
Quality evaluation of two FMD Vaccines Prepared from local Isolates of sero types SAT1 and SAT2 Antigens and Montanide ISA 206 Formulations.

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ABSTRACT

Antibody responses in cattle and guinea pigs vaccinated with montanide ISA 206 adjuvant formulation vaccine were observed. In this study the potency of the inactivated FMD vaccines types SAT1 (Nig 1/98) and SAT2 (Nig 2/97) formulated with montanide ISA 206 adjuvant was determined in guinea pigs and cattle by antibody assay with CF and SN tests and by challenge. The antibody titres obtained with single and repeated inoculations gave good responses and protection from the challenge. The SAT1 146S (Nig. 1/98) antigen maintained a higher titre than SAT2 (Nig 2/97) antigen. The formulated vaccines were stable at 4°C throughout the duration of the study. It was also observed that animals with low and high antibody responses were all protected against FMD by vaccination that may not be, by strictly dependent on high antibody production in the host. The information from this study showed that it might be possible to prepare and use combined or polyvalent montanide ISA 206 FMD vaccines for control of FMD in Nigerian Livestock.

Key words: Vaccine, SAT1, SAT1, Montanide and antibody

INTRODUCTION

Foot and mouth disease virus (FMDV), an aphthovirus in the family, picornaviridae, is the cause of foot and mouth disease, a highly infectious disease of cloven hoofed domestic livestock and all animals of agricultural importance (Sherry et al, 2000). FMD control is mainly implemented by using chemically inactivated whole virus vaccine and immunization with conventional vaccines usually elicit high levels of circulating neutralizing antibody which correlated with protection against the homologous and antigenically related virus (Eric et al; 1998). However, chemically inactivated vaccines have a number of disadvantages which include the requirement for a cold chain for preservation and the risk of virus release during vaccine production (Banco et al 2001). All producers of Foot and mouth disease (FMD) vaccine realize that the ultimate test of their product is its capability of immunizing animals against the disease
Material and Methods

Antigen production

Two immunological distinct FMD virus isolates, type SAT 1 (Nig 1/98) and SAT 2 (Nig 2/97) were separately grown in BHK-21 cells in two replicates in 50 cm3 disposable tissue culture flask, (Nunclon, Inc-, USA). They were harvested separately when the culture attained maximal cytopathic effect (CPE), clarified by centrifugation at 3000 r.p.m. and stored at −30°C until required.

Virus Inactivation

The inactivation of virus infectivity was carried out by making a 1:10 dilution of 0.5% formalin in 0.04m phosphate buffer saline. This was added to the tissue culture harvest and final concentration held at 0.05% v/v. The inactivation reaction were stopped with excess sodium metabisulphite and the pH was maintained at 7.4 – 7.6 with 8.8% sodium carbonate solution. Complete inactivation of the vaccine virus was verified by culturing in fresh BHK-21 monolayer cells (Anderson et al 1970).

Preparation of vaccines

Each vaccine product was obtained as follows: The required volume of the inactivated virus was added to an equal volume of oil adjuvant (Montanide ISA 206). The mixture subsequently homogenized through centrifugation at 3000 r p m for 20 minutes. The homogenate was then kept at 4°C for 30 minutes. The final product was dispensed at 100ml volume in sterile 200ml screw – capped bottles, ready for use.

Sterility Test

The sterility test of the vaccines was tested by culturing the vaccine suspensions in bacteriological nutrient broth, blood agar, McConkey agar and sabouraud dextrose agar plates to determine microbial contamination.

Safety Test

The vaccine safety was carried out by subcutaneous inoculations of guinea pigs and cattle. The animals were observed for 3 weeks for pathological lesions and/or death.
Potency Test

The vaccine potency test was conducted in guinea pigs (at NVRI – Vom) and in cattle (at Alh Umaru’s herd, Rayfield, and Alh. Adamu’s herd, Bokkos). A minimum of 10 guinea pigs and 10 cattle were used per FMD vaccine type.

a. Vaccine test in Guinea Pigs

10 young adult guinea pigs, not less than 500g weight per guinea pig were inoculated subcutaneously with vaccine antigen (0.1ml per guinea pig). After 3 weeks post inoculation, the guinea pigs were killed and exanguinated, the serum separated and heat inactivated at 56°C for 30 minutes. The antisera were stored at -20°C until ready for use.

b. Vaccine testing in cattle

Adult cattle not less than 10 were placed in two separate groups. Each group per FMD vaccine type was inoculated with 3ml of the vaccine per cattle at single and repeated inoculation, subcutaneously. The cattle were bled after 3 weeks post inoculation, the serum was separated and heat inactivated at 56°C for 30 minutes. The antisera were stored at -20°C until ready for use.

Complement Fixation test for Antibody Detection technique was employed. Twenty-five microlitres of the antisera was diluted 2-fold serially in PBS in U-shaped 96 well microlitre plastic plates. Twenty-five microlitres of antigen (inactivated 1465 at 0.001g/ml) was added per well and the mixture was rocked gently and incubated at 4°C for 30-60 minutes. Equal volume of 1:400 complement suspension was added and the reagents were incubated at 4°C for 3 hours. Finally, 25ul of 4% adequately sensitised sheep red blood cell suspension was added. The plates were incubated at 37°C for 1 hour with gentle shaking at 15 minutes interval. CF titers were expressed as the reciprocal of the serum dilution producing 75% haemolysis.

Serum Neutralisation Test

Micro serum neutralisation method was used (Golding et al. 1976). The tests were carried out in microtitre (96 well flat bottom) plates. One hundred TCID<sub>50</sub> virus suspension was inoculated into BHK-21 monolayer cells and incubated at 37°C for 36 hours. The neutralisation titres were expressed as the reciprocal of the final dilution of the serum that neutralised the virus.

Post Vaccination Challenges

In guinea pigs, challenges were done by dipping the foot-pad of the hind legs of the vaccinated guinea pigs in PBS, pH 7.2 for 60 seconds and the challenge virus inoculum was carefully introduced (0.02ml) subcut at the foot-pad using tuberculin
syringe and needle. The infected foot-pads were wetted the second day and then observed for 3 days for the development of vesicles.

In cattle the animals were given 3ml of the field strain of the FMD virus subcutaneous and they were observed for two weeks for symptoms of FMD.

RESULTS

Table 1: Sterility assay by agar plate culturing of FMD Vaccine in ISA 206 Adjuvant.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Growth Media</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nutrient Broth</td>
<td>Blood Agar</td>
<td>MaConkey Agar</td>
</tr>
<tr>
<td>SAT 1 Vaccine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAT 2 Vaccine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inactivated Virus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ISA 206 Adjuvant</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key:  
+ = Microbial Growth  
- = No Microbial Growth

The sterility check of the different reagents after culturing and incubation at 37oc for 72 hours showed no microbial contamination.

Table 2: Complement Fixation Serum Titre Of Guinea Pigs Antisera Inoculated With FMD Vaccines.

<table>
<thead>
<tr>
<th>Vaccine Dose</th>
<th>Animal host</th>
<th>FMD Vaccine type</th>
<th>Prevacination CF titre</th>
<th>CF titre range</th>
<th>CF titre Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Dose</td>
<td>G. guinea</td>
<td>Nig. 1/98/SAT 1</td>
<td>&lt;2</td>
<td>16-128</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nig.2/97 SAT 2</td>
<td>&lt;2</td>
<td>8-32</td>
<td>14</td>
</tr>
<tr>
<td>Repeated Dose</td>
<td>Cattle</td>
<td>Nig.1/98 SAT</td>
<td>&lt;2</td>
<td>8-64</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nig.1/98 SAT</td>
<td>&lt;2</td>
<td>8-32</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>G. pigs</td>
<td>Nig.1/98 SAT 1</td>
<td>&lt;2</td>
<td>32-256</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nig.1/97 SAT 2</td>
<td>&lt;2</td>
<td>16-128</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Nig.1/98 SAT 1</td>
<td>&lt;2</td>
<td>32-512</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nig.1/97 SAT 2</td>
<td>&lt;2</td>
<td>32-128</td>
<td>52</td>
</tr>
</tbody>
</table>
The results showed that antiserum to Nig.1/98 SAT 1 146S antigen produced higher CF antibodies titre range than Nig.2/97 SAT 2 antigen. These were maintained in both guinea pigs and the cattle.

Table 3: Antiserum – Serum neutralisation titer values

<table>
<thead>
<tr>
<th>Vaccine Dose</th>
<th>Animal host</th>
<th>FMD Vaccine type</th>
<th>Pre CF titre</th>
<th>CF titre range</th>
<th>CF titre Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Dose</td>
<td>G. pigs</td>
<td>Nig.1/98 SAT 1</td>
<td>&lt;2</td>
<td>8-128</td>
<td>72</td>
</tr>
<tr>
<td>Inoculation</td>
<td></td>
<td>Nig.2/97 SAT 2</td>
<td>&lt;2</td>
<td>4-32</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Nig.1/98 SAT 1</td>
<td>&lt;2</td>
<td>16-64</td>
<td>32</td>
</tr>
<tr>
<td>Repeated Dose</td>
<td>G. pigs</td>
<td>Nig.2/97 SAT 2</td>
<td>&lt;2</td>
<td>8-32</td>
<td>16</td>
</tr>
<tr>
<td>Inoculation</td>
<td></td>
<td>Nig.1/98 SAT 1</td>
<td>&lt;2</td>
<td>32-512-68</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nig.2/97 SAT 2</td>
<td>&lt;2</td>
<td>16-128-48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Nig.1/98 SAT 1</td>
<td>&lt;2</td>
<td>32-256-68</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nig.2/97 SAT 2</td>
<td>&lt;2</td>
<td>23-128-62</td>
<td></td>
</tr>
</tbody>
</table>

Serum neutralisation titer gave higher titer values in hosts inoculated with Nig 1/98 SAT 1 in both single and repeated dose in guinea pigs and in cattle. It may be considered that the Nig1/98 SAT 1 is more immunogenic than the Nig 2/97 SAT 2, since its elicited high antibody in vaccinated animals.

DISCUSSION

Control of foot and mouth disease (FMD) in endemic regions is partly based on systematic immunization with chemically inactivated vaccines. These vaccines are used widely by many communities where the disease is prevalent. In some disease free countries like United Kingdom, their 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).

The use of inactivated FMD virus types in vaccine preparation for inoculation and production of antisera offers certain advantages. It provides more reproducible methods for their productions. It provides more reproducible methods for their production. Groups of guinea pigs with the same virus antigens can be housed in the same room. Similarly in the absence of either convalescent or post vaccinated antisera from livestock, the use of sera which are more compatible with those induced by vaccines are preferable for the immunological suitability of available vaccine for control of outbreaks (Rweyemamu and pay 1979; Ferris and Donaldson, 1984).

In this study it was found that antisera rose by single and repeated inoculation regime produced CF antibodies and had an acceptable level of CF antisera although often lower than hyper immune sera. The level of serotypic CF reaction was generally low. The difference in the CF antibody ranges may be a reflection of variation in the immunogenic stability of the various antigens used for guinea pigs and the cattle inoculations. Also the different antibody ranges may be linked to the susceptibility of the
animals. This result agreed with the work reported by Ferris et al. (1984) and Garland et al. (1977) on the FMD type A12 vaccine evaluation in guinea pigs and mice. The serum neutralisation result was in line with the studies on the efficacy of inactivated monovalent type A22 FMD vaccine reported by Misra and Lal. (1990).

Serum rose from single dose inoculation of FMD inactivated antigen did not contain VIA – antibody (Cowan and Graves, 1996). However, pinto and Garland (1979) found that multiple vaccination of cattle with formalin inactivated FMDV vaccine, can result in VIA antibody formation. This observation was found in the cross serum neutralisation test which gave <8 titre value from the repeated inoculation serum in cattle but not in guinea pigs serum. Likewise there is no cross reaction from the single dose inoculation serum. The relatedness of the present isolates to the earlier isolates in Nigeria were not tested because of the non-availability of the earlier isolates.

These results on the use of guinea pigs for evaluating inactivated FMDV vaccines may help to reduce the high cost involved in the use of cattle for routine innocuity testing. In conclusion, evidence from this work showed that the vaccine formulated with ISA 206 adjuvant was protective against the current field strain. However, it is suggested that more work be done using monoclonal antibody.

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REFERENCES


