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**.

Insert figure 1: Geographical locations of FMD outbreaks described in this study

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**).

Insert Table 1: Overview of the sampling strategy

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 the different FMDV serotype viral antigen was 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 3,700 RPM 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 3,700 RPM 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 48hrs, 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 3,700 RPM 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 (KX424677-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 (4^{th} 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**.

Insert Table 2: NSP ELISA positive animals by age class

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). 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**).

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

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 2 or more serotypes, and only 2.5% (n=4) generated negative results with the LPBE **(Figure 3** and **Appendix 2)**.

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

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).

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

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**).

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

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 (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**

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

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**).

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

Vaccine Strain Selection

The antigenic relationship between one of the FMDV isolates from Niger (O/NGR/4/2015) and three reference vaccine strains were 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).

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

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 the 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 was that of serotype O (89%), followed by serotypes SAT 1, A and SAT 2. Serotype O was detected in more than 80% of samples from all selected districts where FMD outbreaks occurred. 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 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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Table and figure captions

Table 1: Overview of the sampling strategy

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 correspond to the total number of sera.

Table 2: NSP ELISA positive animals by age class

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.

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

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

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

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

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

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).

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

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

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).

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).

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, SAT1: single response to serotype SAT1, SAT2: single response to serotype SAT2, the remaining are multiple responses to FMDV serotypes (see **Appendix 2**).

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-Est and South Africa.

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 correspond to the total number of sera.

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.

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.

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.

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			3	1st P	O	FMDV-GD	KX424682

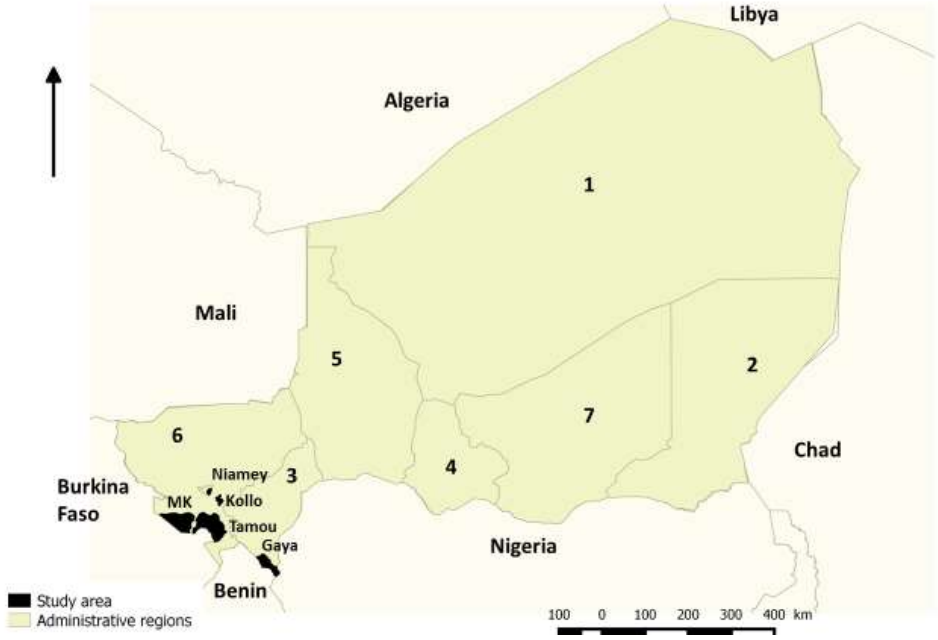
Legend: P: passage; FMDV-GD: FMDV genome detected; Age class: 0: ≤ 2 years; 1: >2 and ≤ 3 years; 2: >2 and ≤ 4 years; 3: > 4 years. Epithelium tissues (n=25) were obtained from clinical FMD cattle originating from seven districts of southwestern of Niger. This table indicates the positive diagnostic (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).

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

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

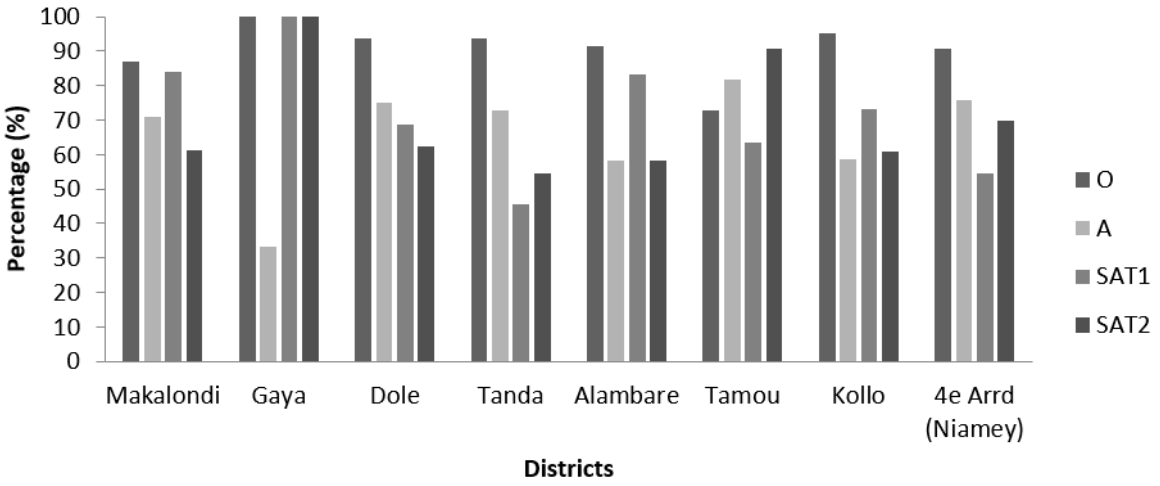
18 Fig. 1


Libya
Algeria
1
Mali
5
2
7
6
Chad
4
Niamey
3
Burkina
Faso
MK
Kollo
Tamou
Gaya
Nigeria
Benin
Study area
Administrative regions
100 0 100 200 300 400 km

19

20

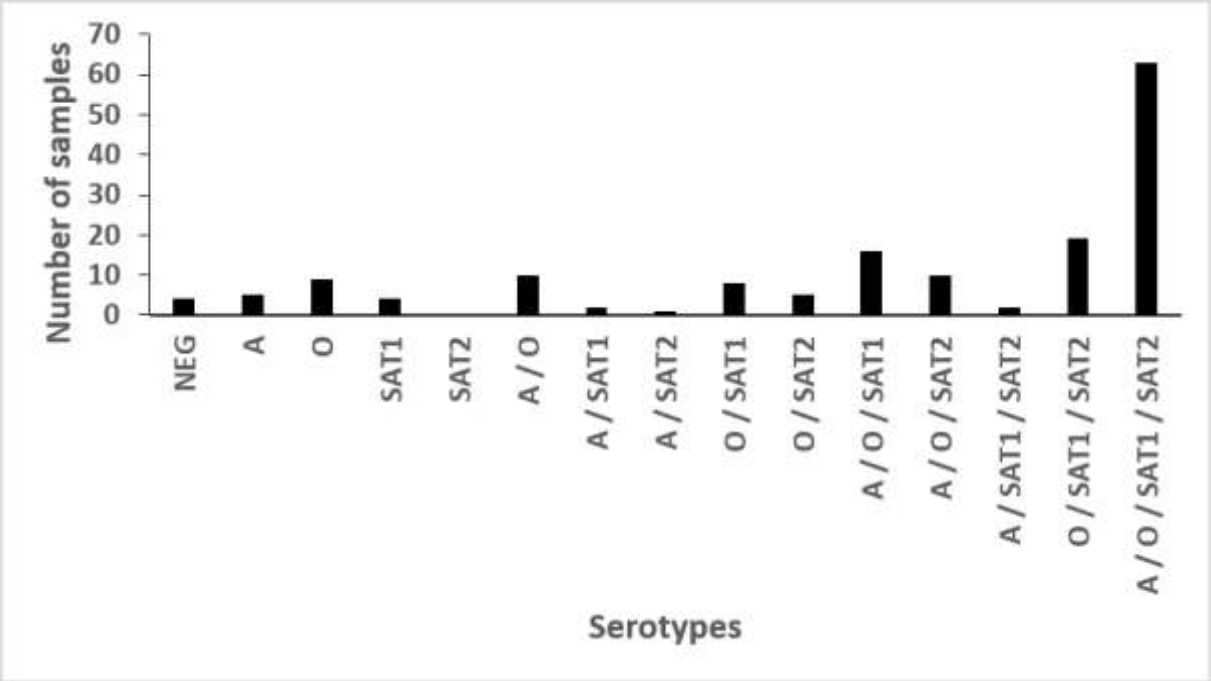
21 Fig. 2


100
90
80
70
60
50
40
30
20
10
0
Percentage (%)
Makalondi
Gaya
Dole
Tanda
Alambare
Tamou
Kollo
4e Arrd (Niamey)
Districts
O
A
SAT1
SAT2

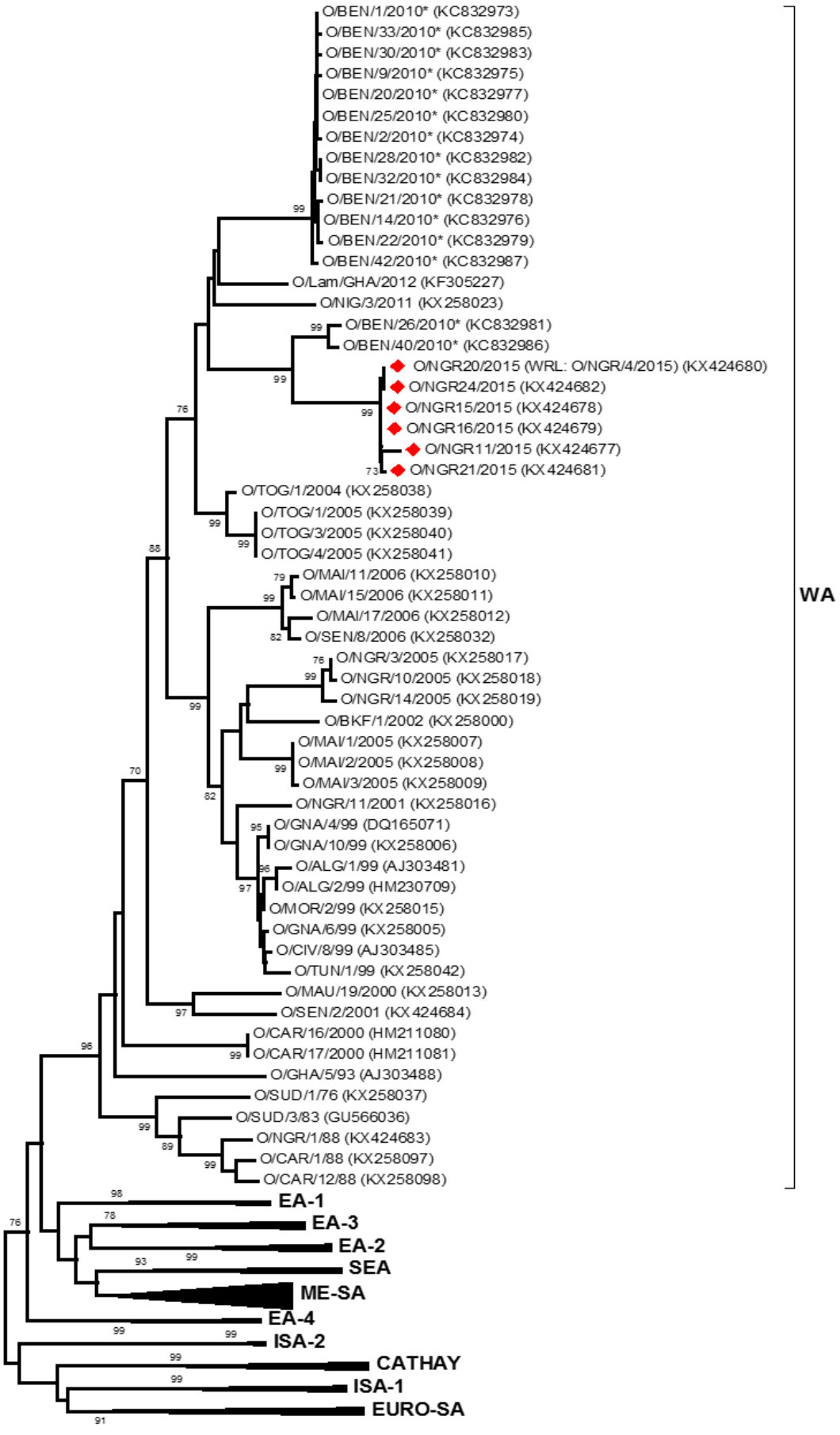
22

23

Fig. 3


Number of samples
70
60
50
40
30
20
10
0
NEG
A
O
SAT1
SAT2
A / O
A / SAT1
A / SAT2
O / SAT1
O / SAT2
A / O / SAT1
A / O / SAT2
A / SAT1 / SAT2
O / SAT1 / SAT2
A / O / SAT1 / SAT2
Serotypes

27 Fig. 4


O/BEN/1/2010* (KC832973)
O/BEN/33/2010* (KC832985)
O/BEN/30/2010* (KC832983)
O/BEN/9/2010* (KC832975)
O/BEN/20/2010* (KC832977)
O/BEN/25/2010* (KC832980)
O/BEN/2/2010* (KC832974)
O/BEN/28/2010* (KC832982)
O/BEN/32/2010* (KC832984)
O/BEN/21/2010* (KC832978)
O/BEN/14/2010* (KC832976)
O/BEN/22/2010* (KC832979)
O/BEN/42/2010* (KC832987)
O/Lam/GHA/2012 (KF305227)
O/NIG/3/2011 (KX258023)
O/BEN/26/2010* (KC832981)
O/BEN/40/2010* (KC832986)
O/NGR20/2015 (WRL: O/NGR/4/2015) (KX424680)
O/NGR24/2015 (KX424682)
O/NGR15/2015 (KX424678)
O/NGR16/2015 (KX424679)
O/NGR11/2015 (KX424677)
O/NGR21/2015 (KX424681)
O/TOG/1/2004 (KX258038)
O/TOG/1/2005 (KX258039)
O/TOG/3/2005 (KX258040)
O/TOG/4/2005 (KX258041)
O/MAI/11/2006 (KX258010)
O/MAI/15/2006 (KX258011)
O/MAI/17/2006 (KX258012)
O/SEN/8/2006 (KX258032)
O/NGR/3/2005 (KX258017)
O/NGR/10/2005 (KX258018)
O/NGR/14/2005 (KX258019)
O/BKF/1/2002 (KX258000)
O/MAI/1/2005 (KX258007)
O/MAI/2/2005 (KX258008)
O/MAI/3/2005 (KX258009)
O/NGR/11/2001 (KX258016)
O/GNA/4/99 (DQ165071)
O/GNA/10/99 (KX258006)
O/ALG/1/99 (AJ303481)
O/ALG/2/99 (HM230709)
O/MOR/2/99 (KX258015)
O/GNA/6/99 (KX258005)
O/CIV/8/99 (AJ303485)
O/TUN/1/99 (KX258042)
O/MAU/19/2000 (KX258013)
O/SEN/2/2001 (KX424684)
O/CAR/16/2000 (HM211080)
O/CAR/17/2000 (HM211081)
O/GHA/5/93 (AJ303488)
O/SUD/1/76 (KX258037)
O/SUD/3/83 (GU566036)
O/NGR/1/88 (KX424683)
O/CAR/1/88 (KX258097)
O/CAR/12/88 (KX258098)
WA
EA-1
EA-3
EA-2
SEA
ME-SA
EA-4
ISA-2
CATHAY
ISA-1
EURO-SA
0.02

29

Appendix 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 separetly: Yes ☐ No ☐

Mixing at watering points: Yes ☐ No ☐

Herd not mixed at watering / watered at different site: Yes ☐ No ☐

- Contact history to wildlife

Herd have contact to wild animals usually: Yes ☐ No ☐

Have contact only rarely: Yes ☐ No ☐

Have 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

86

87

88

89

Appendix 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).