The performance of Southern African Territories serotypes of foot and mouth disease antigen in oil-adjuvanted vaccines *

P. HUNTER **

Summary: The performance of selected oil adjuvants containing Southern African Territories (SAT) serotypes of foot and mouth disease virus was assayed by testing antibody levels elicited in cattle, sheep and goats, and by testing protection of cattle on challenge.

Various oil adjuvant formulations were tested initially in cattle and guinea-pigs, and compared with a standard alhydrogel and saponin-based (AS) vaccine. A commercial double oil emulsion vaccine elicited higher antibody titres and a more prolonged antibody response than the conventional AS vaccine, than a commercial single emulsion oil formulation or an incomplete Freund's adjuvant formulation. Incomplete Freund's adjuvant was selected for the formulation of a trivalent vaccine which was assayed in cattle, sheep and goats for antibody response and local reactions. The double oil emulsion elicited a high level of antibodies, which was maintained for at least six months after a single inoculation. The product was easily injectable and did not cause local or systemic reactions.


INTRODUCTION

Despite the fact that the last recorded case of foot and mouth disease (FMD) occurred in livestock in South Africa in 1983 (1), the disease remains important because Southern African Territories (SAT) serotypes 1, 2 and 3 are endemic in the largest game reserve in the country, the Kruger National Park (KNP), situated in the Mpumalanga. The KNP is populated with African buffalo (Syncerus caffer), which act as reservoirs of SAT virus types (15). Outbreaks of FMD occur regularly in impala antelope (Aepyceros melampus) in the KNP (4), presumably as a result of transmission of SAT type viruses from the buffalo with which the impala come into close contact.

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** Onderstepoort Institute for Exotic Diseases, Agricultural Research Council, P. Bag X6, Onderstepoort 0110, Republic of South Africa.
The KNP serves as a potential source of FMD infection for the surrounding farming areas. As the KNP reserve is a valuable ecological resource, and eradication of the entire buffalo population would be unacceptable, the zone surrounding the reserve is managed as a controlled area, in which stock are vaccinated biannually, inspected regularly and the movement of stock out of the area is subject to prior inspection and the issuing of movement permits.

FMD vaccines adjuvanted with alhydrogel-saponin (AS) have been used successfully to control the disease (6), but the use of these vaccines has some disadvantages in Southern Africa. As stock are farmed extensively in the area in which FMD vaccination is performed, it is logistically difficult to implement the vaccination regimen necessary for achieving optimal antibody levels in immunised animals (13). This situation requires that animals vaccinated for the first time should initially receive two priming inoculations, approximately one month apart, with booster inoculations every four to six months thereafter.

Due to the difficulty of administering the vaccine with the frequency required, the second priming inoculation is seldom given (1). For effective FMD control, a high antibody titre should be maintained in all animals, since, during outbreaks, there is often insufficient time for an anamnestic response to develop (13) (viral replication and invasion occur within 48 hours). Oil-based vaccines have been shown to achieve higher titres and longer persistence of antibody to FMD antigen in cattle, when compared with the AS vaccine (12). These oil-based vaccines have been used extensively in South American countries, where field studies have indicated that they provide better protection than the AS vaccine with fewer inoculations (9).

Since SAT type vaccines have not been assessed in oil formulations in cattle, a preliminary study was designed to compare the performances of SAT serotypes in oil and in AS vaccines. Antibody levels and protection conferred by oil-adjuvanted vaccines were investigated.

**MATERIALS AND METHODS**

**Virus strains used in experimental vaccines**

The monovalent vaccines used in experiment 1 contained SAT 1 strains SAR 09/81 and KNP 196/91. The trivalent vaccine used in experiment 2 also contained these SAT 1 viruses, in addition to SAT 2 strains ZIM 07/83 and KNP 19/89, and SAT 3 strain KNP 10/90.

**Antigen production**

The antigens used in all vaccines were produced as for routine vaccine production on baby hamster kidney (BHK) 21 clone 13 cells, using the monolayer roller bottle system, as described previously (11). The viral harvest was clarified using chloroform (1% v/v) and agitation, and the harvest was filtered through a filter press, using filter pads. The virus was inactivated with two doses of a final concentration of 0.001 molar (M) binary ethyleneimine (14). The inactivated harvest was concentrated by tangential cross-flow filtration, using cassettes with a cut-off point of 100,000 dalton and a surface area of 4.6 m². The antigen was stored in the gas phase of liquid nitrogen pending potency and safety testing.
Serology

Virus neutralisation tests were performed in a microtitre system as described previously (10). The virus strains used in the tests were SAR 09/81 (SAT 1), ZIM 07/83 (SAT 2) and KNP 10/90 (SAT 3).

146S determination

Quantification of 146S particles in the concentrate was performed using an ultraviolet photometric procedure on virus harvest which had been separated by centrifugation through a sucrose gradient (3).

Vaccine formulation

Antigen concentrates were used to formulate experimental vaccines. The 146S mass had been determined for these concentrates, and had a value of > 8PD₅₀ on the potency test carried out in accordance with the method of the British Pharmacopoeia. The vaccines were prepared by thawing the appropriate concentrate and diluting this to the required final concentration of 12 µg antigen per dose per SAT isolate. All vaccines compared in the study therefore contained the same antigen payload. Incomplete Freund’s adjuvant (IFA) was formulated using commercial adjuvants (2). A commercial single oil emulsion (SE) and the IFA emulsion were formulated using a benchtop homogeniser. Successful water-in-oil formulation was confirmed by conductivity measurements of less than 1 microsiemen and microscopic evaluation. A commercial double emulsion (DE) was formulated by stirring on a magnetic stirrer, as recommended by the manufacturer. The AS vaccine was formulated by diluting the concentrate with the required amount of phosphate-buffered saline. The final vaccine contained 25% of a 2% alhydrogel suspension. A saponin solution was added to the vaccine to give a final concentration of 5 mg per 3 ml dose (1.6 mg/ml).

Experimental procedure

An initial experiment was performed comparing the three different oil-adjuvanted vaccines described above, DE, SE and IFA, with an AS vaccine containing the same amount of antigen. The antibody response in cattle and level of protection in guinea-pigs were assayed. Only five animals could be challenged from one oil-adjuvant group. The oil formulation which performed the best with regard to antibody response was selected for the formulation of a trivalent vaccine, which was assayed in the second experiment. Antibody titres to SAT types 1, 2 and 3 were monitored in cattle, sheep and goats over time. Cattle in this group were challenged at seven and eleven months.

Cattle

Seronegative yearling Bonsmara cattle, held at an experimental farm outside the FMD-controlled area, were divided into four groups of ten. Each group received one of the following vaccines: AS vaccine, IFA formulation, a single water-in-oil emulsion and a double emulsion. The antigen used in the vaccines was SAT 1. The dose of the oil vaccines was 5 ml, while a 3 ml dose was used for the AS vaccine. The animals were given a single subcutaneous inoculation and bled at monthly intervals for six months.
Six months after receiving that inoculation, five animals from the IFA group only were available for challenge. These animals were brought into the Onderstepoort Institute for Exotic Diseases (OIED) laboratory and challenged intradermolingually with SAR, using two 0.1 ml inoculations of bovine-adapted SAT 1 strain SAR 09/81, containing $10^4$ infective doses. The cattle were examined daily for ten days post-challenge, for lesions on the feet and in the mouth. Each challenge test incorporated two seronegative controls.

In a second experiment, ten seronegative yearling cattle were inoculated, using a trivalent vaccine with the DE. Initially, the animals were bled at seven and fourteen days, and monthly thereafter for a period of 330 days. The sites of inoculation were examined daily for a month post-vaccination. These cattle were challenged seven and eleven months after immunisation with an SAR 09/81 strain.

Guinea-pigs

A comparative challenge in guinea-pigs was used as a screening method for the IFA, AS, DE and SE vaccines employed in the cattle experiment. The guinea-pigs were challenged using a previously described model (5). In this experiment, groups of ten guinea-pigs were inoculated subcutaneously with a dose of test vaccine so that the same antigen payload was administered for each vaccine. Vaccines were diluted with the appropriate adjuvant so that guinea-pigs received $1/32$ of the cattle dose (0.6 µg). The dilutions were made as follows: the oil vaccines were diluted to the required antigen concentration by adding vaccine to the appropriate oil adjuvant which contained buffer but no antigen in the aqueous phase. The diluent for the AS vaccine was prepared by adding 2% alhydrogel to Eagles' medium, so that the alhydrogel constituted 25% of the suspension. A saponin suspension was added to this, to give a concentration of 1.6 mg/ml. The guinea-pigs were challenged thirty days after vaccination, using a guinea-pig-adapted (GP#7) SAT 1 strain (SAR 09/81), along with five non-inoculated controls of the same sex and weight. In order to reduce the numbers of guinea-pigs used, the PD$_{50}$ was not determined but the percentage of protection was calculated.

Small stock

Forty seronegative adult sheep were held on an experimental station in the North-West Province. The sheep were vaccinated with 2 ml of the trivalent vaccine and monitored for 240 days. The animals were bled every second month for antibody determination. Local reactions were monitored by examining the injection sites and the general habitus of the sheep.

Twenty-four pregnant goats were inoculated subcutaneously, twelve with 1 ml of vaccine and the remaining twelve with 2 ml. The goats were observed for a period of one month for local reactions and deterioration in condition.

Antibody determinations were also performed in sheep and goats (see 'Experimental procedure'). Local reactions at the site of injection were monitored daily for a month post-vaccination.
RESULTS

Experiment 1 (monovalent vaccine)

Cattle

The results of the serology are shown in Figure 1. The antibody level induced by the AS vaccine fell below $\log_{10}^{1.6}$ at between two and three months after inoculation. The mean titres of all three oil preparations were higher than those induced by the AS vaccines. The DE vaccine performed the best, maintaining a titre of $\log_{10}^{2.2}$ antibody for the duration of the six-month period. An homologous challenge was performed on five cattle in the IFA group at the end of the six-month monitoring period. Three of the five animals inoculated were protected.

![Graph showing serology results](image)

**Fig. 1**

Comparative serology of foot and mouth disease vaccines: virus neutralisation titres to Southern African Territories serotype 1

No unacceptable reactions were detected. One animal in the DE and IFA groups showed a swelling of 60-70 mm in diameter, which disappeared within a week. No abcessation occurred in any of the groups. The IFA and SE emulsions were more viscous than the DE vaccine, which could easily be withdrawn in a plastic syringe fitted with a 21 g needle.
Guinea-pigs

No adverse systemic or local reactions were observed in any of the inoculated animals. The protection conferred by the various vaccines at a 1/32 dilution of antigen was as follows:

- DE 100%
- AS 80%
- IFA 66%
- SE 30%.

Experiment 2 (trivalent vaccine)

Cattle

The antibody response elicited by the trivalent DE vaccine over a period of eleven months (330 days) is shown in Figure 2. Antibody levels to all SAT serotypes were \( > \log_{10}^{2.2} \) at day seven post-vaccination and remained \( > \log_{10}^{1.6} \) for up to 330 days. One animal in the group died from babesiosis and could not be challenged. Cattle challenged at seven months showed 75% protection (3/4), and at eleven months showed 40% (2/5). No local or systemic reactions to the vaccine were observed in any of the ten cattle.

![Graph showing antibody levels over time](image_url)

**FIG. 2**

Virus-neutralising antibodies to trivalent double emulsion foot and mouth disease vaccine
Small stock

The antibody response in both sheep and goats remained higher than $\log_{10}^{1.6}$ for up to 240 days of the study, for all three SAT serotypes (Table 1). The inoculated sheep showed no local reactions. In the pregnant goats, neither the 1 ml nor the 2 ml dose caused any local response, loss of condition or abortions during the period of observation.

**TABLE I**

*Mean virus neutralisation titres to trivalent double emulsion vaccine in sheep and goats*

<table>
<thead>
<tr>
<th>Days post-vaccination</th>
<th>SAT 1 sheep/goats</th>
<th>SAT 2 sheep/goats</th>
<th>SAT 3 sheep/goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2.3/2.2</td>
<td>1.7/1.8</td>
<td>2.0/2.4</td>
</tr>
<tr>
<td>150</td>
<td>2.1/1.8</td>
<td>2.2/2.1</td>
<td>2.4/2.1</td>
</tr>
<tr>
<td>240</td>
<td>2.1/1.8</td>
<td>1.9/2.1</td>
<td>1.7/1.8</td>
</tr>
</tbody>
</table>

Titres are expressed as $\log_{10}$ of the reciprocal of the 50% serum end-point dilution

SAT: Southern African Territories

**DISCUSSION**

The findings in regard to the monovalent vaccine were consistent with those from other studies which record a fall in neutralising antibody three to four months after a single inoculation with AS vaccine (13). However, the three oil formulations elicited higher antibody levels after a single inoculation, and the antibody persisted at a level of $>\log_{10}^{1.6}$ for the six-month duration of the experiment.

The serological results obtained in experiment 2 indicate that the DE formulation selected for the trivalent vaccine gave high antibody titres as early as seven days after inoculation. This confirms the findings of other authors using the same commercial adjuvants (8), and demonstrates that oil-based vaccines do not necessarily have a slow-release mechanism or a delay in evoking an antibody response (7). This is important when considering the use of an oil-based vaccine during an outbreak, when a rapid response is required.

Over the eleven-month period of the study, the antibody values for all three serotypes remained above or equal to $\log_{10}^{1.6}$. From data obtained from potency testing at the OIED, it has been determined that the $\log_{10}^{1.6}$ value corresponds to approximately 70% homologous protection in the standard cattle potency test with the SAT isolates in the possession of the Institute (M.D. Gainaru, unpublished findings). The guinea-pig test was used as a screening test, rather than a measure of the performance of individual adjuvants, since there can be species differences in response to different adjuvants.

The antibody response induced by the DE formulation indicated that a single inoculation would suffice for a six-month period, after which a secondary inoculation would probably be necessary in primo-vaccinated animals. The low viscosity of this
vaccine, and the lack of tissue irritation in most groups of animals, make it a possible candidate for FMD vaccines in ruminants.

ACKNOWLEDGEMENTS

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PERFORMANCES DES SÉROTYPES SOUTHERN AFRICAN TERRITORIES DE L'ANTIGÈNE DE LA FIÈVRE APHTEUSE DANS LES VACCINS À ADJUVANT HUILEUX. – P. Hunter.

Résumé : Pour évaluer les performances de certains adjuvants huileux de l'immunité contenant des sérotypes Southern African Territories (SAT) du virus de la fièvre aphteuse, les auteurs ont recherché et titré les anticorps induits chez des bovins, des ovins et des caprins, puis contrôlé la protection conférée aux bovins lors d'une inoculation d'épreuve. Plusieurs formulations d'adjvant huileux ont d'abord été testées sur des bovins et des cobayes, puis comparées à un vaccin de référence à base d'hydroxyde d'aluminium et de saponine (vaccin AS). Un vaccin commercial à double émulsion huileuse a entraîné la production de titres d'anticorps supérieurs et d'une réponse immune plus longue que le vaccin AS classique, que la formulation commerciale à émulsion huileuse simple ou que celle administrée avec l'adjvant incolet de Freund. Cette dernière a été retenue pour la formulation d'un vaccin trivalent ; la réponse immune et les réactions locales induites par ce vaccin ont été étudiées chez des bovins, des ovins et des caprins. La double émulsion huileuse a permis d'obtenir un titre élevé d'anticorps, qui s'est maintenu pendant au moins six mois après simple inoculation. Le produit, facile à injecter, n'a pas entraîné de réactions locales ou systémiques.


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POTENCIA ANTIGÉNICA DE LOS SEROTIPOS SOUTHERN AFRICAN TERRITORIES EN VACUNAS OLEOSAS CONTRA LA FIEBRE AFTOSA. – P. Hunter.

Resumen: Los resultados obtenidos con una serie seleccionada de adyuvantes de aceite que incluían serotipos Southern African Territories de virus de la fiebre aftosa fueron sometidos a examen. Para ello se procedió a la evaluación.
del nivel de anticuerpos inducido en bovinos, ovejas y cabras, así como al estudio de la protección obtenida en los bovinos expuestos al virus.

En un principio se ensayaron sobre ganado y cobayas diversas formulaciones de adyuvantes aceitosos, y se compararon con una vacuna estándar a base de hidróxido de aluminio y saponina (AS). Una vacuna comercial con doble emulsión de aceite proporcionó títulos de anticuerpos más altos y respuesta inmune más prolongada que la vacuna AS convencional, que una fórmula comercial a base de emulsión simple de aceite y que una fórmula incompleta con adyuvante de Freund. Esta última fue elegida para la formulación de una vacuna trivalente, cuyo potencial para la producción de respuesta inmune y reacciones locales se puso a prueba sobre bovinos, ovejas y cabras. La doble emulsión de aceite inducía un alto nivel de anticuerpos, que se mantenía durante al menos seis meses tras una sola inoculación. El producto resultaba fácilmente inyectable y no provocaba reacciones locales o sistémicas.


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REFERENCES


