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https://dx.doi.org/10.4314/nvj.v43i2.4 **ORIGINAL ARTICLE**

SEROLOGICAL DETECTION OF FOOT AND MOUTH DISEASE ANTIBODIES IN APPARENTY HEALTHY PIGS WITHIN FEDERAL CAPITAL TERRITORY, ABUJA.

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SUMMARY

Swine production contributes significantly to the economic wealth of most farmers in developing nations including Nigeria. Foot-and-mouth disease (FMD) has been a highly contagious viral disease of agricultural importance in domestic ruminants and pigs, hampering livestock productivity in countries where the disease is still endemic. The study aimed to determine the swine FMD occurrence in order to elucidate the disease burden within Federal Capital Territory, Abuja and its environs. One hundred (100) sera were collected from Pigs and subjected to 3ABC ELISA test at the FMD Laboratory in National Veterinary Research Institute (NVRI), Vom. The result showed that out of 100 sera samples tested for FMD antibodies, 26 (26%) were positive and 74 (74%) were negative. Sero-positive distribution showed sow/gilt (32.7%), younger pigs (57.7%) and local breeds (50%) were more exposed to FMD. Although, this was not significant ($P>0.05$). Questionnaire report further showed knowledge attitude and practice (KAP) of farmers was good, however, the absence of vaccination could be a contributing factor to FMD spread to other susceptible and between carrier livestock within the study area. This finding suggests an increasing occurrence of FMD in pigs within the Guinea Savanna Zone of North-Central Nigeria. In conclusion, FMD antibodies were detected in apparently healthy, unvaccinated pigs of all breeds, ages and sex slaughtered and reared in the FCT. Control efforts should be put in place to prevent possible transmission of Foot and Mouth Disease between pigs and cattle and other ruminants within and between herds, market and slaughter slabs.

Key Words: Sero-survey, FMD, Swine, FCT

INTRODUCTION

Swine production is a growing important livestock sub-sector contributing towards the overall agricultural production in Nigeria (Ezeibe, 2010). Consequently, providing animal protein sources for the increasing human demand (Igwe *et al*., 2013) through the utilization of a wide range of cheap agro-industrial by-products and crop residues (Tewe and Adeshinwa, 1995). Despite the shortfall in protein intake and eminent need to raise livestock productivity, production of pigs in Nigeria has remained low (Osondu *et al*.,2014) largely due to many factors including disease outbreaks especially those caused by viral diseases such as Foot and Mouth Disease (FMD) (Olabode *et al.,* 2019).

FMD also known as 'aphthous fever' or 'infectious aphthous stomatitis" caused by a highly contagious piconavirus in all cloven-hoofed domestic and wild animals including pigs characterized by trans-boundary transmission (Olabode *et al*., 2014a) with serious economic threat to the livestock industry (MacLachlan and Dubovi, 2011). The disease spread essentially through direct contact involving mechanical transfer of droplets from infected animals to other susceptible animals and indirect spread through contaminated personnel, vehicles and fomites as well as ingestion of contaminated uncooked swill (Alexandersen *et al.,* 2003) and unheated waste food to pigs (Knowles *et al.,* 2001). Although oral transmission is possible, significantly higher doses of virus are required by this route as compared to infection by inhalation (Donaldson, 1987) as pigs further require a much higher infective doses (TCID50) for airborne exposure (Alexandersen *et al.,* 2003) but excretes more aerosolized virus post exposure than cattle and sheep (Gloster *et al.,* 1982). Thus, the consideration of pigs as amplifier hosts (Brown, 2001).

Incubation period is usually 3 to 8 days (Mahima *et al.,* 2012) but in pigs, it is usually two days or more, (OIE, 2008). Clinical signs of FMD are more severe in cattle and intensively reared pigs (Verma et al., 2012) characterized by vesicular formation on the snout, mouth, tongue, and especially the feet with shedding of hooves in severe infections (Kitching and Alexandersen, 2002). Secondary infection occurs on the feet of some swine and leads to chronic lameness. Other pathology includes severe myocardial necrosis in neonatal and young pigs. This often leads to sudden deaths from myocardial failure. This mottled myocardial lesions sometimes are referred to as "tiger-heart" lesions and are useful in diagnosis (Jamal and Belsham, 2013). In Nigeria, Swine FMD is poorly diagnosed and under-reported. Although, little sero-prevalence studies have been conducted in some parts of the country Fakai *et al*., (2015); Aik-Raji *et al.,* (2016), the epidemiology of the disease in pigs is still poorly understood. Therefore, this study was conducted to determine the presence of FMD Non-Structural Protein (NSP) antibodies in apparently healthy and slaughtered pigs as well as to assess the Knowledge, Attitude and Practice of swine farmers in Abuja and its environs. This is to further beware of the role of pigs in FMD epidemiology in the study area.

MATERIAL AND METHOD

Study Area

The Federal Capital Territory is located North of Rivers Niger and Benue confluence. It is bordered by the states of Niger to the West and North Kaduna to the Northeast, Nasarawa to the East and South Kogi the Southwest. Lying between the latitude 8.25 and 9.20 North of the equator and longitude 6.45 and 7.39 East of

Greenwich Meridian (Anon, 2007). Abuja is geographically located in the center of the country with a landmass of approximately 7,315km square, and it is situated within the Savannah region with moderate climatic conditions (Mylne *et al.*, 2015).

Study Design

This purposive sero-survey of NSP antibodies in both local and exotic breeds of pigs raised on semi-intensive and intensive system in farms as well as slaughter slabs within Abuja and its environs (Gwagwalada, Iddo, Karu, Jikwoyi and Masaka) was conducted based on convenient random sampling and willingness of the farmers to provide limited numbers of their pigs for sample collection. The sample size was calculated using the formula described by Daniel (1999) based on a calculated prevalence (P) of 20% from previous study (Fakai *et al.*, 2015) which was 246 samples. However, the study design and sampling was modified to a convienent pilot study sampling to due poor compliance of farmers and butchers which limited the samples collected to one hundred (100) that is 65 (from farms) and 35 (slaughter slabs) over a period of three (3) weeks for analysis at the FMD laboratory, National Veterinary Research Institute (NVRI), Vom. Questionnaire assessment of farmers, farm attendants and pork meat sellers was also conducted.

Sample Collection

Blood samples (5ml) were collected from each restrained pigs through the jugular vein using 10ml needle and syringe for those in the farms and from severed jugular vein samples collected at slaughter points. Blood samples were later transferred into a plane tube and kept at room temperature (37°C) at angle of 45° for 6-8 hours to allow for blood clotting. The sera were aspirated into a new set of eppendorf tube, labeled and stored in cooler containing ice packs and transported to the Veterinary Teaching Hospital, University of Abuja where they were stored at -18°C in a deep freezer prior to the 3ABC ELISA analysis at NVRI, Vom.

FMDV-NS 3ABC blocking ELISA

Blocking ELISA (bELISA) [3ABC ELISA] that is based on recombinant 3ABC protein fusion with a 6-histidine tag was used to detect FMD-NS antibodies in swine sera samples as described by Clavijo et al (2004) at the FMD laboratory, National Veterinary Research Institute, Vom. Briefly, post preparation of the working solutions in advance according to the SOP developed by the FMD laboratory based on manufacturer's recommendation. The antibodies in the sera binds to a specific epitope on the recombinant protein thereby blocking the subsequent binding of an anti-3B monoclonal antibody (mAb) to this specific epitope on the recombinant protein with a result expressed as a percentage of inhibition post addition of a detector antibody (peroxidase conjugated anti-mouse antibody), and chromogenic substrate. Signal emission was inversely proportional to the amount and/or strength of anti-FMDV antibodies in the test sample. The optical density (OD) of the plate was read at 450nm by ELISA and plate reader and Percentage inhibition (PI) was calculated using the formula below:

$$PI = \frac{Q3 - \text{test sample}}{Q3 - Q1} \times 100\ \%$$ for strong positive

$$PI = \frac{Q3 - \text{test sample}}{Q3 - Q2} \times 100\ \%$$ for weak positive

Where: Q1 = Strong positive control, Q2 = Weak positive control, Q3 = Negative control. Samples with PI = ≥ 50% were classified as positive while those with PI < 50% were declared negative.

Questionnaire Survey on Foot and Mouth Disease

Fifty questionnaires (50) were distributed to farmers, farm attendants and pork meat sellers in FCT, Abuja and its environs to evaluate their knowledge, attitude and practice towards management and awareness of foot and mouth disease effects on local and exotic breeds of pigs as described (Olabode *et al*., 2014b).

Statistical Analysis

Data expressed as simple descriptive statistics were evaluate using Microsoft Excel ® and coded for further statistical analysis using SPSS (version 13) set at 95% confidence interval. Chi-square ($\chi 2$) test was used to assess association with FMD seropositivity. Data were also represented graphically and as chart.

RESULTS

The Enzyme Linked Immunosorbent Assay (3ABC ELISA) test for NSP antibody detection showed that 26 (26%) sera were positive out of 100 sera samples tested for FMD antibodies, and 74 (74%) were negative. Secondly, distribution of swine FMD antibodies according to sex showed that out of the 26 infected samples, only 10 (19.6%) were males and while 16 (32.7%) were females (X^2 = 1.127[a,] Df = 1, p= 0.288). In addition, distribution of FMD antibodies according to age indicated 11 (22%) were young and 15 (30%) were older pigs (X^2 = 1.052[a], Df = 2, p= 0.591). FMD antibodies distribution in pigs according to degree of infection and sex showed that 22 (84.6%) were weak positive

and 4 (15.4%) were strong positive. Lastly, FMD antibodies distribution according to breed showed that out of the 26 positive samples 3 (50%) were from local and 23 (24.6%) were of exotic pigs (X^2 = 0.266[a], Df= 1, P= 0.606) as shown in Table I.

TABLE 1. Demographic distribution of swine FMD antibodies prevalence in Abuja and its environs based on sex, age and breed

FMD Status	**Sex**			**Age**			**Breed**			**Degree of Infection**			
	Boar	**Sow/gilt**	**Total (%)=P**	**Young**	**Adult**	**Total (%)=P**	**Local**	**Exotic**	**Total (%)=P**		**Weak positive**	**Strong positive**	**Total (%)=P**
Positive	10 (19.6)	16 (32.7)	26	11 (42.3)	15 (57.7)	26	3 (50.0)	23 (24.5)	26	Male	9 (90.0)	1 (10.0)	10
Negative	41 (80.4)	33 (67.3)	74	39	35	74	3 (50.0)	71 (75.5)	74	Female	13 (81.25)	3 (18.75)	16
Total (n)	**51**	**49**	**100**	**50**	**50**	**100**	**6**	**94**		**Total**	**22 (86.6)**	**4 (15.4)**	**26=P**

Questionnaire result indicated more males (82%) especially farm attendants (58%) participated in this survey which further revealed 85% respondents had previous awareness of FMD that occurred mostly (90%) in their farms characterized by typical FMD sores (80%) especially in younger pigs (90%) amongst exotic breeds predominately (80%). The farmers attitude indicated muti-species breeding practice mostly on intensive (85%) scale with a few on semi-intensive and extensive farm management systems using mostly (80%) portable borehole water exclusive (96%) of other types of livestock (mono-livestock farming system). Most farmers (90%) usually conduct routine vaccinations against bacterial diseases using imported vaccines. However, in an outbreak situation, most farmers (70%) use antibiotics for treatment, while others, cull and slaughter and a very few use local remedies (concussion) as indicated in Table II

TABLE II. Questionnaire response on FMD knowledge, attitude, and practice of swine production handlers in Abuja and its environs

Demographic distribution of respondents							
Gender	**Male**	**Female**	**Total**	**Occupation**	**Farmer**	**Farm attendant**	**Pork meat sellers**
Total (%)	41 (82%)	9 (18%)	**50 (100%)**		15 (30%)	29 (58%)	6 (12%)

Questionnaire response								
Knowledge			**Attitude**			**Practice**		
Variable	**Response**		**Variable**	**Response**		**Variable**	**Response**	
	Yes (%)	**No (%)**		**Yes (%)**	**No (%)**		**Yes (%)**	**No (%)**
Previous acquaintance of FMD? pig farm?	85	15	Availability of Bole water?	80	20	Routine Vaccination?	95	5
Previous outbreak of FMD on farm? pig farm?	90	10	Multi-species rearing?	0	100	Imported vaccines use?	95	5
Lesions characteristic of FMD?	80	20	Treatment and interventions instituted in the phase of FMD outbreaks	Antibiotic use 70%		Types of farming systems	Intensive 85%	
Susceptibility in younger higher than adult pigs?	91	9		Cull & Slaughter 28%			Semi-Intensive 10%	
Susceptibility in exotic higher than local pigs?	80	20		Herbal remedies 2%			Extensive 5%	
			FMD Vaccination	0%	100%			

DISCUSSION

The occurrence of FMD antibodies in 26% of screened pigs in this study is lower than previous reports of 46.2% among pigs in Ibadan by Aiki-Raji *et al.*, (2016). However, this finding is close to reports of 18% in Zuru, Kebbi State by Fakai *et al*., (2015). Variations could be a consequence of larger sample sizes used in these previous reports. In addition, this study finding is not in line with the reports of Lazarus *et al* (2012) that stated 0% seropositivity amongst 90 pigs sampled in Kaduna, North central Nigeria, which indicated pigs were rarely affected with FMD virus. The source of infections in this study pigs could be associated with the availability of shrubs and sagnant water bodies around some of piggery pens sampled as this could possibly attract FMD carrier cattle, sheep and other ruminants that potentially spread the disease directly and indirectly to these pigs. Others factors could include the use of common service providers (livestock medical attendants) (Olabode et al., 2014b) Demographic distribution of swine FMD antibodies detection in this study based on sex, age and breed respectively indicates more sow and gilts (32.7%) shows presence of NSP antibodies than the boars (19.6%) while more adult pigs (57.7%) demonstrates the presence of NSP antibodies than younger pigs (42.3%). Although, the exotic breeds were more numerically exposed to FMD virus than the local breeds, these findings were not significant $P<0.05$ as shown in Table 1. The seropositivity in this survey further indicates more pigs (86.6%) as weak positives suggesting previous FMD infections in more pigs due to passive antibodies and few (15.4%) pigs with PI $\geq$ 70% being considered strong positive and as ongoing infection within a minimum of 40 days (Sorensen *et al*., 1998) showing no clinical signs but have evidence of detectable antibodies. Furthermore, previous reports indicates 3-ABC ELISA for FMD as 100% specific and > 99% sensitive, the percentage prevalence in this study informs true prevalence (Bronsvoort *et al*., 2006). Thus suggesting increasing FMD virus cross infections between cloven hoofed livestock animals as well as environmental contamination especially within and between the North-Central vegetation zones of the country. These potential FMD circulating serotypes and their geo-spatial distribution in pigs within these ecological zones require further clarification as previously conducted by Olabode *et al*., (2014c). Questionnaire findings indicates pig farmers in this study area, mostly engage in mono-livestock keeping with adequate biosecurity practice and good husbandry system as this enhances their ability to know and identify FMD especially in younger pigs. In addition, most (95%) farmers conduct routine vaccinations especially against bacteria diseases such as Erysipelas using mostly (95%) imported vaccines as shown in Table II. The lack of routine vaccination against FMD as indicated by the respondents and detection of NSP antibodies confirms these pigs as unvaccinated (Clavijo et al 2004) which may also be a contributing factor to the observed FMD prevalence in pigs within the FCT. However, most farmers (70%) administer antibiotics treatments in the phase of disease outbreaks such as African Swine Fever (ASF) as well as FMD, while others (28%) cull and slaughter and a few (2%) use herbal remedies.

CONCLUSION

This preliminary study provides a baseline information on the presence of FMD Non-Structural Protein antibodies in all breeds, age and sex of unvaccinated pigs within the FCT.

RECOMMENDATION

Control efforts should be put in place to prevent possible transmission of Foot and Mouth Disease between pigs and cattle and other ruminants within and between herds, market, and slaughter slabs in the FCT. In addition to providing designated points for slaughtering of pigs within the approved abattoirs.

CONFLICT OF INTEREST

Authors declared there is no conflict of interest.

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