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Serological evidence of foot-and-mouth disease virus (FMDV) antibodies in pigs from northeastern Nigeria

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Foot-and-mouth disease (FMD) is endemic in Nigeria with studies conducted in cattle, sheep and goats. A cross-sectional study for foot-and-mouth disease virus (FMDV) antibodies in field sera collected for African swine fever (ASF) surveillance was conducted in order to determine the status of FMDV in swine within the study area. Four hundred and fifty field sera collected as part of National ASF Surveillance Programme from two states of Taraba and Adamawa in Northestern Nigeria were selected and screened for FMDV non-structural proteins using 3ABC ELISA. Positive sera were serotyped using a Solid Phase Competitive ELISA (SPCE) for antibodies specific to FMDV serotype A and O. The results revealed an overall seroprevalence of 1.11% (5/450) with evidence of FMDV serotype A circulating in the swine population within the study area.

Key words: Foot-and-mouth disease (FMD), antibodies, pig, Nigeria.

INTRODUCTION

Foot and mouth disease (FMD) is considered one of the most contagious diseases affecting economically important livestock species such as cattle, sheep and pigs in the 2007 Terrestrial Animal Health Code by the World Organisation for Animal Health (Office-International-des-Epizooties) (Orsel., et al., 2009). Seven immunologically distinct FMDV serotypes have been described, namely serotypes A, O, C (the so-called European types), Asia-1 and three South African Territories (SAT) types 1, 2 and 3. Serotypes A, O, C and Asia-1 constitute a distinct lineage separate from the SAT viruses (Vosloo, et al., 2009). However, it has been established that infection with one serotype of FMD virus does not confer immunity against another (Pereira, 1976). FMD cannot be distinguished clinically from other vesicular diseases, including swine vesicular disease, vesicular stomatitis and vesicular exanthema (OIE, 2012).

FMD is endemic in Nigeria with outbreaks occurring in cattle seasonally. Serotypes O, A, and SAT 2 have been reported as the cause of recent outbreaks in selected locations across the country (Fasina, et al., 2013). FMD affects all cloven-hoofed animals (Alexandersen and Mowat, 2005). However recent studies in Nigeria involving cattle, sheep, goats and pigs (Lazarus, et al., 2012) demonstrated evidence of antibodies in cattle, sheep and goats only with no evidence of antibodies from pig samples. In Nigeria, unlike other parts of Africa where abundant wildlife population exist, no information is available on FMD in wildlife species. However, we cannot

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rule out wildlife-livestock interaction since the husbandry practice in most part of west and central Africa is majority pastoralism.

The role of pigs in the epidemiology of FMD has been recognized in outbreaks in FMD-free countries (Chen, et al., 2008; Gibben, 2011; Hayama, et al., 2012; Knowles, et al., 2001). However in endemic countries of Africa, surveys have demonstrated low to high prevalence of antibodies against FMD virus (Fernandez-Pacheco, et al., 2012; Wekesa, et al., 2014). In a study in Thailand, it has been demonstrated that pigs were not involved in outbreak cases (Chamnanpood, et al., 1995). However, in China and Uganda, FMDV have been isolated from pigs (Yang, et al., 2011; Kerfua, et al., 2013).

In Africa, FMD viruses are maintained in cattle and African buffaloes (*Syncerus caffer*) in domestic and wildlife ecology, respectively (Vosloo, et al., 2004). However, it has been reported that the pig-adapted Cathay strain of FMD virus apparently does not infect large ruminants in the field or experimentally and requires cells of porcine origin for primary isolation (OIE, 2012).

Typical cases of FMD are characterized 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 (OIE, 2012). Even though FMDV outbreaks in pig have been reported in other countries, especially where pig production is intensive, no clinical case of FMD has been reported in swine in Nigeria. In previous related studies, no evidence of antibodies to FMDV was demonstrated in sera of pigs (Lazarus, et al., 2012). However, seroprevalence of FMD antibodies in cattle, sheep and goats were reported (Ehizibolo, et al., 2010; Fasina, et al., 2013; Ishola, et al., 2011; Lazarus, et al., 2012). Nigeria has a limited swine population of 9 million compared to the 17 million heads of cattle (WAHID, 2013). The pig production in most part of the north is backyard piggery system which is on a small scale production. In the country's southwest, more intensive commercialized piggery systems are in place with good market for pork. This study investigated the presence of antibodies against FMDV in pig sera collected as part of a National ASF Surveillance Programme in two states of Taraba and Adamawa north-eastern Nigeria.

MATERIALS AND METHODS

Study area and sample selection

Two States were conveniently selected from a list of states that submitted samples to the National Veterinary Research Institute, Vom for the National ASF Surveillance Programme in 2009.These are Taraba and Adamawa states; they are the second administrative unit in Nigeria administrative structure. These states share land borders with the Adamawa Province of Cameroun where most pastoralist cattle within the Northeast come from. These states also have suitable vegetation and climate that supports the local livestock industry in the country. Farmers in these areas practice the backyard piggery system in addition to keeping other livestock. FMD outbreak is a seasonal occurrence in this area and pigs reared on backyard piggery system normally come in contact with cattle reared within the community. More samples were selected from Taraba relative to Adamawa state, considering the distribution of piggery in Taraba to Adamawa. .

Serology

The ELISA serology was performed according to the manufacturer's instructions for PRIOCHECK FMD-3ABC NS protein ELISA (Sorensen 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 shaken 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 22°C. 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 22°C 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 OD450 values of all samples was expressed as Percentage Inhibition (PI) relative to the OD450 max using the following formula PI = 100 - (OD450 test sample/OD450 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. All samples that tested positive for NSP using the 3ABC ELISA were further typed for structural proteins using a Solid Phase Competitive ELISA (SPCE) for antibodies to FMDV serotype A and O, (IZSLER Brescia, Italy),the test was performed according to the manufacturer's instructions.

RESULTS

The result showed an overall seroprevanlence of 1.11% (5/450) in the study area. 1.26% (5/389) were positive in Taraba State and 0% (0/56) in Adamawa state as indicated in Table 1. Furthermore, serotype specific ELISA test for FMDV serotype A and O revealed 1 out of the 5 positive samples, positive for FMDV serotype A.

DISCUSSION

In most communities within the study area, cattle, sheep, goats and pigs interact freely during grazing and their possible role in the maintenance and epidemiology of important animal pathogens are poorly understood. In this study, we attempted to screen sera of swine from areas that have reported FMD outbreaks for evidence of antibodies to FMDV. In Nigeria, pigs are not vaccinated for FMD and evidence of non-structural protein antibodies might suggest exposure to FMDV. The epidemiology of FMD in swine in Nigeria is poorly understood as a result

Table 1. Seroprevelence of FMDV in pigs and serotype detected.

States	Total number of sera tested	Number positive	Number negative	Seroprevalence % (95%CI)	Serotype O	Serotype A
Taraba	394	5	389	1.26 (0.5 - 3.0)	0	1
Adamawa	56	0	56	0 (0.5 - 21.0)	0	0
Total	450	5	445	1.11 (0.4 - 2.4)	0	1

of the lack of documented evidence from previous studies. This study however, revealed that 1.11% of the samples tested positive for FMD non-structural proteins which might be an indication of exposure to FMDV. In a previous study involving cattle, sheep, goats and pigs from some selected states in Northern Nigeria, samples from swine never tested positive for FMD (Lazarus, et al., 2012). However, in a related study 2% seroprevalence was reported in 869 pig sera collected in sub-Saharan Africa (Fernandez-Pacheco, et al., 2012). In a study in Kenya, serological evidence for SAT 1 FMDV infection in pigs was demonstrated without obvious clinical signs during an outbreak in cattle (Wekesa, et al., 2014). The low seroprevalence observed may be due to under reporting of FMD or as a result of a too small sample size to give a comprehensive picture of the presence of antibodies against FMDV. However, due to the cultural and farming practices in the study area the distribution of pigs is not compared to other livestock species as such few farmers engage in swine production and thus few pig populations. Though FMD has been reported to be severe in pigs (Yoon, et al., 2012), no clinical signs of the disease were observed in the sampled pigs. This may be one of the reasons why FMD is often considered not one of the important diseases of swine in Nigeria. This study has indicated that the pigs in the study area have been exposed to FMDV serotype A. This has been supported by the result of the SPCE serotypes A and O for samples that tested positive at NSP. The inability of the other samples tested positive at NSP to be able to be serotyped might be that they are positive for other serotypes than the A and O antigens in the SPCE. Currently we may not have evidence of how the transmission must have occurred in the swine population, but as a result of the farming system practiced, there may be a need to investigate if this is a result of some inter species transmission from cattle to pigs, since it did not give a clear clinical symptoms in pigs which may suggest an insufficient adaptation in the host. It has been demonstrated that host susceptibility to certain FMDV strains varies (Yoon, et al., 2012), and previous studies have observed pigs to play significant roles in the spread of FMDV serotypes O (Gibbens 2011; Hayama, et al., 2012), Asia 1 (Yang, et al., 2011) and A (Mohamed, et al., 2011). It has been reported that subclinically infected pigs with low level antibody responses may have very limited ability to transmit infection (Kitching and Alexansersen 2002). Hence the findings in this study could indicate infections of pigs acquired from cattle, at low levels that could not develop clinical symptoms.

Conclusion

This is the first report of serological evidence for FMDV serotype A in pigs in Nigeria without obvious clinical signs. Although it has not been reported that pigs may play an obvious role in the epidemiology of FMDV in Nigeria, this study demonstrates that they can be infected and could become important in the epidemiology of the disease when exposed to a virulent strain of FMDV. Therefore, we recommend that contacts between cattle and pigs in communal grazing areas should be limited to avoid interspecies transmission of FMDV and other important animal pathogens. This study is limited by small sample size and incomplete epidemiological data generated. Another major limitation to the study is the SPCE Kit used which was able to detect serotypes A and O. It may be necessary to serotype all NSP positive samples in future studies for all the FMDV endemic serotypes within Nigeria.

RECOMMENDATION

We therefore recommend more in depth research into the possible role of pigs in the epidemiology of FMDV in Nigeria and possibly attempt to isolate and characterize FMDV in pigs.

Conflict of Interest

The authors have not declared any conflict of interest.

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