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Molecular and Serological Epidemiology of Foot-and-Mouth Disease Virus in North Region of Cameroon

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Abstract

The serological prevalence of foot and mouth disease virus (FMDV) among the cattle population in the North region of Cameroon was determined using ELISA (Enzyme Linked Immunosorbent Assays) serological tests for structural as well as non-structural proteins. In these cattle, FMDV RNA was identified, amplified, sequenced and the sequences were used to construct a phylogenetic tree. A sedentary cattle population randomly selected from six veterinary centres in the North region was sampled twice, six months apart. High prevalence of FMDV antibody was recorded in the first (402/466 (85.84%)) and second (358/411 (86.90%)) sampling periods. There was no significant difference ($P > 0.05$) in prevalence of FMDV antibody between the two sampling periods. Goudali and Peulh breeds of cattle and animals of three to five years old were the most infected with FMDV and mostly in the months of May and August. A seroprevalence of 100% (n = 14) of FMDV against serotypes A and O was observed in sera from convalescent animals in the study area. FMDV antigen detection ELISA showed a prevalence of 18/37 (48.65%) for serotypes SAT1 (8.1%), SAT2 (35.1%), A (10.8%) and O (2.7%) among the clinically infected animals. There was no significant difference ($P > 0.05$) in prevalence of FMDV RNA between the sampling periods. A prevalence of

FMDV RNA (17.5% (n = 120) and 16.7% (n = 240)) was observed among the sedentary animals that were sampled four to five months apart. FMDV RNA prevalence of 28/37 (75.6%) among clinically infected animals was also observed, thus confirming all the 12 outbreaks investigated. Sequence analysis of VP1 coding gene of the SAT2 serotype showed that it was homologous to the Libyan isolates (that caused epidemics in northern Africa in 2012) and also clustered with the serotypes isolated from both Nigeria and Sudan in 2007.

Keywords

Foot-and-Mouth Disease, Seroprevalence, Non-Structural Protein, Structural Protein, ELISA, Sedentary Cattle, North Cameroon

1. Introduction

About half of the estimated six million cattle population of Cameroon is found in three Northern regions (Adamawa, North and Far-North regions) alongside small ruminants. These livestocks are managed under transhumance, extensive, intensive and semi-intensive systems [1]. Cattle play an important role in the provision of meat and milk as well as a financial reserve and as an important source of draught power. Cattle rearing is carried out mainly by the Fulanis (known in Cameroon as the Fulbe and Mbororo people) who raise cattle throughout sub-Saharan Africa [2]. In recent years, an increasing number of people from other ethnic groups have begun rearing cattle for either draught power or soil fertility improvement. However, it is hypothesized that the mobility of these animals and the porosity of international borders exposes them to many livestock diseases that include foot-and-mouth disease (FMD) [3]. The highly contagious foot-and-mouth disease (FMD) is caused by a positive-sense; single stranded RNA virus classified in the genus *Aphthovirus* in the family *Picornaviridae*. FMD affects both domestic and wild ruminants as well as swine. It is one of the most economically important diseases of cattle in the tropics [4]. FMD is of concern to the livestock sector in many countries of the world due to its contagiousness and the threat it poses to international trade of livestock and livestock products. As in most parts of Africa, FMD is enzootic in Cameroon. Serological evidence suggestive of circulation of all seven FMDV serotypes in the Adamawa region of Cameroon has been reported [5]. However, virus isolation has only confirmed the circulation of three serotypes (SAT2, O and A) (2). Likewise, more recent studies in the Far North region suggested the presence of five serotypes (A, O, SAT1, SAT2 and SAT3) by serology [6]. However, only two serotypes (O and SAT2) were found by virus isolation causing clinical or subclinical infection [6]. At the time of this study, there was no control program for FMD in Cameroon. A thorough understanding of the local epidemiology of the disease would be beneficial to allow the design of appropriate control measures [7]. Though several studies have been conducted in neighboring regions (Ada-

mawa and Far North), there is no existing report on the circulation of FMD in indigenous cattle populations using serological and high through put molecular techniques in the North region of Cameroon. The current study was designed to inform future control programs regarding the epidemiological pattern and ecology of FMDV in the North region of Cameroon through FMDV antigen and antibody detection and identifying some epidemiological factors associated with the circulation of FMDV in this part of the country to fill this gap.

2. Materials and Methods

2.1. Study Area

Cameroon is a country located in central Africa, in between the Atlantic Ocean and the Lake Chad basin (**Figure 1**). It covers a surface area of about 475.442 km^2 with over 20 million inhabitants [8]. The economy of Cameroon is principally based on agricultural activities. The North region (study area) is bordered to the west by Adamawa state in Nigeria and to the East by The Republic of Chad, to the north by the Far North region of Cameroon and to the south by the Adamawa region of Cameroon (**Figure 1**). Garoua is the administrative capital of the North region, and the region is comprised of four administrative divisions. These divisions are further subdivided into 53 veterinary centers [9]. The sampling sites in the different veterinary centers was because they are found along the transhumance routes for transit animals towards Nigeria, Chad and Central African Republic which represents high risk for indigenous North region herds. So it was interesting to study the occurrence and risk factors of FMD in these indigenous cattle by referring to veterinary centers to facilitate stratified sampling.

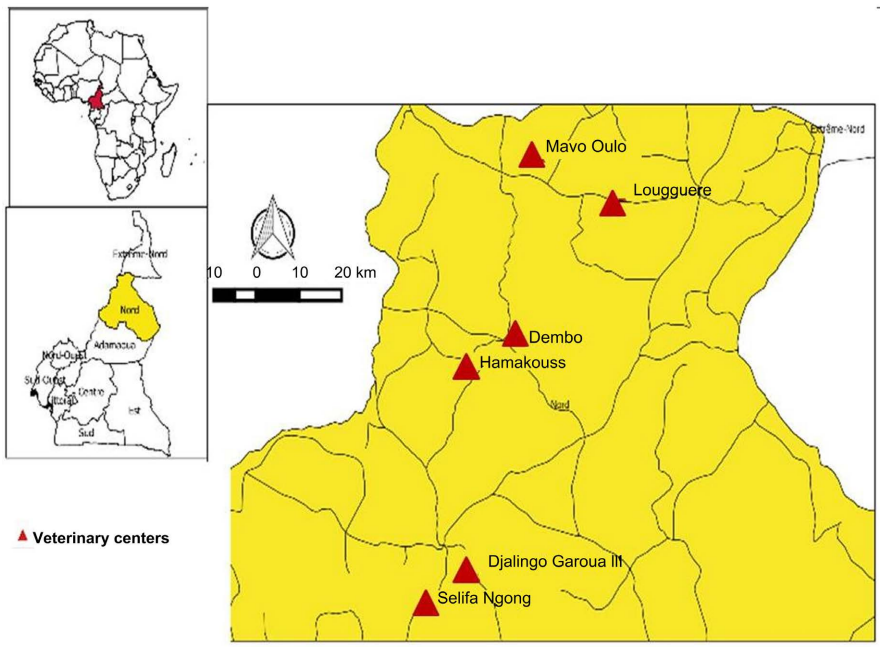

Figure 1. Map of the study area showing the different veterinary centers where sedentary cattle were sampled.

2.2. Selection of Animals and Sampling

A targeted longitudinal study of the sedentary cattle population in the North region of was randomly selected from six veterinary centres in the North region and sampled twice, six months apart. The sample size was calculated using a standard formula [10]:

$$N = P_{exp}(1 - P_{exp})(Z^2/d^2),$$

where n is the sample size;

P_{exp} is the expected prevalence;

d (the desired absolute precision) = 0.05 and Z = 1.96 for a 95% confidence level.

This formula gave a suggested sample size of 466 cattle for accurate estimation of the prevalence from a population-based random sampling. In practice, a nested study design was used, so the number of samples was increased to account for possible correlation. A total of 466 cattle were selected randomly in six randomly-selected veterinary centers out of the 53 veterinary centers in the North region of Cameroon. A sample frame was drawn up of all sedentary herds in each of the six randomly selected veterinary centers using records from the North Regional Delegation of the Ministry of Livestock. At least three herds were selected from each of the veterinary centers. For each selected herd, animals of all age groups were sampled up to a maximum of twenty per herd. The selected animals were ear-tagged and sampled six months apart to produce 877 serum samples (n = 466 at the first sampling period (November to December) and n = 411 at the second sampling period (April to May)). In addition, 37 clinical cases of suspected FMD were also sought in the region (none in the sampled herds). Animals manifesting symptoms of salivation, lameness, and vesicular lesions in the mouth, muzzle, inter-digital space, or on the coronary band of the foot were identified and sampled. Thirty seven sera and 37 epithelial tissue samples from ruptured vesicles were collected from these clinical cases.

2.3. Sample Collection from Study Animals

Blood for serum preparation was collected from the jugular vein of animals using vacutainer tubes and needles (Becton Dickinson, UK). The blood samples were allowed to clot for about 4 hours at ambient temperature and then centrifuged at 2500 rpm for 5 minutes. Serum samples were collected in labeled cryotubes and transported on icepacks to the National Veterinary Laboratory (LANAVET), Garoua for analysis. Epithelial tissues from unruptured or freshly ruptured vesicles (mouth, muzzle, interdigital space, or coronary band) were collected according to a published protocol [11]. Briefly, with the aid of sterile forceps and scissors, a small piece of the epithelial tissue was collected and placed in labeled tubes containing virus transport medium (mixture of 50% Dulbecco's minimum essential medium and 50% glycerol, with 1% antibiotic and antifungal agents). The forceps and scissors were cleaned and disinfected between animals with 75% alcohol, passed over flame and rinsed with water. The samples were transported

on icepacks to LANAVET for analysis.

2.4. Host Related Risk Factors

Two types of structured questionnaires were prepared and administered. One was designed to collect information at herd level while the other was used for individual animals sampled. At herd level, 18 questionnaires were administered to collect the following information: contact of cattle with wildlife, date of last outbreak, number of clinically sick animals, and total number of animals in the herd. At the individual animal level, 466 questionnaires were administered to get information about their age, sex, breed, and color. Where possible, these herder responses were confirmed with visual observations of the animal sex, color and age category.

2.5. Laboratory Analyses

The serum samples were screened for FMDV antibody using an anti-FMDV non-Structural protein (NSP) based Enzyme Linked Immunosorbent Assay (ELISA) (PrioCHECK FMDV NS, Pronics Lelystad B.V. The Netherlands) that detects antibodies directed against the non-structural 3ABC protein of FMDV. The test procedure was carried out according to the manufacturer's protocol. The optical density (OD) of the wells was measured at 450 nm using a Multiscan Ex ELISA plate reader (Thermo Fisher Scientific OY, Vantaa, Finland) within 15 minutes. The mean OD of the wells was calculated. Paired positive and negative control sera (negative, weak-positive, and strong-positive) were also run for confirmation of the procedure. The percentage inhibition (PI) of the controls and test sera were calculated using the following formula:

$$PI = 100 - (OD450\text{test sample}/OD450\text{max}) \times 100.$$

The OD450 values of all samples were expressed as PI relative to the OD450max. The criteria for test acceptance were as follows:

- Mean OD450 of the negative control must be >1.000;
- The mean percentage inhibition of weak positive control must be >50%;
- All samples with PI ≥ 50% were considered positive (*i.e.* antibodies against NS protein of FMDV were present in the test sample).

2.5.1. Structural Protein (SP) ELISA

The FMDV NSP antibody positive serum samples were further serotyped using a commercial Solid Phase Competitive ELISA (SPCE) kit manufactured by IZSLER Biotechnology Laboratory Italy. This ELISA kit detects antibodies directed at structural proteins (SP) of FMDV (*i.e.* capsid proteins). This test is serotype-specific and requires each serum to be run for each serotype tested. The assay was performed according to manufacturer's instructions and by distributing 45 µl of diluent buffer to all wells and followed by adding 5 µl each of negative and positive control sera to wells A1, B1 and C1, D1 respectively; and 5 µl of each test serum was added to remaining wells of five plates. The OD of each well was read at 450 nm

wavelength using a Multiscan-Ex ELISA plate reader (Thermo Fisher Scientific OY, Vantaa, Finland). The criteria for acceptance of the assay were as follows: the OD reading must be greater or equal to 0.8 in wells of negative controls. The positive control serum was expected to give a PI equal to or greater than 90%.

2.5.2. Antigen Detection in Epithelial Tissues

Epithelial tissues were tested for the presence of FMDV antigen using a commercial FMDV antigen detection ELISA kit manufactured by IZSLER Biotechnology Laboratory, Italy. This kit can be used to detect FMDV for serotypes O, A, SAT1 and SAT2. The assay is a sandwich ELISA performed with selected combinations of anti-FMDV monoclonal antibodies (MAbs), used as coated and conjugated antibodies. The samples were prepared as described in OIE diagnostic manual [11]. Briefly, epithelial tissues were ground using sterile pestle and mortar to prepare a 10% - 20% homogenate in PBS. The homogenate was then clarified by centrifuging in a bench centrifuge at 2000 g for 10 minutes and the supernatant was collected and used for antigen detection according to manufacturer's instructions. The ELISA microtitre plates were supplied pre-coated with MAbs for each of the serotypes (O, A, SAT1, SAT2) and with positive and negative controls. The OD of each well was read at 450 nm wavelength using a Multiscan-Ex ELISA plate reader (Thermo Fisher Scientific OY, Vantaa, Finland). The criteria for test acceptance were as follows: The positive controls were expected to give OD values of 1.0 or higher in the type specific reactions and in the pan-FMDV reactions, the negative controls were required to give OD values lower than 0.1. Samples were considered negative for FMDV if the OD value was < 0.1 with all MAbs, after subtracting the OD of the respective negative control. A sample was considered positive for serotype O if the OD value was >0.1 with the type O MAb and with the Pan-FMDV O, A, C and Asia1 MAb. A sample was considered positive for serotype A if the OD value was >0.1 with at least one of the two serotype A Mabs and with the pan-FMDV O, A, C and Asia1 MAbs. A sample was considered positive for SAT1 if the OD value was >0.1 with the serotype SAT1 MAbs, after subtracting the OD of the respective negative control. A sample was considered positive for SAT2 if the OD value was >0.1 with the serotype SAT2 MAbs, after subtracting the OD of the respective negative control. A sample was considered not serotype O, A, SAT1 or SAT2 if the OD value was >0.1 with the pan-FMDV MAb and <0.1 with the all of the serotype-specific MAbs, after subtracting the OD of the respective negative control. OD values between 0.1 and 0.2 were considered suspect and were retested.

2.5.3. Molecular Test

The esophageal and pharyngeal fluids (probang samples) suspensions prepared following the OIE [11] protocol were tested using molecular assay. Positive antigen ELISA samples were further tested using molecular assays. The genomic viral RNA was extracted from 140 µl of 10% suspensions of samples using the

QIAamp viral RNA mini kit (Qiagen, Germany) in accordance with the manufacturer's protocols. RNA was eluted from QIAamp Mini spin columns in a final volume of 60 µl of elution buffer. The RNA samples were stored at −80˚C until used. Five microliters (5 µl) of each test sample was used as template. The RNA was reverse-transcribed and amplified using the one step classical PCR technique using the universal primer sets: 1Forward 5'-gCCTgg TCT TTC CAggTC T-3'/1Reverse 5'-CCA gTC CCC TTC TCA gAT C-3' at a working concentration of 10 µM. The process was carried out using one step RT-PCR kit (Qiagen, Germany). The amplification cycling was conducted using IAH-Pirbright's protocol as follows: reverse transcriptase at 50˚C for 30 minutes, starting denaturation at 95˚C for 15 minutes, denaturation at 94˚C for 30 seconds and annealing at 52˚C for 30 seconds and elongation at 72˚C for 30 seconds and carried out for 40 cycles. There was an extra elongation (final elongation) step of 72˚C for 10 seconds. This process was carried out using Applied Biosystem 9700 PCR thermocyler. The amplicons (PCR product) were migrated on 2% agarose gel and the expected size of band was 327 bp. Amplicons were sent to the National Agency for Sanitary Food security (ANSES), France for sequencing and phylogeny since the required facilities were not available at LANAVET.

The sequencing reactions and phylogeny based on FMDV VP1 coding gene were carried out on three amplicons (ZIBOU 09, VELE 03 and VELE 09). Phylogeny was carried out using the MEGA 5.1 software by comparing the sequence data obtained with reference nucleotide sequences available in the National Center for Biotechnology Institute (NCBI) Gene Bank., USA. The molecular epidemiological pattern of FMD virus was deduced using the phylogenetic information obtained.

2.5.4. Statistical Analyses

A software called Statistical Package for the Social Sciences (SPSS) 16.0 manufactured by SPSS Inc. Chicago, IL was used for data analysis (SPSS is currently distributed and maintained by IBM Corp, Armonk, New York). Prevalence was estimated and compared between sampling rounds and locations using the Chi-square tests and statistically significant level was set at $P \leq 0.05$.

3. Results

The non-structural protein based ELISA revealed the prevalence of FMDV antibodies of 86.3% and 87.1% for the first and second sampling periods respectively (Table 1). Seventy NSP-positive sera were picked from either sampling round to determine serotype specificity. All the veterinary centers with serotype-specific testing conducted had animals reactive to both serotypes A and O (Table 2). The majority of positive sera 94% and 93% in the first sampling and second sampling period respectively reacted with high PI (61% - 100%) indicating high titres. For the prevalence with color, grey color animals had high percentage positivity (90%) (Figure 2). Concerning the test positive proportion with breed of cattle, the Goudali breed had the highest percentage of positives (95.7%) for FMDV

Table 1. Spatio-temporal distribution of FMDV NSP-ELISA Antibody among Veterinary Centers in North Region of Cameroon.

Veterinary Center	1st sampling		2nd sampling	
	No. tested	No. (%) pos.	No. tested	No. (%) pos.
Selifa	45	38 (84.4)	40	37 (92.5)
Mayo-Oulo	100	100 (100)	99	99 (100)
Lougguere	100	94 (94)	56	50 (89.3)
Djalingo	30	30 (100)	27	26 (96.3)
Dembo	97	76 (78.4)	93	74 (79.6)
Hamakoussou	94	64 (68.1)	96	72 (75.0)
Total	466	402 (86.3)	411	358 (87.1)

Table 2. Results of analyses of epithelial tissues from infected cattle for presence of FMDV antigen.

veterinary center	Total samples Tested	No. (%) of positives	Distribution of antigenic serotypes	No. of outbreaks reported	No. (%) of outbreaks confirmed
Dembo	10/4	3 (30)*	SAT2 (2), SAT1 (1), O (1)	4	4 (100)
Djalingo	22	12 (54.6)[a,b]	SAT2 (8), A (4), SAT1 (2)	4	4 (100)
Pitoa (Zibou)	3	1 (33.3)	SAT2 (1)	1	1 (100)
Velé	2	2 (100)	SAT2 (2)	2	2 (100)
Total	37	18 (48.7)		11	11 (100)

*One animal had co-infection with 3 serotypes (SAT1, SAT2 and O) 13 SAT2; [a]One animal had co-infection with 3 serotypes (SAT1, SAT2 and A); [b]One animal had co-infection with 2 serotypes (SAT1 and A).

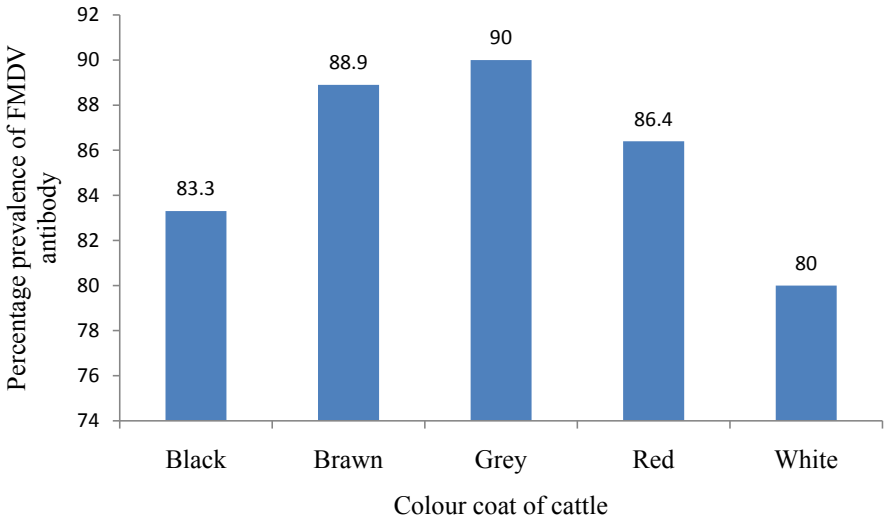

Figure 2. Distribution of FMDV NSP-ELISA Antibody based on colour coat of Cattle.

NSP-ELISA antibody followed in decreasing order of by Peulh (93.9%), Bokolo (88.7%), Foulfouli (83.7%) and Fulani (78.5%) breeds of cattle (Figure 3). Cattle tested from herds that had contact with wildlife showed that 84.54% were positive for NSP-ELISA antibody while those animals tested from herds that never had contact with wildlife had 84.41% positives for NSP-ELISA antibody (Figure 4). Out of the 272 female animals tested, 83.82% were positive as against the 85.88% males that tested positive for NSP-ELISA antibody (Figure 5). The period of last outbreak of FMD in a herd was considered as indicative of the period of the year when the disease outbreak occurred. Analysis of the monthly distribution of the prevalence rates of FMDV NSP-ELISA antibody revealed in decreasing order of prevalence the month of May (97.3%), followed by August (91.4%), July (83.0%) and September (82.0%) (Figure 6). Cattle in the age group of 3 < 5 years had highest prevalence (90.4%), followed by age group of 1 - <3 years with 88.31%, but age group < 1 year had lowest percentage positive of 72.29% (Figure 7). The prevalence rate increased with the age of animals studied.

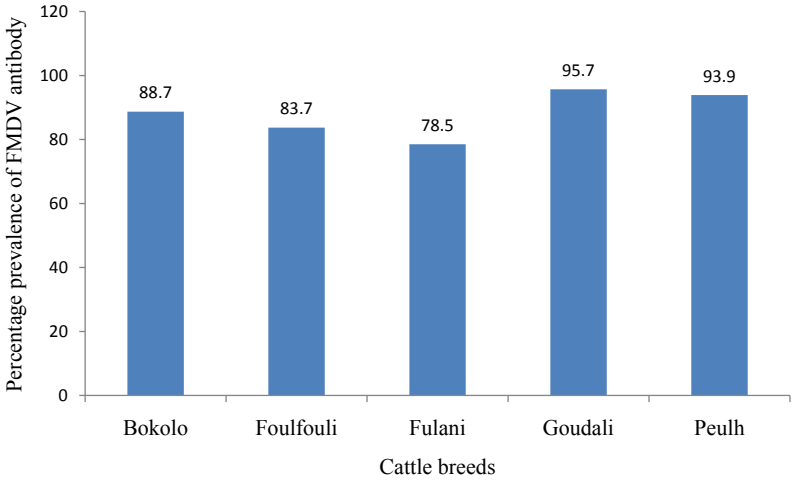

Figure 3. Distribution of FMDV NSP-ELISA antibody based on breed of cattle sampled.

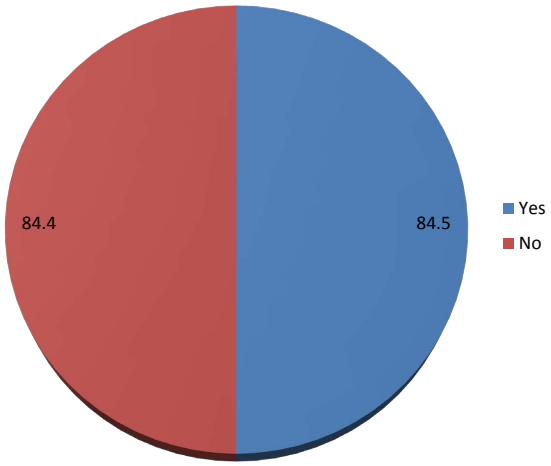

Figure 4. Distribution of percentage prevalence of FMDV NSP-ELISA antibody based on the response of farmers on contact of their animals with wild life.

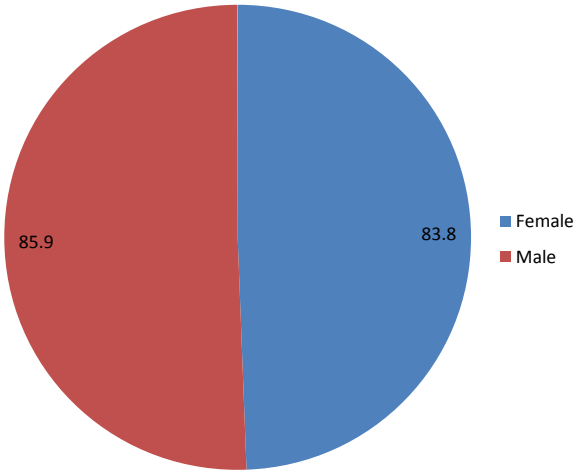

Figure 5. Prevalence of FMDV NSP-ELISA antibody based on gender of cattle studied.

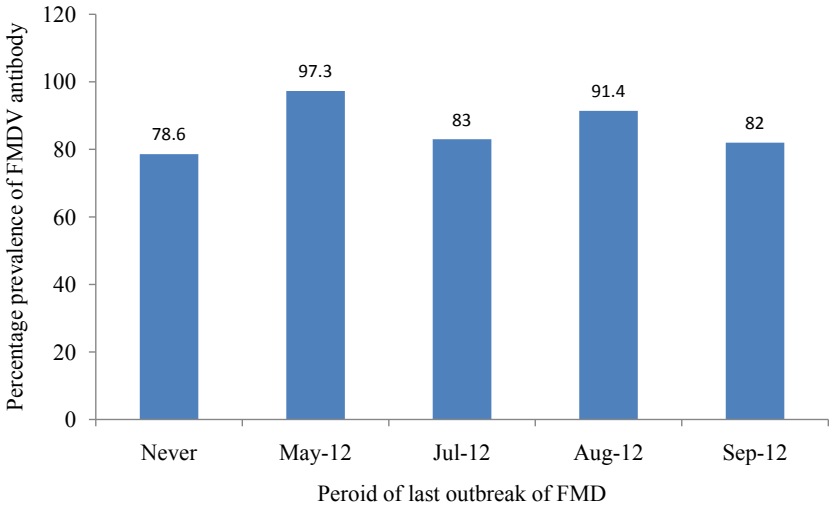

Figure 6. Distribution of FMDV NSP-ELISA antibody based on period of last outbreak of FMD of cattle sampled.

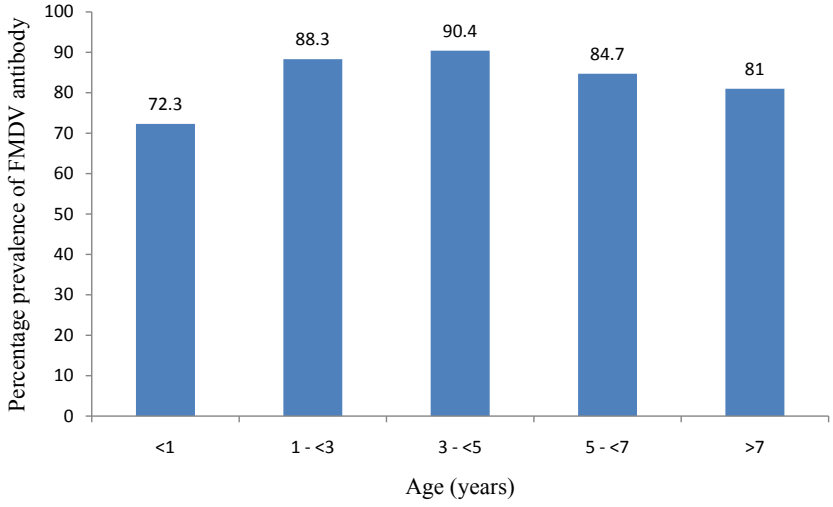

Figure 7. Age distribution of FMDV NSP-ELISA antibody in cattle studied.

Out of the 37 epithelial tissue samples tested, 18 (48.7%) were positive for the presence of FMDV antigens. The serotypes detected were SAT2, SAT1, O and A (Table 2). One animal each from Dembo and Djalingo had multiple infection or cross-reaction with 3 serotypes (SAT1, SAT2 and O) and (SAT1, SAT2, A) respectively; while another animal in Djalingo had a co-infection or cross-reaction with 2 serotypes (SAT1 and A). Serum samples collected from clinical cases with lesions more than two weeks old (convalescent sera) were tested using NSP ELISA and positive samples were further tested using SP ELISA for serotypes O and A (Table 3). For the serotype prevalence with site, Dembo had 100% for serotype A and 80% for serotype O, Hamakoussou had 0% for serotype A and 42.9% for serotype O, Lougguere and Mayo-Oulo both had 100% for serotype A and 0% for serotype (Table 4). All the tissue and fluid samples collected during clinical outbreaks reported during this study were confirmed by PCR with a percentage positivity of 75.6%. A prevalence of FMDV RNA (17.5% (n = 120) and 16.7% (n = 240)) was observed among the sedentary animals that were sampled four to five months apart. The gel obtained from the PCR products revealed the expected band size for the disease (Figure 8). Genotyping results of the SAT 2 isolates (CAM VELE03, CAM VELE 05 and CAM ZIBOU 09), revealed that the three isolates were similar with phylogenetic distance of <3% between CAM VELE viruses and <10% between CAM VELE and CAM ZIBOU viruses (Figure 9). These viruses were homologous to the published sequences from Libya, JX570633.1

Table 3. Distribution of FMDV NSP-ELISA antibody positive sera into different serotypes (A and O) based on non-structural protein positive samples.

veterinary center	Total No. tested	No. (%) positive for:	
		Serotype A	Serotype O
Dembo	15	7 (46.7)	10 (66.7)
Hamakoussou	22	16 (72.7)	19 (86.4)
Djalingo	18	12 (66.7)	11 (61.1)
Mayo-Oulo	15	10 (66.7)	13 (86.7)
Total	70	45 (64.3)	53 (75.7)

Table 4. Analyses of convalescent sera from infected cattle for FMDV NSP-ELISA antibody and distribution into serotypes.

Sampling Unit	Total No. tested	No. (%) positive	No. (%) positive for:	
			Serotype A	Serotype O
Dembo*	5	5 (100)	5 (100)	4 (80.0)
Hamakouss	7	7 (100)	0 (00.00)	3 (42.9)
Lougguere	1	1 (100)	1 (100)	0 (0.0)
Mayo Oulo	1	1 (100)	1 (100)	0 (0.0)
Total	14	14 (100)	7 (50.0)	7 (50.0)

*Four of the samples tested for both serotypes.

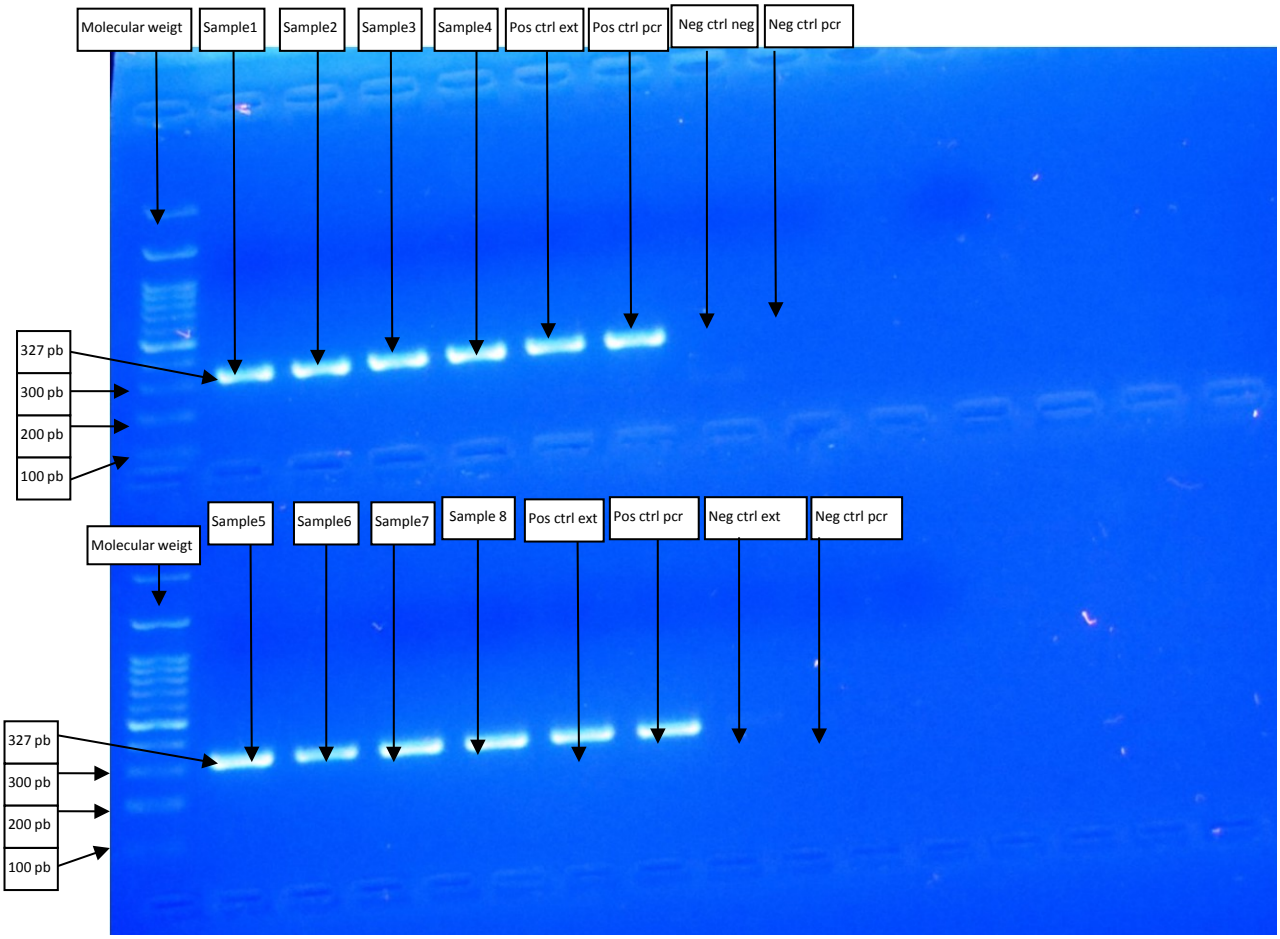

Figure 8. PCR Gel showing the bands of FMDV amplicons from classical PCR using Universal Primers 1F and 1R.

LIB/39/2012, (2012 epidemic) with a phylogenetic distance of <1%. The viruses also clustered with the published sequences from Nigeria isolates (JX570636.1, NIG/2/2007) and Sudan isolates (GU566071.1 SUD/1/2007) submitted to NCBI database by Knowles in 2012 and 2010 respectively with phylogenetic distance of <7%.

4. Discussion

Cameroon as a whole and the North region in particular offer a unique opportunity in the epidemiological study of FMD, as the country did not vaccinate against the disease until recently, which facilitates study of the natural spread of the infection. In this study, some aspects of the epidemiology of FMD in North region of Cameroon were investigated with the goal of formulating effective prevention and control strategies. Results of the longitudinal study carried out on sedentary animals in randomly selected veterinary centers, revealed a high prevalence of FMDV antibody of 86.3% and 87.1% among the cattle population studied during the first and second sampling periods respectively. This is an indication of natural exposure of the animals to FMDV over a long period of time because the NSP-ELISA differentiates antibodies due to vaccination from that of

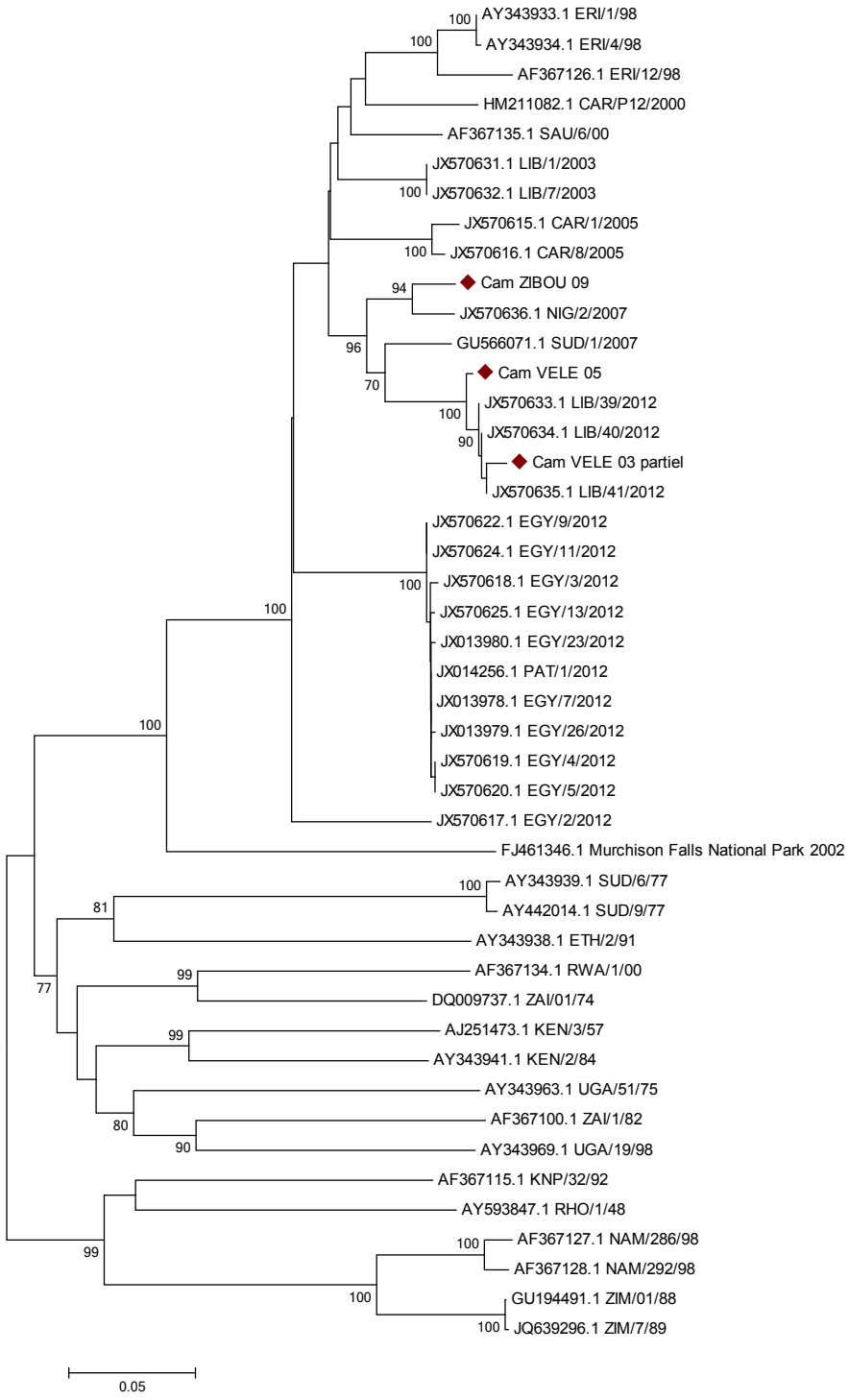

Figure 9. Phylogenetic tree of the SAT 2 isolates from the North region of Cameroon.

natural infection. A majority of the NSP-ELISA-positive sera had inhibitions from 81% to 100%. There was no significant difference in seroprevalence between the veterinary centers or between the two sampling periods. To the best of our knowledge this is the first serological study on FMDV in cattle population in the North region of Cameroon. The findings in this study are similar to the 75%

seropositivity reported by Ludi *et al.* [6] in the Far North region of Cameroon. The results of the current study showed that two veterinary centers (Djalingo and Mayo-Oulo) had the highest seroprevalence during the sampling periods. This might be because these centers are located along a transhumance route for cattle and are also frequently used for the illegal movement of animals from neighbouring countries [9]. The differentiation of serological response to natural infection from that due to vaccination is sometimes difficult [11]. Serotyping of the NSP-ELISA positive sera into serotypes A and O revealed a high activity of the two serotypes in the study area with one of the centers (Dembo) exhibiting apparent co-circulation of both serotypes. Serum samples collected from convalescent animals were also reactive to serotype A and O from all the localities sampled. This is consistent with the findings of Bronsvoort *et al.* [12] and Ludi *et al.* [6] who detected serotypes O and A in addition to other serotypes from the Adamawa and Far North regions of Cameroon, respectively. In this study the serotypes SAT1, SAT2, O, and A were detected in clinical samples with some animals showing evidence of multiple infections (SAT1, SAT2 and O), (SAT1, SAT2 and A), (SAT1 and A). This is suggestive of co-circulation of multiple serotypes. This could be due to poor cross protection between the serotypes and short duration of immunity [13] indicating that animals having immunity against one serotype (either by vaccination or infection) will not be protected against infection with the other serotypes or it could suggest a cross-reaction in the serotype-specific ELISA test. This observation is similar to the findings of Ludi *et al.* [6] from the Far North region of Cameroon and Ehizibolo *et al.* [14] in Nigeria, who reported prevalence of FMDV serotypes SAT1, SAT2, SAT3, O and A. The FMDV exists in the form of seven serologically and genetically distinguishable types, namely, O, A, C, Asia1, SAT1, SAT2, and SAT3, but several subtypes have evolved within each serotype [15]. However, apart from Asia1, the other six FMDV serotypes have each been reported in the African continent at some time [16].

Analysis of results of NSP-ELISA in relation to some possible risk factors showed age, breed and period of outbreak to be significantly associated with prevalence of FMDV. The Goudali and Peuhl breeds, the months of May and August and the 3 - <5 year old were found to have higher FMDV NSP-ELISA antibodies. The most plausible explanation for this could be that the month of May is the beginning of rainy season and most animals that went on transhumance usually return to their normal grazing areas bringing along the virus from their migratory areas. The month of August is the peak of rainy season and the season appeared to favour outbreaks of FMD. The age group 3 - <5 is a susceptible age group. Morbidity and mortality in FMD depend upon the breed and age of the animal. The mortality in adult animals is very low (2%) as compared to 20% in young counterparts [17].

From the results of this study, it could be concluded that FMD is endemic and has high seroprevalence among the cattle population in the North region of Came-

roon. The most prevalent FMDV serotypes are the SAT2 (35.1%), A (10.8%), SAT1 (8.1%), and O (2.7%); occurring in single or in double and triple co-infections. The basic epidemiological factors influencing the circulation of FMDV in the study area are age, breed and period of last outbreak.

Analyses of probang samples from the NSP-ELISA positive animals tested by PCR showed low positivity rate for the first (17.5%) and second (15.83%). This finding is similar to that of Bronsvoort *et al.* [12] who reported herd-level estimate of apparent prevalence of probang-positive herds of 19.5%. These PCR positive animals could be considered as carriers since the samples were collected from apparently clinically healthy animals. Usually, after resolution of clinical signs, ruminants can become persistent carriers. Zhang and Alexandersen [18], defined persistent carrier animals as having FMDV present (usually detected in their esophageal-pharyngeal fluid) beyond 28 days post infection. In this study, it was observed that few animals lost their carrier status 4 - 5 months after the initial sampling and some animals only became positive during the second sampling which could be an indication of a new infection. In natural FMDV infection, the main route of virus entry is the respiratory tract where the initial virus multiplication usually takes place (epithelium of the pharynx), producing primary vesicles or "aphthae" [19]. All the clinical outbreaks reported during this study were confirmed by PCR with percentage positivity of 75.6%.

In this study, three SAT2 isolates were genetically characterized, from VP1 sequence of three amplicons (CAM VELE03, CAM VELE 05 and CAM ZIBOU 09). All the three isolates were similar with phylogenetic distance of <3% between CAM VELE viruses and <10% between CAM VELE and CAM ZIBOU viruses. These viruses were homologous to the published sequences from Libya, JX570633.1 LIB/39/2012, (2012 epidemic) with a phylogenetic distance of <1%. The viruses also clustered with the published sequences from Nigeria isolates (JX570636.1, NIG/2/2007) and Sudan isolates (GU566071.1 SUD/1/2007) submitted to NCBI database by Knowles in 2012 and 2010 respectively with phylogenetic distance of <7%. This is suggestive that either the viruses were exported from Cameroon to Libya or from Libya to Cameroon. Another possibility could be that the viruses isolated in Nigeria and Sudan in 2007 could still be circulating and causing outbreaks in the central and Northern African countries where there are several transboundary cattle trade routes. Cattle originating from Chad and Sudan are usually moved on foot across the Far North and North regions of Cameroon on their way to being sold in Nigeria [6]. We were not able to characterize the serotypes A and O virus isolates because these serotypes were detected late when the SAT2 serotypes amplicons were already sent to ANSES for sequencing and phylogeny. Future phylogenetic studies will be carried out on serotypes A and O isolates.

5. Conclusion

High prevalence of FMD occurs among the cattle population in the North region

of Cameroon. The FMDV serotypes identified in this region are SAT1, SAT2, A and O; occurring in single or in double and triple co-infections. Phylogenetic analysis of the SAT2, the most common serotype, showed that this serotype is homologous to the 2012 Libyan isolate and clustered with the 2007 isolates from Nigerian and Sudan.

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Authors' Contribution

SJD, LR, ADY, SSB, RG, NTA and JAN—research concept and design; SJD and RG, MMM, SSL—collection and/or assembly of data. MMM—data analysis and interpretation; SJD and SSL—writing the article; CBR, AW, JAN, NTA and SSL—critical revision of the article; SJD, JAN and NTA—final approval of the article.

Conflicts of Interest

Authors declared no potential conflict of interest exists.

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