Asian J. Med. Biol. Res. 2015, 1 (2), 163-168; doi: 10.3329/ajmbr.v1i2.25606

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Serological response of commercial dairy cattle to inactivated foot-and-mouth disease vaccine (type-O & A) in Nigeria

David Dazhia Lazarus[1*], Yiltawe Simwal Wungak[1], Mohammed Ignatius Adah[2], John Okpapi Ibu[3], Jerry Ngutor Abenga[3], Pius Stephen Ekong[4] and Hussaini Gulak Ularamu[1]

[1]Foot-and-Mouth Disease Laboratory, National Veterinary Research Institute, Vom Nigeria
[2]Department of Medicine, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria
[3]Department of Microbiology and Pathology, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria
[4]Central Diagnostic Laboratory, National Veterinary Research Institute, Vom Nigeria

*Corresponding author: David Dazhia Lazarus, Foot-and-Mouth Disease Laboratory, National Veterinary Research Institute, PMB 01, Vom Nigeria. E-mail: lazdav2003@yahoo.co.uk

Received: 15 July 2015/Accepted: 30 July 2015/ Published: 30 September 2015

Abstract: An observational study was conducted in a peri-urban dairy establishment in Jos South, Plateau State Nigeria to determine immune response of dairy cattle to commercial inactivated foot-and-mouth disease vaccine serotypes (O and A). Thirty seven Friesian cattle aged ≥2years old with their crosses (15 selected pre-vaccination and 22 selected 21 days post-vaccination) were investigated for immune response to vaccination with an inactivated trivalent FMD vaccine containing serotypes O, A and SAT 2). Sera collected on day 0 pre-vaccination and 21 days post-vaccination was tested for structural protein antibodies to FMD serotypes O and A using the Solid Phase Competitive ELISA assay. The mean OD value for serum end point titre of FMD serotype O pre-vaccination was 11.64% with 6.67% (95%CI: 0.33 – 28.73) of the selected cattle being seropositive, at 21 days post-vaccination the mean OD value in selected cattle was 52.83% with 68.18% (95%CI: 46.95 – 84.89) of the selected cattle seropositive. For the FMD serotype A, 26.67% (95%CI: 9.10 – 52.53) of the selected cattle were seropositive pre-vaccination with a mean OD value of 29.21% and by 21 days post-vaccination, 72.73% (95%CI: 51.67 – 88.13) of the selected cattle were seropositive with a mean OD value of 61.70%. Serological response to vaccination improved in most selected cattle by 21 days post-vaccination. This study result has indicated that commercial inactivated FMD vaccines used for the prophylactic control of FMD in commercial dairy farm in Nigeria provoked immune response after a single shot.

Keywords: commercial; dairy; foot-and-mouth-disease; inactivated; response; serological; vaccine

1. Introduction

In Foot-and-mouth disease (FMD) endemic countries, as a prophylactic measure to prevent against outbreaks in susceptible cloven-hoofed farm animals, routine vaccination with inactivated foot-and-mouth disease virus (FMDV) vaccines is recommended (Doel, 2003). However, in most cases clear policies for prophylactic vaccination of farm animals do not exist. According to the Office International des Epizooties (OIE), FMD vaccines used for the prophylactic control of FMD in endemic settings should be vaccines of standard potency formulated to contain sufficient antigen and appropriate adjuvant to ensure that they meet the minimum potency level required (recommended as 3 PD_{50} [50% protective dose in vaccinated candidates]) (Elnekave *et al.*, 2013). Administering at least 2 doses one month apart when cattle are vaccinated for the first time against FMD with such vaccine is recommended, however due to cost and logistical reasons this is not routinely practised in most endemic countries (Knight-Jones *et al.*, 2014a). FMD structural protein antibody levels are strongly correlated

with protection in vaccinated cattle (Reeve *et al.,* 2011; Robiolo *et al.,* 2010). Therefore, when testing FMD protection from antibody titre, the serological test used should be correlated with protection (OIE, 2013), because when the test antigen used is different from the one evaluated in the study, the extent of the protection may be uncertain.

Globally, it has been reported that large scale FMD vaccines are being applied to control FMD in cloven-hoofed livestock with over 2 billion doses being used annually (Knight-Jones *et al.,* 2014a). However, despite the sustained efforts in the progressive control of FMD, little is done to evaluate the field performance of FMD vaccines (Knight-Jones *et al.,* 2014a). About 2-5% of cattle globally have been reported to be affected by FMD each year with incidence in other cloven-hoofed species reported slightly lower (Knight-Jones *et al.,* 2013). Losses associated with export of livestock commodities have been reported to be of huge importance to countries with developed livestock industries, which has placed a trade barrier restricting economic growth of such countries (Rich and Perry, 2011; Knight-Jones *et al.,* 2013).

In Nigeria, FMD virus is endemic with outbreaks occurring seasonally in most pastoral settings and established farm holdings. To date FMD serotypes O, A, SAT 1 and SAT 2 have been reported in sedentary cattle since the first report of FMD in Nigeria in 1924 (Libeau, 1960; Fasina *et al.,* 2013; Lazarus *et al.,* 2012). Recently between 2009 – 2014, outbreaks of FMD serotypes O, A and SAT 2 have been reported in Jos South, a municipal area which is the second administrative unit for a state structure in Nigeria (FAO, 2010a; FAO, 2010b, FAO, 2014a, FAO, 2014b, FAO, 2014c). Although cattle are not routinely vaccinated for FMD in the country, some established dairy farms vaccinate cattle to prevent against seasonal outbreaks. However, the field effectiveness of such vaccines during outbreak is hardly evaluated. As observed in human vaccination campaign programmes, where seroconversion is often studied using pre and post-vaccination sera to access the vaccine response, a similar survey using sera collected post-vaccination are common in livestock (Knight-Jones *et al.,* 2014c), in which case the proportion of livestock with antibody titre above a specific threshold associated with protection is then determined (Robiolo *et al.,* 2010). In this study we present a preliminary report of serological response of Holstein-Friesian cattle and their crosses vaccinated with inactivated trivalent (O, A and SAT 2) FMD vaccines to antigen for serotypes O and A only.

2. Materials and Methods

2.1. Animals

Holstein Friesian cattle and their crosses belonging to a private commercial dairy farm in Jos South, Plateau State, Nigeria were used for this study. The dairy farm accommodated over a thousand cattle with their calves. From history, there was no introduction of cattle from other localities into the dairy farm and the farm is situated at the outskirt of a local community. As a routine prophylactic programme, all animals on the farm are vaccinated against FMDV serotypes; A, O and SAT 2 annually.

2.2. Vaccination and sample collection

During August, 2012, 300 cattle on the farm were vaccinated with alhydrogel-saponin adjuvanted inactivated FMD vaccine against FMD types O, A and SAT 2 (Aftovax®, Merial Animal Health Ltd/Botswana Vaccine Institute Gaborone). Cattle were individually injected with 3 ml of the vaccine subcutaneously in the neck region using a hypodermic syringe system. Prior to vaccination, 15 cattle aged ≥2years old were randomly selected and whole blood collected from the jugular vein, using plain evacuated tubes to determine the antibodies status prior to vaccination. Blood was allowed to clot at ambient temperature in the field and transported to the laboratory where it was centrifuged at 1450 x g for 10 min and serum collected and stored at -20°C until testing. After 21 days post-vaccination, 22 cattle were randomly selected and bled to determine seroconversion and immune response to vaccination.

2.3. Serological test

Samples were analysed for FMD-specific antibodies using a Solid-phase Competitive ELISA (SPCE) as previously described elsewhere (Grazioli *et al.,* 2008) for serotypes A and O at the Foot-and-Mouth Disease Laboratory, National Veterinary Research Institute, Vom. The assays were performed using the antibodies FMDV ELISA kits for serotypes A and O produced by IZSLER Biotechnology Laboratory, Brescia-Italy. Briefly, 96 wells pre-coated with FMDV antigens captured by FMD serotypes A and O specific MAb flat-bottomed plates were used. Four dilutions at 1/10, 1/30, 1/90 and 1/270 were performed for the sera. Without washing, the conjugate (Horse radish peroxidase) was added and incubated at room temperature for 1 hour. The

plates were then washed, after which the substrate/chromogen solution (Tetra-methyl-benzidine) was added and kept in the dark for 20 minutes. The reaction was later stopped by the addition of a stop solution and the plates were read on a MultiSkan® spectrophotometer ELISA plate reader (Thermo Scientific, USA) at 450 nm wavelength. Serum end-point titre was expressed as the highest dilution producing 50% inhibition, with serum having end point titre ≥50% being classified as positive.

2.4. Statistical analysis

The data was entered into Microsoft Excel® and exported in R software (version 3.0.2) for analysis. The percentage of seropositive cattle for FMD serotypes O and A were determined and the exact 95% confidence intervals (CI) were calculated using the Pearson-Klopper method. The mean serum end point titre (optical density [OD]) for FMD serotype O and A pre- and post-vaccination were calculated. Differences in the mean OD for each serotype, pre- and post-vaccination, with their 95% CI were calculated using the t-test.

3. Results

In total 37 cattle were included in the study of which 15 were selected pre-vaccination and 22 were selected 21 days post-vaccination respectively. The cattle were randomly selected from a commercial dairy farm that vaccinates routinely against FMD using a trivalent inactivated vaccine for serotypes (O, A and SAT 2). The percentage of cattle positive for serotype O, pre- and post-vaccination were 6.67% (95% CI: 0.17–31.95%) and 68.18% (95% CI: 45.13–86.14%), respectively (Table 1). There was a significant difference (P<0.001) in the mean OD pre- and post-vaccination (mean difference = 41.19% (95% CI: 26.74–55.65%). The mean OD for serotype O and A pre- and post-vaccination among selected cattle is presented in Figure 1. The percentage of cattle positive for serotype A, pre- and post-vaccination were 26.67% (95% CI: 7.79–55.10%) and 72.73% (95% CI: 49.78–89.27%), respectively (Table 1). There was a significant difference (P<0.001) in the mean OD pre- and post-vaccination (mean difference = 32.49% (95% CI: 17.51–47.47%).

Table 1. Distribution of tested and positive samples pre- and post- FMD vaccination of dairy cattle with inactivated types O, A and SAT 2 vaccine.

	Pre-vaccination		Post-vaccination	
Serotype	**O**	**A**	**O**	**A**
No. Tested	15	15	22	22
No. Positive	1	4	15	16
% Positive (95% CI)	6.67 (0.17–31.95)	26.67 (7.79–55.10)	68.18 (45.13–86.14)	72.73(49.78–89.27)

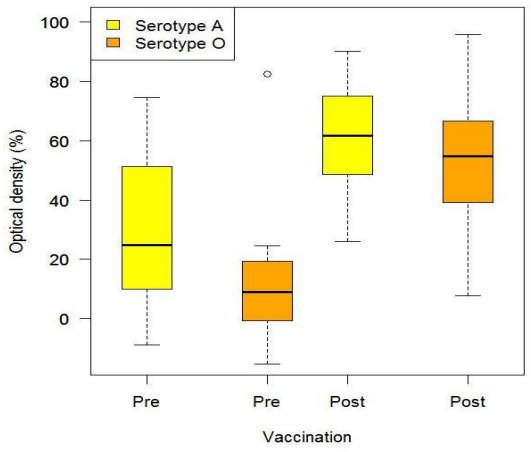

Figure 1. Boxplots showing the mean optical density (OD) values for FMD serotype O and A pre-vaccination and 21 days post-vaccination. Boxplots shows the median value (horizontal line), interquartile range (box) and range (whiskers).

4. Discussion

Vaccination of dairy cattle with a trivalent FMD vaccine (type O, A, and SAT 2) is practiced in some selected dairy farms in Nigeria. However, several previous reports have indicated that imported commercial FMD vaccines have performed sub-optimally under field situation in Nigeria (Nicholls *et al.*, 1983). In this study, we attempted to select a subset of vaccinated population of cattle in order to evaluate immune response to FMD vaccine, testing for serotypes O and A only. To our knowledge, this is about the first time an attempt was made to investigate immune response to commercial FMD vaccines containing serotypes O and A in Nigeria. In a previous study involving FMD serotypes SAT 1 and SAT 2 isolated from Nigeria, it was demonstrated that cattle responded satisfactorily to vaccination irrespective of age, breed, or simultaneous vaccination with Contagious Bovine Pleuro-pneumonia (CBPP) vaccine (Nicholls *et al.*, 1983). Although it is a known fact that FMD vaccines need to be adequately matched to the field virus to ensure sufficient protection against a challenge with a field virus, little is done in most developing countries to match vaccines before implementing campaign programmes. This may not however, be unconnected with the fact that FMD vaccines are not always readily available and farmers would have to make do with what they have. In most cases, this ends up with outbreaks occurring even after animals have been vaccinated against FMD.

This study has demonstrated that the current vaccination programme practiced on the farm does not provide sufficient protection of the herd against FMD should an active outbreak occur within the inter-vaccination interval. In most cases, it is believed that once animals are vaccinated against a disease they are adequately protected. However, with the increased incidence of disease in vaccinated population the need for post-vaccination monitoring is being highlighted. Also contrary to the standard recommendation by the manufacturers that cattle in endemic settings should be vaccinated at least twice within a period of 2-8 weeks and thereafter every 4-6 months depending upon the epidemiological situation within the region, most regions in developing countries adopt a single course of an annual mass vaccination campaign proceeding periods of suspected outbreaks.

In this study few animals had evidence of pre-existing antibodies to the serotypes being tested for pre-vaccination which might be as a result of the rapid decline of antibodies to the previous vaccination as reported previously for alhydrogel-saponin adjuvanted vaccines (Hunter, 1996; Hunter, 1998; Cloete *et al.*, 2008). However, by 21-days post-vaccination there was a marked improvement in immune response of vaccinated cattle for both serotypes O and A, which is indicative of seroconversion to vaccination. It has been reported that vaccinated livestock respond rapidly to the first dose of vaccine and produce peak antibody titres between 14 and 28 days depending on the vaccine composition (Doel, 2003). Immune response against FMDV includes circulating humoral antibody has been shown to correlate with protection (Reeve *et al.*, 2011; Robiolo *et al.*, 2010; Mackowiak *et al.*, 1962; van Bekkun, 1969; Pay and Hingley, 1987; McCullough *et al* 1992a; McCullough *et al.*, 1992b). Therefore serological evidence of FMD antibodies in vaccinated cattle in the absence of circulating field virus might be an indicator of protection against challenge with a homologous virus. This study is limited due to the number of cattle sampled pre and post-vaccination period which might underscore the level of the herd immunity. Another limitation to the study is the inability to test for serotype SAT 2 antigen in the serum of vaccinated cattle which would have provided a better understanding of the immune response to the SAT 2 antigen contained in the vaccine. Since we have not been able to monitor the herd longitudinally for duration of immunity post-vaccination, it will not be possible to demonstrate the duration of antibodies response that persists in vaccinated cattle which will be a guide to designing an effective vaccination regimen. Therefore, we recommend that a detailed and structured study should be designed with the aim of investigating immune response and duration of immunity in Nigerian cattle to commercial vaccines containing relevant FMD serotypes representative of the region. Efforts should also be intensified to develop vaccines using indigenous isolates targeting representative field viruses in Nigeria. Designing an effective vaccination programme with a quality vaccine against FMD will be a welcome development for Nigeria at this moment when the global oil price is declining and the national population is increasing with high demand for animal proteins.

5. Conclusions

In this study, we have demonstrated that a commercial FMD vaccine with a standard antigen payload used according to the manufacturer's instruction for the inoculation of dairy cattle in Nigeria produced measurable level of anti-FMD antibodies against serotypes O and A by 21 days post-vaccination.

Conflict of interest
None to declare.

References
Cloete M, B Dungu, L Van Staden, N Ismail-Cassim and W Vosloo, 2008. Evaluation of different adjuvants for foot-and-mouth disease vaccine containing all the SAT serotypes. Onderstepoort. J. Vet. Res., 75: 17-31.

Doel T, 2003. FMD vaccine. Virus Res., 91:81.

Elnekave E, LY Li, B Zamir Even-Tov, P Hamblin, B Gelman, J Hammond and E Klement, 2013. The field effectiveness of routine and emergency vaccination with an inactivated vaccine against foot and mouth disease. Vaccine, 31: 879-885.

FAO, 2010a. World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) Genotyping Report, 7 July 2010, FMD type O, Nigeria, 2009. WRLFMD/2010/000022

FAO, 2010b. World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) Genotyping Report, 5 July 2010, FMD type A, Nigeria, 2009. WRLFMD/2010/000022

FAO, 2014a. World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) Genotyping Report, 10 July 2014, FMD type O, Nigeria, 2011-2014. WRLFMD/2010/00016

FAO, 2014b. World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) Genotyping Report, 10 July 2014, FMD type A, Nigeria, 2011-2013. WRLFMD/2010/00016

FAO, 2014c. World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) Genotyping Report, 10 July 2014 FMD type SAT 2, Nigeria, 2011-2012. WRLFMD/2010/00016

Fasina FO, DR Connell, OA Talabi, DD Lazarus, GA Adeleke, TP Olusanya and JA Fernandez, 2013. Foot-and-mouth disease (FMD) virus strains and examination of exposure factors associated with FMD seropositivity of cattle herds in Nigeria during 2007-2009. Prev. Vet. Med. 109: 334-342.

Grazioli S, E Brocchi, V Tranquillo, S Parida and D Paton, 2008. Development of solid phase competitive ELISAs based on monoclonal antibodies for the serology of FMDV serotypes SAT 1 and SAT 2. The Global control of FMD – Tools, ideas and ideals – Erice, Italy 14-17 October 2008, 267-276.

Hunter P, 1996. The performance of southern African territories serotypes of foot and mouth disease antigen in oil-adjuvanted vaccines. Rev. sci. tech. Off. Int. Epiz., 15: 913-922.

Hunter P, 1998. Vaccination as a means of control of foot-and-mouth disease in sub-Saharan Africa. Vaccine, 16: 261-264.

Knight-Jones TJD and J Rushton, 2013. The economic impact of foot and mouth disease- What are they, how big are they and where do they occur. Prev. Vet. Med., 112: 161-173.

Knight-Jones TJD, 2014c. Field evaluation of foot and mouth disease vaccination in Turkey. PhD Thesis, Royal Veterinary College, University of London. pp. 1-217.

Knight-Jones TJD, AN Bulut, S Gubbins, KDC Stärk, DU Pfeiffer, K Sumption and DJ Paton, 2014a. Retrospective evaluation of foot and mouth disease vaccine effectiveness in Turkey. Vaccine, 32: 1848-1855.

Lazarus DD, WJG Schielen, Y Wungak, D Kwange and FO Fasina, 2012. Sero-epidemiology of foot-and-mouth disease in some Border states of Nigeria. Afr J. Microbol. Res., 6: 1756-1761.

Libeau J, 1960. Foot and mouth disease in Africa South of the Sahara- the present position. Bull Epiz Dis Afr., 8: 152-158.

Mackowiak C, R Lang, J Fontaine, R Camand and HG Petermann, 1962. Relation entre titre d'anticorps neutralisants et protection des animaux après vaccination anti-aphteuse. Annales de l Institut Pasteur, 103: 252-61.

McCullough K, F De Simone, E Brocchi, L Capucci, Crowther J and Kihm U, 1992a. Protective immune response against foot-and-mouth disease. J. Virol., 66: 1835-1840.

McCullough KC, L Bruckner, R Schaffner, W Fraefel, HK Müller and U Kihm, 1992b. Relationship between the anti-FMD virus antibody reaction as measured by different assays, and protection in vivo against challenge infection. Vet. Microbiol., 30: 99-112.

Nicholls MJ, MM Rweyemamu, EN Okeke, NN Shidali and AG Lamorde, 1983. The control of foot and mouth disease by vaccination: Consideration for Nigeria. Rev. sci. tech. Off. Int. Epiz., 2: 771-780.

OIE, 2013. Manual of diagnostic tests and vaccines for terrestrial animals, Paris: World Organization for Animal Health.

Pay T. and Hingley P, 1987. Correlation of 140S antigen dose with the serum neutralizing antibody response and the level of protection induced in cattle by foot-and-mouth disease vaccines. Vaccine, 5: 60-64.

Reeve R, S Cox, E Smitsaart, CP Beascoechea, B Haas, E Maradei, DT Haydon and P Barnett, 2011. Reducing animal experimentation in foot and mouth disease vaccine potency tests. Vaccine, 29: 5467-5473.

Rich KM and BD Perry, 2011. The economic and poverty impacts of animal diseases in developing countries: New roles, new demands for economics and epidemiology. Prev. Vet. Med., 101: 113-147.

Robiolo B, J La Torre, E Maradei, CP Beascoechea, A Perez, C Seki, E Smitsaart, N Fondevila, E Palma, N Goris, K De Clerq and N Mattion, 2010. Confidence in indirect assessment of foot and mouth disease vaccine potency and vaccine matching carried out by liquid phase ELISA and virus neutralization tests. Vaccine, 28: 6235-6241.

van Bekkun, JG, 1969. Correlation between serum antibody level and protection against challenge with FMD-virus. European Commission for the Control of FMD, Session of the Research Group of the Standing Technical Committee, 24-26 September 1969, Brescia Italy.