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Full Length Research Paper

Sero-epidemiology of foot-and-mouth disease in some Border States of Nigeria

Lazarus, D. D.*[1,4], Schielen, W. J. G.[2], Wungak, Y.[1], Kwange, D.[3] and Fasina, F. O.[4]

[1]Viral Research Division, National Veterinary Research Institute, Vom, Nigeria.
[2]Prionics Lelystad B. V. Platinastraat 33, 8211 AR Lelystad, the Netherlands.
[3]National Animal Disease Information System, Federal Department of Livestock, Abuja, Nigeria.
[4]Department of Production Animal Studies, University of Pretoria, Onderstepoort, South Africa.

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A serological survey was conducted between 2009 and 2011 in six Border States and two other states that lie on the major cattle trek routes in Nigeria with the objective of determining the seroprevalence of foot and mouth disease (FMD) in cattle and demonstrate the evidence of antibodies in sheep, goats and pigs. Four hundred and forty-eight (448) sera were screened for FMD antibodies using the Enzyme-linked immunosorbent assay (ELISA) including samples collected during suspected field outbreaks. Statistics was conducted by using the modified Wald method and two-by-two contingency table. Higher seroprevalence was recorded in cattle samples from Yobe State (82%), followed by those from Plateau (80%), Ogun (77.77%), Taraba (73.50%), Adamawa (68%), Borno (67%), Sokoto (63%) cattle and Bauchi (27.84%), is only in sheep and goat. None of the pig sera obtained from Kaduna was positive. There is no difference in seropositivity between cattle sampled at the border and those from the trek routes. The result confirmed that FMD is still an important cattle disease in Nigeria since the diagnostic procedure employed in this analysis only detect positive serum in FMD infected animals and no history of vaccination was declared for any of the surveyed animals. Based on these results, it will be important to determine the recently circulating virus strains and factors responsible for the widespread seropositivity in order to design appropriate control strategies to limit the effect of FMD particularly on the Nigerian cattle.

Key words: Foot and mouth disease (FMD), Nigeria, cattle, sheep, goats, pigs, sero-epidemiology.

INTRODUCTION

Foot and mouth disease virus (FMDV) belongs to the genus *Apthovirus* within the family *Picornaviridae* and is the causative agent of foot-and-mouth disease (FMD), a highly contagious infection of all ruminants that has great potential for causing severe economic losses in susceptible cloven hoofed animals. The spread of the disease is mainly through direct and indirect contact, the former involving mechanical transfer of droplets from infected animals to other susceptible animals while the latter route is through contaminated personnel, vehicles and fomites (Alexandersen et al., 2003). Airborne transmission over long distances has been implicated under certain climatic and meteorological conditions, particularly in respect to domestic pigs that exhale the highest quantities of airborne virus (Alexandersen and Donaldson, 2002). This is easily passed onto in-contact ruminants that are highly susceptible to infection by the respiratory route.

There are seven known serotypes of the virus namely: A, O, C, SAT 1, SAT 2, SAT 3 and Asia 1. Infection with one serotype does not confer immunity against another and in addition, there are topotypic differences between one location and another. Within serotypes, many

*Corresponding author. E- mail: lazdav2003@yahoo.co.uk . Tel: +27717042261.

subtypes can be identified by biochemical and immunological tests (OIE, 2009).

Typical cases of FMD are characterised by high fever, loss of appetite, salivation and vesicular condition of the feet, buccal mucosa and, in females, the mammary gland (Thomson, 1994). Clinical signs can vary from mild to severe, and fatalities may occur, especially in young animals.

FMD is endemic to most of sub-Saharan Africa, except in a few countries in Southern Africa, where the disease is controlled by the separation of infected wildlife from susceptible livestock as well as by vaccination. In most parts of Africa, FMD outbreaks are often underreported either because of its endemicity as well as the fact that it is not associated with high mortalities in adult susceptible animals, as such it is not perceived as an important livestock disease among herdsmen.

The precise current situation of FMD in Nigeria is unknown yet alarming, as there are regular outbreaks, no national control strategy, no enforcement of legislation for disease reporting to veterinary authorities, and animal movement control are poor. Since the cattle population in Nigeria are from within the country and from the neighbouring countries of West and Central Africa, the animals are at perpetual risk of infection from the endemic strains as well as antigenic variants prevalent in neighbouring countries. At different times between 1924 and 1981, serotypes O, A, SAT 1 and SAT 2 were identified in outbreaks that occurred in Nigeria. However, recent sampling conducted between 2007 and 2009 have indicated that serotypes O, A and SAT 2 are still circulating (Lazarus et al., 2010).

FMD serology is particularly important because it can detect antibodies in a range of livestock infected as well as in animals with mild infection, where collection of oral lesions is not feasible and has been recommended as standard test (Brocchi et al., 2006).

Similarly, the demonstration of specific antibodies to structural proteins in non-vaccinated animals is indicative of prior infection with FMD. This is particularly useful in mild cases or where epithelial tissues cannot be collected (OIE, 2009). Tests for antibodies to some non-structural proteins of FMD are useful in providing evidence of previous or current viral replication in the host, irrespective of vaccination status. The non-structural proteins unlike the structural proteins are highly conserved and are not serotype specific. Since the inactivated vaccine in FMD used partially purified virus antigen (free of NSP), antibody response to NSP in a non-vaccinated cattle serum is indicative of an infection status rather than response to vaccination. As such the prevalence of FMD can be detected serologically by measuring the antibody level to 3-ABC non-structural protein (Diego et al., 1997).

Since there is no recent data on the prevalence of FMD in Nigeria, the present study aims at determining the prevalence of FMD in certain areas of Nigeria which may serve as baseline data for subsequent research and planning purposes.

MATERIALS AND METHODS

Study area and sample selection

Six Border States were conveniently selected from a list of Border States that submitted samples for Rinderpest sero-surveillance (Figure 1). A state is a second administrative structure of government after the National or Federal Government Administration. The selection of states was done to exclude states that do not have prominent trans-national cattle trek routes (for example, states that border water-bodies, creeks and impassable alleys). Two states with multiple cattle trek routes (Bauchi and Plateau) were also purposively included to determine the possible effect of intra-national cattle trek routes. Finally, Kaduna state was included in the survey to assess seroprevalence to FMD in pigs since it has the largest pig market in West Africa.

These states serve as major routes for pastoralist herds that traverse countries mainly within the West and Central African subregion including Cameroon, Niger, Chad and Benin Republic. The inclusion of small ruminants from Bauchi state was to understand the dynamics of FMD epidemiology between small and large ruminants in Nigeria since these small ruminants are closely grouped or are together with cattle (Figure 1). Selected states (all sera from each of these states) were also included in the survey due to the perceived higher risks of infections with FMD in those states based on history. Nigeria has traditionally been infected with FMD through the borders as trans-national nomadic pastoralism is the most important type of cattle management system. The study populations include cattle from Adamawa, Plateau, Borno, Yobe, Taraba, Sokoto and Ogun states; sheep and goats from Bauchi state; and pigs from Kaduna state.

Serology

The ELISA serology was performed according to the manufacturer's instructions for PRIOCHECK FMD-3ABC NS protein ELISA (Sørensen et al., 1998; Brocchi et al., 2006). Briefly described, 80 µl of the ELISA buffer and 20 µl of the test sera were added to the 3ABC-antigen coated test plates. Negative, weak positive and strong positive control sera were added to designated wells on each test plate, gently shook and incubated overnight (18 h) at 22°C. The plates were then emptied and washed six times with 200 µl of washing solution and 100 µl of diluted conjugate was added to all wells. The test plates were sealed and incubated for 60 min at 22ºC. The plates were then washed six times with 200 µl of the washing solution and 100 µl of the chromogen (Tetra-Methyl Benzidine) substrate was dispensed to all wells of the plates and incubated for 20 minutes at 22ºC following which 100 µl of stop solution was added to all the wells and mixed gently. Readings were taken on a spectrophotometer Multiskan® ELISA reader (Thermo Scientific, USA) at 450 nm and the OD_{450} values of all samples was expressed as Percentage Inhibition (PI) relative to the OD_{450} max using the following formula PI = 100 – [OD_{450} test sample/OD_{450} max] × 100. Samples with PI = ≥ 50% were considered positive, while those with PI < 50% were declared negative. Since the 3-ABC ELISA for FMD was = 100% specific and > 99% sensitive, the percentage prevalence was taken as true prevalence (Van Aarle, 2001).

Data analysis

All descriptive statistics were computed for seroprevalence at 95% confidence interval using the Modified Wald Method (Agresti and

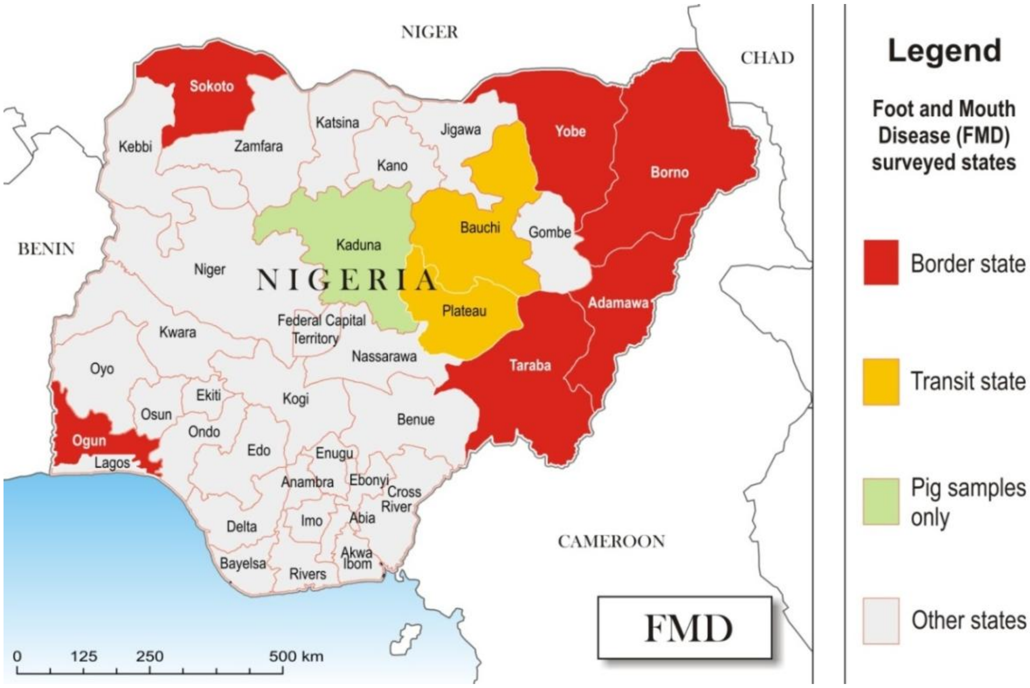

Figure 1. Map of Nigeria showing the border and transit states where samples were collected.

Table 1. Seroprevalences of FMD in selected states of Nigeria.

State	No of sera tested	No of seronegative	No of seropositive	Seroprevalence rate (%)	Lower and upper limits at 95% CI
Adamawa	50	16	34	68.00	54.13, 79.30
Plateau	40	8	32	80.00	64.99, 89.76
Borno	100	33	67	67.00	57.28, 75.46
Yobe	72	13	59	82.00	71.38, 89.27
Taraba	68	18	50	73.50	61.92, 82.62
Sokoto	30	11	19	63.33	45.45, 78.19
Ogun	9	2	7	77.77	44.28, 94.66
Bauchi	79*	57	22	27.84	19.12, 38.63
Kaduna	90**	90	0	0.00	0.00, 0.04
Total (excluding pigs)	448	158	290	64.73	60.20, 69.02

* represents sheep and goat sera alone while ** represents pig sera.

Coull, 1998; Newcombe, 1998). A2 × 2 contingency table was employed to test for association of risks of seropositivity among groups of cattle, sheep and goats. Mid-P exact values and Condition Maximum Likelihood Estimate (CMLE) Odd Ratio and other risk-based estimates were computed using Taylor Series at 95% confidence intervals ($CI_{95\%}$) (Martin and Austin, 1991). The OpenEpi ® software was used for all statistics.

RESULTS

In this study, 448 sera of cattle, sheep and goats were examined from six border states and two other states with major cattle trek routes for seroprevalence of antibodies to FMDV using the 3-ABC ELISA test. The overall prevalence of FMD in the study areas was 64.73% (290/448); $CI_{95\%}$: 60.20 to 69.02%. The location specific seroprevalence was 82.00% (59/72); $CI_{95\%}$: 71.38 to 89.27% in Yobe; 80.00% (32/40); $CI_{95\%}$: 64.99 to 89.76% in Plateau; 77.77% (7/9); $CI_{95\%}$: 44.28 to 94.66% in Ogun; 73.50% (50/68); $CI_{95\%}$: 61.92 to 82.62% in Taraba; 68.00% (34/50); $CI_{95\%}$: 54.13 to 79.30% in Adamawa; 67.00% (67/100); $CI_{95\%}$: 57.28 to 75.46% in Borno; 63.33% (19/30); $CI_{95\%}$: 45.45 to 78.19% in Sokoto and 27.84% (22/79); $CI_{95\%}$: 19.12 to 38.63% (for sheep and goats) in Bauchi (Table 1). Specific seroprevalence in sheep and goats were 41.66 and 21.81%, respectively

Table 2. Seroprevalences of FMD in small ruminants.

Small ruminants	No sampled	No of seropositive	Seroprevalence rate (%)	95% CI
Sheep	24	10	41.66	24.44, 61.20
Goat	55	12	21.81	12.80, 34.52
Total	79	22	27.84	19.12, 38.63

All tests were conducted using Modified Wald Method (Agresti and Coull, 1998; Newcombe, 1998).

(Table 2).

Cattle is 2.61 times more likely to be seropositive compared to sheep and goat combined (OR = 6.84; $p < 0.0001$) while cattle will almost be twice as seropositive compared to sheep alone (OR = 3.70; $p < 0.005$). Cattle will be 3.33 times more likely to be seropositive compared to goat (OR = 9.45; $p < 0.0001$) and cattle and sheep will be 3.24 times more likely to be seropositive compared to goat (OR = 8.62; $p < 0.0001$). Although sheep will be twice more likely to be seropositive compared to goat, the data is not significant ($p = 0.084$). Similarly, there is no significant difference in the seropositivity between cattle sampled in the Border States and those sampled from states that lies within the cattle trek routes($p = 0.274$) (Table 3). For pigs, none of the 90 sera tested was positive for FMD.

DISCUSSION

Based on the result of this sero-survey, FMD remains a significant disease of ruminants in Nigeria with an overall prevalence rate of 64.73%. Of the total sample analysed for cattle alone, 72.62% showed evidence of FMD non-structural protein antibodies. Ehizibolo et al. (2010) had earlier reported a comparable result (64.30%) in nomadic herds from Plateau state. The consistence of this finding confirmed that FMD is still endemic in Nigeria. Although routine vaccination for FMD using inactivated purified virus antigen is not done in Nigeria, we are aware that certain farmers may import such vaccines illegally and use on cattle. In this situation, viral replication and antibody to Non-structural Protein (NSP) may be suppressed and undetected. It may be possible that there is higher level of infection than those reported in this study. It is also possible that such vaccinated animals may have been introduced from outside the Nigerian border.

For the small ruminants (sheep and goat), a seroprevalence of 41.66 and 21.81% were obtained respectively. Other workers from Nigeria reported a significantly lower seroprevalence of FMD (9.3%) and (15%) in sheep and goat respectively using virus-infection associated antigen (Ehizibolo et al., 2010). These variations are likely to be due to a higher level of specificity (= 100%) and sensitivity (> 99%) of the protocol used in our analysis (Van Aarle, 2001; Brocchi et al., 2006). Furthermore, this protocol has been validated for testing variety of sera including those of cattle, sheep, goats and pigs (Sørensen et al., 1998; Van, 2001; Brocchi et al., 2006).

Despite reports that the small ruminants (sheep and goat) are susceptible, represents a risk of infection to cattle and plays an epidemiological role in FMD, our analysis indicated that cattle have a greater risk of infection than sheep and goat (Table 3) (Donaldson, 1999, 2000; Barnett and Cox, 1999; Balinda et al., 2009). However, the high degrees of seropositivity found in small ruminant is not unlikely to be connected with infections and possible viraemia in sheep and goats and these represent significant risks to naïve cattle population since these small stocks move together and often lead the cattle along the trek-route and can shed the virus for long time without showing apparent clinical signs while infecting other animals (Burrows, 1968; Arzt et al., 2011). It will be important to determine the specific role of sheep and goat in the epidemiology of FMD in West and Central Africa.

The Nigerian pigs do not seem to be at risk of infection by FMD since none of the pig sera tested was positive for FMD NS antibodies. The logical reason for this is that while commercial pigs are mostly kept under intensive operation, cattle, sheep and goats are usually managed under pastoralist operations. Similarly, most of the pastoralists practice religion that forbid contacts with pigs and these create some natural barrier between pigs and these other livestock.

Based on the risks of infection, in a typical population of cattle, sheep and goat in Nigeria, cattle will be at the highest risk of infection by FMD followed by sheep while goats remain the least risk group. Using the state by state analysis, it will appear that there is no difference between the risk of infections at the Border States compared to those states along the trek-routes ($p = 0.274$) and this is likely associated with the selective removal and sale of sick animals for slaughter along the trek route, although such removal does not prevent the spread of infection which may have occurred during the window period between infection with the virus and the display of clinical signs. In addition, since there are many livestock markets along the borders and the animals from different sources are often pulled together for marketing purposes and

Table 3. Risk of seropositivity in cattle, sheep and goats from selected states of Nigeria.

Category (population evaluated)		Percentage risk	Range	p-value
Risk in cattle versus sheep and goat (n = 448)	Cattle (268/369)	72.63%	67.86, 76.93 (%)	<0.0001
	Sheep and goat (22/79)	27.85%	19.12, 38.63 (%)	
Overall risk in the population		64.73%	60.20, 69.02 (%)	
Risk ratio in cattle/sheep and goat		2.61	1.82, 3.74	
CMLE Odd Ratio		6.84	4.01, 11.96	
Risk in cattle versus sheep (n = 393)	Cattle (268/369)	72.63%	67.86, 76.93 (%)	<0.005
	Sheep (10/24)	41.67%	24.44, 61.20 (%)	
Overall risk in the population		70.74%	66.05, 75.02 (%)	
Risk ratio in cattle/sheep		1.74	1.08, 2.81	
CMLE Odd Ratio		3.70	1.58, 8.89	
Risk in cattle versus goat (n = 424)	Cattle (268/369)	72.63%	67.86, 76.93 (%)	<0.0001
	Goat (12/55)	21.82%	12.80, 34.52 (%)	
Overall risk in the population		66.04%	61.40, 70.39 (%)	
Risk ratio in cattle/goat		3.33	2.01, 5.51	
CMLE Odd Ratio		9.45	4.88, 19.34	
Risk in sheep versus goat (n = 79)	Sheep (10/24)	41.67%	24.44, 61.20 (%)	0.084
	Goat (12/55)	21.82%	12.80, 34.52 (%)	
Overall risk in the population		27.85%	19.12, 38.63 (%)	
Risk ratio in sheep/goat		1.91	0.96, 3.80	
CMLE Odd Ratio		2.53	0.88, 7.27	
Risk in cattle and sheep versus goat (n = 448)	Cattle and sheep (278/393)	70.74%	66.05, 75.02 (%)	<0.0001
	Goat (12/55)	21.82%	12.80, 34.52 (%)	
Overall risk in the population		64.73%	60.20, 69.02 (%)	
Risk ratio in cattle and sheep/goat		3.24	1.96, 5.37	
CMLE Odd Ratio		8.62	4.46, 17.57	
Risk in cattle sampled at the border states versus those sampled in the transit states and markets (n = 369)	Cattle from border states (236/329)	71.73%	66.63, 76.33 (%)	0.274
	Cattle from transit states and markets (32/40)	80.00%	64.99, 89.76 (%)	
Overall risk in the population		72.63%	67.86, 76.93 (%)	
Risk ratio in cattle sampled at the border states/those sampled in the transit states and markets		0.90	0.76, 1.06	
CMLE Odd Ratio		0.64	0.27, 1.39	

Bold P-values indicated significant difference. Mid-P Exact values were taken for p-values. CMLE = Condition Maximum Likelihood Estimate. All the risk-based estimates were used using Taylor Series at 95% confidence intervals (Martin and Austin, 1991).

redistributed among the farmers, the role of these markets in FMD epidemiology cannot be underestimated.

Although the role of wildlife has been advocated and these has been documented from other parts of Africa (Bastos et al., 1999; 2000; Barnett and Cox, 1999; Arzt et al., 2011), in West Africa and part of Central Africa, the population of wild ungulates has experienced significant depreciation compared to the situation in the Southern and East Africa, and these wildlife are hardly encountered along the trek routes, thus, the evidence of antibodies to FMD non-structural proteins in this study cannot possibly be linked to these wild animals.

In this study, we did not consider the risk associated with age, sex and differences in management conditions because of the following: the overall majority of Nigerian cattle population are pastoralist/trade/transhumance cattle and the sedentary herds primarily originate from these animals. Similarly, age was excluded because FMD cause significant death in young population as compared with the older ones and a comparison between the groups will introduce bias since it will not be a true reflection of seroprevalence among the young animals. Previous study had also shown that there is no difference in risk associated with FMD transmission between male and female animals (Jenbere et al., 2011).

The outcome of this serosurvey has provided a template for subsequent research into the epidemiology of FMD in Nigeria.

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