8.5 kbp genome, which encodes for structural proteins (SP) as well as non-structural proteins (NSPs) (Carroll et al., 1984; Forss et al., 1984, Grubman et al.,1984). This virus exhibits a high potential for genetic and antigenic variation which has led to the classification of seven serotypes recognised worldwide, known as serotypes A, O, C, SAT 1, SAT 2, SAT 3 and Asia 1 (Domingo et al., 2003) and all of these occur in Africa except Asia 1 (Vosloo et al., 2002). FMD is considered endemic in most of sub-Saharan Africa, with the exception of disease free zones in Southern Africa (Bronsvoort et al., 2006). It is usually spread by the movement of infected animals which excrete large amount of virus.

The presence of large numbers of migrating nomads with their livestock may play a major role in spreading infection across borders. This observation is supported by the results of genetic studies of serotype A, O and SAT 2 which show that a single epizootic often affects two or more neighbouring countries in West Africa (Knowles et al., 1998; Bastos and Sangare, 2001; Sangare et al., 2001). Also, pigs usually become infected with the virus by eating FMDV-contaminated products, by direct contact with infected animals, or by being placed in a heavily contaminated environment (e.g. a pen, an abattoir lairage or previously contaminated transport vehicle) (Kitching and Alexandersen, 2002). Infected pigs initially show mild signs of lameness, blanching of the skin around the coronary bands and may develop fever (Kitching and Alexandersen, 2002). The hoof lesions are usually severe with vesicles on the coronary band, heel and interdigital space. These lesions are the most consistent finding in pigs (Kitching and Alexandersen, 2002). Vesicles may also be seen on the snout. FMD virus poses a negative economic implication in the pig industry due to high morbidity and loss of production (Brown, 2001).

The detection of antibody to the NSPs of FMDV has been used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated (Berger et al., 1990; De Diego et al., 1997; Sørensen et al., 1998a) . The NSPs are expressed only by replicating viruses. Inactivated vaccines are purified to remove cellular proteins and NSPs, and therefore only animals that have been infected with live virus should develop antibodies to these proteins (Berger et al., 1990; Bergmann et al., 1993). Conventionally, the detection of antibody to NSPs has been carried out by measuring antibody to the virus infection-associated antigen (VIAA; the viral RNA polymerase protein 3D) using agar gel immunodiffusion (AGID) (Dawe and Pinto, 1978; McVicar and Sutmoller, 1970).

Due to the insensitivity of AGID, the VIAA test has now largely been superseded by assays that measure antibody to FMDV NSPs produced by recombinant techniques in a variety of *in vitro* expression systems (Mackay et al., 1997; Meyer et al., 1997). Currently, the polyproteins 3ABC and 3AB appear to be most promising as diagnostic antigens (Mackay et al., 1997). Antibody to these polyproteins is the single most reliable indicator of infection (De Diego et al., 1997; Sørensen et al., 1998b).

Foot-and-mouth disease was first reported in Nigeria in 1924 as sporadic outbreaks attributed to serotype O FMDV incursions (Libeau, 1960). Serotypes A, SAT 1 and SAT 2 FMDV outbreaks have subsequently been reported in the country (Owolodun, 1971; Nawathe and Goni, 1976; Durojaiye, 1981). In Nigeria, there is a dearth of information on the status of FMD in the swine species; recent available literature has been generally limited to ruminant animals (Knowles, 2008; Lazarus et al., 2012; Ehizibolo et al., 2014). This survey was conducted to investigate the presence of FMD in the domestic pig population in two states (Plateau and Enugu) of Nigeria

MATERIALS AND METHODS

Study area

This study was conducted in Plateau State (Latitude 10° 26N, 10° 40E and Longitude 8° 22N, 8° 35E) located in the North Central geopolitical zone and Enugu State (Latitude 6° 30N, 6° 50E and Longitude 7° 30N, 7° 50E) located in the South Eastern geopolitical zone of Nigeria (Figure 1). Both States are actively involved in pig production with several pig farms with at least 15 pigs in their holdings under semi- intensive to intensive management system. Serological evidence and FMD outbreaks have been reported previously in ruminants in Plateau State (Ehizibolo et al., 2014), so far to our knowledge, no previous report is on FMDV in any species of livestock from Enugu State. Also, no preventive vaccination is routinely applied for FMDV in the livestock population in the study areas. Pig farms were selected based on the pig population in the study areas, while the central abattoir in the states was considered for sampling.

Sample collection

Blood samples (n = 822) were collected in a cross sectional study of piggeries and abattoirs in Plateau and Enugu States of Nigeria in 2014. Systematic sampling method was employed to collect blood sample from one of every three pigs slaughtered daily in the abattoirs, and the simple random sampling was applied in selected pig farms. Every piggery with more than fifteen pigs in their holdings and are accessible were eligible and those whose owners did not agree to participate in the study were excluded. At least five pigs were sampled from each pig farm based on the pig population per holdings. A total of 450 serum samples were obtained from Plateau State comprising sera (n=350) from pigs slaughtered in Jos abattoir and those (n=100) collected from pig farms (n=18) in three districts (Gyel, Kuru and Vwang) in Jos South Local Government Area (LGA). On the other hand, 372 serum samples was obtained from Enugu State comprising of sera (n=305) from 27 piggeries in Enugu east and Enugu south LGA and samples (n=67) from the abattoir located in Enugu north LGA. Five milliliters of blood was collected post-slaughter at the abattoirs into well labeled tube from each selected pig, and from the anterior vena cava of each selected pig in the pig farms sampled using syringe and needle. The blood samples were allowed to clot, and then centrifuged in the laboratory at 4000 rpm for 5 min. Sere were separated into properly labelled cryovials, and stored at -20°C until tested at the FMD laboratory, National Veterinary Research Institute, Vom, Nigeria.

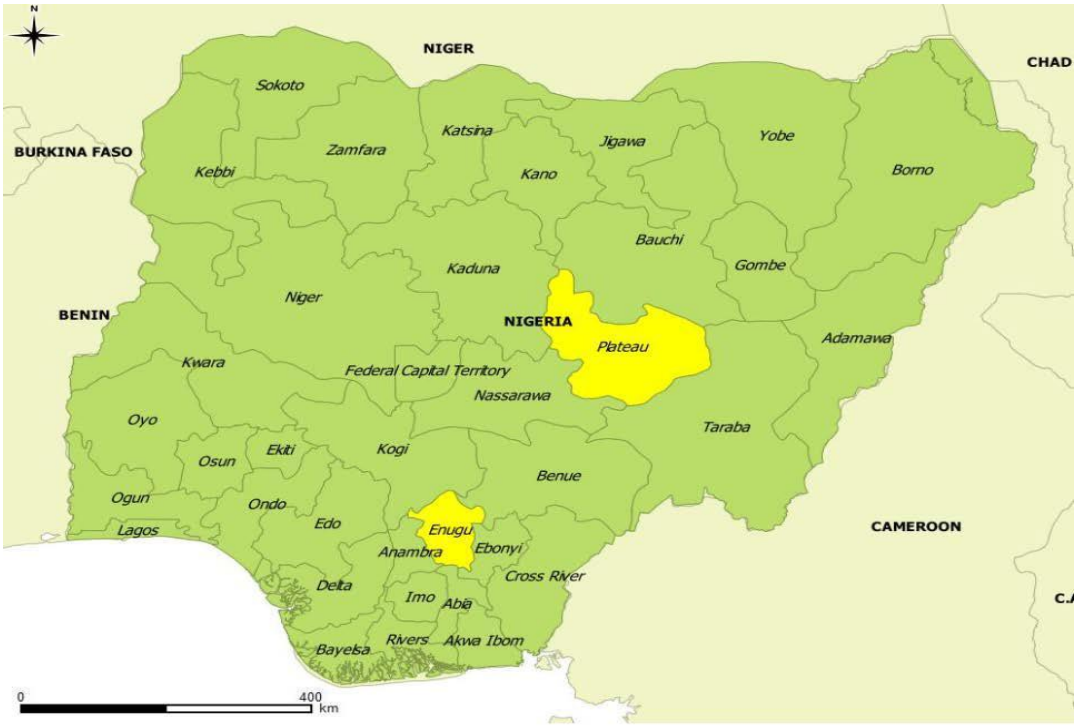

Figure 1. Map of Nigeria showing the study area.

Serological analysis

FMD 3-ABC ELISA

The PrioCHECK® FMDV NSP antibody test ELISA kit (Prionics Lelystad B.V., The Netherlands, product code 7610440), was used according to the manufacturer's instructions as previously described (Sørensen et al., 1998b) for detection of antibodies to the highly conserved non-structural 3ABC protein of FMDV.

Solid phase competitive ELISA

The 3-ABC ELISA positive sera were tested on solid phase competitive ELISA (SPCE) for virus serotyping. The SPCE was performed using a commercial FMDV-SPCE test kits (IZSLER Biotecnology, Brescia, Italy). Test kits specific for serotype O, A and SAT 2 were used according to manufacturer's instructions. Briefly, the ELISA micro plates were supplied pre-coated with FMDV specific antigen captured by the homologous monoclonal antibody (MAb). FMDV specific antigen used includes O1 Manisa, A22 Iraq and SAT2 (virus strain not provided) for serotypes O, A and SAT2 ELISA kits, respectively. Appropriately, diluted test sera were incubated with the trapped antigen. The anti-FMDV specific MAb, conjugated with peroxidase was added; its reaction with the homologous antigen will be inhibited by antibodies of positive sera previously bound to the virus, while the conjugated MAb can bind to the FMDV antigen in the case of negative sera. After incubation, the unbound conjugate was washed away and the substrate/chromogen was added into the wells. A colorometric reaction develops. A stop solution was added and the optical density was measured on a MultiScan® spectrophotometer (ThermoScientific, USA) at 450 nM wavelength. A positive result was determined by percent inhibition of ≥70% at the 1/10 dilution.

RESULTS

The results obtained (Table 1) shows that out of a total of 822 sera collected from pigs across two states (Plateau and Enugu States) in Nigeria, 12 (1.5%) tested positive for antibodies to the highly conserved non-structural 3-ABC protein of FMDV. Four (0.9%) of the 450 sera collected from Plateau State were recorded positive by FMDV 3-ABC ELISA, while 8 (2.2%) of 372 sera from Enugu State also tested positive by the 3-ABC ELISA. Seven (58.3%) of the 12 reactors were from sera collected from abattoirs in the two States sampled. The results also indicated that the 3- ABC ELISA reactors were positive for FMDV serotypes O and SAT 2 by the serotype-specific SPCE.

DISCUSSION

Foot and mouth disease is one of the most economically important diseases of animals in the world, and it is extremely difficult to control (Brown and Slenning, 1996). In FMD-free countries, the role of pigs in the epidemiology of the disease in outbreaks is well recognized (Chen et al., 2008; Gibbens, 2011; Hayama et al., 2012), but in FMD -endemic countries like Nigeria, little is known about the epidemiology of the disease in pigs. The inherent potential in pig farming in Nigeria has manifested in the rapid increase in the number of pig

Table 1. FMDV non-structural protein 3-ABC and serotypes detection in pigs.

Location	Number sampled	Vaccination history	3 ABC ELISA Positive	Serotype-specific SPCE		
				O	A	SAT 2
Plateau State						
Jos abattoir	350	Unknown	4	+	-	-
Kuru	39	NV	0	-	-	-
Gyel	31	NV	0	-	-	-
Vwang	30	NV	0	-	-	-
Enugu State						
Enugu east	235	NV	4	-	-	+
Enugu south	70	NV	1	-	-	+
Enugu north	67	Unknown	3	-	-	+
Total	822		12 (1.5%)			

NV- not vaccinated.

farms witnessed in peri- urban and urban areas. FMD can cause significant losses that could threaten the entire pig production industry. The detection of antibodies to the non-structural protein (NSP) of FMDV in the sera of pigs sampled is an indication of FMD infection, since only animals infected with live virus develops antibodies to NSPs (Berger et al., 1990; Bergmann et al., 1993). Currently, the polyprotein 3-ABC is said to be the single most reliable indicator of FMDV infection (De Diego et al., 1997; Sørensen et al., 1998b) . This is suggestive of FMD viral replication in the positive pigs despite the absence of obvious clinical lesion. Pigs are not considered to be carriers of FMD virus unlike cattle (Salt, 1993). Lin et al. (2000) carried out an experimental study to support the general view that pigs are not persistently infected by FMD virus. These authors demonstrated that the typical FMD virus, O/TWN/97 that infected Taiwanese pig farms was eliminated from pigs 21 days post infection and antibodies positive reactors could not be detected on some pig farms in their study, which was an indication of FMDV disappearance from the infected herds. This experimental report supports our opinion that antibody reaction to the NSP ELISA in the present study is an indication of recent exposure of the tested pigs to FMD virus. In this survey, we have therefore shown that the NSP ELISA test can be useful to monitor evidence of the circulation of FMDV in apparently healthy pig population in Nigeria. Though the mode of transmission of infection to these pigs is not well understood, probable exposure via interspecies transmission from cattle, the predominant FMD-susceptible species in Nigeria was advanced. However, this requires further investigation.

Studies have shown that NSP ELISA test could be used to detect present or past FMD infection in pig population even while FMD vaccination program is ongoing (Chen et al., 2011). The seroprevalence level in this study is quite low. Similarly, low seroprevalence (2%) have been reported in pigs in sub-Saharan Africa (Fernandez-Pacheco et al., 2012) and in regions of the world where swine are considered as the predominant FMD-susceptible species (Chung et al, 2003; Chen et al., 2011). Recently, a much higher seroprevalence (58%) was reported in Kenya (Wekesa et al., 2014). Our present finding does not corroborate the views asserted in a previous study (Lazarus et al., 2012) that pigs in Nigeria do not seems to be at risk of FMD due to the fact that they could not detect FMD virus antibody from 90 pig sera tested in their study.

Most often, pig farmers sell out weak and sick animals, and also as a result of fast turnover (6-8 months interval), pigs sold from farms are often slaughtered at the abattoir. Sample collection from the slaughter house could give a good indication of disease condition of most pig farms. Seven of the 12 NSP-ELISA reactors were detected from sera sample collected from abattoir in the two states. Therefore, we suggest that sample collection from the abattoirs should be explored, as it could be very effective active surveillance method.

Serological evidence of two FMDV serotypes, O and SAT 2 were detected by the SPCE in Plateau and Enugu States, respectively. These serotypes have been documented to be circulating in ruminants particularly in Plateau State (Ehizibolo et al., 2014), however, there is no documented report (serological or outbreak) on FMDV in Enugu State. Hence, we could not explain the level of seropositivity detected in Enugu State. Although, the specific history of these animals was not available at the time of sampling, we suspect that the observed reactors could have been procured from exposed/infected herd from other States. More recently in East Africa, serological evidence of FMDV SAT1 have been reported in domestic pigs in Kenya (Wekesa et al., 2014), and serotype O was isolated and characterized in Uganda (Kerfua et al., 2013) providing further information on the

role of pigs in the epidemiology of FMDV in endemic settings of some parts of sub-Saharan Africa.

Conclusion

Currently, there is no routine prophylactic vaccination programme for FMD in Nigeria due to prohibitive cost of vaccines, and available data on the diseases have centered mainly on ruminant animals (cattle and sheep). Little is known about the status of the disease in pigs. This preliminary report suggests that FMD viruses are present in domestic pigs in Nigeria. In order to implement any effective FMD control programme, it is imperative not to ignore the role of pigs in the overall epidemiology of the disease since pigs represent an important part of FMD-susceptible species in Nigeria. Continued surveillance of FMD in swine species is recommended.

Conflict of interests

The authors did not declare any conflict of interest.

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