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Quality and Toxicity Assessments of Foot and Mouth Disease Virus Vaccine in Inoculated Guinea Pigs

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Abstract
The quality and toxicity assessment of foot and mouth disease virus vaccine was carried out in inoculated guinea pigs. The vaccine was developed from local isolates for the control and prevention of foot and mouth disease in Nigerian cattle. All the vaccine inputs tested were sterile and the high mean titre levels of complement fixing antibodies (92.8 and 147.2) and serum neutralizing antibodies (1.68 and 2.15), either as single dose or repeated doses inoculations. The blood immune cells parameters showed a positive immunological response due to activation by the vaccine antigens. There were increases in their population as compared to the controls. The blood enzyme profiles did not show any significant evidence of tissue or organ damage as all the results obtained were within the reference standard ranges of normal healthy guinea pigs. The findings from the study show that the vaccine could be used for the control and prevention of foot and mouth disease in Nigerian livestock.

Keyword: Foot and Mouth Disease, Antibody, Quality, Toxicity, Vaccine.
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Introduction
Foot and mouth disease (FMD) is a very important economic disease of cloven hoofed animals both domestic and wild (Chukwuedo et al., 2007). It is a communicable, highly acute and infectious disease (Sherry et al., 2002) caused by a single stranded, non segmented, positive sense, RNA genomic virus of Aphthovirus genus (Kitching, 2002). FMD is endemic in Nigeria and the causative virus agents have been reported and found to be circulating among Nigerian cattle (Chukwuedo and Nimzing, 2012). The control of FMD with the use of potent inactivated vaccines in endemic regions has been recommended (Nawathe and Majiyagbe, 1981; Asagba, 1982; Abegunde et al., 1987).

Currently effort has been made to develop inactivated FMD vaccine for the control of the disease in Nigerian cattle (Chukwuedo and Nimzing, 2011). This vaccine has undergone some laboratory and field tests. The results obtained were quite good and the challenged animals with field strains of FMD virus survived post-vaccination tests. In this study, efforts were made to assess the quality and toxicity of FMD vaccine developed with local isolates in an inoculated guinea pigs.

Materials and methods
Guinea Pigs: Ten (10) guinea pigs were used in this study and they were purchased from the small animal unit of the National Veterinary Research Institute, Vom. The animals were certified fit by a veterinarian. The guinea pig weighed 470 to 520g. Both males and females were used as the animal sex do not affect the aim of the study. The animals were shared into two groups of five in each group. Group1 was used as test animal for the vaccine while group2 was used as controls.

Vaccine: The locally developed vaccine contained FMD serotypes, southern African Territories (SAT) 1 and 2 blended with Incomplete Seepic Adjuvant (ISA) montanide 206, which produced a milky suspension. The vaccine was maintained at +4℃ fridge temperature.

Serum: sera samples obtained from pre-vaccinated and post vaccinated guinea pigs were heat inactivated at 56℃ in a water bath for 30min and stored at -20℃ (deep freezer) ready for assay.

Sterility Test: The samples of the vaccine suspension, buffered saline, serum, adjuvant and cell culture medium was cultured by dropping 1.0ml of the samples on each of the bacteriological media and spread with a flamed wire loop. The plates and medium were incubated for 72hrs at 37℃ and observed each day for microbial growth.

Safety and Potency Test: The group 1 set of 5 guinea pigs were used for safety and potency tests. The animals were subcutaneously inoculated with 0.5ml of the vaccine per guinea pig at the left thigh of the hind leg. The test and control animals were observed for any pathological moribund or death for 14 days with adequate supply of feed and water. They were bled after 21 days and the sera were separated and stored at -20℃ ready for use.

Complement Fixation Test: The micro complement fixation method of Rweyemamu (1984) was used. The positive result was determined by over 75% nonhemolytic reaction in the test wells of 96 u-shaped disposal polystyrene microlitre plates while the titre was expressed as the reciprocal of the dilution.

Serum Neutralization Test: The micro serum neutralization test method was used in accordance with Rweyemamu and Pereira (1978) method. The test was carried out in a 96 well flat bottom sterile disposal microtitre plates. The neutralization titres were expressed as the reciprocal of the final dilution of the serum that neutralized the virus.

Blood Parameter Assay: Five milliliters (5ml) of blood was collected from the test and control guinea pigs into disposable sample bottles containing Ethylene Diamine Tetra Acetic Acid (EDTA). Blood sample were analyzed using mindray BC 2800 Vet Auto analyzer (Mindray Med Int. Ltd. Shensan 518057, PR China) it analyzes 19 parameters and 25 samples of whole blood or prediluted blood.

Serum Enzyme Assay: The serum of the test and control guinea pig was assayed for Aspartate amino transferase, Alanine amino transferase, bilirubin, serum urea and creatinine. The test methods of Schmidt and Schmidt (1963) and Water and Gerald (1980) were used. It involved reagent blank and sample blank. The reagent blank is measured against the sample blank and the results were determined at Hg 546nm wavelength.

Post Vaccination Challenge Test: Vaccine challenge method of Nicholls et al. (1990) was adopted. The foot pad of the hind leg of the guinea pig was dipped into phosphate buffered saline (PBS), pH 7.2 for 60 seconds and the challenge virus suspension was carefully inoculated (0.02ml) subcutaneously at the foot pad using tuberculin syringe and needle. The infected foot pad was wetted the second day and then observed for 14 days.

Results

Table 1 shows the results of the sterility tests of the vaccine and other vaccine inputs cultured on artificial bacterial media. The results obtained showed that the materials were sterile and none of the items were toxic to the BHK-21 cell monolayer. The data obtained from the guinea pigs inoculated with single and repeated does of the vaccine were presented in Table 2. No antibody to FMD virus was detected in their pre-vaccination sera (<2 or <0.3). The booster dose gave the expected immunological reaction with increase in antibody production from 92.8 CF mean titre of the single dose to 147.2CF mean titre of the repeated vaccination. Both the single and repeated SN titres were above the required 1.51 minimum neutralization titre (Table 2).

Table 1: Sterility tests of the vaccine and other vaccine inputs cultured on artificial bacterial media

Samples	BA	MCB	TGB	BHK	Cells	PPLO (Agar)
Vaccine	-	-	-	-	-	-
Serum	-	-	-	-	-	-
Buffered Saline	-	-	-	-	-	-
Montanide ISA 206	-	-	-	-	-	-
Cell culture Medium	-	-	-	-	-	-
Cell culture Harvest	-	-	-	-	-	-

Key: BA= Blood Agar, MCA = Mac Conkey Agar, SDA – Sabouraud Dextrose Agar, TGB = Thioglycholate Broth, - = No growth, + = There is growth, BHK = Baby hamster kidney, PPLO = Pleuro-pneumonia like Organism

Table 2: Complement fixation and Serum Neutralization antibody titres in G. pigs inoculated with single and repeated Doses of FMD vaccine

Animal Host	Vaccine Dose (ml)	CF Test			SN Test		
		Pre Vacc Titre	Pre Vacc Titre range	Mean Titre	Pre Vacc Titre	Post Vacc Titre range	Mean Titre
G. Pigs	Single dose (0.3)	<2	16-128	92.8	<0.3	1.21-2.11	1.68
G. Pigs	Repeated dose (0.3)	<2	32-256	147.2	<0.3	2.11-2.41	2.15
Control	No Vaccination	<2	<2	<2	<0.3	<0.3	<0.3

Key: CF = Complement Fixation, SN = Serum Neutralization

Table 3 shows the results of some blood immune cell parameters of the immunized and control guinea pigs. There was an increase in the population of the white blood cell, Red blood cells, Platelets, monocytes, granulocytes and lymphocytes. The control and vaccinated animals survived till the end of the experiment and none showed moribund or have any pathological damage at the site of vaccination. The data obtained from blood enzyme profiles of the vaccinated and control guinea pigs are presented in Table 4. The figures from the immunized and control guinea pigs were within the reference standard range for normal healthy guinea pigs. There was slight increase in the data obtained from the immunized test animals as compared to the control animals but not up to 2-fold rise as to be significant for any evidence of toxicity or organ damage and the guinea pigs were healthy till the end of the test period.

Table 3: Blood Immune Cell Parameters of Vaccinated and Control Guinea Pigs

Sample	WBC ($x10^9$)	RBC ($x10^{12}$/l)	HCT (%)	PLT ($x10^9$/l)	Mono ($x10^9$)	Gram ($x10^9$)	Lym ($x10^9$)
Test	76.62	5.69	48.84	434.80	1.55	63.10	5.82
SD	±3.10	±0.10	±0.23	±0.00	±0.12	±0.03	±0.00
Control	73.64	5.21	45.80	315.30	1.35	52.10	4.39
SD	±7.21	±0.58	±0.08	±0.04	±0.01	±0.98	±0.00

Key: SD = Standard Deviation. WBC = White Blood Cells ($x10^9$/L) ; RBC = Red Blood Cells ($x10^{12}$/L) HCT (PCV) = Pack Cell Volume(%); PLT = Platelet ($x10^9$/L); Mono = Monocytes ($X10^9$), Gran=Granulocyte($XI0^9$); Lym= Lymphocytes ($X10^9$)

Table 4: Data of Blood Enzyme Profiles in Vaccinated and Control Guinea Pigs

Sample	ALT (µ/l)	AST (µ/l)	Serum Urea (mmol/l)	Creatinine (mg/dl)	Bilirubin (mmol/l)
Tests	24.40	33.81	6.45	0.7222	3.34
SD	±1.09	±0.20	±0.18	±0.00	±0.00
Controls	23.75	32.75	6.08	0.713	3.13
SD	±0.00	±0.00	±0.08	±0.002	±0.56
Ref. Std.	21.45165.35	25.25-349.25	2.04-11.28	0.023-0.735	2.00-17.60

Key: AST = Aspartate Amino Transferase, ALT = Alanine Amino Transferase, SD = Standard Deviation
Reference Standard Source: Rabe, H. (2011) Vet test®8008 blood analyzer.

Discussion

Foot and mouth disease is a global economic disease of livestock especially among the cloven hoofed domestic animals. Its devastating consequences are seriously felt in the beef and dairy animals, resulting to huge

income loss (Chukwuedo et al, 2005). The use of FMD vaccines for the control and prevention of foot and mouth disease has been advocated especially in the disease endemic regions of the world (OIE, 2009). However, in some designated FMD free countries like United States of America, Canada, Mexico and United Kingdom, livestock are never vaccinated but they prefer the use of strict movement controls and slaughter of infected and contact animals when outbreak occur (Doel et al., 1994; Alexandersen et al., 2002).

In this present study, the locally developed FMD vaccine with the indigenous strains elicited FDM specific antibody to 146s virus antigen contain in the vaccine. The repeated vaccination of the test animals presented a booster immunological reactions. Both complement fixing and neutralizing antibodies were produced which are diagnostic in FMD vaccinated or naturally infected animal (Table 2). This finding has also been reported by Mowat et al. (1980) and Ferris and Donaldson (1984).

In some countries of Asia, South America and Africa, vaccination with chemically inactivated FMD vaccines has been adopted as the immediate protective measures to safe guard their animals during FMD virus outbreak (WOAH, 2008). The difference in the CF and SN antibody titers may be as a result of variation in the immunogenic stability of the various vaccine antigens used for the guinea pigs inoculations, also the different antibody ranges may be attributed to the susceptibility of the guinea pigs. It could be linked to other undetectable biological and environmental factors. This result agreed with the work reported by Ferris et al; (1984) and Mowat et al. (1995) on the FMD type A12 vaccine evaluation in guinea pigs and mice.

The serum neutralization test result was in-line with the studies on the efficacy of inactivated monovalent type A22 FMD vaccine reported by Misra and Lai (1990). The results in this study on the use of guinea pigs for evaluating inactivated FMD virus vaccines may help to reduce the high cost involved that makes the use of cattle and other higher ruminant an impractical proposition for routine innocuity testing. The choice of guinea pigs for the test was due to their harmless nature, cheap, easy to handle and maintain. They are as sensitive and susceptible with clinical signs as the target hosts (cattle, sheep, goats and pigs). Housing guinea pigs has the economy of space and has less tendencies for escape (Rweyemamu and Pereira, 1978; Abegunde et al., 1987) The adjuvant (ISA206) incorporated into the vaccine was a good excipient as no pharmacological or pathological reactions or damages were observed at the site of inoculations. These observations agreed with the findings of Nawathe and Majiyagbe (1981), Nicholls et al. (1990), Mowat et al. (1995) and Chukwuedo and Nimzing (2011) in their various attempts made in the development of foot and mouth disease vaccines using ISA 205 and 206.

Conclusion

Findings and information from this study showed that the formulated FMD vaccine, containing the indigenous FMD isolates with ISA 206 adjuvant was protective against the current field FMD virus strain. The vaccine can help to prevent the re-occurrence of the FMD outbreaks, reducing uncertainty and allowing farmers to plan better ahead. Vaccination would also help prevent the adverse effects on the welfare of the entire livestock owners. The use of FMD vaccines in the control of FMD outbreaks especially in endemic regions of the developing countries like Nigeria will help to boost the livestock sector and the industries as mortality due to FMD will reduce and increase animal population, alleviate poverty, improve animal protein supplies and ensure food security. It may help to stop the importation and in future encourage the export of beef and some animal products to other countries. The production and sales of the vaccine will generate employment and revenue for the country.

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