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Serological evidence of foot-and-mouth disease virus (FMDV) in camels (*Camelus dromedaries*) in Nigeria

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Foot-and-mouth-disease (FMD) is one of the most important trans-boundary animal diseases in Africa with outbreaks occurring mostly in cattle. However, there is scarcity of information on the potential role of camels in the epidemiology of FMD virus in West Africa. A total of 360 camel sera collected from abattoir in Nigeria from different geo-political zones (North, West and East) were screened for the presence of antibodies produced against 3ABC non-structural proteins (NSP) for foot-mouth-disease virus (FMDV) using a commercially available kit prioCHECK® FMDV NS. Thirty nine, (10.83%) out of the 360 sera samples were tested positive for 3ABC NSP ELISA. The 39 positive samples were further subjected for sero-typing using solid-phase competitive ELISA (SPCE) for antibodies to FMDV serotype A and O (Solid-Phase Competitive ELISA, IZSLER Brescia-Italy). Two out of the 39 sera samples were positive for serotype A and the remaining were negative for both serotype A and O. This appears to be the first report of evidence of FMD antibodies in dromedaries in West Africa and that dromedaries may be susceptible to FMDV infection.

Key words: 3ABC; camel, ELISA, foot-and-mouth-disease (FMD), non-structural proteins (NSP), sera.

INTRODUCTION

Foot-and-mouth disease (FMD) is the most economically important disease of cloven hoofed animals. The virus is highly contagious, affecting almost exclusively among animals such as cattle, sheep, goats, Bactrian camels and pigs (Wernery and Kinne, 2012). The disease affects both domestic and wild animals. The disease is characterized by lesions in hairless area and myocardial degeneration in calves has been observed (Wernery and Kinne, 2012). Many countries around the globe have been certified free for FMD by Office International des Epizooties (OIE) (Wernery and Kaaden, 2004). FMD virus has continued to circulate in other continents of the World like Asia, South America, Middle East and Africa. This has affected the economy of such continents significantly due to the effects on international trade of susceptible animals and their products.

The Camelidae are found in countries like North and East Africa, Middle and East Asia as well as South America where FMD is endemic (Du et al., 2009). The dromedary camels are also found in West Africa, Nigeria

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in particular because of its significance in trade and meat products. There are divergent opinions as to whether Camelidae family are susceptible to FMD or not, or they may serve as a reservoir host of the virus (Yousef et al., 2012). The two closely related camel species of Bactrian and dromedary camels possess noticeably different susceptibility to FMD virus (Larska et al., 2009). Bactrian camels under experimental studies can easily develop obvious clinical sign of FMD (Larska et al., 2009), while several investigation appear to suggest that dromedaries are less susceptible to inoculation with FMD virus serotype O but that they do not present a risk in transmitting the disease to susceptible animals (Wernery and Kaaden, 2004; Alexandersen et al., 2008). However, Kumar et al. (1983) have described the isolation of FMDV serotype O from one of two randomly selected dromedaries in India, and Moussa et al. (1987) in Egypt described a strain of type O FMD virus isolated in Giza from a camel with vesicular, ulcerative stomatitis and they suggested that dromedaries are susceptible to natural FMD infection (Yousef et al., 2012).

FMD is caused by an RNA aphthovirus of the family picornaviridae. There are seven immunological serotypes (A, O, C, SAT 1, 2, 3 and Asia1) that exist and over 60 subtypes of the virus are circulated Worldwide (Wernery and Kinne, 2012). Four (A, O, SAT 1 and 2) out of this seven serotype exist in Nigeria (unpublished data). Foot-mouth-disease virus (FMDV) is a small non-enveloped virus and has a genome of 8.5 kb which encodes for structural as well as non-structural proteins (NSPs) (Yousef et al., 2012). The viral capsid composed of four structural proteins, VP1, VP2, VP3 and VP4 (Fry et al., 2005).

A structural protein produces antibodies to FMDV in vaccinated animals, whereas infected animals produce antibodies to both the structural and non-structural proteins (Yousef et al., 2012). Meanwhile, assays to demonstrate antibodies against non-structural proteins have potential to differentiate infected animals from vaccinated (Berger et al., 1990; Rodriguez et al., 1994; De Diego et al., 1997; Clavijo et al., 2004). The disease is endemic in some parts of Europe, Africa, Middle East and Asia, but places like North America, Australia, New Zealand and many countries in Western Europe are free from the disease and have stringent regulations preventing the introduction of the virus (Wernery and Kinne, 2012).

The first reported case of FMD outbreak in Nigeria was in 1924, which was attributed to type O virus (Libeau, 1960). Subsequently, other serotypes (A, SAT 1 and SAT 2) were reported, and each of these introductions was associated with trade cattle entering Nigeria from neighboring countries, (Nawathe and Goni, 1976; Owolodun, 1971; Durojaiye, 1981; Abegunde et al., 1988). It should be understood that trans-humance production system is the predominating system of animal management in the Sub-Saharan Africa and many of these individuals traverses national borders (especially those of Central African Republic, Chad, Niger, Cameroun, Benin and Nigeria) in search of feed and water resources for their livestock without any recourse to quarantine and control measures. Furthermore, camels are frequently moved across the desert of Niger, Chad, Benin and Central African Republic to Nigeria across areas that FMD outbreak is endemic in cattle and other small ruminants. Therefore, it is possible that camels may play a possible role in the maintenance and transmission of FMDV and may carry FMDV over a very long distance and across the borders (Yousef et al., 2012). However, because of the limited information concerning FMDV in camels in West Africa, Nigeria in particular, this study was aimed to investigate the serological evidence of natural exposure of camels *(Camelus dromedaries)* to FMD virus. The investigation was to detect the presence of antibodies against non-structural proteins (NSP) using competitive ELISA and solid-phase competitive ELISA (SPCE) for serotype specific FMDV antibodies, to evaluate the role of camels in the epidemiology of FMD in Nigeria and by extension West Africa.

MATERIALS AND METHODS

A total of 360 abattoir camel sera samples were collected from North Western Nigeria (Kano and Sokoto States) and North Eastern Nigeria (Borno State) over a period of one year (November, 2010 to October, 2011). The sera were collected from all the slaughtered camels in the said abattoir without evidence of any clinical signs of FMD, even though the camels had unrestricted contact with susceptible ruminants (cattle, sheep and goats) that had history of infection with FMD. Whole blood was collected in a wide mouth sample collection bottles which was allowed to clot at room temperature for about 3 to 4 h. The serum was harvested and transferred into a cryovials for storage at -20°C until testing. All positive sera were serotype for antibodies against FMDV serotypes A and O. The prioCHECK® FMDV NS commercial ELISA kit (Prionics Lelystad B.V, The Netherlands) for detection of antibodies against non-structural proteins (NSP) of FMDV was used for testing serum samples of cattle, sheep, goats camels and pigs. The assay was performed according to the manufacturer's protocol and the optical density (OD) value was read using Multiskan® spectrophotometer (Thermo Scientific, USA) at a 450 nm wavelength and results expressed as a percentage inhibition (PI) of the controls and the test sera which was calculated using the formula in the protocol (Sorensen et al., 1998).

Solid-phase competitive ELISA (SPCE) for antibodies to FMDV serotype A and O

Commercial SPCE kit (IZSLER Brescia, Italy) was used for detection of serotype-specific antibodies (A and O) to foot-and-mouth disease virus according to the manufacturer's instructions. The criteria for the validity of the test are that the spectrophotometric readings must be ≥1 OD in the wells of the negative control while the positive control serum is expected to give ≥90% inhibition at 1/10 dilution and >50% inhibition at the second dilution (I/30). For screening purposes, the test sera is considered positive when it produces an inhibition ≥70% at the 1/10 dilution and negative when

Table 1. Abattoirs from where camel samples were collected and results of samples tested.

Abattoir location	No. of animals tested	No. of samples tested positive	NSP prevalence (%)	95% CI
Maiduguri	68	1	1.47	0.07 - 7.04
Kano	260	37	14.23	10.37 - 18.88
Sokoto	32	1	3.13	0.15 - 14.46
Total	360	39	10.83	7.93 - 14.37

Table 2. Solid-Phase Competitive ELISA (SPCE) for antibodies specific to FMDV serotypes O and A.

Abattoir location	NSP positive	FMD serotype O	FMD serotype O % (95%CI)	FMD serotype A	FMD serotype A% (95%CI)
Maiduguri	1	0	0 (0 – 95)	0	0 (0 – 95)
Kano	37	0	0 (0 – 7.78)	2	5.4 (0.92 – 16.73)
Sokoto	1	0	0 (0 – 95)	0	0 (0 – 95%)
Total	39	0	0 (0 – 7.39)	2	5.1 (0.86 – 15.93)

producing an inhibition of <70% at the dilution of1/10.

RESULTS

The overall result indicated 10.83% (95% CI: 7.93 to 14.37) of the total serum samples collected from Maiduguri, Kano and Sokoto abattoirs to be positive for antibodies against FMD NSP (Table 1). All sera that tested positive for FMD NSP were further analyzed for antibodies against FMD structural proteins for serotypes A and O using a SCPE and the results is presented in Table 2. The optical density of camel sera was read on a MultiSkan spectrophotometer reader at 450 nm wavelength.

DISCUSSION

FMD is endemic in most of Sub-Saharan Africa, in particular with unrestricted movement of susceptible animals across the border with no or less control measure instituted to the susceptible animals. Because of the poor veterinary services in most African countries and limited information on the role of camels (dromedary camels) in the epidemiology, few documented evidence exists on its epidemiology of FMD. Camels however, move frequently across the Sahara desert for grazing and trade purposes thereby mixing freely with susceptible animals in endemic countries like Niger, Nigeria, Chad and Central African Republic. These results indicate serological evidence of FMD non-structural proteins in dromedaries camel which may be as a result of exposure to FMDV since camels are not vaccinated against FMDV. It could be that during FMD outbreak, camels come in contact with infected susceptible animals like cattle, sheep and goats which are often herded together by most pastoralists.

These results contradict several reports by researchers that tested camel sera in Africa and the United Arab Emirates for evidence of FMD with negative results (Wernery and Kaaden, 2004). Also, the finding of Moussa and Yousef (1998) that the antibodies identified by Richard (1979) were non-specific inhibitory substances frequently observed in camel sera may be correct but with the use of ELISA test, it is confirmed that dromedaries develops antibodies against FMDV and this results is in agreement with studies by Moussa et al. (1987) in Egypt which indicated the susceptibility of dromedaries to natural FMD infection. Similarly, Metwally et al. (1986) did experimental studies in dromedaries in Egypt with FMD serotype O1/2/72 Egypt with no clinical signs observed, but the virus was re-isolated from one camel between one to three weeks post-inoculation (PI), both dromedaries sero-converted but with low titers, which lasted for six weeks and this contradicted the studies by Moussa et al. (1987) where the dromedary did not develop any antibodies to the artificial infection which suggest the lack of antibodies development is as a results of route of inoculation.

From these results, it is clear that dromedaries can contract the FMDV by contacts with FMD infected animals, but may not pose risk of transmitting the disease to susceptible animals. According to Wernery and Kaaden (2004) it seems that dromedaries do not become FMD carriers because FMDV has not been isolated from oesophageal-pharygeal fluid (OPF) 14 days after viral exposure and the statement agrees with the study of Farag et al. (1998) where they were unable to isolate FMDV from 30 probang samples harvested from

dromedaries on different farms in Saudi Arabia where FMD was said to be endemic. This study was limited because of the availability of the ELISA kit that could test only for two FMD structural protein serotypes A and O. Therefore, other serotypes might have been remained undetected as a result of the non-availability of a kit that could test for other FMDV serotypes that circulates within this region.

However, from the results obtained dromedaries appear to be susceptible to infection with FMDV but may likely not play a significant role in the epidemiology of FMD. However, further research on the epidemiology of FMD in dromedaries in West Africa is necessary where the disease is endemic.

Conflict of Interest

The authors have not declared any conflict of interest.

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