

Original Research Article

Serological Epidemiology of Foot-and-Mouth Disease (FMD) in Sedentary Mixed-species Herds in Adamawa-Cameroon

ABSTRACT

Foot-and-mouth disease (FMD) is an economically important viral disease of domestic animals and wildlife. High circulation occurs during outbreaks but little is known about the current transmission dynamics in sedentary cattle herds with sheep. To investigate on this, samples from cattle and sheep located in the Vina Division of the Adamawa region of Cameroon, during FMD outbreaks were collected. Non-Structural Protein (NSP) and antigen detecting/serotyping FMD ELISAs were used for sample screening. We used NSP serological data of cattle to estimate transmission parameters in catalytic and reverse catalytic models. The Akaike's Information Criteria (AICs) revealed the reverse catalytic model as the most parsimonious for our NSP serological data and was used to estimate the force of infection (FOI), the rate of waning immunity and to estimate periods of sustained transmission. Four serological types of FMD notably O, A, SAT1 and SAT 2 were identified from cattle vesicular epithelia tissues. Seroprevalence findings revealed 65.14% and 15.71% FMDV antibodies in cattle and sheep respectively with highest prevalence in both populations occurring in Mbidjoro. The FOI (λ) in sedentary herds was constant; the rate of waning immunity (ω) was 0.32 meaning cattle are generally immune for 3.12 years post natural infection. The reproductive number (R_t) was 7.33, meaning approximately 87% of cattle always need to be effectively immunized to prevent outbreaks. Therefore, FMD circulates in sedentary cattle and sheep populations with four serological types detected in cattle.

Key words: Seroprevalence; serotypes; Foot-and-mouth disease; reverse catalytic model; cattle; sheep.

1. INTRODUCTION

Foot-and-Mouth Disease (FMD) is one of the most contagious viral disease in mammals and has a great potential for causing severe economic losses in susceptible animals. This disease is caused by an Aphthovirus of the family Picornaviridae with seven serological types (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1) already known [1] and there is no cross immunity between the seven serotypes [2]. Although FMD is a disease of low mortality in adults, the frequency of outbreaks and the large numbers of animals and species affected in each outbreak results in a high and on-going impact for FMD in endemic countries [3] such as Cameroon. Mortality is elevated in animals up to one year of age [4] causing some visible impact for herders. Due to the huge economic consequences of the disease, it precludes international trade in areas where it circulates. In Cameroon there is no vaccination for this disease, but herders mix their uninfected cattle with infected cattle to speed transmission. Although the report of Bertram et al., [5] on the efficacy of the trivalent vaccine (SAT 2, O, A) for FMD in Ngaoundere was positive, a commercial vaccine remains unavailable for the disease in Cameroon.

The Adamawa region of Cameroon is major cattle rearing region where over 12,500,000 stocks reside [6] and greater than 90% of the herds are sedentary. Mixed farming in this region involving cattle, goats and sheep is common but pigs are rare. Despite representing the majority of the world's FMD-susceptible livestock, sheep and goats have generally been neglected with regard to their epidemiological role in the spread of FMD [7]. One of many complexities in FMDV epidemiology is the occurrence of seven different serotypes [1] that cause indistinguishable clinical disease [8]. Four serological types: O, A, SAT 1 and SAT 2 circulate in Cameroon which is found in pool 3 of the FMD world distribution map [9]. According to the working document entitled 'Strategic Plan for FMD Control in Cameroon' published on February 2015, three of the types A, SAT 1 and SAT 2 occur in the Adamawa Plateau of Cameroon but only SAT 1 is known to exist in Vina Division. Bronsvort et al., [10] diagnosed serotype O in sheep from Ngaoundere and realised that the type O pig isolate was not closely related to that recovered from cattle in the same area. The seropositivity with respect to serotype O in in-contact sheep with infected cattle and pigs was greater than that in in-contact sheep with non-infected cattle and pigs in

the report of Phyoe et al., [11], indicating the risk of infected cattle and pigs in FMD transmission in sheep populations. There is no information on ovine FMD as well as serotypes circulating among this population co-existing with cattle in Cameroon.

For future control plans to be successful, it is important to make predictions about future epidemics, by understanding the epidemiology of FMDV in much greater detail and by quantifying transmission and immunodynamics in the endemic setting. A quantity of particular interest is the force of infection (FOI), which measures the rate at which susceptible individuals acquire infection. The FOI can inform epidemic behavior, identify heterogeneities in transmission, and specify targets for disease control [12-13]. Standard approaches to estimating the FOI fit accurate counts of both susceptible and infected individuals to dynamical catalytic models. However, this information is difficult to obtain, especially for endemic disease when there is little or inconsistent surveillance, or when the disease can be subclinical. In these cases, the lack of necessary information prohibits the design and implementation of disease control programs.

A possible solution to quantifying disease dynamics in the absence of counts of infected and susceptible individuals is to fit dynamical models to serology data [14]. These data classify an individual based on the presence of host antibodies against a pathogen, which indicates past exposure to the pathogen. Here, we show that serology data matched with host age can be fitted to dynamical models to provide a record of historical exposure, even when animals are sampled only in a single event such as a cross-sectional study. The serology data also allow us to estimate the duration of immunity, an important epidemiological parameter that is poorly known for FMD.

To address uncertainty in the duration of a host immune response against FMDV in the absence of vaccination, we fit contrasting models of FMDV transmission and immunity to the serology data: the catalytic model and reversible catalytic model [15-16]. The catalytic model describes transmission followed by lifelong seroconversion, suggesting that the antibody response lasts for the lifespan of the animal. Alternatively, the reverse catalytic model assumes that infection leads to temporary seroconversion, and that the immune memory wanes over time. We also discriminate between two hypotheses about variation in the FOI. Previous studies have shown

that the FOI is age-specific, since animals have contact rates that vary with age, in an endemic steady state [12-17]. Other studies have shown that the FOI may be time-varying, because incidence often exhibits cycles or local extinction due to depletion of susceptible individuals which does not support the idea of a disease steady state [18]. We distinguish between age-specific and time-varying differences in the FOI by estimating the FOI of FMDV irrespective of the serotype. In this way, we test between two sets of alternative hypotheses: first, between lifelong seroconversion versus waning immunity and second, between age-specific FOI versus time-varying FOI.

The objectives of this study were 1) to identify the serological types of FMD circulating in some herds during the 2016 FMD epidemic in Ngaoundere, 2) to determine the prevalence of NSP antibodies of the FMDV and associated risk factors, 3) to determine the force of infection and duration of immunity in sampled cattle population.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The Adamawa region is in the northern part of Cameroon and is one of the major cattle rearing region of Cameroon [6-19]. The Vina division of this region, our study area, has a surface area of about 17196 km² and is sub-divided into two administrative and geographical units: the plateau: the districts of Ngaoundere and Belel and the plain (district of Mbe). Ngaoundere where our study herds were located is sub-divided into three sub-districts: Ngaoundere 1, Ngaoundere 2 and Ngaoundere 3 (Fig.1). The vegetation of this region is mainly wooded savanna. Small holder cattle farmers (people who have less than 300 cattle) own an estimated 40 percent of the cattle population in the Adamawa region. The rest of the cattle are kept by ranchers who stock their ranches with thousands of cattle. The cattle breeds consist of Zebu cattle (White and Red Fulani and Goudali or Peuhl of the Adamawa), cross breeds such as Charlorais x Goudali, Fulani x Goudali and the metis are kept under traditional extensive mixed husbandry systems with communal herding including other species like sheep, goats, dogs, poultry and rarely pigs. Among the cattle diseases of this region, FMD is considered a serious threat to cattle and incurs huge

economic losses to herders, due to annual death or reduced production [19-20]. This disease occurs every year and for the past years, primary outbreaks occurred between the months of August and September in the rainy season [19]. While vaccine trials have been done in a few herds since 2014, vaccine is not widely available for FMD in Cameroon. Boundaries remain porous for trade and transhumance herds from neighboring countries like Chad, Central Africa, Gabon, Sudan, Nigeria and Niger potentially exposing the local cattle population to exotic strains of FMD.

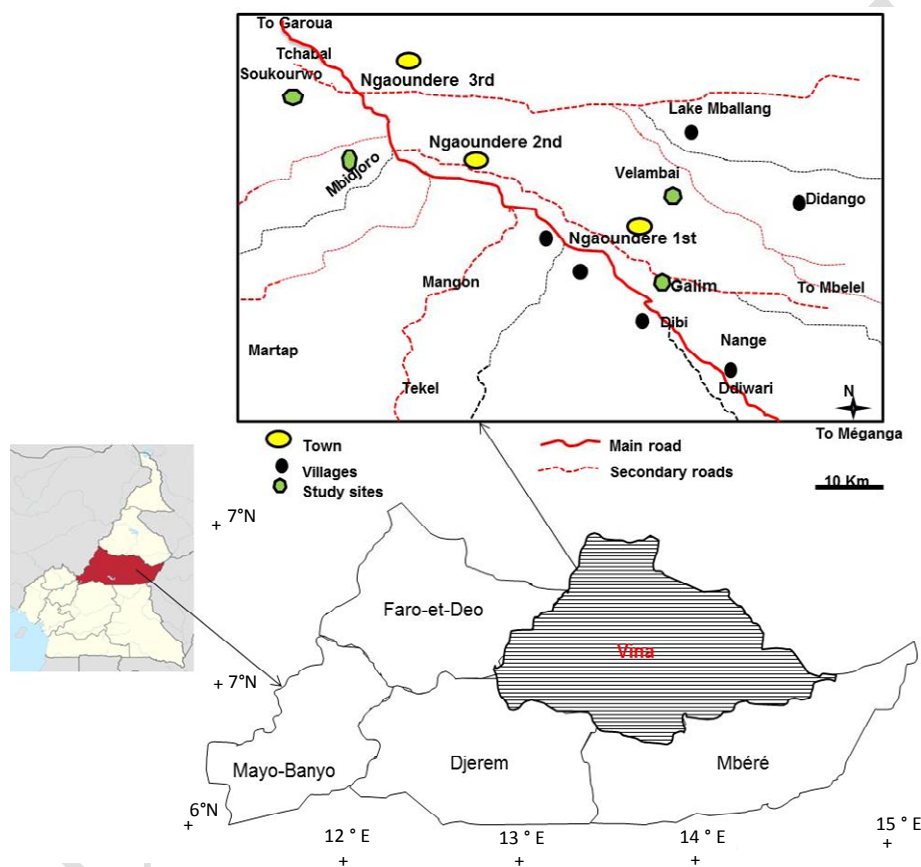


Fig.1. Map of sampled sites (green dot) in Vina Division

2.2 Statistical Analysis

Statistical analysis was carried out using the R-statistical software (R version 3.4.0). The likelihood estimations were performed using the “optim” function in R [21]. The catalytic and reverse catalytic model fitting was determined as described in Pomeroy

et al., [14] and Moreno-Torres et al., [22]. The chi-square test was used to compare the seroprevalence with hosts related health parameters like age and sex as well as study sites.

2.3 Epithelial tissue collection from cattle

Epithelial tissues from unruptured or freshly ruptured vesicles (mouth, muzzle, interdigital space, or coronary band) were collected according to a published protocol [23]. 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 Blood Collection from Cattle and Sheep

Blood was collected from 315 non-vaccinated animals in the four herds *i.e.* for cattle: Herd 1 (n=39), Herd 2 (n=44), Herd 3(n=45) and Herd 4 (n=47) (N= 175) and sheep: Herd 1 (n=36), Herd 2 (n=33), Herd 3(n=34) and Herd 4 (n=37) (N= 140). The sampled herds were chosen based on the fact that they consisted animals with FMD clinical signs and on the willingness of animal owners. More than 80% of the target herds for the two ruminant species were sampled. Blood collection was carried out via the jugular vein into anticoagulant free sterile tubes and labeled. The blood was transferred to the laboratory where it was centrifuged at 4000 revs/5mins. Sera was recovered and transferred into well labeled cryotubes prior to serological analysis.

2.5 NSP ELISA test

Cattle sera were screened using the FMDV NSP-ELISA kit (PrioCHECK FMDV NS, Pronics Lelystad B.V. the Netherlands). Test sera, negative, weak positive and strong positive reference sera were added to 96-well microtitre plates, pre-coated with 3ABC-antigen and test was conducted according to manufacturer's instructions.

Optical density of test samples measured at 450nm using a MultiSkan plate reader (Thermo Fisher Scientific OY, Vantaa, Finland) within 15 minutes and results were expressed as index derived by dividing the absorbance value of test serum by cut-off control value, according to manufacturer's instruction. Samples with percentage inhibition (PI) $\geq 50\%$ were classified positive and samples with $PI < 50\%$ were considered negative respectively. The PI of the controls and the test sera were calculated according the following formula:

$$PI = 100 - \left[\frac{OD_{450} \text{ test sample}}{OD_{450} \text{ max}} \right] \times 100$$

2.6 The FMDV Antigen Detection ELISA and Serotyping using Bovine Vesicular Epithelia tissue

The epithelial tissues collected from cattle were tested for presence of FMDV antigen using a commercial FMDV antigen detection ELISA kit produced by IZSLER Biotechnology Laboratory, Italy. This kit can be used to detect FMDV for serotypes O, A, SAT1, 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 [23]. 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 2,000g 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 450nm 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 MAb. A sample was considered positive for SAT1 if the OD value was > 0.1 with the serotype SAT1 MAb, 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 MAb, 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 MAb, after subtracting the OD of the respective negative control. OD values between 0.1 and 0.2 were considered suspect and were retested.

2.7 Model Selection, Parameterization and Sensitivity

The FMDV NSP serological data of sampled cattle was used to estimate transmission parameters in catalytic and reverse catalytic models. The catalytic and reverse catalytic models were compared and the best-fit model was selected using Akaike's Information Criterion (AIC) [24]. To find estimates for the FOI and rate of waning immunity, we estimated values for the b-splined force of infection and rate of waning immunity by maximizing the binomial likelihood of seropositivity by age according to Pomeroy et al., [14]. Likelihood estimations were performed using the `optim` command in R. Plots of the time-varying forces of infection was compared across ages and herds, local minima and maxima were visually determined. If local minima and maxima occurred at the same ages regardless of serotype, we can assume that the FOI is age or herd-related and FMD dynamics are at or near a steady state. If local minima and maxima occurred at different ages across herds, we can assume that the FOI is time-varying and FMD dynamics are not at a steady state.

2.8 Model Derivation

The catalytic and reverse catalytic models were compared, and the best-fit model was selected using Akaike's Information Criterion (AIC) [24]. To find estimates for the FOI and rate of waning immunity, we estimated values for the b-splined force of

infection and rate of waning immunity by maximizing the binomial likelihood of seropositivity by age according to equation 1:

$$y_a \sim \text{Bin}(N_a, P_a) \dots\dots\dots \text{Equation 1}$$

Where N_a is the total number of animals sampled at age a and p_a is the proportion of animals that are seropositive.

Catalytic model: consider a two-state disease system, in which individuals are classified relative to their disease status. Let S and P represent proportions of the total population, such that $S + P = 1$ (Fig.2).

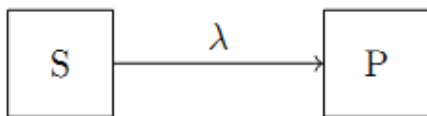


Fig.2. Catalytic Model

Susceptible individuals (S) test negative for disease and are susceptible to contracting it. Positive individuals (P) show evidence of previous or current infection. The force of infection (λ), is the rate at which individuals acquire disease and convert from susceptible to positive disease status.

For the catalytic model, and assuming an age-specific FOI represented by $\lambda(a)$,

$$\frac{dP(a)}{da} = \lambda(a)(1 - P(a)) \dots\dots\dots \text{Equation 2}$$

Solving for $P(a)$ gives

$$P(a) = 1 + Ce^{-\int_0^a \lambda(a) da} \dots\dots\dots \text{Equation 3}$$

Assume that all individuals are susceptible at birth such that $P(a)=0$, when $a = 0$.

Then, $C = 1$ and

$$P(a) = 1 - e^{-\int_0^a \lambda(a) da} \dots\dots\dots \text{Equation 4}$$

The catalytic models above assume lifelong immunity implying that conversion to the seropositive state is permanent and individuals cannot convert back to the sero-negative state. This may or may not be a valid assumption for FMDV serostatus.

Reverse catalytic model: it relaxes the assumption of lifelong immunity, so that the duration of immunity may be considered at variable intervals. Again, let S and P represent proportions of the total population, such that $S + P = 1$ (Fig.3).

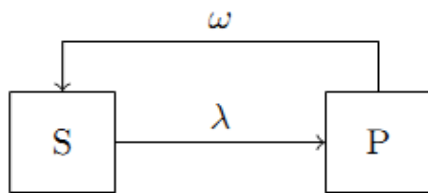


Fig.3. Reversible Catalytic Model

Susceptible individuals (S) test negative for disease and are susceptible to contracting it. Positive individuals (P) show evidence of previous or current infection. The FOI, or, is the rate at which individuals acquire disease and convert from negative to positive disease status and the rate of waning immunity (ω), permits re-entry into the sero-negative serostatus.

For the reversible catalytic model, again assuming an age-specific FOI represented by (a) ,

$$\frac{dP(a)}{da} = \lambda(a)(1 - P(a)) - \omega P(a) \dots \dots \dots \text{Equation 5}$$

Solving for P (a),

$$P(a) = \frac{\lambda(a)}{\lambda(a) + \omega} + C e^{-\int_0^a (\lambda(a) + \omega) da} \dots \dots \dots \text{Equation 6}$$

Again, assume that all individuals are susceptible at birth such that $p(a) = 0$ when $a=0$.

Then, $C = \frac{\lambda(a)}{\lambda(a) + \omega}$ and,

$$P(a) = \frac{\lambda(a)}{\lambda(a) + \omega} (1 - e^{-\int_0^a (\lambda(a) + \omega) da}) \dots \text{Equation 7}$$

Age-specific FOI versus time-varying FOI: Let I represent the number of infectious animals and let N represent the total population size. Consider that the FOI (λ) depends on the rate of contact between animals (c), the probability that contact will be with an infectious individual (I/N), and the probability that contact with an infectious individual produces a new infection (p) such that

$$\lambda = c \frac{I}{N} P \dots \text{Equation 8}$$

Following Begon [25]. Previous work has indicated that λ can vary with age and/or with time, such that the force of infection for the i^{th} serotype can be written as:

$$\lambda_i(a, t) = c(a) \frac{I_i(t)}{N} P \dots \text{Equation 9}$$

If the variation in λ_i is due to age-related variation in the contact rate, then the age(s) at which each serotype exhibits maximum and minimum λ_i occur(s) at

$$\frac{d\lambda_i(a)}{da} = \frac{dc(a)}{da} = 0 \dots \text{Equation 10}$$

Given an age-specific λ_i , the maximum and minimum would occur at the same ages regardless of serotypes. If the variation in λ_i is due to time-varying abundance of infectious individuals, then setting

$$\frac{d\lambda_i(a)}{da} = \frac{dI_i(t)}{dt} = 0 \dots \text{Equation 11}$$

Would result in a curve for λ_i in which the age at which each serotype exhibits maximum and λ_i varies by serotype, assuming asynchrony in $I_i(t)$

Ages were calculated from birth months and years reported in interviews during group discussions. Therefore, the accuracy of reported ages may affect model selection and parameterization. We investigated the impact of inaccuracy in age

reporting using the Morris Method for parameter sensitivity [26] to find the deviation from the reported age at which the model selected would change.

3. RESULTS

3.2 Seroprevalence in Cattle and Sheep Populations

From the 315 sera screened with NSP-ELISA, 175 were from cattle and 140 from sheep in the same mixed farming style in all the four herds. One hundred and fourteen (114) cattle were positive giving a seroprevalence of 65.14%. 15.71% (22 out of 140) of sheep were detected with FMDV NSP antibodies (Table 1).

3.3. Seroprevalence based on hosts-health related parameters.

Age seroprevalence in sheep population showed that young sheep were mostly detected with FMDV NSP antibodies as compared to their adult counterparts but the difference was not significant ($\chi^2=0.149$, $df= 1$, $P=0.699$). This trend was different for cattle where adults were most detected with the FMDV antibodies than the young ones but again, the difference was not significant ($\chi^2 =3.4483$, $df= 1$, $P= 0.06332$). Similarly, there was no statistically significant difference between the seroprevalence of the middle and last cohorts of cattle, but were significantly different from the first age cohort. (Table 1).

Table 1. Seroprevalence of FMD with respect to age of sheep and cattle

Species	Parameter	Negative (%)	Positive (%)	Total (%)	df	χ^2	P-value
	Age (years)						
Sheep	Young (1 and 1,5)	110 (78.62)	20 (14.30)	130 (92.90)			
	Adult (>1, 5)	8 (5.70)	2 (1.40)	10 (7.10)	1	0.149	0.699
Cattle	<2	20 (68.96)	9 (31.03)	29 (16.57)			
	2 to 3	18 (26.47)	50 (73.52)	68 (38.85)			
	>3	23 (29.48)	55 (70.51)	78 (44.57)	1	3.4483	0.06332

Seroprevalence with respect to sex in the cattle population revealed that female were more often detected with the FMDV antibodies than males with a statistically significant difference ($\chi^2 =24.853$, $df= 7$, $P= 0.0008057$). Similarly, female sheep were

more likely to have FMDV antibodies as compared to their male counterparts even though the difference was not significant ($\chi^2 = 2.371$, $df = 1$, $P = 0.124$) (Table 2).

Table 2. Seroprevalence of NSP FMD antibodies with respect to sex of cattle and sheep

Species	Sex	Negative (%)	Positive (%)	Total (%)	df	χ^2	P-value
Cattle	Female	28 (30.10)	65 (69.89)	93 (53.14)	7	24.853	0.0008057
	Male	65 (57.01)	49 (42.98)	114 (65.14)			
Sheep	Female	93 (66.40)	14 (10.00)	107 (76.40)	1	2.371	0.124
	Male	25 (17.90)	8 (5.70)	33 (23.60)			

Seroprevalence based on study herds revealed that sheep in the cattle herd in Mbidjoro had a higher prevalence of FMDV NSP antibodies than all the other herds of sheep in the other sites with no statistically significant difference ($\chi^2 = 2.371$, $df = 1$, $P = 0.124$). Generally, there was a significant statistical differences with FMDV NSP antibody prevalence with cattle herds of the different sites ($\chi^2 = 12.309$, $df = 3$, $P = 0.006395$). However, cattle in herds in Mbidjoro and Soukourwo were most detected with the FMDV NSP antibodies as compared to those in Velambai and Galim (Table 3).

Table 3. Seroprevalence of NSP FMD antibodies in sheep and cattle with respect to site

Species	Site	Negative (%)	Positive (%)	Total (%)	df	χ^2	P-value
Sheep	Mbidjoro	24 (17.1%)	9 (6.40)	33 (23.6)	3	12.309	0.006395
	Galim	33 (23.6%)	4 (2.90)	37 (26.4)			
	Velambai	32 (22.9%)	4 (2.90)	36 (25.70)			
	Soukourwo	29 (20.7%)	5 (3.60)	34 (24.30)			
Cattle	Mbidjoro	10 (22.72)	35 (77.77)	44 (25.14)	3	12.309	0.006395
	Soukourwo	10 (22.22)	34 (72.27)	45 (25.71)			
	Velambai	18 (46.15)	21 (53.84)	39 (22.28)			
	Galim	23 (48.93)	24 (51.06)	47 (26.85)			

3.4. Serotype-specific Distribution in the Study Herds

Four serotypes: O, A, SAT1 and SAT 2 were detected in the VETs of cattle from the sampled herds. The distribution of the FMD serotypes in cattle population in the various herds sampled revealed that serotype O was most frequent. In the herd

found in Mbidjoro all the four serotypes were recovered from cattle and SAT 2 was the most frequent. In Soukourwo, type O, A and SAT 2 were recovered from cattle with type O being the most common. In Velambai, type O, A and SAT 1 were recovered from cattle, but type A was recovered from more than one case. In Galim, type O was the only serological type recovered from cattle (Table 4).

Table 4. FMD serotypes detected from the VET of cattle in the sampled sites

Species	Sites	Total serotyped	Serotype			
			O	A	SAT 1	SAT 2
Cattle	Mbidjoro	15	1	1	1	4
	Soukourwo	17	3	1	0	1
	Velambai	11	1	2	1	0
	Galim	7	3	0	0	0

3.5 Model Results

The reverse catalytic model (one with waning immunity) and with maternal immunity was the preferred one for FMD (in general, not specific serotypes) in the Ngaoundere herds (see the summary table 5 of AICs-lowest is best).

Table 5. Presenting the AICs of the two models

Base Model	B-Spline Age	Maternal Immunity	AIC
Catalytic	No	No	43.8
Reverse Catalytic	No	No	37.3
Catalytic	No	Yes	42.1
Reverse Catalytic	No	Yes	33.0
Catalytic	Yes	No	47.0
Reverse Catalytic	Yes	No	46.6
Catalytic	Yes	Yes	36.2
Reverse Catalytic	Yes	Yes	140.8

From the reverse catalytic model, the FOI (λ) was quantitatively equal to 0.92yr^{-1} and appears to be constant. The rate of waning immunity denoted by ω was 0.32, meaning cattle are generally immune for 3.12 years post natural infection. Maternal immunity duration was 1.5 years, if the average lifespan of cattle on these farms is about 8 years, from λ , we calculated the reproductive number (R_t) to be 7.33 and that

approximately 87% (0.86) of cattle always need to be effectively immunized to prevent outbreaks.

Seroprevalence trends in the four herds sampled for this study had the same pattern where cases occurred mostly in three years old cattle and continued steadily to the eldest in the herds (Fig.4). Generally, Seroprevalence was directly proportional to age, meaning FMD antibodies mostly occurred with age (Fig.5).

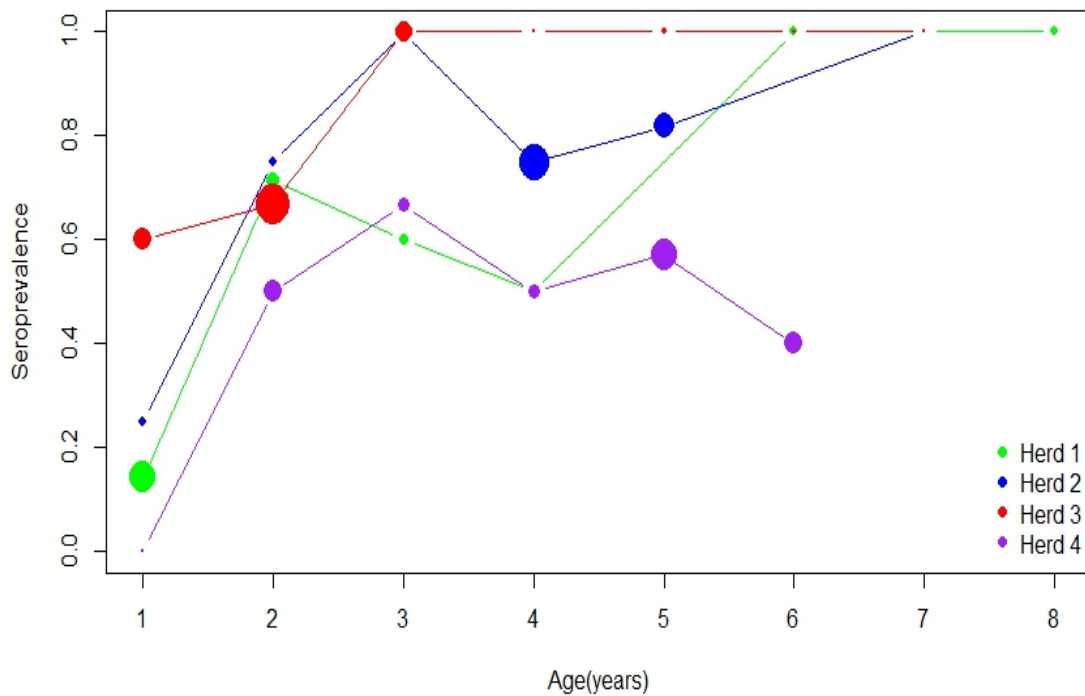


Fig.4. Variation of seroprevalence with age in the different herds of cattle
Herd 1, Velambai, Herd 2, Mbidjoro, Herd 3 Soukourwo and Herd 4 Galim

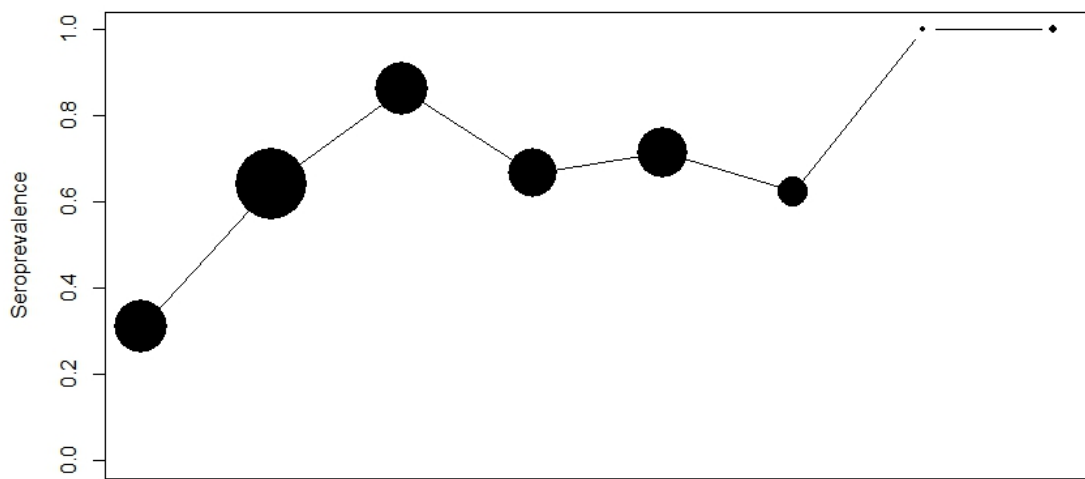


Fig. 5. General FMD seroprevalence trend with age of cattle

4. DISCUSSION

The overall NSP serological prevalence in cattle was 65.14% which was higher as compared to 15.71% in sheep population. This finding is like that of Eldaghayes et al., [27] in Lbya. The present NSP FMD seroprevalence status of sedentary herds in Ngaoundere was higher than 20.70% obtained by Kuate [28] in the same study area, but lower than 87.50% obtained by Oliva [29] in non-vaccinated cattle herds in the same study region. The mixed animal husbandry farming system in Ngaoundere where cattle graze together with sheep might be a risk factor in FMD spread among the two populations during an epidemic. This phrase is partly supported by Karim Al-Rukibat et al., [30] who reported that in an FMD endemic setting; apparently healthy Awassi sheep report high FMD antibodies and contaminate other stocks. Also, Phyo et al., [11] found that seropositivity was highest in in-contact sheep with infected cattle and pigs than in-contact sheep with non-infected cattle and pigs.

Seropositivity distribution based on age in both cattle and sheep populations were observed. In cattle, the adult cohort recorded the highest NSP FMD antibodies than the younger one even though the difference was not statistically significant. This finding is in consonance to that of Mohamoud et al., [31] and this might be because adults have acquired the infection through repeated exposure to the different serotypes of the virus and could get access to mixing with other herds at watering points and communal grazing areas. Conversely, the younger ones especially calves have low frequency of exposure to the virus and prevailing passive maternal immunity can give them protection against the disease; in addition, farmers in the study area keep their calves around the homestead, where there is less contact with other herds. Moreover, other attributes might be that the sample size of the three age groups was not proportional. The increasing seroprevalence of FMD with age

disagrees with previous report by Isholo et al., [32] who noted that cattle within the age of 1-2 years are more prone to FMD. In the sheep population, young cohort reported highest NSP FMD antibodies than adults with no significant difference. This finding is contrary to that of Saif-ur-Rehman et al., [33] who rather reported highest cases in sheep of >36months of age. This can be attributed to the disproportional sample size numbers in the different age categories as young were most frequent than adult in our study area.

Seroprevalence with sex in the cattle population resulted in higher detection of NSP FMD antibodies in females than their male counterparts with a statistically significant difference. This finding is like that obtained by Mohamoud et al., [31] and Abubakar et al., [34]. This finding was not parallel with the findings of Jenbere et al., [35] that showed that sex has no difference in risk association with FMD transmission. It also contradicts the findings of Fakai et al., [36] on pigs; Esayas et al., [37] and Megersa et al., [38] in Ethiopia. Similarly, concerning sheep populations in the same herds, females were most detected with the NSP FMD antibodies than males with a non-statistical significant difference. This finding is like that of Saif-ur-Rehman et al., [33]. Also, in another epidemiological investigation in Ethiopia, the prevalence rate of FMD among female animals was found higher (15.7%) than in males (8.27%) [39]. The greater percentage in females might be due to the physiological stresses that include oestrus, pregnancy and lactation which are known to affect their resistance to infection [40]. Lower seroprevalence in males could also be attributed to the fact that male cattle and sheep are usually provided with better nutrition than female in studied areas. Females are kept for an extended duration of time as compared to males to promote production exercises, which may ultimately result in high prevalence of antibodies to FMDV in female animals. Another possible reason is that sick males may be sold making their numbers lower than that of females in the sampled population.

The highest frequency of FMD NSP antibodies in cattle and sheep populations was noticed in Mbidjoro which is a village around Ngaoundere town where the main cattle market is located. This indicates the risk of such areas in FMD transmission. Distance from the study farm to the closest market was associated with FMD. Markets represent a source of adequate direct or indirect contact between susceptible livestock and FMD-infected stock. In the Northern regions of Cameroon,

producers move cattle from one market to the next in search of better prices especially the Ngaoundere market which happens to be the largest market in this region. Therefore, the rate of contact between herds, and markets are important variables that can affect FMD virus transmission [41-42-19].

Four FMD serological types A, O, SAT1 and SAT2 were circulating in Ngaoundere during the 2016 epidemic. This present serotype constitution is like that reported by Bronsvort et al., [10] except for SAT1 which was absent in their report. The study of Bertram et al., [5] on the circulating serotypes in Ngaoundere reported three serotypes (SAT 2, O, A). Five serotypes A, O, SAT1, SAT2 and SAT3 have been reported to be in circulation in the Far North region of Cameroon [43]. The report on the FMD situation of Cameroon from 2011 to 2016 indicates four serotypes *i.e.* A, O, SAT1 and SAT2 [9] and the occurrence of SAT 3 is still doubted. The high frequency circulation of type O in cattle population indicate its importance in FMD epizootiology in Ngaoundere. Bronsvort et al., [10] found type O in a pig case in Ngaoundere and in cattle but realised that the strain in pig and cattle were assimilar.

From our reverse catalytic model, the FOI was 0.19yr^{-1} , waning immunity was 0.32, maternal immunity was 1.5yrs and the risk of transmission was 7.33. These numbers are like those reported by Pomeroy et al., [14] for Type O in the Far North with a slightly higher FOI. Cunkiffe [44] reported that a single animal was protected against secondary infection, 4.5yrs after the primary FMDV infection. Another study by Garland [45] revealed that 8 others were protected 5.5yrs after the first infection. A previous study of cattle in the Adamawa region by Bronsvort et al., [46] rather indicated that antibody prevalence might wane for serotype O. The FOI in the herds studied was constant, cattle were generally immune for 3.12 yrs, maternal immunity was 1.5yrs, from the reproductive number, 87% of the herd need to be immunized at all times to prevent outbreaks and sheep like other small ruminants should not be neglected in case of any FMDV mass vaccination program in the region.

5. CONCLUSION

Four FMDV serological types: O, A, SAT1 and SAT2 were circulating in cattle herds in Ngaoundere during the 2016 outbreak with type O being the most common in

cattle population. A major source of transmission could be cattle markets. In a mixed husbandry system including cattle, sheep and pigs, the role of each in disease transmission should not be neglected in case there is vaccination. Mathematical models are important in predicting the future disease situation in order to organize intervention strategies. In the case of sedentary herds of Ngaoundere, the FOI was constant, the waning immunity was 3.12yrs, maternal immunity was 1.5yrs and based on the reproductive number, 87% of the cattle needs to be immunized to prevent outbreaks. FMD NSP antibodies highly circulates in sedentary cattle and sheep populations during outbreaks.

ETHICAL APPROVAL

Animal use protocols were reviewed and approved by the Ohio State University Institutional Animal Care and Use Committee (Protocol Number: 2012A00000154). Herders were present to carefully restrain the animals and only minimum blood required for the study was collected.

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