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Occurrence of Foot and Mouth Disease serotypes in trade cattle sold in Kwara state, Nigeria

Olabode, Hamza Oatunde K[1,2*], Kazeem, Haruna Makanjuola[2], Raji, Mashood Abiola[2], and Ibrahim, Najume Doguwar Gignya[2]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, Nigeria.
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
Email: olabodeok@yahoo.com

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ABSTRACT

Aims: Serological detection of Foot and Mouth Disease Virus (FMDV) serotype specific antibodies in trade cattle was conducted to determine occurrence of FMDV serotypes.
Methodology and results: Cross-sectional study based on randomly sampled sera in cattle with unknown FMD vaccination history was carried out in five cattle markets in Kwara state over a period of 3 months (August-September-October) in 2011. The sera were screened for antibodies to FMDV non-structural protein (NSP) using NS-Blocking ELISA Kit (PrioCHECK®) and serotype specific antibodies determined by a Solid-Phase Competitive ELISA. Out of 253 sera positive for FMDV serotypes, 74.3% (188) sera were from recovered and 25.7% (65 sera) infected trade cattle. The percentage serotype distribution was 18.6%, 46.6%, 21.3%, and 13.4% for serotype A, O, SAT1 and SAT2 respectively. However, combination of O and A, A and SAT2 as well as O and SAT2 occurred in some cattle. Serotype distribution according to location showed serotype O and SAT2 was highest in Ajasse and serotype A and SAT1 was highest in Bode Sadu and Ilesha baruba markets respectively.
Conclusion, significance and impact study: Specific antibody detection confirmed FMD serotypes endemicity and ongoing infections in trade cattle within the Nigerian local markets. The identification of these circulating FMDV serotypes is necessary as a baseline for vaccination control strategy, in conjunction with international livestock trade regulation to enhance potentials of national dependence on beef exportation.

Keywords: Sero-survey, Foot and Mouth Disease virus, serotype specific antibodies, trade cattle, Kwara State

INTRODUCTION

Foot-and-mouth disease (FMD) is a transboundary animal disease (TAD) (FAO-OIE, 2004) with significant economic, trade and food security importance for a considerable number of countries. The disease easily spreads to other countries and reaches epidemic proportions and their control including exclusion, requires cooperation between several countries (Rweyemamu *et al.*, 2008b). FMD virus (FMDV) consists of a single-stranded, positive-sense RNA genome that belongs to the family Picornaviridae and encodes a large polyprotein, which cleaves into structural proteins and nonstructural proteins (Shao *et al*., 2010). The seven distinct serotypes include Type A, O, C, Southern African Territories (SAT 1, SAT 2, SAT 3) and Asia 1. Cumulative incidence of FMD serotypes showed that six of the seven serotypes (O, A, C, SAT 1, SAT 2, SAT 3) have occurred in Africa (Rweyemamu *et al.*, 2008a). In Nigeria, previous reports confirm FMD endemicity with serious economic losses due to serotypes A, and SAT 2 (Nawathe and Goni, 1976; Durojaiye, 1981), serotypes A, SAT 1 and SAT 2 (Abegunde, 1987), serotypes SAT 1 and SAT 2 (Chuwuedo *et al*., 2008) and serotypes O, A, SAT 1 and SAT 2 (Olabode, 2012). Serological demonstration of specific antibodies to structural proteins in non-vaccinated animals, where a vesicular condition is present is sufficient for a positive diagnosis. This is particularly useful in mild cases or where epithelial tissue cannot be collected (Hamblin *et al*., 1986). The detection of antibodies to non-structural proteins (NSP) 3ABC of FMDV has been shown to be sensitive and specific method to differentiate between infection and vaccination (Sorensen *et al.*, 1998; Clavijo *et al.*, 2004a, b). NSPs, unlike structural proteins are highly conserved and therefore, not serotype specific (Ferris and Dawson, 1988). The solid phase competitive ELISA,

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***Corresponding author**

(SPCE) provides reliable, fast results (Paiba *et al.*, 2004) hence, the use of SPCE is of great benefit in areas, where FMD prevention, control, eradication programs and regulation of international livestock trade are carried out (Mackay *et al.*, 2001). In Nigeria, the latter is rarely enforced due to lack of national dependence on beef exportation and inefficient disease reporting system (Chukwuedo *et al*., 2003) and this has made planning for disease prevention and control very difficult (Chukwuedo *et al*., 2008). The quantitative indication of FMD serotype burden amongst trade cattle in this gateway state is required to provide assessor with sufficient data on FMD virus serotype entry into the country through cattle trade. Therefore, this study seeks to establish FMD and FMDV serotypes occurrence in trade cattle found in Kwara state.

MATERIALS AND METHODS

Study area

Ilorin is the administrative division and capital of Kwara state located on longitude N 8° 30' 0" and latitude E 5° 0' 0" 8.5 / 5 (Geo Name Id: 2332785) (Anonymous, 2010). Kwara state shares common boundaries with Niger and Kebbi States to the North, Oyo, Ondo and Edo States to the South, Benue, Plateau and Federal Capital Territory to the East. It maintains an international boundary with Republic of Benin to the West. Because of its location between the Northern and Southern parts of Nigeria, Kwara State is referred to as the 'gateway' with 15 Local Government Areas namely: Asa, Baruten, Edu, Ekiti, Ifelodun, Ilorin East, Ilorin West, Irepodun, Isin, Kaiama, Moro, Offa, Oke-Ero, Oyun, and Pategi.

Sampling technique and study design

A cross-sectional study of FMD serotype occurrence was conducted using random sampling of bovine blood from major markets in five local government areas on a forth night basis over a period of 3 months between August - September- October, 2011. The choice of sampling locations was based on their proximity (borders) with other neighboring states to this study area. Ninety whole blood samples per location in each of the five LGAs were collected for sera used in this study. Interactive discussion and Interviews was conducted with marketers during sampling. Two hundred and fifty three (253) positive sera from the preliminary NSP-blocking ELISA were packaged in accordance with international standard practice for further analysis (serotyping) at the FMD laboratory, National Centre for Animal Diseases, Winnipeg, Canada.

Sample collection

The blood (5 mL) was aseptically collected through the jugular vein of well restrained cattle with sterile hypodermic 18G $1^{1}/_{2}$ needle and 10 mL syringe during market sampling. This blood was transferred into clean labeled 5 mL plastic bottles (ANTEC®) without anticoagulant and allowed to stand at room temperature at an angle of 45° for sera separation from cellular component. These sera were transferred into cryovials, labeled and properly packed on ice for transportation to the laboratory, stored at 4 °C and later –70 °C until use.

Serological assay using FMDV-NS ELISA

Detection of antibodies to FMDV NSP in bovine sera was conducted using PrioCHECK® FMDV-NS blocking ELISA following the manufacturers' protocol [Prionics Lelystad B.V.: The Netherlands]. This assay was conducted at the FMD laboratory, National Veterinary Research Institute, Vom. Briefly, ELISA plates wells were coated with 3ABC specific monocolonal antibody (mAb) using 80 µL ELISA buffer. Twenty microliter (20 µL) negative and positive controls were dispensed in designated wells. The test was performed by dispensing 20 µL of test samples to the remaining ELISA test plate wells, then sealed with enclosed plate sealer and gently rocked prior to overnight incubation at 22 ± 3 °C. After incubation, plates were washed six times with 200-300 µL washing solution and 100 µL conjugate was added to all wells. The test plates were sealed and incubated for 60 min at 22+3 °C. FMDV NS specific antibodies, directed against the non-structural proteins that may be present in test samples bond to the 3ABC protein and hence blocked mAb-HRPO binding. After incubation, plates were washed six times with 200-300 µL washing solution and 100 µL chromogen (TMB) substrate was dispensed into all wells. Post-incubation at room temperature (22 ± 3 °C) for 20 min the color development was stopped, by adding 100 µL of stop solution before optical density measurement at 450 nm wavelength to indicate antibodies directed against FMDV.
A percentage Inhibition (PI) of < 50% was considered negative and it was interpreted that the animal tested had not been exposed to FMD for 40 days. A PI of ≥ 50% was considered positive and recent exposure <40> days to FMD was assumed. More specifically, a PI value of ≥ 50% but < 70% was considered a weak positive result and a PI value of ≥ 70% was considered a strong positive result (Sorensen *et al.*, 1998).

Serological assay for FMDV NSP using 3ABC Competitive (cELISA)

A recombinant 3ABC protein fused to 5-histidine tag Competitive ELISA (cELISA) was used to assay for FMD antibodies as described by Clavijo *et al.* (2004a). In this 3ABC cELISA, FMDV antibodies in serum samples compete with anti-3B monoclonal antibody (mAb) for specific protein recombinant epitopes. Post addition of detector antibody, peroxidase conjugated anti-mouse antibody and chromogenic substrate to complete the reaction produced signal emission inversely proportional to the amount and/or strength of anti-FMDV antibodies in the test sample.

Result were expressed as percentage of inhibition (PI) and calculated based on mean optical density (OD) values of a duplicate sample, compared with a standard negative reference serum corrected for background signal

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Table 1: Occurrence of Foot and Mouth Disease serotypes amongst sero-positive trade cattle in Kwara state.

Test ELISA	Serotypes						Total
	NSP	A	O	SAT1	SAT2	SAT3	
Blocking	+	47	118	54	34	-	253/338
3ABC	-	13	31	7	14	-	65 (25.7%)
$_c$ELISA		34	87	47	20	-	188 (74.3%)
%		18.6	46.6	21.3	13.4	-	253

by subtracting the OD of the high positive reference serum. Test results were derived by the formula:

PI = [(negative reference serum OD-test sample OD) / (negative reference serum OD - positive reference serum OD)] X 100%.

Test validation: Based on the testing of over 500 bovine, ovine and porcine field sera from an FMDV-free country (Canada) and distribution frequency of their PI values, samples were considered positive if they inhibited 50% or more of standard negative control signal strength.

Serotying assay using Solid Phase Competitive blocking ELISA

Solid-Phase competitive enzyme-linked immunosorbent assay was conducted for foot and mouth disease virus antibody detection as described by Clavijo *et al.* (2004b). FMDV antigen was bound to anti-FMDV antibodies on a solid support, then simultaneous incubation of test sera with guinea pig anti-FMDV antibodies. Positive test sera that contained anti-FMDV antibodies showed various inhibition degrees to the binding of guinea pig anti-FMDV antibody. Post addition of detector antibody, peroxidase conjugated anti-guinea pig antibody and chromogenic substrate, signal emitted was inversely proportional to the amount and/or strength of anti-FMDV antibodies in test sample.

Test sera results were expressed as percent inhibition of FMDV antigen capture assay signal strength. The PI value for each sample was calculated by formula:

PI = (100 - mean OD of 2 replicate test samples / (mean OD of the 4 replicate target standards) X 100.

Test validation: Based on testing of over 900 cattle field sera and over 600 porcine field sera from an FMDV-free country (Canada) and the frequency distribution of their PI values, samples were considered positive if they inhibited 50% or more of standard vesicular FMDV antigen capture assay signal strength.

Participatory appraisal

Participatory appraisal using designed interview questions as described by (McCracken, 1988; Catley, 2005) was conducted in accordance with authors' agreed term of reference to evaluate cattle marketers' FMD knowledge, management practice and cattle source

Statistical analysis

The number of positive serotypes was expressed in simple descriptive statistics such as percentage as described by (Mahajan, 1997).

RESULTS

Occurrence of FMD serotypes observed amongst sero-positive trade cattle in Kwara state using Solid-Phase Competitive enzyme-linked immunosorbent assay as shown Table 1 indicated presence of serotypes A, O, SAT 1, and SAT 2. However, some cattle showed multiple serotype combinations of O and A, A and SAT 2 as well as O and SAT 2.

FMD serotype distribution amongst trade cattle according to different markets in Kwara state (Table 2) showed highest occurrence of serotype O and SAT 2 in Ajasse, serotype A in Bode Sadu and SAT1 in Ilesha baruba markets respectively.

Participatory appraisal with cattle marketers revealed FMD was commonly regarded as Chabu or Boro. FMD affected all sex, age and breed of cattle brought to the markets for sales. Typical signs of FMD were noticed in trade cattle in both dry and rainy seasons as responded by the marketers. Infected cattle were usually treated locally and un-responsive cattle are sold to butchers with no occurrence of human case as stated by the marketers. Further discussion with cattle dealers revealed these cattle were brought from states such as Bauchi, Borno, Taraba (Bali), Oyo (Ibgeti, Kishi), other parts of Kwara (Jebba, Bode Sadu, Ilesha baruba, Kiama, Ilapa, Ajasse) states and the borders of Cameroon (Kafanchin). The Ilesha baruba international cattle marketers also indicated cattle were brought from Mali, Togo, Benin, Cote de voire/Burkina Faso, Senegal and others from neighboring Local Government Areas in Kwara and Oyo states.

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Table 2: Distribution of Bovine Foot and Mouth Disease serotypes according to market locations in Kwara state.

Test	Serotypes					
	A	O	SAT1	SAT2	SAT3	ASIA-1
Offa	12	13	5	6	-	-
Bode Sadu	14	19	9	6	-	-
Ilesha Baruba	8	24	16	4	-	-
Ilorin	9	28	13	8	-	-
Ajasse	4	34	11	10	-	-
Total	47	118	54	34	-	-

DISCUSSION

Preliminary screening and detection of non-structural antibodies using FMDV-NS blocking (PrioCHECK®) ELISA indicates 75.11% sero-prevalence as reported by the authors (Olabode *et al*., 2013) prior to serotyping using SPC-ELISA. FMDV serotype occurrence and distribution in this present study indicates 188 (74.40%) sera out of the total 253 serotyped sera were from recovered cattle positive for either one or more of serotypes A, O, SAT1 and SAT 2 as shown in Table 1. However, combinations of serotypes O and A, A and SAT 2 as well as O and SAT 2 is of diagnostic value as the high positivity for one or more types in this ELISA indicates interferon or cytokines (virus growth inhibitors) were not responsible for this false positive reaction but perhaps the antibodies might be from vaccinated cattle with low levels of FMDV specific antibodies as previously reported by Moonen *et al*. (2000) and Moonen *et al*. (2004). However, virus neutralization test (VNT) to clarify this present finding was not conducted.

The serotype antibody detection in this study is similar with previous reports of Abegunde (1987); Chuwuedo *et al*. (2008). However, multiple serotypes occurrence in this study could be attributed to cross reactivity absence and multiple infections from within various herds where these trade cattle originate and or during transit as these cattle travel long distances across 2-3 ECOWAS countries before entering Nigeria through Kwara State. Also intra and inter market transmission of FMD multiple serotype infections is a possibility at cattle concentration points during cattle transit within this study area and between other states.

The observed 65 (25.69%) FMD carriers testing positive for serotype specific 3ABC ELISA but negative for NSP ELISA, [serotype A (13 of which were negative for the NSP ELISA), serotype O positive (31 of which were NSP negative), SAT 1 positive (7 of which were NSP negative) and SAT 2 (with 14 being NSP negative)] as shown in Table 1 indicates positive attributes to vaccination or an indication of NSP-specific antibody decay following infection. Considering FMD vaccination is uncommon in Nigeria, the latter would be the most likely scenario. Hence, these cattle are regarded as active FMD infections (without typical lesions) associated with serotype A, O, SAT1 and SAT2 spread across the study area sampled during survey. However, despite non FMD vaccination in Nigeria, there exist possibility for the former scenario as these trans-border trade cattle transit Nigeria and the study area from countries known for FMD vaccination against either of the obtained serotypes. This data therefore, provides preliminary information for disparities correction between prevalence based outbreak (infection) report and animal level prevalence based on serological detection of recovered animals.

The occurrence of active FMD infections and recovered cattle within the various markets during in-intra and inter-state cattle trade activities may facilitate carrier status which can aggravate disease spread and endemicity within the study area. In addition, occurrence of recovered, actively infected and or doubtful vaccinated cattle in these markets indicates also a scenario of economic burden on the demand for trans-border cattle through uncontrolled trade and routes of North-East and South-Western Nigeria as substantiated by cattle marketers.

The serotype percentage occurrence shown in Table 1 indicates distribution of 18.6%, 46.6%, 21.3% and 13.4% for serotype A, O, SAT1, and SAT2 respectively. This serotype distribution amongst trade cattle in different markets in Kwara state further indicates highest occurrence of serotype O and SAT2 in Ajasse, serotype A in Bode Sadu and SAT1 in Ilesha baruba market as shown in Table 2. This Ilesha baruba international market which takes delivery of cattle from Cote d'Ivoire, Mali, Liberia, Senegal and Burkina Faso through Benin as evident by interaction with cattle traders is a potential entry portal for FMD serotypes into the study area and in deed Nigeria.

Serotype O predominance in this study is line with previous reports of Samuel and Knowles (2001) as this serotype is cosmopolitan in nature with occurrence in

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Algeria, Cote d' Ivoire Guinea, Morocco, Niger, Ghana, Burkina Faso, Tunisia and Sudan. Other cattle trade participating neighboring countries with Nigeria where serotype A have occurred include Mauritania, Mali, Cote d' Ivoire, Ghana, Niger, Cameroon, Chad, Senegal and Gambia (Vosloo *et al*., 2005). Previous reports indicates SAT1 occurrence in Sudan and Niger (Sangare *et al.*, 2003) and SAT 2 in Senegal, Liberia, Ghana, Mali, and Cote d' Ivoire (Vosloo *et al*., 2005). These countries represent potential sources of the observed serotypes in this study area. Although, SAT3 occurrence was limited to East and South Africa Vosloo *et al.* (1995); Reid *et al*. (2001); Bastos *et al.* (2003), recent report of Ehizibolo *et al*. (2014) indicates preliminary detection of SAT3 in Nigeria while Asia 1 is still restricted to Asia (FAO, 2007) but the incursion of the latter serotype is a possibility following intense trade activity due to increasing meat demand in Nigeria.

CONCLUSION

This study provides information on FMD serotype occurrence, distribution and endemicity amongst trade cattle in Kwara State of Nigeria.

RECOMMENDATION

Base on the existing circulating serotypes, control strategy to limit FMD negative impacts is required. Thus a cocktail vaccine made from circulating serotypes is hereby recommended. In addition, continuous disease surveillance and cattle quarantine and movement control within and between states and national borders should be encouraged. The need to incorporate cattle traders as FMD control agents is also advocated as previously documented (FAO, 2002).

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ETHICAL APPROVAL

The research procedure was approved by the staff and postgraduates seminar committee, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Kaduna State of Nigeria. The samples were collected, stored and analyzed as approved in accordance with FMD World Reference Laboratory standard and recommendations.

All authors declare no conflict and competing interests and gave their consent prior to inclusion in this study.

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