
Mali	2005	O	WA	-	
Mali	2006	O	WA	-	
Mauritania	2006	A	AFRICA	G-IV	WRLFMD, 2016
Mauritania	2000	O	WA	-	Ularamu et *al*, 2016
Mauritania	2001	O	WA	-	
Mauritania	2014	SAT2	VII	-	
Niger	2001	O	WA	-	
Niger	2005	O	WA	-	
Niger	2015	O	WA	-	Souley Kouato *et al*., 2017WRLFMD, 2016
Niger	2005	SAT2	VII	Lib-03	Ularamu et al, 2016
Nigeria	2009	A	AFRICA	G-IV	Ehizibolo *et al*., 2014; Fasina *et al*., 2013; Ularamu *et al*., 2016
Nigeria	2011	A	-	-	Olabode *et al*., 2014
Nigeria	2011	A	AFRICA	G-IV	Ularamu et *al*, 2016; WRLFMD, 2016
Nigeria	2012	A	AFRICA	G-IV	
Nigeria	2013	A	AFRICA	G-IV	
Nigeria	2015	A	AFRICA	G-IV	Ehizibolo *et al*., 2017a
Nigeria	2007	O	EA-3		Fasina et al, 2013
Nigeria	2009	O	EA-3		
Nigeria	2007	O	EA-3	-	Fasina et *al*, 2013; WRLFMD, 2016
Nigeria	2009	O	EA-3	-	
Nigeria	2011	O	EA-3	-	Ularamu et *al*, 2016; WRLFMD, 2016
Nigeria	2014	O	EA-3	-	Ehizibolo *et al*., 2017a
Nigeria	2011	O	WA	-	Ehizibolo *et al*., 2017a ; Ularamu et *al*, 2016 ; WRLFMD, 2016
Nigeria	2012	O	WA	-	
Nigeria	2013	O	WA		

Nigeria	2014	O	WA	-	
Nigeria	2015	SAT1	X	-	Ehizibolo *et al*., 2017b
Nigeria	2011	SAT2	-	-	Olabode et *al*, 2014
Nigeria	2008	SAT2	VII	-	Ularamu et *al*, 2016 ; WRLFMD, 2016
Nigeria	2011	SAT2	VII	-	Ularamu et *al*, 2016
Nigeria	2012	SAT2	VII	-	Ularamu et *al*, 2016 ; WRLFMD, 2016
Nigeria	2007	SAT2	VII	-	Fasina et *al*, 2013; WRLFMD, 2016
Nigeria	2008	SAT2	VII	-	
Nigeria	2013	SAT2	VII	-	Ehizibolo *et al*., 2017a
Senegal	2006	O	WA	-	Ularamu et *al*, 2016
Senegal	2009	SAT2	VII	-	
Togo	2004	O	WA	-	
Togo	2005	O	WA	-	
Togo	2005	A	AFRICA	G-IV	

S5 Table. FMDV isolated from East Africa for the period between 1997 and 2016

Country	Year	Serotype	Topotype	Genotype/Strain	Reference
Eritrea	1997	A	AFRICA	G-IV	WRLFMD, 2016
Eritrea	1998	A	AFRICA	G-IV	
Eritrea	2006	A	AFRICA	G-IV	
Eritrea	2007	A	AFRICA	G-IV	
Eritrea	2008	A	AFRICA	G-IV	
Eritrea	2009	A	AFRICA	G-IV	
Ethiopia	2000	A	AFRICA	G-VII	Ayelet *et al*., 2009; WRLFMD, 2016
Ethiopia	2001	A	AFRICA	G-VII	
Ethiopia	2002	A	AFRICA	G-VII	WRLFMD, 2016
Ethiopia	2005	A	AFRICA	G-VII	Ayelet *et al*., 2009
Ethiopia	2007	A	AFRICA	G-VII	
Ethiopia	2008	A	AFRICA	G-VII	Negusssie *et al*., 2011; WRLFMD, 2016
Ethiopia	2009	A	AFRICA	G-VII	WRLFMD, 2016
Ethiopia	2015	A	AFRICA	G-VII	
Kenya	1998	A	AFRICA	G-I	
Kenya	2003	A	AFRICA	G-I	
Kenya	2005	A	AFRICA	G-I	
Kenya	2005	A	AFRICA	G-VII	
Kenya	2006	A	AFRICA	G-I	
Kenya	2008	A	AFRICA	G-I	
Kenya	2009	A	AFRICA	G-I	

Kenya	2012	A	AFRICA	G-I	
Somalia	2006	A	AFRICA	G-IV	
Somalia	2011	A	AFRICA	G-IV	
Somalia	2013	A	AFRICA	G-IV	
Sudan	2006	A	AFRICA	G-IV	Habiela *et al*., 2010
Sudan	2011	A	AFRICA	G-IV	WRLFMD, 2016
Sudan	2013	A	AFRICA	G-IV	
Tanzania	2008	A	AFRICA	G-I	Kasanga *et al*., 2015; Sallu *et al*., 2014; WRLFMD, 2016
Tanzania	2009	A	AFRICA	G-I	
Tanzania	2011	A	AFRICA	G-I	Sallu et *al,* 2014
Tanzania	2012	A	AFRICA	G-I	Sallu et *al,* 2014; WRLFMD, 2016
Tanzania	2013	A	AFRICA	G-I	WRLFMD, 2016
Uganda	2013	A	AFRICA	G-I	Namatovu *et al*., 2015b
Eritrea	2011	O	EA-3	-	WRLFMD, 2016
Ethiopia	1999	O	EA-2	-	Balinda *et al*., 2010b
Ethiopia	2000	O	EA-2	-	
Ethiopia	2001	O	-	-	Sallu et *al,* 2014; WRLFMD, 2016
Ethiopia	2003	O	EA-3	-	WRLFMD, 2016
Ethiopia	2004	O	EA-3	-	Ayelet *et al*., 2009; WRLFMD, 2016
Ethiopia	2005	O	EA-3	-	
Ethiopia	2005	O	EA-4	-	
Ethiopia	2006	O	EA-3	-	
Ethiopia	2007	O	EA-3	-	
Ethiopia	2008	O	EA-3	-	Negusssie *et al*., 2011; WRLFMD, 2016
Ethiopia	2009	O	EA-3	-	
Ethiopia	2010	O	EA-3	-	
Ethiopia	2011	O	EA-3	-	
Ethiopia	2012	O	EA-3	-	

Ethiopia	2013	O	EA-4	-	WRLFMD, 2016
Ethiopia	2013	O	EA-3	-	
Ethiopia	2014	O	EA-3	-	
Ethiopia	2015	O	EA-3	-	
Ethiopia	2016	O	EA-4	-	
Kenya	1998	O	EA-3	-	Wekesa *et al*., 2015a; WRLFMD, 2016
Kenya	1999	O	EA-3	-	
Kenya	2000	O	EA-2	-	Balinda *et al*., 2010b; WRLFMD, 2016
Kenya	2001	O	EA-2	-	
Kenya	2002	O	EA-2	-	Wekesa *et al*., 2015a; WRLFMD, 2016
Kenya	2003	O	EA-2	-	
Kenya	2004	O	EA-2	-	Balinda *et al*., 2010b; WRLFMD, 2016
Kenya	2005	O	EA-2	-	WRLFMD, 2016
Kenya	2007	O	EA-2	-	Balinda *et al*., 2010b; WRLFMD, 2016
Kenya	2008	O	EA-2	-	
Kenya	2008	O	EA-1	-	WRLFMD, 2016
Kenya	2009	O	EA-2	-	
Kenya	2009	O	EA-1	-	
Kenya	2010	O	EA-1	-	
Kenya	2010	O	EA-4	-	Wekesa *et al*., 2015a; WRLFMD, 2016
Kenya	2010	O	EA-2	-	
Kenya	2011	O	EA-2	-	
Somalia	2007	O	EA-3	-	WRLFMD, 2016
Sudan	2005	O	EA-3	-	Habiela et *al,* 2010; WRLFMD, 2016
Sudan	2008	O	EA-3	-	
Sudan	2009	O	EA-3	-	
Sudan	2010	O	EA-3	-	
Sudan	2011	O	EA-3	-	

Sudan	2012	O	EA-3	-	WRLFMD, 2016
Sudan	2013	O	EA-3	-	
Rwanda	2004	O	EA-2	-	
Tanzania	1996	O	EA-2	-	Nsamba *et al*., 2015
Tanzania	2004	O	EA-1	-	Kasanga et *al*, 2015; WRLFMD, 2016
Tanzania	2008	O	EA-2	-	
Tanzania	2009	O	EA-2	-	Kasanga et *al*, 2015
Tanzania	2012	O	EA-2	-	WRLFMD, 2016
Tanzania	2014	O	EA-2	-	
Uganda	1998	O	EA-4	-	Ayelet *et al*., 2009; WRLFMD, 2016
Uganda	1999	O	EA-4	-	Balinda *et al*., 2010b; WRLFMD, 2016
Uganda	2002	O	EA-2	-	Asfor *et al*., 2014; Ayelet *et al*., 2009; WRLFMD, 2016
Uganda	2003	O	EA-2	-	Balinda et *al*, 2010b
Uganda	2004	O	EA-2	-	
Uganda	2005	O	EA-2	-	Nsamba et *al,* 2015
Uganda	2006	O	EA-2	-	Balinda *et al*., 2010c
Uganda	2006	O	-	-	Mwiine *et al*., 2010
Uganda	2007	O	EA-2	-	WRLFMD, 2016
Uganda	2008	O	EA-2	-	Kasambula *et al*., 2012
Uganda	2009	O	EA-2	-	
Uganda	2011	O	EA-2	-	Namatovu *et al*., 2015a
Burundi	1999	SAT1	I	-	Reid *et al*., 2010
Ethiopia	2007	SAT1	IX	-	Ayelet *et al*., 2009; WRLFMD, 2016
Ethiopia	2007	SAT1	IX	-	
Ethiopia	2007	SAT1	IX	-	
Kenya	1998	SAT1	I	-	Nsamba *et al*., 2015
Kenya	1999	SAT1	-	-	Nsamba *et al*., 2015; Sangula *et al*., 2010a
Kenya	2004	SAT1	I	-	Wekesa *et al*., 2015b; WRLFMD, 2016

Kenya	2005	SAT1	I	-	Sangula et *al*, 2010; WRLFD, 20016
Kenya	2006	SAT1	I	-	
Kenya	2006	SAT1	III	-	Sangula et *al*, 2010
Kenya	2008	SAT1	I	-	WRLFMD, 20016
Kenya	2009	SAT1	I	-	
Kenya	2010	SAT1	I	-	
Kenya	2011	SAT1	I	-	
Kenya	2013	SAT1	I	-	
Tanzania	1999	SAT1	III	-	Nsamba et *al,* 2015 ; Salhe et *al*, 2007
Tanzania	2012	SAT1	I	-	Sallu et *al,* 2014WRLFMD, 2016
Tanzania	2013	SAT1	I	-	WRLFMD, 2016
Tanzania	2014	SAT1	I	-	
Uganda	1997	SAT1	IV	-	Salhe et *al,* 2007
Uganda	1999	SAT1	IV	-	
Uganda	2007	SAT1	IV	-	Ayebazibwe *et al*., 2010
Uganda	2013	SAT1	IV	-	Dhikusooka *et al*., 2016
Eritrea	1998	SAT2	IV	-	Bastos *et al*., 2003
Eritrea	1998	SAT2	VII	-	Nsamba et *al,* 2015 ; Salhe et *al*, 2007
Ethiopia	2007	SAT2	XIII	-	Ayelet *et al*., 2009; WRLFMD, 2016
Ethiopia	2009	SAT2	XIII	-	Hall *et al*., 2013; WRLFMD, 2016
Ethiopia	2010	SAT2	XIII	-	
Ethiopia	2014	SAT2	VII	Alx-12	Ularamu *et al*., 2016; WRLFMD, 2016
Ethiopia	2015	SAT2	VII	Alx-12	
Kenya	1998	SAT2	-	-	Sahle *et al*., 2007
Kenya	1999	SAT2	I	-	Bastos *et al*., 2003
Kenya	2002	SAT2	IV	-	Sangula *et al*., 2010b
Kenya	2004	SAT2	IV	-	

Kenya	2005	SAT2	IV	-	Sangula et al, 2010b ; WRLFMD, 2016
Kenya	2006	SAT2	IV	-	Sangula et al, 2010b
Kenya	2007	SAT2	IV	-	Sangula et al, 2010b ; WRLFMD, 2016
Kenya	2008	SAT2	IV	-	WRLFMD, 2016
Kenya	2009	SAT2	IV	-	
Kenya	2011	SAT2	IV	-	
Kenya	2012	SAT2	IV	-	
Rwanda	2000	SAT2	V	-	Bastos et *al*, 2003b
Rwanda	2001	SAT2	VIII	-	Nsamba et *al*, 2015
Rwanda	2004	SAT2	VIII	-	Hall et *al*, 2013; WRLFMD, 2016
Sudan	2007	SAT2	VII	-	Habiela et *al*, 2010; WRLFMD, 2016
Sudan	2008	SAT2	XIII	-	
Sudan	2010	SAT2	VII	-	Hall et *al*, 2013
Sudan	2012	SAT2	VII	Alx-12	WRLFMD, 2016
Sudan	2013	SAT2	VII	Alx-12	
Sudan	2014	SAT2	VII	Alx-12	
Tanzania	2009	SAT2	IV	-	Kasanga *et al*., 2015; WRLFMD, 2016
Tanzania	2011	SAT2	IV	-	WRLFMD, 2016
Tanzania	2012	SAT2	IV	-	
Uganda	1998	SAT2	X	-	Sahle *et al*., 2007
Uganda	2002	SAT2	VII	-	Christensen et *al*., 2004
Uganda	2002	SAT2	XII	-	Nsamba et *al*, 2015
Uganda	2004	SAT2	-	-	Balinda *et al*., 2010a
Uganda	2007	SAT2	X	-	Ayebazibwe et *al*, 2010
Uganda	2013	SAT2	X	-	
Uganda	1997	SAT3	v	-	WRLFMD, 2016
Uganda	2013	SAT3	v	-	Dhikusooka *et al*., 2015; WRLFMD, 2016

S6 Table. FMDV isolated from southern Africa for the period between 1997 and 2016

Country	Year	Serotype	Topotype	Genotype/Strain	References
Botswana	1998	SAT1	II	-	Bastos *et al*., 2001; WRLFMD, 2016
Botswana	2006	SAT1	III	-	O'Leary *et al*., 2016; WRLFMD, 2016
Botswana	2014	SAT1	III	-	
Botswana	2015	SAT1	III	-	
Malawi	2001	SAT1	-	-	
Mozambique	2002	SAT1	III	-	Nsamba et *al*, 2015
Mozambique	2002	SAT1	I	-	WRLFMD, 2016
Mozambique	2010	SAT1	III	-	O'Leary *et al*., 2016
Mozambique	2010	SAT1	I	-	Kasanga *et al*., 2014
Namibia	1998	SAT1	II	-	Bastos *et al*., 2001
Namibia	2010	SAT1	II	-	O'Leary *et al*., 2016
Namibia	2015	SAT1	III	-	WRLFMD, 2016
Southern Africa	2010	SAT1	I	-	Kasanga *et al*., 2014
South Africa	1998	SAT1	I	-	Bastos *et al*., 2001
South Africa	2000	SAT1	I	-	O'Leary *et al*., 2016
South Africa	2001	SAT1	-	-	Vosloo *et al*., 2007; Vosloo *et al*., 2002
South Africa	2002	SAT1	I	-	O'Leary *et al*., 2016
South Africa	2003	SAT1	I	-	
South Africa	2009	SAT1	-	-	
South Africa	2010	SAT1	I	-	
Swaziland	2000	SAT1	II	-	WRLFMD, 2016

Swaziland	2015	SAT1	II	-	
Zambia	2004	SAT1	I	-	
Zambia	2005	SAT1	I	-	
Zambia	2006	SAT1	III	-	O'Leary *et al*., 2016
Zambia	2008	SAT1	I	-	WRLFMD, 2016
Zambia	2009	SAT1	I	-	
Zambia	2012	SAT1	III	-	
Zimbabwe	1997	SAT1	-	-	Hargreaves *et al*., 2004
Zimbabwe	1998	SAT1	III	-	O'Leary *et al*., 2016
Zimbabwe	2003	SAT1	-	-	
Zimbabwe	2015	SAT1	II	-	O'Leary *et al*., 2016; WRLFMD, 2016
Botswana	1998	SAT2	II	-	O'Leary *et al*., 2016
Botswana	1998	SAT2	I	-	Bastos *et al*., 2003b
Botswana	2002	SAT2	-	-	Brito *et al*., 2016
Botswana	2003	SAT2	-	-	Baipoledi *et al*., 2004
Botswana	2005	SAT2	III	-	WRLFMD, 2016
Botswana	2006	SAT2	II	-	Brito *et al*., 2016
Botswana	2006	SAT2	III	-	WRLFMD, 2016
Botswana	2007	SAT2	III	-	
Botswana	2008	SAT2	III	-	
Botswana	2009	SAT2	III	-	
Botswana	2010	SAT2	III	-	
Botswana	2011	SAT2	III	-	
Botswana	2011	SAT2	I	-	
Botswana	2012	SAT2	III	-	
Botswana	2013	SAT2	III	-	
Botswana	2015	SAT2	III	-	
Malawi	2008	SAT2	I	-	

Malawi	2010	SAT2	I	-	
Malawi	2014	SAT2	I	-	
Malawi	2015	SAT2	I	-	
Mozambique	2002	SAT2	I	-	
Mozambique	2010	SAT2	I	-	Kasanga *et al*., 2014; WRLFMD, 2016
Mozambique	2014	SAT2	I	-	WRLFMD, 2016
Mozambique	2015	SAT2	I	-	
Namibia	1998	SAT2	I	-	Bastos *et al*., 2003b
Namibia	2007	SAT2	III	-	WRLFMD, 2016
Namibia	2007	SAT2	II	-	O'Leary *et al*., 2016
Namibia	2008	SAT2	III	-	Brito et *a*l, 2016; WRLFMD, 2016
Namibia	2008	SAT2	II	-	O'Leary *et al*., 2016
Namibia	2011	SAT2	-	-	Brito et *al*, 2016
Namibia	2015	SAT2	III	-	WRLFMD, 2016
South Africa	1998	SAT2	III	-	Brito et *al*, 2016
South Africa	2001	SAT2	I	-	Phologane *et al*., 2008
South Africa	2003	SAT2	-	-	Brito et *al*, 2016
South Africa	2006	SAT2	-	-	
South Africa	2007	SAT2	I	-	O'Leary *et al*., 2016
South Africa	2008	SAT2	I	-	
South Africa	2010	SAT2	I	-	
South Africa	2011	SAT2	I	-	Brito et *al*, 2016
South Africa	2012	SAT2	I	-	
Zambia	2007	SAT2	III	-	WRLFMD, 2016
Zambia	2008	SAT2	III	-	
Zambia	2009	SAT2	III	-	Van *et al*., 2016; WRLFMD, 2016
Zambia	2012	SAT2	IV	-	Banda *et al*., 2014; WRLFMD, 2016

Zambia	2015	SAT2	IV	-	WRLFMD, 2016
Zimbabwe	1997	SAT2	I	-	Hargreaves et *al*, 2004
Zimbabwe	1998	SAT2	I	-	Bastos *et al*., 2003b
Zimbabwe	2000	SAT2	I	-	
Zimbabwe	2001	SAT2	-	-	Opperman *et al*., 2012
Zimbabwe	2003	SAT2	I	-	Brito et *al*, 2016
Zimbabwe	2002	SAT2	I	-	
Zimbabwe	2010	SAT2	II	-	WRLFMD, 2016
Zimbabwe	2010	SAT2	I	-	
Zimbabwe	2014	SAT2	II	-	
Zimbabwe	2014	SAT2	I	-	O'Leary *et al*., 2016; WRLFMD, 2016
Zimbabwe	2015	SAT2	II	-	
Botswana	1998	SAT3	II	-	Bastos *et al*., 2003a
Botswana	2010	SAT3	II	-	WRLFMD, 2016
Mozambique	2010	SAT3	VI	-	O'Leary *et al*., 2016
Namibia	1998	SAT3	II	-	Bastos *et al*., 2003a
South Africa	1997	SAT3	-	-	Bastos *et al*., 1999; Vosloo *et al*., 2001
South Africa	1998	SAT3	I	-	Bastos *et al*., 2003a
South Africa	2001	SAT3	I	-	Vosloo *et al*., 2001
South Africa	2006	SAT3	I	-	WRLFMD, 2016
South Africa	2010	SAT3	I	-	Jori *et al*., 2016
Zambia	2006	SAT3	II	-	WRLFMD, 2016
Zambia	2015	SAT3	II	-	
Zimbabwe	1999	SAT3	I	-	Bastos *et al*., 2003a
Zimbabwe	2010	SAT3	-	-	Jori *et al*., 2016
South Africa	2000	O	ME-SA	Pan Asia	Knowles *et al*., 2005; Mason *et al*., 2003; Sangare *et al*., 2001
Zambia	2010	O	EA-2	-	

Zambia	2015	A	AFRICA	G-I	WRLFMD, 2016

Legend: EA (East Africa), ME-SA (Middle East-South Asia), WA (West Africa); - Not Known or not mentionned.

S7 Table. Overview of molecular epidemiology studies on foot-and-mouth disease virus at regional or continental level in Africa

Study purposes	Origin of isolates	Key findings / conclusion	References
Assessment of genetic diversity of FMDV SAT2	Southern Africa	11 FMDV SAT2 lineages were revealed by phylogenetic analysis. 4 lineages corresponded to southern African FMDV, 2 to west Africa and 5 to central and east Africa	Bastos *et al*., 2003b
To assess the genetic heterogeneity of FMDV SAT3	Southern and eastern Africa	Six distinct FMDV SAT3 lineages evolving independently in different geographical localities topotypes were identified. Topotypes I-IV occur in southern Africa, whilst topotypes V and VI were found east Africa	Bastos *et al*., 2003a
Assessment of the genetic heterogeneity of FMDV SAT1	Southern Africa	3 FMDV SAT1 topotypes have been found: topotype I in South Africa and southern Zimbabwe, topotype II from Namibia, Botswana and western Zimbabwe, and topotype III found in Zambia, Malawi and northern Zimbabwe. The results of the phylogenetic analyses further showed that the genetic characterization of contemporary buffalo viruses is applicable to determining the origin of historical FMD outbreaks.	Bastos *et al*., 2001
Investigation of FMDV SAT2 transmission between wildlife and livestock	Southern Africa	Results from this study suggested that the probable FMDV transmission from cattle to buffalo. Further the results have shown that the genetic diversity of FMDV SAT2 has decreased in buffalo and cattle population during the last decade.	Brito *et al*., 2016
To update the picture of SAT2 phylogenetic	SSA, North Africa and Middle East	Relevant conclusions emerged from this comprehensive study on FMDV SAT2: 1 The phylogenetic analysis has shown that FMD SAT2 outbreaks that have occurred in North Africa from 2012, appear to have origins in countries immediately south of the Sahara whereas those in the Middle East were likely related to those from East Africa. 2 FMDV SAT2 spread is most probably caused by relatively short-distance movements of animals across national borders.	Hall *et al*., 2013

To determine the genetic relationship of FMDV serotypes SAT1, 2&3	Southern Africa	FMDV SAT Serotypes were mainly involved in outbreaks in livestock-wildlife interface areas of these countries. FMDV SATs recently detected in Tanzania and Zambia were genetically related to lineages and topotypes from East and South Africa, with a newly emerged unassigned type SAT1 topotype in Mozambique.	Kasanga *et al*., 2014
Phylogenetic comparison of African FMDV serotype O	SSA	Two previously unrecognised genetic lineages of FMDV O were identified in East Africa (Salhe et al, 2004), each having a distinct geographic distribution. The result of this study demonstrated a plausible recombination near the 3' end of VP1 of the virus that may have played a role in the evolution of the EA-2 topotype.	Knowles *et al*., 2004
Genetic comparison of PanAsia FMDV serotype O	Asia, Africa and Europe	Taken together, analyses of the complete genome sequence data reveal a remarkable conservation among the PanAsia virus isolates, which appear to be much more stable than other type O viruses circulating in Asia during the same period. This analysis provided confirmation of the close relationship between the viruses responsible for the South Africa and UK outbreaks, but failed to identify any genetic characteristic that could account for the unprecedented spread of this strain.	Mason *et al*., 2003
To elucidate the genetic variation among Ethiopian FMDV O	East, South and West Africa. Middle east, Asia and South America	Three FMDV serotype O lineages have been identified: 1 African/Middle East-Asia, 2 Cathay and 3 South American. Within lineage I African/Middle East, three topotypes were defined such as. East and West Africa and the Middle East-Asia together with the South African isolate. The Ethiopian isolates clustered as part of topotype I, the East African topotype. Additionally, two clades based on < 12 % nucleotide difference A and B were identified within the East African isolates, with clade A being further classified into three significant branches, A1, A2 and A3. Clade B consisted of two Kenyan isolates.	Sahle *et al*., 2004
Phylogenetic analysis of FMDV SAT1	East, South and West Africa.	This study demonstrated the presence of at least 6 lineages and 11 genotypes within SAT1 serotype in SSA. Differences were observed between isolates from countries in East Africa, with individual countries suffering outbreaks from isolates belonging to various genotypes, which according to the authors suggested evidence of reintroduction of strains and long-term circulation of outbreak viruses.	Sahle *et al*., 2007a
Phylogenetic analysis of FMDV SAT2	East, South and West Africa.	Fourteen genotypes were identified of which three were newly identified and belonged to East Africa, bringing the total number of genotypes for that region to eight. The genotypes clustered into three lineages that demonstrated surprising links between East, southern and south-western Africa. One lineage lineage II was unique to West Africa. These results established numerous incursions across country borders in East Africa and long term conservation of sequences for periods up to 41 years.	Sahle *et al*., 2007b

Genetic analysis of FMDV serotype O	North Africa, Middle East and Europe	The results of this study have shown that the FMD viruses isolated from North Africa and the Middle East were very different from the classical European vaccine strains. All the viruses isolated during earlier FMD outbreaks in North African epidemic 1989-1992 formed a cluster differing by no more than 6% from each other.	Samuel *et al*., 1999
To determine the extent of genetic diversity within FMDV type O	Isolates of FMDV O worldwide	This analysis identified eight major genotypes with cut-off value of 15 % nucleotide difference. They were named Cathay, Middle East-South Asia ME-SA, South-East Asia SEA, Europe-South America Euro-SA, Indonesia-1 ISA-1, Indonesia-2 ISA-2, East Africa EA and West Africa WA. These eight genetic lineages fell within geographical boundaries, since this finding enabled the approval of topotype concept to describe these viruses.	Samuel & Knowles, 2001
To determine the number of FMDV serotype O	West and South Africa	Results showed three discrete evolutionary lineages correspond to different geographical regions as follows: Lineage I: Africa–Asia; Lineage II: Asia; and Lineage III: Europe–South America. Within each of these lineages, further clusters or genotypes were similarly identified and labelled A–G in accordance of topotype concept described above Samuel & Knowles, 2001. Among these, the genotype A occurred in Asia, the Middle East and South Africa and corresponds to the ME-SA topotype while genotype B were found in east Africa; and C west and north Africa. The results confirmed continued circulation of viruses in the field as well as trans-boundary and inter-continental transmission.	Sangare *et al*., 2001
To elucidate regional genetic relationships of SAT-2	SSA	This study identified Eight major genotypes A - H and they constituted four major evolutionary lineages I–IV that were associated with geographically distinct regions. Lineages I and II were constituted with viruses from of West Africa exclusively suggesting that the existence of two distinct West African topotypes within FMDV SAT2. Viruses from West Africa Nigeria and East Africa Eritrea constituted lineage III, whilst lineage IV, comprising viruses from Central and East Africa.	Sangare *et al*., 2004
Assessment of genetic variation of FMDV SAT1	SSA	The result of this study has identified six major evolutionary lineages I–VI with two separate lineages I and II observed in West Africa while the remaining lineages III–VI were previously identified as FMDV SAT1 topotypes found in East and southern Africa. Lineage I was constituted with viruses involved in outbreaks in Nigeria 1975–1976 and those responsible for the disease in Niger in 1976, indicating a likely spread of this virus from Nigeria to Niger.	Sangare *et al*., 2003

Description of the emergence of FMDV SAT1 diversity	SSA	Results have shown the existence of two virus groups with probable independent introductions from southern Africa. One group was exclusive to Uganda while the other was present within Kenya and Tanzania. According to the authors, their results suggested that Kenya and Tanzania appear to experience a much greater exchange of viruses at their respective southern and northern borders through the trans-boundary livestock and wildlife movements than with Uganda.	Sangula *et al*., 2010
Genetic analysis of FMDV SAT1 serotype	Southern Africa	Results of this study confirmed the existence of the three main topotypes I, II and III previously described for SAT1 viruses in southern Africa. Although the role of buffalo in the epidemiology of FMD has been previously emphasised, this study has demonstrated that other wildlife species such as Impala can also play an important intermediary role in disseminating FMDV.	Vosloo *et al*., 2006

Legend : FMDV: Foot-and-Mouth Disease Virus, SSA: Sub Saharan Africa, VP1: (FMDV) structural protein 1.

Part two

Chapter 6: Outbreak investigations and molecular characterization of foot-and-mouth disease viruses circulating in southwest Niger

Chapter 6: Outbreak investigations and molecular characterization of foot-and-mouth disease viruses circulating in southwest Niger

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Abstract

In Niger, the epidemiological situation regarding foot-and-mouth disease is unclear since many outbreaks are unreported. This study aimed i) to identify FMDV strains currently circulating in cattle herds, and ii) to identify risk factors associated with FMD seropositive animals in clinical outbreaks. Epithelial tissues (n=25) and sera (n=227) were collected from cattle in eight districts of the southwestern part of Niger. Testing of clinical material revealed the presence of FMDV serotype O that was characterised within the O/WEST AFRICA topotype. The antigenic relationship between one of the FMDV isolates from Niger (O/NGR/4/2015) and three reference vaccine strains was determined by the two-dimensional virus neutralization test (2dmVNT), revealing a close antigenic match between the field isolate from Niger and three FMDV serotype O vaccine strains. Serological analyses using a non-structural protein (NSP) test provided evidence for previous FMDV infection in 70% (158/227) of the sera tested. Multivariate logistic regression analysis revealed that only the herd composition (presence of both cattle and small ruminants) was significantly associated with FMDV seropositivity as defined by NSP positive results (P-value = 0.006). Of these positive sera, subsequent testing by Liquid Phase Blocking ELISA (LPBE) showed that 86% (136/158) were positive for one (or more) of four FMDV serotypes (A, O, SAT 1 and SAT 2). This study provides epidemiological information about FMD in the southwestern part of Niger, and highlights the complex transboundary nature of FMD in Africa. These findings may help to develop effective control and preventive strategies for FMD in Niger as well, as other countries in West Africa.

Keywords: Foot-and-Mouth Disease Virus; Identification, Molecular Characterization; Serology; Risk factors, southwestern Niger.

Introduction

Foot-and-mouth disease (FMD) is a highly contagious transboundary disease of cloven-hoofed domestic and wild animals caused by FMD virus (FMDV) belonging to the *Aphthovirus* genus within the *Picornaviridae* family. FMDV is a small, non-enveloped, icosahedral virus that has a positive-sense, single-stranded RNA genome of approximately 8.5 kb that encodes a single polyprotein which is cleaved into four structural proteins (SP) and 10 non-structural proteins (NSPs) by virus encoded proteases (Belsham, 1993). FMDV exists in seven immunologically distinct serotypes, O, A, C, Asia 1, SAT (Southern African Territories) 1, SAT 2 and SAT 3, each with a wide range of antigenically distinct subtypes. (Gleeson, 2002; Kasambula *et al*., 2012; Knowles & Samuel, 2003).

FMD is endemic in Niger where clinical disease has been reported mainly in cattle (Couacy-Hymann *et al*., 2006; Sangare *et al*., 2001; Sangare *et al*., 2004a. FMD was first reported in Niger in 1945, when samples corresponding to serotype C were typed by the Laboratoire Central de Recherches Vétérinaires of Maisons-Alfort in France (Pagot, 1948). According to a retrospective study that reviewed FMD outbreaks occurring between 1971 and 2001 (Couacy-Hymann *et al*., 2006), four FMDV serotypes (namely O, A, SAT 1 and SAT 2) were suspected to be present in West African countries including Niger. Other published studies support the circulation of these four FMD serotypes in the region (Fasina et al., 2013; Gorna et al., 2014; Olabode et al., 2014; Sangare et al., 2001; Sangare et al., 2003; Sangare et al., 2004a, Sangare et al., 2004b; Ularamu et al., 2016), although a comprehensive understanding of FMD epidemiology that can be used to inform disease control programs is currently lacking. Unfortunately, livestock in Niger have never been vaccinated against FMD. Moreover, as the livestock production system is mostly characterized by transhumance, nomadism and trade with neighbouring countries, there are no restrictions on animal movements in the country or elsewhere in West African. Therefore, the objectives of this study were to identify FMDV strains responsible for outbreaks in the southwestern part of Niger that occurred in cattle in 2014, as well as to describe risks factors associated with FMDV seropositivity in animals from these herds.

Materials and methods

Study area

In this article, sampling locations were defined at the district-level (Niger is administratively divided into 8 regions, 63 departments and 265 districts). The study was conducted in eight districts in the southwestern part of the country that included three regions namely Niamey (the capital), Tillabery and Dosso.

It is in the region of Tillabery that the largest numbers of samples were obtained in four districts: Kollo located 35 km from Niamey, Makalondi, Tamou and Alambaré bordering with Burkina Faso. In addition, Tamou and Alambaré are located near the W Regional Park which is a major national park in West Africa (Niger, Burkina Faso and Benin) around a meander in the River Niger shaped like a "W". In the Dosso region, three districts were involved in the study, including Dole, Tanda and Gaya, which share a common border with Nigeria and Benin. In Niamey, one district (called the fourth Arrondissement) was involved. Except for Niamey's district, these localities are located either on the transhumance route towards Benin and Nigeria (districts of Tanda, Dole and Gaya), or towards Burkina Faso and Benin (districts Tamou, Alambaré and Makalondi). This zone covers an area of more than 29,000 km² with a cattle population of about 500,000 animals (representing 5% of the cattle population at national level) based on the latest livestock census in 2007. Agriculture and livestock are the main activities of the resident population. The study area is depicted in **Figure 1**.

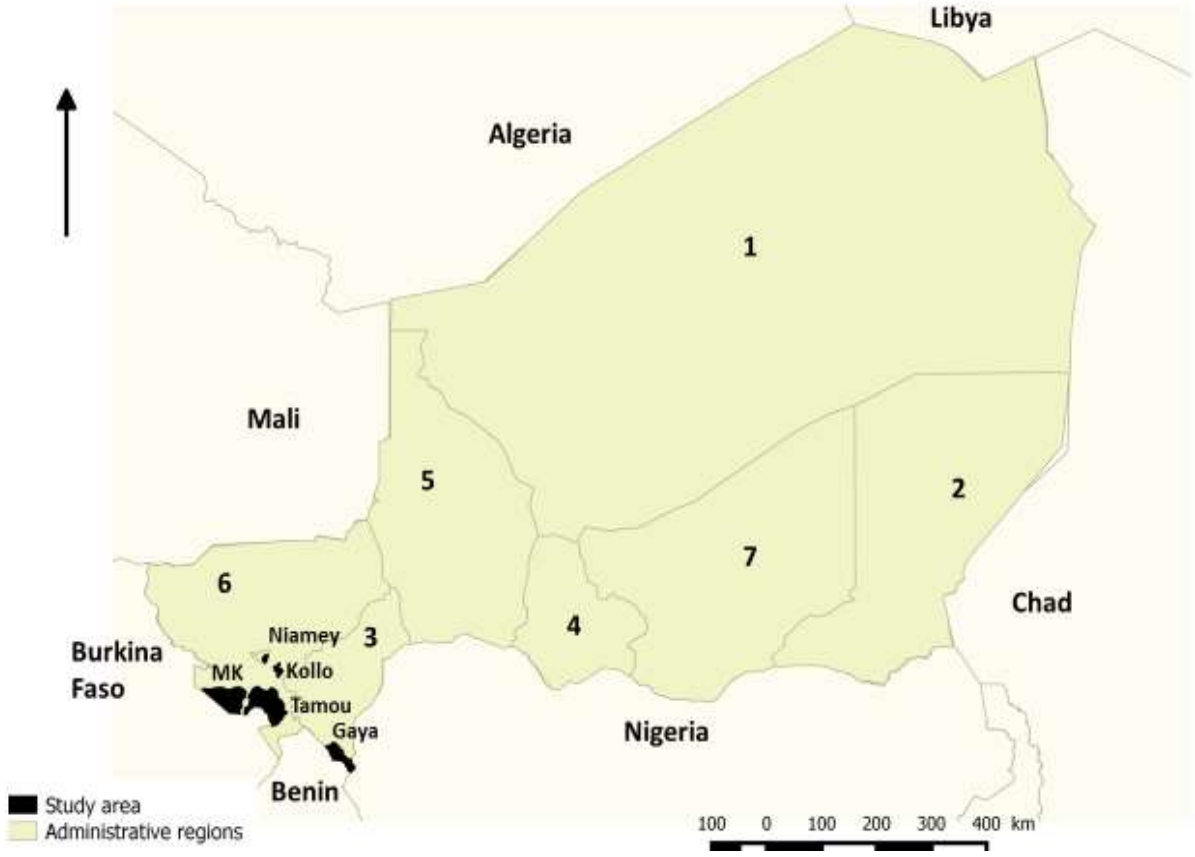


Figure 1: Geographical locations of FMD outbreaks described in this study

Legend:
Administrative regions: 1: Agadez, 2: Diffa, 3: Dosso, 4: Maradi, 5: Tahoua, 6: Tillabery, 7: Zinder and Niamey (capital city)
Study area (Eight sampling districts described in this study): Niamey, Kollo, MK: Makalondi, Gaya (that covers administratively the district of Tanda, Dole) and Tamou (covering administratively Alambaré).

Sampling design and disease investigation

In this study, an outbreak was defined as a district from which one or more clinical cases of FMD were reported by the district animal health service and/or by the farmers themselves. During September to October 2014, all reported outbreak sites were visited as soon as possible after notification; epithelium and serum samples were collected from cattle in the described study area.

As far as we are aware, no FMDV vaccination or other control measures were implemented at the study sites as in other parts of the country. The animals were first examined for evidence of

salivation and lameness. Salivating and/or lame animals were restrained in a crush pen for thorough examination and sampling. The oral cavity of salivating animals was examined for evidence of intact and/or ruptured vesicles, erosions and ulcers on the tongue, dental pad and mucosa. The hooves of lame animals were thoroughly washed with water and carefully examined for lesions, particularly on the coronary bands and interdigital spaces of the hooves. The epithelium samples were taken from sick animals showing suspected clinical signs and lesions of FMD, while the sera were taken from all examined animals during the herd visit, including those on which epithelium samples were collected (**Table 1**).

Table 1: Overview of the sampling strategy

Sampling site	**Number of herds visited**	**Number of sick animals** [i]	**Number of apparently healthy animals** [j]	**Number of Samples collected**	
				Epithelium[a]	Serum[b]
Makalondi	6	32	13	7	45
Gaya	1	4	3	2	7
Dolé	4	8	12	1	20
Tanda	2	9	9	1	18
Alambaré	2	11	8	5	19
Tamou	3	2	10	2	12
Kollo	5	26	29	7	55
4e Arrd (Niamey)	5	27	24	0	51
Total	28	119	108	25	227

Legend: [a]: Epithelium samples collected from sick animals with existing oral and foot lesions, [b]: sera collected from all examined animals during the herd visit, including those on which epithelium samples were collected. i + j = total number of sampled animals during the herd visit that corresponds to the total number of sera.

Sample and data collection

Twenty-five epithelium tissues were collected from oral and foot lesions from suspected FMD-infected cattle in seven separate districts: Makalondi (n=7), Gaya (n=2), Dolé (n=1), Tanda (n=1), Kollo (n=7), Alambaré (n=5) and Tamou (n=2). After collection, the tissues were immediately placed in a virus transport media composed of equal amount of sterile glycerol (50% v/v) and 0.04 M phosphate buffered saline (PBS) at a pH between 7.2 and 7.6.

At the same time, 227 blood samples were collected from apparently healthy and from clinically affected cattle. Sera were collected in eight districts (seven mentioned above and in one of the districts of Niamey): Makalondi (n=38), Gaya (n=5), Dolé (n=19), Tanda (n=17), Kollo (n=48), Alambaré (n=14), Tamou (n=10) and Niamey (n=51). In the last district, Niamey, the FMD outbreak was notified at least three weeks after the occurrence of the active outbreak and at the

time of the visit there were neither clinical signs nor lesions in affected cattle. The samples (serum and epithelium) were transported to the National Veterinary Laboratory of Niamey (LABOCEL) on dry ice. At LABOCEL, samples were stored at -20°C (serum) and at -80°C (epithelium) until their shipment to the Botswana Vaccine Institute (BVI) laboratory for analyses. All specimens were packaged as described by Kitching and Donaldson (1989) and shipped in a transport media to the BVI laboratory in Gaborone, Republic of Botswana. Among the epithelium tissues, positive samples diagnosed at BVI were submitted for confirmation to the World Reference Laboratory for FMD (WRLFMD) at The Pirbright Institute, UK.

Data were collected using a questionnaire (see **Appendix 1**), which was used to interview farmers responsible for 28 herds (with a total of 227 sampled animals) selected on the basis of FMD outbreak notification. The recorded data included animal age, sex and location, and the presence or absence of clinical signs and lesions in cattle. In addition, the interview collected information regarding FMD risk factors such as the number of animals in the herd, the herd composition, the grazing and watering habits, the herd management (transhumance nomadic or sedentary), and the potential contact with wildlife.

Serological analysis

Detection of antibodies against FMDV non-structural proteins (NSP-ELISA)

Serological diagnostics were performed at the Botswana Vaccine Institute (BVI) in accordance to the established standards and practices of this OIE reference laboratory for Sub-Saharan Africa. Sera were initially screened for antibodies against the highly conserved NSP of FMDV using the PrioCHECK® FMDV NS Enzyme-Linked Immunosorbent Assay (ELISA) test kit (Prionics AG, Switzerland), following the manufacturer's protocol. The Optical Density at 450nm (OD450) values of all samples were expressed as Percentage of Inhibition (PI) relative to the OD450 max. Positive results were defined as samples that generated a PI value of ≥50, whereas a strong positive result was set at a PI value of ≥70.

Detection of serotype-specific antibodies against FMDV Liquid-phase blocking ELISA (LPBE)

NSP ELISA positive reactive sera were further assessed using the Liquid-Phase Blocking ELISA (LPBE) modified from Hamblin et al. (1986). Briefly, ELISA plates NUNC Maxisorp (Gibco, Cat#4-39454A) were coated with FMDV serotype-specific rabbit hyperimmune sera (serotypes O, A, SAT1 and SAT2 suspected to be present in Niger), and left overnight in a

humid chamber at room temperature. In carrier plates, 2-fold series of each test serum were prepared, from 1/16 to 1/128. Control sera (strong and weak positive, and negative) were diluted at 1/16. To each well of the carrier plate, 50 µl of the different FMDV serotype viral antigen were added at a pre-determined working dilution, resulted in a ratio of sera with FMD antigen starting from 1/32 to 1/256. The following day, the rabbit antiserum-coated ELISA plates were washed three times with phosphate buffered saline containing 0.05% Tween 20 (PBST) (pH 7.4), and serum/antigen mixtures were transferred from the carrier plates to the rabbit-serum–coated ELISA plates and incubated at 37ºC for 1 hour on a rotary shaker. The plates were then washed three times as previously and FMDV serotype-specific guinea pig antiserum was added to each well at a predetermined working concentration and incubated at 37 °C for 1 hour on a rotary shaker. After incubation and washing step as previously, rabbit anti-guinea pig immunoglobulin conjugated to horseradish peroxidase was added to each well at a predetermined working concentration. The plates were washed after 1 hour of incubation and substrate solution (orthophenylene diamine [OPD] + 0.05% H_2O_2) was added to each well. The reaction was stopped by adding 50µl of 1 M sulfuric acid. The plates were read at 492 nm on a Thermo Scientific™ Multiskan™ FC Microplate Photometer and antibody titres were expressed as the final dilution of the tested serum giving 50% of the mean absorbance value in the virus control wells where test serum was absent. Titres of less than 1/40 (or 1.6 in reciprocal $\log_{10}$ form) were considered as negative while titres more than 1/40 were considered positive (Hamblin et al., 1986).

Analysis of epithelium tissues

Virus isolation

The epithelium tissues were processed by the standard WRLFMD/World Organisation for Animal Health (OIE) procedure for virus isolation (OIE, 2012). The composition of the media used for virus isolation and culture of cells is as follows: 10% Minimum Essential Medium 10X (MEM 10X), 10% Lactalbumin Hydrolysate 10X, 4.5% Sodium Bicarbonate, 1% Negative Calf serum, 0.2% Penicillin and top up to 100ml with sterile distilled water. The epithelium samples were first taken from the PBS/glycerol, and blotted dry on absorbent paper. A suspension was prepared by grinding 1 gram of the sample in sterile sand in a sterile pestle and mortar with a small volume of tissue culture medium. Medium was added until a final volume of nine times that of added epithelial sample was reached, giving a 10% suspension. The suspension was clarified on a bench centrifuge at 2000 g for 10 minutes at 4°C. The clarified suspensions suspected to contain FMDV were inoculated onto primary lamb kidney cell cultures (Rein de

Mouton [RM]: at BVI) or primary bovine thyroid cell cultures (BTy: at WRLFMD) and incubated for 1 hour at 37 °C. Fresh cell culture medium was then added (15 ml); the cultures were incubated at 37 °C and monitored for cytopathic effect (CPE) for 48 hours. If no CPE was observed after 48 hours, the sample was considered as 'no virus detected' the culture was frozen at -70°C, then thawed and centrifuged at 2000 g for 10 minutes at 4°C to collect supernatant for second passage (P2), this was repeated for third passage (P3) and if no CPE was observed at 48h, then the sample was considered negative for FMDV. The first passage (P1) and the second passage (P2) were subject to one freeze-thaw cycle. If CPE was observed, the culture medium was pooled and cleared by centrifugation at 2000 g for 10 minutes at 4°C. A sample of supernatant was tested by RT-PCR following RNA extraction. However, it should be noted that the samples were examined for virus isolation nine months after they had been collected in the field.

Conventional RT-PCR assay for VP1 analysis

RNA was extracted from the ground tissue suspension samples using ZR Viral RNA kit (ZymoResearch, USA) following the manufacturer's instructions. Extracted nucleic acid samples were analysed for FMDV RNA using conventional reverse transcription-polymerase chain reaction (RT-PCR) using oligonucleotide forward primer O-1C244F (5'-GCAGCAAAACACATGTCAAACACCTT-3') and reverse primer EUR 2B-52R (5'-GACATGTCCTCCTGCATCTGGTTGAT-3′) targeting the VP1 gene within the FMDV RNA genome (Knowles et al., 2016). At the BVI, the RT-PCR was set and ran as following: reverse transcription at 48ºC for 30 minutes; the initial denaturation at 94ºC for 1 minute; 40 cycles (denaturation at 94ºC for 15 seconds; annealing at 60ºC for 30 seconds; extension at 68ºC for 1 minute); a final extension at 68ºC for 5 minutes and then hold at 4ºC. Amplification products were separated on a 1.5% agarose gel and visualised by Gel Red staining and UV irradiation. One-step RT-PCR at the WRLFMD was performed as previously described (Knowles et al., 2016).

Sequencing and phylogenetic analysis

The RT-PCR amplicons were sequenced on both strands as previously described (Knowles et al., 2016). The sequences were assembled and verified using SeqMan software (DNAStar, Lasergene v.8). VP1 nucleotide sequences were aligned by using BioEdit version 7.2.5 (Hall, 1999) and Clustal W (Thompson *et al*., 1994).

The comparison and midpoint-rooted Neighbor-joining trees of FMDV VP1 sequences from Niger with those from Africa available in the NCBI GenBank database (www.ncbi.nlm.nih.gov) were performed using MEGA 6.06 (Tamura *et al*., 2013). The robustness of tree topology was assessed with 1000 bootstrap replicates by using the model in MEGA 6.06. Bootstrap values of >70 are shown at the relevant major nodes. Sequences showing 100% nucleotide identity in VP1 were classified as a single genetic variant. The complete VP1 nucleotide sequences generated in this study corresponding to each genetic variant but also collected from a specific geographic location were submitted to the NCBI GenBank database under the accession numbers (KX424677to KX424682).

Vaccine Strain Selection

Vaccine strain selection for serotype O isolates was performed at WRLFMD by two-dimensional virus neutralization test (2D-VNT). The vaccines used in this study were provided by international vaccine manufacturers (Merial Animal Health and Merck Animal Health). The 2D-VNT test was carried out using the pooled post-vaccination monovalent bovine vaccine sera (BVS) collected after 21 days post-vaccination of naïve animals. Briefly, the BVS was tested against both the homologous (vaccine strains) and the heterologous (field strain). Antibody titres of the reference serum against the homologous (reference) and heterologous (field) viruses for five virus doses were calculated, and a linear regression line was drawn (Minitab program) to allow the $\log_{10}$ reciprocal antibody dilution required for 50% neutralization of 100 tissue culture infective units ($TCID_{50}$) of virus to be calculated. The antigenic relationship between the field strain and the reference strain was then expressed as an 'r_1' value based on the following equation: *"Reciprocal $\log_{10}$ of (heterologous titre / homologous titre)"* (Rweyemamu *et al*., 1976). An r value of > 0.3 suggests that the vaccine virus may protect against the field strain (Paton *et al*., 2005).

Statistical analysis

In a first step, a multilevel mixed-effects model was used to take into account the possible herd and/or district levels as random effects. Because random effects were not observed, logistic regression was used to model the odds of being NSP positive as a function of investigated potential exposure risk factors. Initial screening of potential risk factors for FMD was performed by univariate regression (Hosmer & Lemeshow, 2000). Secondly a multivariate logistic regression using backward stepwise analysis was used to check the relationship between NSP positive results and explanatory variables (Petrie, 2006). The following explanatory variables and their respective reference classes were used: province of origin of the

herd (4th Arrondissement as reference), herd type (nomadism or transhumance as reference), herd size (continue variable), herd composition (only cattle as reference), contact with wildlife (rare as reference), transhumance destination (inside the country as reference), detection for FMD cases after the transhumance (yes as reference), gender (male as reference), age (≤ 2 years as reference), animal origin (birth inside the herd as reference), clinical signs (presence as reference) and lesions (presence as reference). In addition, to assess the collinearity, a backward elimination of variables was performed (Preux, 2005). If a variable induced a modification of the odds ratio of more than 20%, this variable was retained in the final model where the interaction was tested in case of biological relevance. Goodness of fit was assessed using the Hosmer–Lemeshow goodness-of-fit test. Statistical analyses were performed using STATA/SE Acad. 14 (Stata Corp., College Station, Texas, USA).

Results

Characteristics of sampled animal

A total of 227 cattle including 93 males (41%) and 134 females (59%) belonging to 28 herds (20 transhumant or nomadic herds and 8 sedentary herds) were sampled during the period between September 4, 2014 and October 16, 2014. Most of the sampled animals were relatively young as the age of 58% (n=132) was estimated between 0 and 2 years, while 42% (n=95) had an estimated age between 3 and 4 years or more. Only 15% (n=33) of the sampled animals were introduced into their respective herds from outside, via purchase from livestock markets. With respect to animal species composition, 7 out of the 28 of the sampled herds were composed only of cattle, while the 21 of the other herds were mixed (8 herds with cattle and small ruminants and 13 herds with cattle, small ruminants and other animals such as poultry, camels and horses). In Makalondi District, a single mixed herd included pigs. All the sampled animals of the selected herds mixed with animals of other herds of neighbouring districts during grazing and access to water points. According to herdsmen, in more than half of the selected herds (54%, n=15), clinical cases of FMD were reported when the cattle came back from transhumance. Of the total of 227 animals tested, 38 animals (17%) exhibited both clinical signs and lesions of FMD. Accordingly, it was among these 38 animals that sufficient epithelium samples were taken from 25 sick cattle.

Serological analysis

Using the NSP ELISA test, 70% (158/227) of sera were positive for the presence of antibodies against FMDV. There was random distribution of positive animals among age classes (Chi-

square (3 df) = 6.12; p = 0.11). The seroprevalence of animals of the age group between 3 and 4 years (83%) was not significantly higher than the prevalence of animals of other age categories (70%, 62% and 65% for ≤ 2 years, > 2 and ≤ 3 years and > 4 years respectively) **Table 2**.

Table 2: NSP ELISA positive animals by age class

Age category	Number of tested animal	Number of NSP ELISA positive	Seroprevalence (%)
≤ 2 years	74	52	70
> 2 and ≤ 3 years	58	36	62
> 3 and ≥ 4 years	47	39	83
> 4 years	48	31	65
Total	**227**	**158**	**70**

Legend: Sampled cattle were classified into 4 age group, this table shows the seroprevalence of animals of each age class, 70% represent the overall seroprevalence yielded by NSP ELISA

Among the NSP ELISA positive sera tested by LPBE, 86% (136/158) were positive for two or more serotypes (A, O, SAT 1 and SAT 2). Based on the distribution of seroprevalence by sampling site, the highest serological prevalence was for serotype O observed in 7/8 districts (except the district of Tamou) (**Figure 2**).

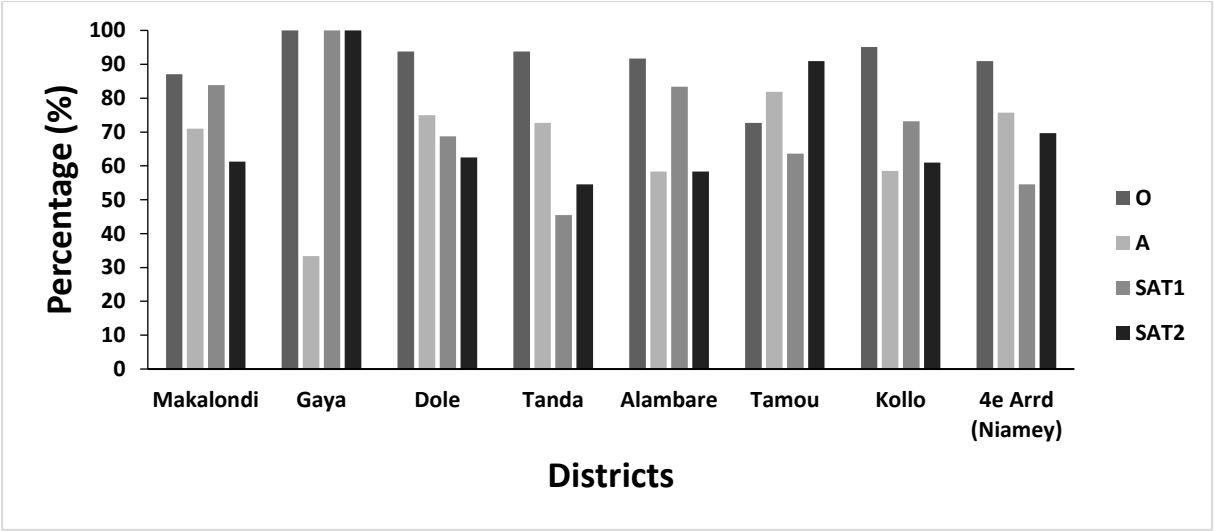


Figure 2: Liquid Phase Blocking ELISA results based on geographical locations of FMD outbreaks

Legend: 4e Arrd is one the district of Niamey called the fourth Arrondissement. Sera (n=227) were collected in 8 districts of southwestern of the country. LPBE test was performed on NSP ELISA positive samples (n=158).

In addition, either as single or as multiple serological reactions, there was a clear dominance of serotype O followed by serotypes A and SAT1. However, only 11.3% (n=18) of NSP ELISA positive samples yielded positive results for a single serotype: against serotypes A (5 samples), SAT1 (4 samples) or O (9 samples), while 86.1% (n=136) were positive for two or more serotypes, and only 2.5% (n=4) generated negative results with the LPBE **(Figure 3** and **Appendix 2)**.

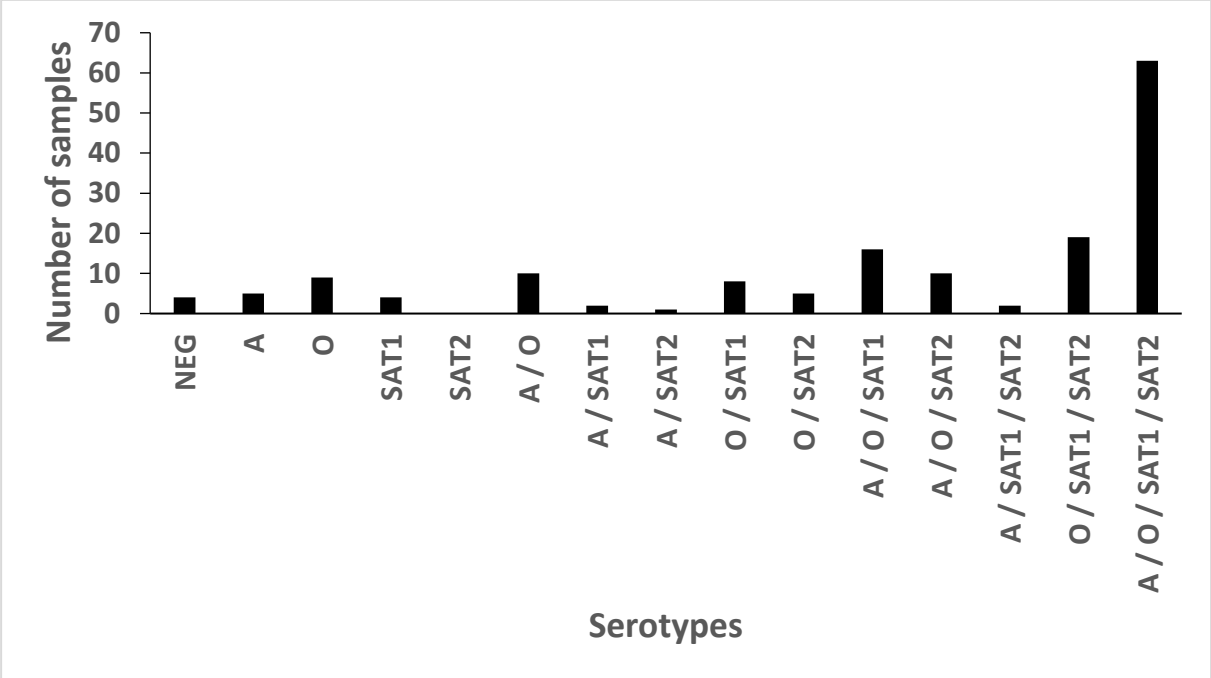


Figure 3: Prevalence of single or multiple FMDV serotypes detected in LPBE

Legend: The LPB ELISA test was performed on NSP ELISA positive samples (n=158). The total sera represent 227 samples from both subclinical and clinical cattle. Neg: Negative, A: single response to serotype A, O: single response to serotype O, SAT 1: single response to serotype SAT 1, SAT 2: single response to serotype SAT 2, the remaining are multiple responses to FMDV serotypes (see Supporting information).

Factors associated with FMDV seropositivity based on a logistic regression analysis

The results of univariate regression analysis for odds of being NSP ELISA positive as a function of investigated potential exposure risk factors showed that only the herd composition (presence of both cattle and small ruminants) was highly significantly associated with FMDV seropositivity ($p = 0.002$; **Table 3**). The remaining variables were not significantly associated with FMDV seropositivity at the 5% level, but those with a p-value ≤ 0.2 were considered as

potential risk factors and therefore entered in the multivariable analysis model (herd composition, district of origin and age of animals).

Table 3: Potential risk factors associated with FMDV seropositivity based on a univariate logistic regression model

Variable	Modality	OR	95% CI	*P*-value
Commune	4th Arrondissement	Ref.	-	-
	Alambaré	0.94	0.31-2.79	0.90
	Dolé	2.18	0.63-7.52	0.22
	Gaya	0.41	0.08-2.03	0.28
	Kolo	1.60	0.69-3.68	0.27
	Makalondi	1.21	0.51-2.84	0.67
	Tamou	6.00	0.72-50.30	0.10
	Tanda	0.86	0.28-2.60	0.79
Herd type	Nomadism or transhumance	Ref.	-	-
	Sedentary	0.94	0.44-1.98	0.86
Herd size (continue variable)	Size	1.001.827	0.99-1.01	0.48
Herd composition	Only cattle	Ref.	-	-
	Cattle and small ruminants	3.60	1.58-8.22	0.002*
	Other	1.60	0.78-3.27	0.20
Contact with wildlife	Rare	Ref.	-	-
	No	0.92	0.49-1.74	0.80
Transhumance destination	Inside the country	Ref.	-	-
	Outside the country	0.71	0.14-3.75	0.69
	Inside and outside the country	0.87	0.17-4.56	0.87
	No	0.56	0.10-3.36	0.53
Detection of FMD cases after the transhumance	Yes	Ref.	-	-
	No	0.72	0.37-1.40	0.33
Gender	Male	Ref.	-	-
	Female	0.97	0.55-1.74	0.94
Age	≤ 2 years	Ref.	-	-

	Between 2 and 3 years	0.69	0.33-1.43	0.32
	Between 3 and 4 years	2.06	0.83-5.12	0.12
	≥ 4 years	0.77	0.36-1.67	0.51
Animal origin	Birth inside the herd	Ref.	-	-
	Birth outside the herd	0.85	0.39-1.87	0.69
Clinical signs	Presence	Ref.	-	-
	Absence	1.17	0.66-2.06	0.60
Lesions	Presence	Ref.	-	-
	Absence	1.24	0.64-2.39	0.52

Legend: * P-value less than 0.05, OR: Odds Ratio, CI: Confidence Interval.

Multivariate analysis including all variables (with a p-value less than 0.20 after univariate analysis) exploited a final model that included district and herd composition as variables. Herd composition was significantly associated with FMDV positivity (p = 0.006). The Hosmer–Lemeshow test showed that this final model fitted the data well (Chi-square = 1.81; df = 6, P-value = 0.94). The interaction between the two retained variables was not tested because of the lack of biological relevance (**Table 4**).

Table 4: Final model of risk factors associated with FMDV seropositivity based on a multivariate logistic regression model

Variable	Modality	OR	95% CI	*P*-value
Commune	4th Arrondissement	Ref.	-	-
	Alambaré	0.79	(0.24-2.54)	0.70
	Dolé	2.02	(0.51-8.07)	0.32
	Gaya	1.09	(0.15-7.80)	0.93
	Kolo	1.49	(0.47-4.77)	0.50
	Makalondi	1.96	(0.57-6.72)	0.29
	Tamou	7.04	(0.70-70.97)	0.10
	Tanda	0.86	(0.28-2.60)	0.79
Herd composition	Only cattle	Ref.	-	-
	Cattle and small ruminants	3.99	(1.47-10.82)	0.006*
	Other	2.66	(0.85-8.34)	0.10

Legend: * P-value less than 0.05, OR: Odds Ratio, CI: Confidence Interval.

Isolation and identification of FMDV

Thirteen of the 25 epithelial samples produced CPE during one, two or three passages on primary lamb kidney cell cultures at BVI. These samples were from the districts of Tamou (3), Gaya (2), Makalondi (2) and Kollo (6). By antigen ELISA designed to detect all seven serotypes of FMDV and SVDV (performed at the WRLFMD), FMDV serotype O was identified in cell culture harvests from seven epithelia collected in Gaya (n=1), Makalondi (n=2) and Kollo (n=4) districts. Based on the sequence comparison using BLAST, the serotype identification of these samples was in concordance with the Ag-ELISA results. The other six samples (from the 13 CPE positive samples) were detected negative by both antigen ELISA and PCR tests. Sequences were obtained for six of the seven isolates of FMDV serotype O, and these are included in the phylogenetic analysis and listed in **Table 5.**

Table 5: Diagnostic results on epithelium samples collected in Niger in 2014 and the GenBank accession numbers of VP1 sequences

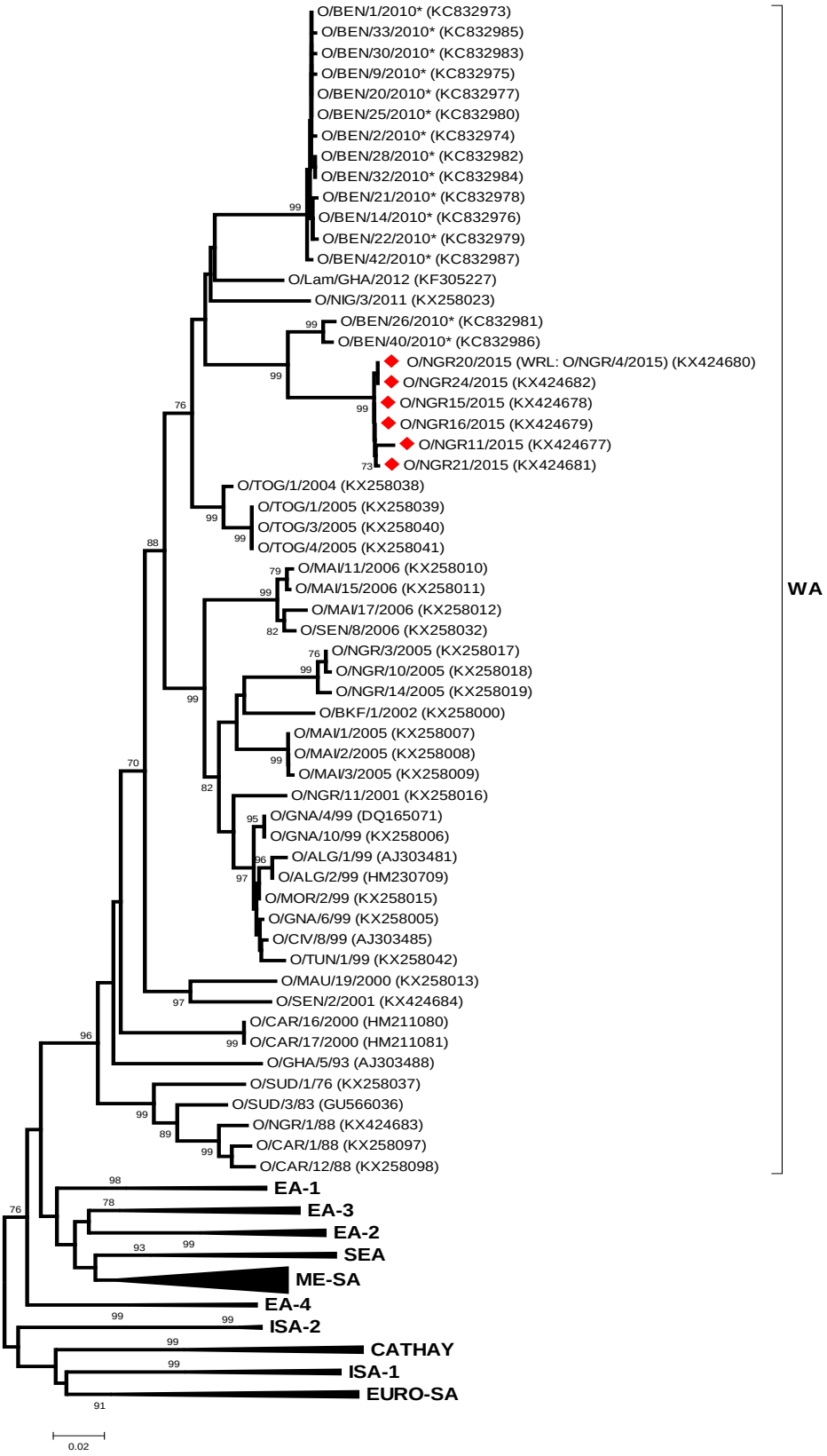
Sample ID	BVI code	WRLFMD code	Outbreak Location	Animal age class	Cell Culture passage	Serotyping by Ag-ELISA	PCR	GenBank accession No.
GY7	NGR/11/2015		Gaya	1	3rd P	O	FMDV-GD	KX424677
MK7	NGR/15/2015		Makalondi	0	1st P	O	FMDV-GD	KX424678
MK17	NGR/16/2015		Makalondi	0	2nd P	O	FMDV-GD	KX424679
KL2	NGR/20/2015	NGR/4/2015	Kollo	0	1st P	O	FMDV-GD	KX424680
KL44	NGR/21/2015		Kollo	2	1st P	O	FMDV-GD	KX424681
KL3	NGR/24/2015		Kollo	3	1st P	O	FMDV-GD	KX424682

Legend: P: passage; FMDV-GD: FMDV genome detected; Age class: 0: ≤ 2 years; 1: >2 and ≤ 3 years; 2: >2 and ≤ 4 years; 3: > 4 years.
Epithelium tissues (n=25) were obtained from clinical FMD cattle originating from seven districts of southwestern of Niger. This table indicates the positive diagnostic (virus isolation, Ag-ELISA and PCR) results with high quality sequences (n=6). These positives samples were from the following districts: Gaya (GY), Makalondi (MK) and Kollo (KL)

Phylogenetic analysis

From FMDV isolates collected in 2014, amplicons corresponding to the complete VP1 coding region were generated by RT-PCR and sequenced for six of the virus isolates. These sequences were compared with others from NCBI, GenBank and results from phylogenetic analyses revealed that they all belonged to the topotype O/West Africa (WA). Those isolated from Kollo district (NGR/4/2015, NGR/21/2015 and NGR/24/2015) had pairwise alignment (nt) identities of 99.3% - 99.7% with each other while the viruses isolated from Makalondi (NGR/15/2015 and NGR/16/2015) had 100% nt identity with each other. The FMDV isolate from Gaya (NGR/11/2015) had pairwise nt identity of 99.0% - 99.4% with other isolates from other districts. The VP1 sequences from Niger were compared to those available in the GenBank database (**Figure 4**). The analysis revealed that the Niger isolates are mostly related to the FMDV from Benin [O/BEN/40/2010 (KC832986) with 95.2% to 95.8% nt identity and O/BEN/26/2010 (KC832981) with 94.2% to 95.8% nt identity], Togo [O/TOG/1/2004 (KX258038) with 90.3% to 92.3% nt identity and O/TOG/1/2005 (KX258039) with 92.1% nt identity] and from Ghana [O/Lam/GHA/2012 (KF305227) with 90.3% to 90.9% nt identity] all being classified within the type O/WA topotype. However, the Niger FMDV isolates show lower relationship values with other earlier West African FMDV serotype O isolates from Côte d'Ivoire [O/CIV/8/99 (AJ303485) with 88.9% to 90.4% nt identity] and from Ghana [O/GHA/5/93 (AJ303488) with 85.8% to 87.4% nt identity] (**Figure 4**).

Figure 4: Midpoint-rooted Neighbor-joining tree showing the relationship between the VP1 sequences of serotype O isolated in Niger



Legend: WA=West Africa; ME-SA= Middle-East and South Africa.

Vaccine Strain Selection

The antigenic relationship between one of the FMDV isolates from Niger (O/NGR/4/2015) and three reference vaccine strains was determined by the two-dimensional virus neutralization test (2D-VNT). The results presented (**Table 6**) revealed that there is a close antigenic relationship between the three FMDV serotype O vaccine strains and Niger's FMDV serotype O field isolate. The calculated 'r_1' value was greater than the minimum requirement (> 0.3) for especially the two vaccine strains (O3039 and O/TUR/5/2009).

Table 6: 'r1' values obtained between FMDV serotype O field isolates and vaccine strains

2D-VNT r1 value			
	Vaccines strains		
	O3039	O Manisa	O/TUR/5/2009
Field isolate (O/NGR/4/2015)	0.63	0.36	0.6

An 'r_1' value greater than 0.3 indicates the existence of close antigenic relationship between the vaccine strain and the field isolate

Discussion

This study reports on serological and molecular information for FMD outbreaks in southwest Niger based on samples collected from cattle in September and October 2014. FMD is endemic in most parts of Africa and only few countries in the south of the continent have managed to control the disease (Brito *et al*., 2015; Vosloo *et al*., 2002), while only sporadic cases of FMD are regularly reported (Brito *et al*., 2015; Tekleghiorghis *et al*., 2016). Niger with an area of 1,267,000 km^2, is one of the largest West African countries. Based on the general census of agriculture and livestock in 2007, the cattle population was estimated at more than 7 million of heads. However, despite the important role of the livestock sector in Niger (La Rovere *et al*., 2005; Turner & Williams, 2002), this industry is continuously challenged with multiple constraints such as the persistence of animal diseases, including FMD. Although FMD outbreaks have been reported every year, the veterinary authorities and farmers have placed little emphasis to FMD. Hence, even though FMD is on the list of monitored animal diseases in epidemio-surveillance networks, there is still an under-reporting of FMD outbreaks. The main purpose of this study was to characterize FMD viruses responsible for clinical cases and additionally to have an overview of circulating FMDV antibodies in livestock associated with risk factors analysis. This was only the justification of the adopted sampling method that can

be designated as a "convenience sampling" consisting therefore to sample suspected sick animals (for epithelial tissues) and both the suspected sick animals and apparently healthy animals (for sera) in all reported infected herd (as soon as possible after the rare notification of outbreaks). However, despite the limited nature of sampling, this study could certainly have the value to update data on FMD in a country where the epidemiological status of the disease is poorly understood.

The serological results indicate that FMDV is endemic within the livestock population in the study area, suggesting that multiple FMDV serotypes (such as A, O, SAT 1 and SAT 2) may be involved as has been shown elsewhere in the West African region (Brito *et al*., 2015; Di Nardo *et al*., 2011; Ehizibolo *et al*., 2014; Fasina *et al*., 2013; Gorna *et al*., 2014). Using the budget available for this study, serological testing (by LPBE) was designed to detect four different FMDV serotypes (A, O, SAT 1 and SAT 2) suspected to be present in Niger. Further studies may be warranted to also include serotypes C and SAT 3, although serotype C has not been detected in any country since 2004. SAT 3-specific antibodies have been recorded in sera from west and central Africa (Ehizibolo *et al*., 2014; Ludi *et al*., 2016) and from eastern Africa (Ayebazibwe *et al*., 2010; Dhikusooka *et al*., 2015; Mwiine *et al*., 2010; Namatovu *et al*., 2015), although this serotype has not previously been detected in Niger. Although the sampling strategy is different to that implemented by Ludi et al, (2016), our results appear to be similar regarding the presence of different serotypes in unvaccinated animals. Serological tests also reveal that antibodies to four FMDV serotypes were present among the animals sampled although only one FMDV serotype (O) was detected by viral isolation and sequencing. The presence of animals with single serological reactivity to serotypes A and SAT 1 (Figure 3) may indicate either past exposure to these FMDV, or may arise as a result of cross-reactivity among serotypes in the LPBE (Hedger et al, 1982; Jackson et al., 2007). Future serological studies are warranted to these results.

Since 2005, only O and SAT 2 serotypes have been isolated in Niger, serotype A having been isolated for the last time in 1973 and SAT1 in 1976 (WRLFMD, 2016b). In this study, the highest serological prevalence (single and multiple responses to FMDV serotypes) was that of serotype O (89%), followed by serotypes SAT 1, A and SAT 2. Based on geographical locations of FMD outbreaks, serotype O was detected in more than 70% of samples from all selected districts. Furthermore, for individual districts, serotype O was most frequently detected, except in Gaya and Tanda Districts where serotype A (at 33%) and serotype SAT1 (at 45%) were

found, respectively. Interestingly, specific response to serotype O was obtained in cattle from 3/7 districts, namely Tamou, Kollo and Niamey. Additionally, in Niamey where the epithelium sampling was not possible due to the delay in the notification of the FMD outbreak, five sera were specifically positive to serotype O. The serological results for serotype O, could be interpreted as significant for this study because the serotype O was the only FMDV detected positive through viral isolation test. However, there is no evidence about any conclusion regarding the serological responses by the fact that the adopted sampling scheme is not consistent to make an accurate statement on statistical inference of results.

There was no association between seropositivity and age. Generally, keeping young animals around the homestead or in areas separated from adult animals helps to decrease their exposure to FMDV (Bayissa *et al*., 2011; Bronsvoort *et al*., 2006; Molla *et al*., 2010). However, the relative high seropositivity of FMDV antibodies in cattle of all age groups as observed in this study, combined with the spatial distribution of the herds over all of the districts in the study area, suggests that there is frequent infection with FMDV in this part of Niger.

In epidemiological settings, such as Niger with the existing livestock management practices, all potential risk factors could contribute to FMD infection. However, the statistical analysis showed that only the herd composition (cattle mixed with small ruminants) was highly significantly associated with FMDV seropositivity in FMD outbreaks. Despite these results, the role of other factors should not be ignored. The role of transhumance in FMD spread has been shown to play an important role elsewhere in sub-Saharan Africa (Rweyemamu *et al*., 2008). Furthermore, significant buffalo populations exist in West and Central Africa, including the W park (trans-border area shared between Benin, Burkina Faso and Niger). Notably, two districts in the study area (Alambaré and Tamou) are located at the interface zone between domestic animals and wildlife through the national park W of Niger. To what extent types of FMDV prevalent in domestic ruminants infect wildlife is unknown, and this important pattern of the FMD transmission dynamics remains to be more explained (Ayebazibwe *et al*., 2010; Di Nardo *et al*., 2015; Vosloo *et al*., 2002; Anderson *et al*., 1993; Fevre *et al*., 2006). Furthermore, there are important rural livestock markets in the study area (for example Alambaré), where contact between animals increases by absence of any quarantine measure and where subsequently the transmission of FMD virus and other animal diseases is enhanced (Dean *et al*., 2013; Garland & de Clercq 2011). It is obvious that the effect of the potential risk factors would be more

clearly reflected with a comprehensive random sampling in domestic animals as well as in wildlife.

Out of the total analysed epithelium samples (n=25), only six VP1 sequences were obtained for phylogenetic analysis. This relatively low rate (6/25) of sequence recovery could be explained by several factors such as the insufficient quality of the samples with degradation of the genome, due to a long time of storage of samples - about 10 months - and to poor shipping conditions or, on the other hand, by the lower analytical sensitivity of the sequencing VP1 RT-PCRs or primer mismatches. Furthermore, the relative lower quality of epithelium tissue samples could likewise be the reason that one FMDV isolate was recovered among the four samples sent to the WRLFMD. The failure to isolate FMDV from more samples restricted the extent of vaccine matching work that could be performed at the WRLFMD. Further work is urgently required to expand these vaccine-matching studies to more field isolates from the country. Furthermore, these in vitro results would benefit from results of in vivo pilot studies that evaluate the performance of the vaccine in the target host species.

During the last ten years, serotype O field isolates have been characterized in Benin, Burkina Faso, Togo, Nigeria, Ghana, Cameroon, Senegal, Mali and Niger. VP1 sequence analysis undertaken in this study indicates that these FMD viruses from Niger are closely related to strains previously isolated in West Africa. These isolates display the closest relationship with the strains from Benin (O/BEN/40/2010 and O/BEN/26/2010), Togo (O/TOG/1/2004 and O/TOG/1/2005), and from Ghana (O/Lam/GHA/2012). This close genetic relationship supports the role of cross-border animal movements are a major route by which FMD spreads in the region (Brito *et al*., 2015; Bronsvoort *et al*., 2004b; Couacy-Hymann *et al*., 2006; Di Nardo *et al*., 2011; Ehizibolo *et al*., 2014; Fasina *et al*., 2013; Gorna *et al*., 2014; Knowles & Samuel, 2003; WRLFMD, 2016a). In addition to the uncontrolled movement of animals along the border, to our knowledge, countries such as Benin and Togo do not practice mass vaccination against FMD.

In conclusion, the serological and molecular observations of this study urge for continuous surveillance of FMD enabling to monitor the infection status and the spread of FMDV serotypes in livestock as well as in wildlife populations in Niger. It is anticipated that the results of this study despite its limited sampling design will motivate further work to characterise FMDV from field outbreaks in the country where the epidemiological status of the disease is poorly

understood. In addition, regarding to transboundary animal movements and international animal trade, an integrated control approach at regional or continental level is strongly recommended.

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The authors declare no conflict of interest.

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Appendixes

Additional file 1: Sampling sheet for FMD

(Translated from French to English)

Background information

- **Number of Sample: .....Date:…../……/……**

- Region:……..Department:……..……Commune:……..... Locality:….
- Geographic coordinates: Longitude:……Latitude:……
- Owner's name:

Animal identification

- Sexe: Male ☐ Female ☐

- Age

Age category			
<2 years	[2 - 3 years[	[3 - 4 years[	> 4 years

- Animal origin

Born in the herd: Yes ☐ No ☐
Introduced from other area: Yes ☐ No ☐

- Herd composition

Herd of only cattle: Yes ☐ No ☐
Herd of cattle, sheep and goat: Yes ☐ No ☐
Herd of cattle, sheep, goat and other domestics animals: Yes ☐ No ☐

- Grazing habit of livestock

Grazing all neighbors livestock together as one herd: Yes ☐ No ☐
Grazing house hold herd separetely: Yes ☐ No ☐
Mixing at watering points: Yes ☐ No ☐
Herd not mixed at watering / watered at different site: Yes ☐ No ☐

- Contact history to wildlife

Herd has contact to wild animals usually: Yes ☐ No ☐
Has contact only rarely: Yes ☐ No ☐
Has no contact at all: Yes ☐ No ☐

- Do you usually conduct your herd to transhumance:

Yes ☐ No ☐

If so, what is the main destination of transhumance?

☐ Neighboring region ☐ Neighboring district ☐ Some where in the country (Niger) ☐ Neighboring country (Which one?)

- After returning from transhumance in your district, have you had some FMD cases?

Yes ☐ No ☐

- Clinical signs, type of lesions observed and samples taken

Clinical signs						**Type of lesions**					**Samples taken**	
Lameness	Fever	Salivation	Foot	Mouth	Teats	Intact vesicle	Recently ruptured vesicle	Raw eroded area	Ulcer with fibrinous scab	Ulcer with fibrosis	Whole blood	Epithelium tissue

Additional file 2: Individual serological response to NSP ELISA and LPBE tests

Sample ID	PI (NSP)	LPB ELISA titration results				
		A	O	SAT1	SAT2	Result interpretation
MK1	67	1.54	1.93	1.84	1.94	OSAT1SAT2
MK2	81	1.40	1.92	1.69	1.95	OSAT1SAT2
MK3	91	1.76	1.93	1.78	1.94	AOSAT1SAT2
MK4	95	1.93	1.92	1.94	1.95	AOSAT1SAT2
MK5	94	1.78	1.91	1.85	1.94	AOSAT1SAT2
MK7	91	1.94	1.92	1.62	1.89	AOSAT1SAT2
MK8	87	1.74	1.90	1.71	1.95	AOSAT1SAT2
MK9	72	1.77	1.90	1.85	1.56	AOSAT1
MK10	85	1.88	1.93	1.93	1.95	AOSAT1SAT2
MK11	95	1.85	1.87	1.80	1.94	AOSAT1SAT2
MK12	91	1.93	1.90	1.88	1.93	AOSAT1SAT2
MK13	87	1.71	1.93	1.81	1.94	AOSAT1SAT2
MK15	95	1.91	1.93	1.87	1.84	AOSAT1SAT2
MK16	65	1.41	1.92	1.77	1.92	OSAT1SAT2
MK24	83	1.76	1.79	1.75	1.73	AOSAT1SAT2
MK25	91	1.51	0.93	1.84	1.28	SAT1
MK26	65	1.22	1.56	1.43	1.57	Negative
MK27	97	1.93	0.82	1.87	1.17	ASAT1
MK28	90	1.93	1.93	1.95	1.52	AOSAT1
MK30	62	1.94	1.92	1.84	1.58	AOSAT1
MK31	79	1.93	1.48	1.90	1.46	ASAT1
MK32	96	1.94	1.93	1.86	1.95	AOSAT1SAT2
MK33	56	1.82	1.95	1.91	1.91	AOSAT1SAT2
MK35	79	1.90	1.88	1.91	1.32	AOSAT1
MK36	97	1.94	1.93	1.94	1.95	AOSAT1SAT2
MK38	92	1.90	1.87	1.78	1.92	AOSAT1SAT2
MK39	72	1.74	1.84	1.78	1.04	AOSAT1
MK40	78	1.87	1.89	1.87	1.86	AOSAT1SAT2
MK42	80	0.99	1.49	1.71	0.51	SAT1
MK44	73	1.58	1.92	1.75	1.93	OSAT1SAT2
MK45	56	1.32	1.90	1.91	1.45	OSAT1
GY2	61	1.49	1.92	1.79	1.73	OSAT1SAT2
GY3	95	1.56	1.94	1.86	1.97	OSAT1SAT2
GY5	93	1.74	1.91	1.76	1.95	AOSAT1SAT2
GY8	88	1.62	1.82	1.67	1.97	OSAT2
GY10	76	1.94	1.94	1.72	1.78	AOSAT1SAT2
GY12	88	1.93	1.94	1.93	1.58	AOSAT1
GY13	93	1.93	1.92	1.88	1.76	AOSAT1SAT2
GY14	90	1.82	1.87	1.85	1.88	AOSAT1SAT2
GY15	84	1.49	1.94	1.76	1.49	OSAT1
GY16	66	1.93	1.95	1.72	1.82	AOSAT1SAT2
GY17	78	1.93	1.95	1.93	1.58	AOSAT1

GY20	87	1.79	1.92	1.92	1.80	AOSAT1SAT2
GY21	76	1.93	1.94	1.81	1.72	AOSAT1SAT2
GY22	69	1.84	1.93	1.37	1.51	AO
GY23	86	1.83	1.94	1.91	1.81	AOSAT1SAT2
GY24	94	1.91	1.93	1.53	1.78	AOSAT2
GY25	94	1.88	1.93	1.52	1.22	AO
GY26	89	1.54	1.58	0.57	1.01	Negative
GY27	78	1.53	1.95	1.89	1.71	OSAT1SAT2
GY28	92	1.83	1.93	1.59	1.54	AO
GY29	89	1.70	1.93	1.57	1.73	AOSAT2
GY30	91	1.94	1.31	1.59	1.06	A
GY32	68	1.75	1.93	1.43	1.26	AO
GY37	92	1.41	0.86	1.49	1.27	Negative
GY38	73	1.72	1.94	1.52	1.49	AO
GY39	92	1.95	1.94	1.92	1.58	AOSAT1
GY40	51	1.84	1.94	1.52	1.26	AO
GY41	84	1.36	1.94	1.71	1.82	OSAT1SAT2
GY42	67	1.91	1.93	1.89	1.93	AOSAT1SAT2
GY45	98	1.57	1.56	1.77	1.10	SAT1
TM2	78	1.05	1.56	1.79	1.42	SAT1
TM3	95	1.94	1.92	1.92	1.89	AOSAT1SAT2
TM4	93	1.90	1.93	1.85	1.95	AOSAT1SAT2
TM5	95	1.94	1.93	1.85	0.72	AOSAT1
TM6	70	1.44	1.90	1.80	1.52	OSAT1
TM11	88	1.59	1.89	1.56	1.57	O
TM12	92	1.94	1.93	1.58	1.75	AOSAT2
TM13	59	1.47	1.91	1.91	1.70	OSAT1SAT2
TM15	68	1.90	1.85	1.91	1.82	AOSAT1SAT2
TM16	95	1.92	1.93	1.81	1.97	AOSAT1SAT2
TM18	96	1.28	1.93	1.79	1.97	OSAT1SAT2
TM19	69	1.78	1.94	1.89	1.85	AOSAT1SAT2
TM20	83	1.95	1.94	1.89	1.90	AOSAT1SAT2
TM21	51	1.71	1.14	1.51	1.52	A
TM22	74	1.93	1.93	1.93	1.78	AOSAT1SAT2
TM23	63	1.77	1.94	1.89	1.71	AOSAT1SAT2
TM24	63	1.90	1.23	1.56	1.51	A
TM25	89	1.82	1.75	1.90	1.97	AOSAT1SAT2
TM26	94	1.94	1.48	1.91	1.85	ASAT1SAT2
TM27	91	1.76	1.92	1.47	1.84	AOSAT2
TM28	76	1.29	1.92	1.46	1.81	OSAT2
TM29	67	1.34	1.91	1.84	1.86	OSAT1SAT2
TM30	96	1.87	1.93	1.82	1.86	AOSAT1SAT2
KL51	74	1.45	1.92	1.87	1.85	OSAT1SAT2
KL53	89	1.40	1.91	1.89	1.84	OSAT1SAT2
KL52	75	1.89	1.80	1.90	1.54	AOSAT1
NY52	76	1.38	1.82	1.51	1.46	O

NY54	87	1.44	1.88	1.87	1.85	OSAT1SAT2
NY53	80	1.52	1.93	1.44	0.79	O
KL1	55	1.13	1.89	1.83	1.87	OSAT1SAT2
KL2	71	1.40	1.93	1.89	0.54	OSAT1
KL3	85	1.80	1.46	1.49	1.29	A
KL4	87	1.48	1.93	1.86	1.85	OSAT1SAT2
KL6	65	1.78	1.92	1.83	1.55	AOSAT1
KL7	68	1.38	1.88	1.84	1.42	OSAT1
KL8	54	1.91	1.94	1.81	1.82	AOSAT1SAT2
KL9	87	1.36	1.88	1.77	1.84	OSAT1SAT2
KL10	69	1.93	1.90	1.72	1.79	AOSAT1SAT2
KL11	77	1.93	1.93	1.86	1.39	AOSAT1
KL12	95	1.84	1.93	1.88	1.94	AOSAT1SAT2
KL13	86	1.87	1.92	1.85	1.83	AOSAT1SAT2
KL14	78	1.94	1.87	1.85	1.80	AOSAT1SAT2
KL16	59	1.87	1.92	1.92	1.76	AOSAT1SAT2
KL17	64	1.93	1.91	1.83	1.41	AOSAT1
KL18	95	1.29	1.91	1.44	1.94	OSAT2
KL19	77	1.95	1.92	1.83	1.83	AOSAT1SAT2
KL20	85	1.18	1.93	1.51	0.68	O
KL21	77	1.30	1.89	1.84	0.99	OSAT1
KL23	74	1.52	1.92	1.86	1.77	OSAT1SAT2
KL25	96	1.90	1.92	1.91	1.94	AOSAT1SAT2
KL26	95	1.85	1.91	1.76	1.27	AOSAT1
KL28	95	1.75	1.94	1.88	1.87	AOSAT1SAT2
KL31	69	1.90	1.92	1.82	1.72	AOSAT1SAT2
KL32	66	1.52	1.91	1.76	1.17	OSAT1
KL33	78	1.49	1.92	1.55	1.47	O
KL34	80	1.79	1.85	1.75	1.17	AOSAT1
KL35	81	1.31	1.92	1.11	1.54	O
KL37	63	1.56	1.93	1.31	1.84	OSAT2
KL38	93	1.91	1.93	1.41	1.91	AOSAT2
KL39	65	1.90	1.92	1.86	1.84	AOSAT1SAT2
KL40	70	1.76	1.90	1.46	0.60	AO
KL45	71	1.25	1.86	1.90	1.89	OSAT1SAT2
KL46	69	1.80	1.90	1.92	1.85	AOSAT1SAT2
KL48	83	1.86	1.91	1.56	1.86	AOSAT2
KL49	90	1.91	1.87	1.83	1.36	AOSAT1
KL50	99	1.86	1.88	1.90	1.84	AOSAT1SAT2
NY1	91	1.95	1.94	1.93	1.97	AOSAT1SAT2
NY2	69	1.86	1.95	1.83	1.88	AOSAT1SAT2
NY3	54	1.74	1.93	1.91	1.71	AOSAT1SAT2
NY4	67	1.82	1.94	1.84	1.77	AOSAT1SAT2
NY5	77	1.46	1.94	1.81	1.49	OSAT1
NY6	67	1.85	1.93	1.89	1.88	AOSAT1SAT2
NY7	95	1.71	1.93	1.91	1.97	AOSAT1SAT2

NY10	64	1.93	1.93	1.85	1.79	AOSAT1SAT2
NY12	93	1.93	1.91	1.87	1.86	AOSAT1SAT2
NY13	69	1.77	1.94	1.44	1.97	AOSAT2
NY15	86	1.93	1.95	1.94	1.93	AOSAT1SAT2
NY16	91	1.94	1.94	1.77	1.80	AOSAT1SAT2
NY17	63	1.78	1.94	1.55	1.54	AO
NY18	66	1.79	1.93	1.83	1.76	AOSAT1SAT2
NY19	90	1.84	1.93	1.40	1.28	AO
NY20	78	1.79	1.92	1.80	1.83	AOSAT1SAT2
NY21	67	1.93	1.94	1.75	1.85	AOSAT1SAT2
NY22	67	1.56	1.95	1.45	1.98	OSAT2
NY25	78	1.74	1.94	1.42	1.16	AO
NY27	71	1.94	1.50	1.39	1.56	A
NY29	84	1.78	1.94	1.72	1.73	AOSAT1SAT2
NY32	57	1.54	1.87	1.49	1.52	O
NY33	74	1.40	0.95	1.46	1.18	Negative
NY34	80	1.85	1.31	1.84	1.78	ASAT1SAT2
NY35	71	1.79	1.92	1.41	1.82	AOSAT2
NY37	67	1.93	1.94	1.86	1.89	AOSAT1SAT2
NY38	57	1.84	1.94	1.46	1.86	AOSAT2
NY40	57	1.20	1.94	0.25	1.33	O
NY41	61	1.38	1.94	-1.05	1.44	O
NY42	87	1.90	1.42	1.24	1.84	ASAT2
NY45	50	1.84	1.94	1.56	1.85	AOSAT2

Legend: Sera were collected in 8 districts of south-western of Niger: Makalondi (MK), Gaya (GY), Kollo (KL), Tamou (TM) and Niamey (NY). Sera from Dolé and Tanda were included as originating from Gaya (administrative subdivision that covers these districts), likewise, sera collected in Alambaré were considered as from Tamou that is the administrative subdivision covering this district. Antibody titres were expressed as the final dilution of the tested serum giving 50% of the mean absorbance value in the virus control wells where test serum was absent. Titres of less than 1.6 (in inverse $\log_{10}$ form) were considered as negative while titres more than 1.6 were considered positive (Hamblin *et al*., 1986).

Part three

Chapter 7: General discussion, conclusions and perspectives

Chapter 7: General discussion, conclusions and perspectives

7.1. General discussion

Achieving a better understanding of the epidemiology is of particular relevance for the prevention and control of diseases including FMD. This was the main purpose of this thesis focused on the disease situation in Niger. Correspondingly, it was important to determine the high-risk areas and the factors associated with the onset of FMD outbreak and to get insight into the economic impact of the disease. On the other hand, as for many West African countries, the current FMDV strains circulating in Niger are not well known (Couacy-Hymann *et al*., 2006; Rweyemamu *et al*., 2008b). This has important implications for the choice of a suitable vaccine and it is essential that the selection is made on the basis of the relationship with current field strains rather than with historical or other previous viruses (Doel, 1996; Doel, 2005). Accordingly, through the research of this thesis intended to generate accurate information, which could be used to implement a future preventive and control plan for FMD in cattle as well as in other susceptible animal species.

To address the above-mentioned issues and to make a state of knowledge of FMD in Africa, it was necessary to first carry out an imperative bibliographic research through two systematic reviews. The first systematic review was related to epidemiological risk modelling and showed that the main risk factor of FMDV transmission is the uncontrolled animal movement (Allepuz *et al*., 2015; Ayebazibwe *et al*., 2010b; Hamoonga *et al*., 2014; Jemberu *et al*., 2016b). Although, other transmission factors were identified in some articles included in this review. These factors were associated with the livestock system (mixing animals around water points and pastures), as well as with the environment (presence of wildlife) and the climate. In addition, this first review showed that epidemiological modelling studies are not common in Sub Sahara Africa. Indeed, most FMD risk assessment studies (for the introduction and/or spread of FMDV) were carried out in developed free FMD countries. Although, some reviewed studies demonstrated the feasibility of implementing epidemiological modelling in endemic settings even based on simulations (Dion & Lambin, 2012; Jori & Etter, 2016; Mokopasetso, 2005; Pomeroy *et al*., 2015). Further development of such modelling should strongly be encouraged. However, this review highlighted also the obvious need to have good-quality data to perform such studies in order to improve the disease reporting system and to plan efficient disease control. The second systematic review was related to the molecular

epidemiology of FMD in Africa. Despite the relatively few molecular investigations of FMD carried out in some African countries, notably located in Central and West Africa, this review showed that over the last two decades there was a growing interest in performing more epidemiological studies for identification and molecular characterization of FMDV strains in Africa. This review provided an overview of the occurrence and distribution of different serotypes and topotypes of FMDV across the continent. It showed also that in Africa, FMDV serotypes are not uniformly distributed, and that each serotype results in different epidemiological patterns and consequently in a complex FMD epidemiology in endemic Sub Saharan African countries. Interestingly, the articles included in this second review highlighted as main finding that the transboundary and uncontrolled livestock mobility was the main source of FMDV spread from one country or region to another (Balinda *et al*., 2010; Bastos *et al*., 2003b; Knowles *et al*., 2005; Knowles *et al*., 2016; Ularamu *et al*., 2016). This is consistent with the conclusions made in the first systematic review. Finally, these two systematic reviews highlighted the ultimate need for an integrated regional approach to effectively combat FMD in Africa and in the world.

In the following subchapters, the findings of the different studies carried out within the framework of this thesis as well as the used methodology are discussed.

7.1.1 Spatiotemporal distribution of FMD in Niger

Based on a retrospective analysis of nine years (from 2007 to 2015) outbreaks data, this study indicates that over 700 FMD outbreaks were reported throughout the country. All the eight regions were affected and out of these regions, only two regions, namely Agadez (North) and Diffa (South east), were less affected by the disease. On the other hand, much more outbreaks were reported mainly in three regions, explicitly Dosso and Tillabery (South west) and Zinder (Centre east). This geographical distribution suggests that the disease has been more reported in regions with a high cattle population. In addition, clinical FMD cases were most detected at the borders of neighbouring countries, notably Burkina Faso, Benin and Nigeria. These three countries represent the main destination of the seasonal transhumance carried out by a large number of herdsmen of Niger.

The study also showed that FMD is prevalent not only in any region but also at any time. Each year, more than fifty FMD outbreaks are recorded throughout the country. An annual and

monthly variation in the occurrence of the disease was observed. According to our results, more FMD outbreaks were recorded in 2007 and 2015 than in other years. There is no scientific evidence to support these annual variations. However, it is known that livestock production in the Sahel adapts to seasonal and inter-annual variations in plant biomass and water resources availability (Boutrais, 2007; Touré *et al*., 2009). This has a consequence in the animal concentration which can promote the transmission of infectious diseases including FMD. Additionally, when no control measure is applied, highly contagious diseases like FMD tend to occur with regular epidemic cycles related to the increase of the susceptible population through time (Thrusfield, 2005). In Niger, it was often believed that FMD has a seasonal pattern, but this statement has never been supported by scientific evidence. Our study confirmed the seasonal trend of the disease by demonstrating that a high number of outbreaks were mostly recorded in the cold and dry season (from October to January) and at the end of the rainy season (September). This seasonal trend corresponds adequately to the timing of transhumance in Niger. Indeed, during the dry and warm season (corresponding to March-April-May), transhumance is practiced either to the south of the country or to neighbouring countries, in search of water and grazing and then returns to the settlements at the start of rainy season (June-July). However, to avoid conflicts between crop farmers and herdsmen, the livestock authorities regulate the pastoral movements and allow transhumant herdsmen (especially those implementing cross border transhumance) to come back in their localities only after the end of the rainy season (thus after crop production, corresponding to September and October). This seasonal pattern of occurrence of clinical FMD has been also reported in other African countries by several authors (Bayissa *et al*., 2011b; Bronsvoort *et al*., 2003b; Genchwere & Kasanga, 2014; Habiela *et al*., 2010; Molla & Delil, 2015; Rufael *et al*., 2008b). A high incidence of FMD during the cold and rainy seasons was reported in Mali (Sangare *et al*., 2004b), a neighbour country of Niger with almost the same climatic conditions. Additionally, in Niger, from October to January, the annual vaccination campaign against contagious bovine pleuropneumonia (CBPP), *peste des petits ruminants* (PPR) and other animal diseases such as pasteurellosis is usually implemented. These periods correspond to the onset of clinical FMD in most herds and is consistent with our observation indicating that contact due to animal density is one of the main predictors of FMD occurrence in Niger. On the other hand, although FMD outbreaks appear less frequent in the dry and warm season (from late February to late June) than in the dry and cold season, when FMD appears during the dry and hot season, animals seem to suffer much more than in another period (Habou, 1976). This could be explained by the stress due the high temperature (with an average of maximum temperature of around 40 °

C) but also because of the undernourishment of animals at this period when it is recurrent to observe a less pasture and water scarcity.

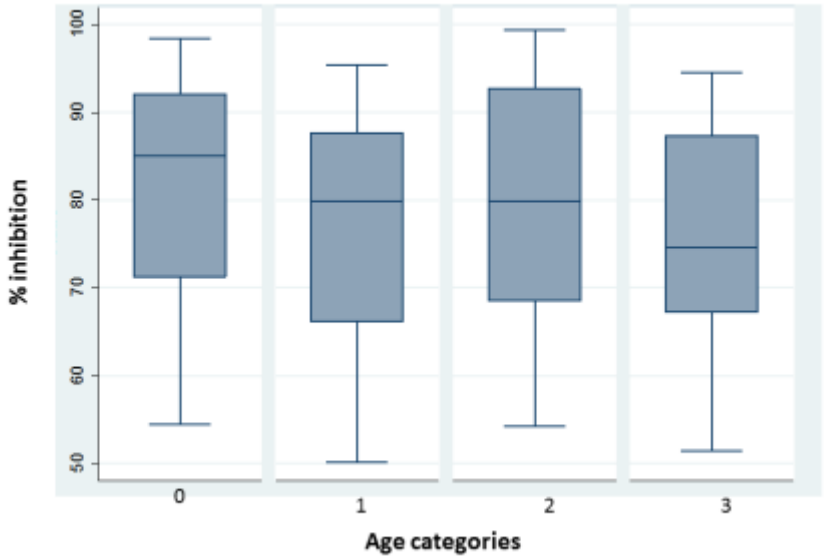
The role of seasonal migration or uncontrolled animal movement as an important factor of disease transmission, will be further discussed in this section, more specifically in relation with one of our study presented in this thesis. Globally, it was demonstrated for the first time that there are some areas in Niger that would be more prone to FMDV transmission but also that the disease would follow a seasonal trend. This provides an indication on where and when priority should be given when applying control measures, especially in a context of limited resources. However, the findings of this study need to be interpreted with caution because of the likely bias of underreporting of FMD outbreaks which is inherent in a passive surveillance system (Ouagal *et al*., 2010). This may result in an underestimation of the number of outbreaks as well as of the clinical and economic impacts of the disease However, underreporting of animal disease outbreak is a common feature in most developing countries with poor disease reporting system where the majority of animals are held by rural livestock farmers (Sumption *et al*., 2008). In addition, one of the limitations of this study is the lack of laboratory confirmation of clinical suspicions of diseases recorded in the Ministry of Livestock's database. However, in Niger, like in some other endemic countries, veterinary officers and livestock farmers are aware of the clinical picture of FMD (Bronsvoort *et al*., 2003b; Bronsvoort *et al*., 2004a; Morgan *et al*., 2014). Therefore, we considered in this study that the clinical suspicions were legitimate to be used in the analyses. The study revealed the imperative need to improve the passive surveillance system as well as to develop capacities to conduct laboratory tests to confirm clinical suspicions. Despite the limited nature of these retrospective data, it was possible to estimate for the first time the economic impact of FMD in Niger.

Nevertheless, understanding of geographic distribution of the disease and identification of the involved FMDV serotypes are important inputs required to initiate any control program. In this regard, to the best of our knowledge, there are no published data in Niger on field seroprevalence as well as on potential risk factors that are likely to modify the disease incidence. To obtain such data, it should be preferably to carry out a cross-sectional survey, but instead of this an FMD outbreak investigation of FMD has been carried out including the use of serological tests associated with risk factors analysis, virus isolation and subsequently with molecular characterization of isolates.

7.1.2 Risk factors associated with FMD seropositive animals in clinical outbreaks

During 2014, numbers of FMD outbreaks were reported in southwestern Niger including three regions namely Niamey, Dosso and Tillabery. A total of 227 sera were tested using NSP ELISA and among these, 158 positive samples were further analysed using a liquid-phase blocking ELISA (LPBE) to detect antibodies against FMDV structural proteins. The study confirmed in 86% samples (136/158) the presence of FMDV specific antibodies against one or more serotypes (A, O, SAT 1 and SAT 2) with the highest serological prevalence observed for serotype O (Figure 10).

Figure 9: Box plot of NSP ELISA according to the age category

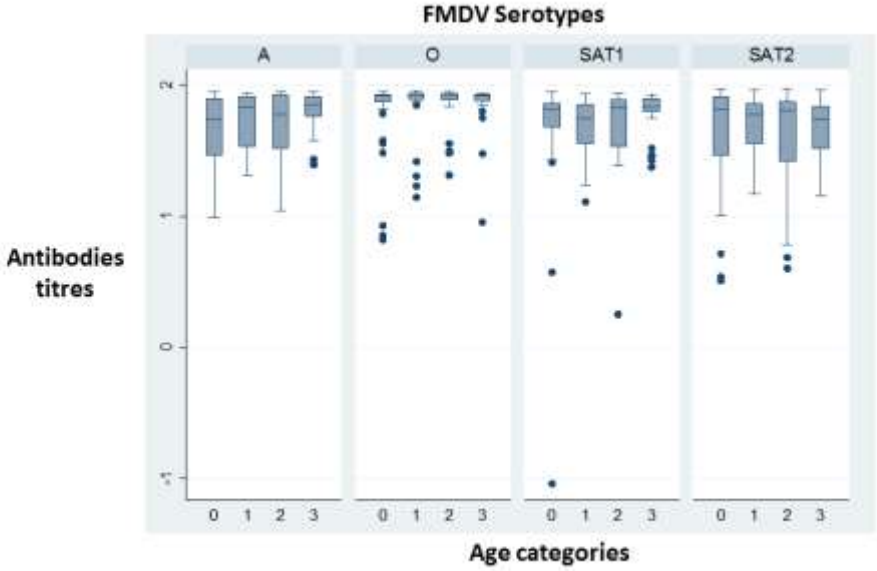


Legend: Sampled cattle were classified into 4 age groups (0: animals ≤ 2 years; 1: animals between 2 and 3 years; 2: animals between 3 and 4 years; and 3: animals ≥ 4 years), this figure shows the seroprevalence of animals of each age class. Cut off was set at Percentage of Inhibition (PI) value of ≥ 50% being positive sample and ≥ 70% being strong positive samples).

This relative high seropositivity of FMDV antibodies in cattle of all age groups, combined with the spatial distribution of the herds over the study area, indicates that infection is likely permanent in the area (Figures 9 and 10). Indeed, these serological results together with the spatiotemporal distribution of FMD confirm the endemic nature of the disease in Niger. The endemicity of the disease could alternatively be explained by numerous factors including

the lack of any control measures such as vaccination. Consequently, FMD serotypes continually spread within already infected areas (local animal population) or re-infected regions (due to animal movements including transhumance), and periodically give rise to FMDV serotypes that "break immunity" in susceptible animal and cause periodic regional epidemics (Sumption *et al.*, 2007).

Figure 10: Distribution of FMDV antibodies titres according to the age category



Legend: antibody titres were expressed as the final dilution of the tested serum giving 50% of the mean absorbance value in the virus control wells where test serum was absent. Titres of less than 1.6 (in inverse $\log_{10}$ form) were considered as negative while titres more than 1.6 were considered positive (Hamblin *et al.*, 1986).

On the other hand, from the various risk factors investigated (see **Chapter 6**), only herd composition was found to be significantly associated with FMDV seropositivity. As mentioned above, no seroprevalence study has been previously reported for Niger for comparison. However, with a different sampling strategy, Ludi *et al.* (2016) found in the Far North Region of Cameroon, serological evidence of FMDV infection in over 75% of the animals sampled with no significant differences of prevalence observed among the sampled groups. Moreover, regarding to the risk factors associated with the seropositivity of the sampled animals, another study performed in Nigeria, showed that cattle herds sharing water points with other cattle herds

along the trek routes had higher odds of being classified as seropositive to FMD (Fasina *et al*., 2013). However, it should be acknowledged that our study has a possible data collection bias due to the use of a convenience sampling rather than a random sampling. Although the transhumance practice (as well as some other risk factors like the contact with wildlife) did not appear to be a significant factor for FMD risk in our study, the role of transhumance in FMD spread should not be ignored (Bronsvoort *et al*., 2003b; Bronsvoort *et al*., 2004b; Bronsvoort *et al*., 2004a; Di Nardo *et al*., 2011; Ehizibolo *et al*., 2014; Rweyemamu *et al*., 2008b). In addition, the effect of the potential risk factors would be more clearly reflected using a comprehensive random sampling suggesting the need for more serological investigation associated with risk factors analysis. Furthermore, even if the sampling strategy used in this study needs to be consider cautiously, these results provide useful indications on the presence in cattle of FMDV serotypes already suspected to be present in West Africa, justifying therefore the attention that should be given to this when planning FMD control, for instance by vaccination.

Nevertheless, the persistence of FMDV can still only be definitively confirmed by virus isolation from samples of epithelium tissues or oesophagopharyngeal (probang) fluid (OIE, 2016). Moreover, it is well accepted that variation between FMDV strains within a given serotype may result in poor coverage and may necessitate matching of one or more vaccine strains against the circulating FMDV (Paton *et al*., 2009), which is still a challenge in endemic African countries (Namatovu *et al*., 2013).

7.1.3 Molecular characterization of FMDV circulating in Niger and vaccine matching test

The study was conducted also in the same three regions of the southwest of the country mentioned above where FMD outbreaks occurred from September to October 2014. From a total of 25 epithelium tissue samples, 52% (n=13) showed cytopathic effect (CPE) indicating that almost half of the samples were negative for this test. Further molecular analysis identified the recovered virus as serotype O. This was subsequently confirmed by the WRLFMD in Pirbright, UK. These results associated with the high seroprevalence for serotype O confirm that the occurred FMD outbreaks were caused by FMDV serotype O. This could be expected as during the last ten years, serotype O was frequently isolated in several West African countries including Benin, Burkina Faso, Togo, Nigeria, Ghana, Cameroon, Senegal and Mali

(WRLFMD, 2016). Specifically, FMDV serotype O from Niger was often recorded from past FMD outbreaks and genetically characterised in the period of 1988 (Sangare et al., 2001) and 2005 (WRLFMD, 2015a).

From the total of samples (n=25), only six VP1 sequences were obtained for phylogenetic analysis. This might be due to inadequate samples transportation from the field first to the Nigerien national veterinary laboratory (LABOCEL) in Niamey and secondly to the Ghana central veterinary laboratory from where the samples were shipped to BVI. Indeed, there were many constraints encountered during samples collection and shipment to BVI for analyses. During sample collection, we faced, among others, constraints related to the recurrent problem of underreporting of FMD outbreaks. Due to the lack of abilities for detection and characterisation of FMDV field strains in Niger, the alternative was to send the samples to the BVI laboratory which is the reference laboratory for FMD for Sub-Saharan Africa. In fact, OIE recommends FMD diagnosis to be carried out in OIE class 4 facilities (Namatovu *et al*., 2013) and this is generally found in FMD-free countries or in few African countries such as Botswana, South Africa and recently in some East African countries. Furthermore, the reason for sample shipment from Accra is that no airline companies in Niger admitted dry ice for transportation as the sending of biological materials such serum samples collected from field is not yet clearly regulated in the country. Although, the collected samples were transported from field to LABOCEL and from LABOCEL to Accra respecting the cold chain. However, the Niamey-Accra trip was by road and customs officers very often requested to open the package containing the samples, which could have eventually lead to a break in the cold chain. This situation undoubtedly reflects the problem of lack of adequate infrastructure for FMD diagnosis in most African countries and particularly in Niger.

However, with the six-good quality VP1 sequences obtained, the results of phylogenetic analyses showed that the Niger's field isolates belong to West African topotypes (WA). These phylogenetic results show a strong relation amongst and between collected samples from Niger suggesting a single FMDV strain circulating throughout the study area, possibly countrywide. In addition, these isolates are closely related to strains previously isolated in some other West African countries as Benin, Togo and Ghana. These results are in agreement with those on spatiotemporal patterns of FMD transmission, which confirms once more the hypothesis that FMD in Niger is mainly linked to the uncontrolled animal movement, to cross borders transhumance and to live animal trade. A number of examples illustrate the impact of

uncontrolled livestock movements and transhumance in the transmission of FMDV in Africa, especially in the Sahel region where a large majority of countries are concerned with cross-border transhumance either as a country of departure, or as receiver or transit country (Abiola *et al*., 2005). In the context of such a livestock production system, contact between infected and healthy animals and between potentially infected wildlife could pose a significant risk of disease spread, FMD included (Bronsvoort *et al*., 2003a; Couacy-Hymann *et al*., 2006; Dean *et al*., 2013). Taking into account that restricting contacts between herds either on pasture area or at drinking places would be practically impossible, one of the most effective control and prevention strategy is vaccinating the animals. To implement such a vaccination campaign, extensive knowledge and understanding of FMDV dynamics and epidemiology are still required. On the other hand, to ensure an effective vaccination, it is fundamentally needed to conduct vaccine matching studies to establish a relationship between prevalent field isolates with available vaccine. To this effect, an attempt was made at Pirbright institute with the isolated FMDV from Niger. The results of the vaccine matching test revealed that there is a close antigenic relationship between three FMDV reference vaccine strains and Niger's FMDV serotype O field isolate. This also indicated that the selected field isolate could be used as strain for a possible candidate FMD vaccine for the country as well as for the neighbouring countries. However, it is undeniable that vaccine match on just one sample is highly limited data. This could therefore be considered as a preliminary study of crucial importance for Niger. These initial results could be interpreted as scientific evidence to stimulate further research and planning FMD vaccination in a country where officially there has never been a vaccination against FMD. In addition, the genetic diversity of FMDV associated with its endemic nature in West Africa implies the necessity of the use of an at least trivalent vaccine which contains the serotypes A, O and SAT 2 currently dominant in the region (Tekleghiorghis *et al*., 2016). Despite multiple restrictions, this study provides interesting data from FMD outbreaks in Niger, a country where such data is scantily reported. Before the data provided by our study, the latest report (published by the WRLFMD) on genotyping of FMDV strains from Niger was related to FMD outbreak which occurred since 2005 (WRLFMD, 2016). However, apart from the constraints linked to the genetic diversity of FMDV, there are undoubtedly economic constraints which may explain why systematic animal FMD vaccination has not become widespread a strategy of disease control, especially in developing countries.

7.1.4 Economic impact of FMD in Niger

In Niger, like in many other West African countries, the veterinary services and farmers neglected FMD because of the dominant subsistence oriented livestock production. Since the 1990s, with the privatization of the veterinary profession, the animal health situation deteriorated by the withdrawal of the government of Niger to entirely support diseases control programs (MEL, 2012). For example, among the many existing animal diseases, the mandatory vaccination is only against CBPP (for cattle) and against PPR (for small ruminants). Other vaccinations (Pasteurellosis, anthrax, etc.) are optional. Notwithstanding FMD is endemic in the country with several outbreaks reports every year, there has never been an official control plan against the disease. However, there is an increasing interest to launch a national control program against FMD to mitigate the impact of the disease on production and even on international trade. Additionally, the control of FMD should be economically viable under the existing livestock production systems. It was therefore of great importance to determine the economic impact of FMD but also to economically assess the feasibility of future vaccination plan.

Our study confirms that FMD is a disease with a highly significant clinical incidence. Indeed, at outbreak level, in average more than fifty cattle are clinically affected by the disease and around four animals were estimated to die. This has an important economic repercussion because clinical FMD leads to losses of milk production, draft power loss and mortality, especially of young cattle (Rushton, 2009). In this study, only the milk production losses and mortality of young animals were estimated. In agreement with other studies performed in other endemics area, our estimates showed that FMD outbreaks caused financial losses at herd level and likely at the national level (Baluka, 2016; Barasa *et al*., 2008; Bayissa *et al*., 2011a; Jemberu *et al*., 2014; Jemberu *et al*., 2016a; Rufael *et al*., 2008a). Although FMD is commonly considered as mild in indigenous animals in traditional productions systems (James and Rushton, 2002; Vosloo et al., 2002; Thomson and Bastos, 2005), our study demonstrates that FMD outbreaks have a huge negative impact on cattle farming performances due to losses related to a relative high mortality of young cattle with an estimated cost per outbreak being at more than 300 euros. The total cost of FMD at herd level was estimated at more than 700 euros i.e. more than 450,000 CFA francs (the local currency). These losses are enormous for a country where nearly half of the population (48.2%) is below the poverty line (UNDP, 2017). Although the cost of milk production only represents 23.09% of the total losses, this could have serious

economic consequences as well as social impact by affecting human nutrition. In Niger, milk and by-products constitute the main food source for at least 20% of the population (essentially pastoralist) and important supplementary food for the remaining 80% (White, 1997; Blench *et al*., 2003).

This study is the first to estimate the economic impact of FMD in Niger. Nevertheless, albeit the negative impact of FMD is widely recognized since a while, policy makers still need empirical evidence to get convinced. Controlling FMD, like for any other disease, requires the availability of financial and human resources. In a context of restricted financial resources, it is often a rule to set priorities in relation to opportunities. Hence, one of the addressed issues considered in this study was to determine whether investment in control of FMD is economically beneficial or not. Accordingly, based on CBPP vaccination program, our study estimated the overall vaccine cost by animal at 0.11 euros which in some respect agrees with that estimated in Ethiopia by Jemberu *et al*. (2016a). In addition, our study showed that the estimated cost benefit ratio is more than 2 suggesting the total cost of losses due to FMD outbreaks being more than twice the cost to be allocated for FMD vaccination. Indeed, the economic costs due to FMD outbreak were found to be lower if there is regular vaccination with a trivalent vaccine (O, A and SAT 2) imported from BVI and the costs further decreases if FMD vaccination is done during the annual livestock vaccination campaign. This is not surprising because this study leads to the same findings as other studies assessing the cost-benefit of FMD vaccination. Perry et al. (2003) demonstrated the benefit of FMD control even for smallholder farming in the southern African region through a national economic growth that would create a suitable base for poverty reduction. On the other hand, even when the total cost of FMD inclined to be lower than the cost of control, vaccination should be continued until burden of clinical outbreaks of FMD disease is substantially reduced for a sufficient time period as stated by OIE and FAO about one of the objectives of FMD vaccination, especially in endemic countries. In fact, in many endemic countries, several studies highlighted the effectiveness of FMD vaccination programme (Govindaraj *et al*., 2015; Jemberu *et al*., 2016a; Rast *et al*., 2010; Young *et al*., 2013). Therefore, despite the limited used data mentioned above, the provided estimates from our study can serve as prior knowledge for future implementation of FMD vaccination.

Finally, these research contributions performed in the framework of the present thesis attempted to fill the existing knowledge gaps by generating epidemiologic and economic

information on FMD with the aim to formulate some realistic recommendations regarding its control in Niger.

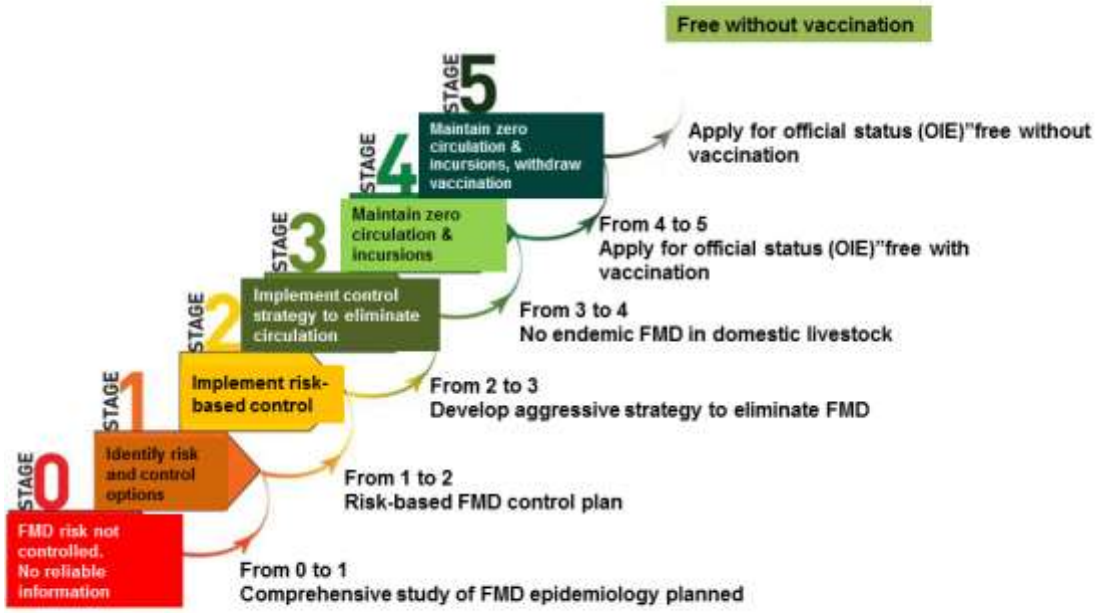
7.2. Conclusions, implications and perspectives

Foot-and-mouth disease in Niger was neglected or at less not considered as a priority animal disease because it occurred mostly on rustic and less productive domestic animals. In such animals, the losses due to FMD were considered as less severe and consequently full attention was paid to control other more "dramatic" epizootic disease such as Rinderpest and CBPP. Although FMD is not a fatal disease, its economic impact is real and not negligible. The current policy of the government of Niger includes, among others, the intensification of animal production and the support to the livestock and by-products marketing process to ensure remunerative income for farmers. The development of livestock through the improvement of animal health is therefore undeniably an essential part of a pro-poor enhanced poverty reduction strategy for the benefit of vulnerable populations in developing countries such as Niger. This urges to deal also with epizootic constraints like FMD.

However, decision-making regarding the most effective control strategy of animal disease should emphasize, among others, an ecosystem approach, the identification of primary sources of infection, and climate and animal husbandry practices (Stephen *et al*., 2005). In this respect, many countries are embarking on the stepwise Progressive Control Pathway (PCP) approach proposed by OIE and FAO to improve their FMD control capacity in a sustainable manner (**Figure 11**). The Different regions of Sub-Saharan Africa are at different developmental stages of control and thus face multiple challenges and priorities in terms of FMD control. Unfortunately, Niger as many West African countries are at the stage zero of the FMD-PCP (**Figure 12**). Therefore, generating more and accurate epidemiological information on FMD is strongly required to move forward in this global dynamic. Accordingly, the present thesis is presented as a contribution to the improvement of the knowledge of the epidemiology of FMD in Niger.

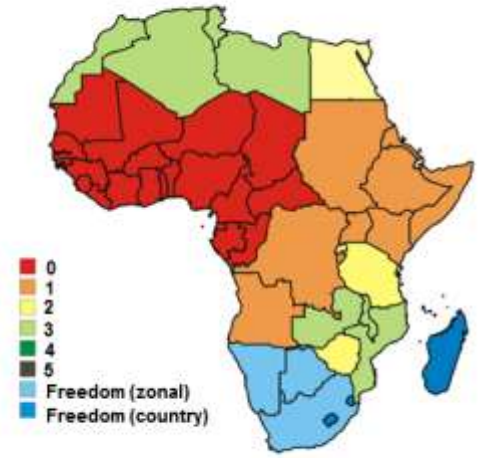
Figure 11: FMD Progressive Control Pathway stages

(Adapted from http://www.fao.org/ag/againfo/commissions/eufmd/commissions/eufmd-home/progressive-control-pathway-pcp/en/)



Legend: 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 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: (i) countries not free from FMD (PCP stages 0–3), (ii) FMD-free countries or zones practicing vaccination (PCP stage 4), and (iii) FMD-free countries or zones where vaccination is not practiced (PCP stage 5).

Figure 12: Map indicating the different FMD-PCP stages of African countries
(Adapted from Maree, 2014)



Based on retrospective data, it was demonstrated that FMD is endemic in all parts (regions) of Niger with variable outbreak occurrence in the different geographic zones of the country. The highest number of occurrence was observed in areas accounting relative high cattle density. Within these areas, FMD outbreaks are more recorded at the borders with neighbouring countries. Temporal analysis showed two periods of importance in clinical disease occurrence with a cold and dry season from October to January and at the end of rainy season starting from September. The conclusion of these findings is that when resources are limited, a control policy has first to target high risk areas or to determine where and when the control could have a higher impact on reducing the disease transmission as well as its economic losses.

- ☞ Paradoxically to this situation of endemicity of the disease, data on the prevalence regarding the whole country is lacking. Consequently, in order to create a database on FMD (sero) prevalence, research should be carry out countrywide to determine the prevalence of the disease and its associated risk factors. However, for budgetary reasons, studies described in this thesis were restricted to cattle. Although in Niger, as in many African countries, the tradition animal husbandry practice involves rearing cattle, sheep and goats in close proximity. Similarly, communal grazing is practiced in most of the areas, and both small and large ruminants use the same pasture land and water sources. Accordingly, the silent and discrete feature of FMD infection of small ruminants could pose a potential risk of virus dissemination to cattle and other susceptible animals. Moreover, herd composition (cattle and small ruminants) was significantly associated with FMD outbreaks in Niger through one of our studies. Hence, surveillance activities

as well as epidemiological researches addressed to small ruminants alongside cattle population must be strengthened. Although dromedaries are estimated at more than one million five hundred thousand heads according to the last census of livestock in 2007, they are not susceptible to FMD and do not transmit infection (Wernery and Kinne, 2012).

This study showed stochastically with the estimated data that beyond the empirical assumptions, FMD remains a disease with huge economic impact as well as a significant negative effect on rural livelihoods. The study proved also that FMD control by vaccination is expected to generate positive economic returns by reducing production losses irrespective of any motivating the export of animals and animal products at international markets.

- ☞ Based on the systematic review of FMD risk modelling performed in this thesis, we highlighted the need of risk assessment to assist the decision-makers to rapidly react during FMD outbreaks by implementing appropriate measures in due time. The obvious prerequisites of good-quality data to perform modelling were also emphasised. In our study for quantitative assessment of the economic impact of FMD, one of the challenge faced, was the limited availability of data. Additionally, underreporting of disease has been a frequent constraint encountered during this study. Accordingly, it would be appropriate to improve the network of epidemio-surveillance of animal diseases as well as to set up a vigilance committee and a monitoring unit. This should be accompanied by the development of capacities to conduct laboratory tests to confirm clinical suspicions. This network system would enable collecting more reliable data that enable the development of epidemiological and economical modelling.
- ☞ Other supplementary actions such as education and awareness campaigns for farmers, livestock traders and other stakeholders should be also foreseen and carried out. These actions will certainly allow the stakeholders to fully understand the importance of disease reporting for effective surveillance and disease control.
- ☞ Government strategy in FMD control through regular vaccination should be implemented. For effective FMD vaccination there is a need to use at least a trivalent or quadrivalent vaccine given the circulating viruses in the West African region. Hence, given the relatively exorbitant cost of FMD vaccines we propose that vaccination should initially intended for lucrative farms such as state ranch, dairy farms. After five years of

success, the government should subsidize the vaccine for the implementation of an annual vaccination campaign similar to that for CBPP.

Through the implemented FMD outbreaks investigation, FMDV serotypes O, SAT 1, A, and SAT 2 in order of decreasing seroprevalence were identified as circulating viruses in cattle in southwestern Niger. The main associated risk factor for seropositive FMDV was the herd composition meaning cattle mixed with small ruminants. The study showed that these outbreaks were caused by FMDV serotype O belonging to West African topotype. These isolates are closely related to strains previously isolated in neighbouring countries suggesting that cross-border animal movements including transhumance, are the main factor of FMDV spread in the region. Additionally, one of these isolates showed a close antigenic relationship with three FMDV reference vaccine strains.

- ☞ Given the serotype diversity in African countries, an extensive regular surveillance and serotyping of the outbreak isolates throughout the country should be conducted to check the introduction and circulation of potential new serotype in the country and to ensure that circulating viruses would be protected by current manufacture's vaccines. Additionally, molecular characterization of the FMDV should be carried out following each FMD outbreak. Subsequently the vaccine strain and field strains can be assessed frequently.
- ☞ Although cross-border animal movement bounded to transhumance appears to be a decisive factor in the spread of FMDV, there is so far, a lack of reliable data on the role of transhumance in the transmission of the disease. Therefore, it would be advisable to promote a comprehensive study to provide more clarification on that by implementing serological and clinical surveillance along the borders. In a context of nomadism and transhumance systems, controlling animal movement would not be a realistic option. But, where an outbreak has occurred, strict quarantines should be enforced to avoid the spread of the disease to new FMD free areas.
- ☞ On the other hand, even though West Africa has a relatively smaller African buffalo's population compared to southern Africa, there are still some national reserves of wild animals such as the W park at the junction between Benin, Burkina Faso and Niger. It is very common for domestic animals such as cattle to graze near these reserves. Hence, it would be interesting to conduct studies to understand the interaction between wild animals and domestic animals in the maintenance and spread of FMD.

- ☞ For transboundary animal diseases like FMD, it is imperative to implement a control plan based on an integrated regional approach. Through this thesis, one of the relevant recommendations addressed to policy makers is about the establishment of a West African research centre for FMD devoted to conduct regional epidemiological studies and, subsequently, to launch a program for vaccine production, specifically against West African strains.

Despite its limitations, our research contributes largely to a better knowledge of the epidemiology of FMD in Niger, although there are additional fields of research in the epidemiology of the disease that need to be explored to improve the decision support process in the disease's control. The recommended actions mentioned above are based on two fundamental axes: research and disease control. The above-mentioned recommendations aim to reduce the impact of FMD in the country and to sustainably mitigate all identified risk factors in order to attain PCP stage 2 after 5 to 10 years.

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