Immuno-serological studies on a foot and mouth disease virus type A strain involved in breakdown of vaccine immunity

L.M. BELWAL, A.P. KALANIDHI, K. NAGAIAH, B.C. RAMANNA and V.A. SRINIVASAN*

Summary: A foot and mouth disease virus type A strain, isolated from an outbreak in a vaccinated herd, was compared serologically with the vaccine virus by the two-dimensional microneutralisation test and immunologically through a cross-immunity test in cattle. The antigenic diversity of the field virus from the vaccine virus as revealed by serological analysis was substantiated by lack of cross-protection in primo-vaccinated animals. The anamnestic response consequent to infection was compared with that obtained through booster vaccination in terms of antibody levels and heterologous immunity. It was observed that anamnestic response had little effect on heterologous neutralisation, owing to the wide antigenic divergence.

KEYWORDS: Aphthovirus - Cattle diseases - India - Serological techniques - Serotypes - Vaccination.

INTRODUCTION

Immunity breakdown in vaccinated herds in the case of foot and mouth disease (FMD) is not uncommon. It has been attributed to factors such as quality and usage of the vaccine, severity of infection (incidence rate), immunity of the animals at the time of infection and antigenic variation among FMD virus strains (9, 10, 11). The present report gives an account of FMD in animals 45 days after vaccination with a quadrivalent vaccine containing BEI-inactivated type O, A, C and Asia 1 strains of Indian origin adsorbed on aluminium hydroxide gel and adjuvanted with saponin. The antigenic diversity of the type A strain involved in the outbreak from vaccine virus was investigated by the serum neutralisation test and heterologous challenge in vaccinated cattle. Rweyemamu (11) and Pay (10) have reviewed enhancement of heterologous immunity by revaccination in the face of an outbreak due to a variant strain. This phenomenon was investigated by comparing homologous and heterologous virus neutralisation by anamnestic response sera obtained after challenge and after booster vaccination.

MATERIALS AND METHODS

Viruses

The field virus referred to as type A KAB 66/84 was adapted to a baby hamster kidney (BHK 21 C1.13) cell line and maintained in a virus seed bank system with

* Indian Immunologicals, 11-4-657 Lakdi-Ka-Pul, Hyderabad 500 004, India.
passage history: A KAB 66/84 BHK 2 E/T BHK 7 (E/T = ether treated). Two vaccine virus strains and one candidate vaccine virus strain were employed for reference (Table I).

**Table I**

*Details of reference virus strains employed to differentiate the field virus by two-dimensional microneutralisation tests*

<table>
<thead>
<tr>
<th>Reference virus</th>
<th>Passage history</th>
<th>Place of origin</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;22&lt;/sub&gt; India 57/59</td>
<td>BHK E/T BHK 8 Susp. 5</td>
<td>Dharmapuri, Tamilnadu, India</td>
<td>Vaccine strain incorporated in the quadrivalent vaccine containing A&lt;sub&gt;22&lt;/sub&gt; subtype for A valency.</td>
</tr>
<tr>
<td>A (Holland)*</td>
<td>BHK 6 Susp. 9</td>
<td>FMD Laboratory, Amsterdam. Obtained from Indian Veterinary Research Institute, Bangalore, as BHK cell.</td>
<td>Vaccine strain incorporated in the quadrivalent vaccine not containing A&lt;sub&gt;22&lt;/sub&gt; subtype.</td>
</tr>
<tr>
<td>A India 7/82</td>
<td>E/T Bty 2 BHK 3 Susp. 6</td>
<td>Kaira, Gujarat, India. Obtained from Animal Virus Research Institute, Pirbright, UK, as bovine thyroid cell culture isolate.</td>
<td>Candidate vaccine strain identified and under study at Indian Immunologicals, Hyderabad.</td>
</tr>
</tbody>
</table>

BHK — Baby hamster Kidney cell line; E/T — ether treatment; Susp. — BHK suspension cells; Bty — Bovine thyroid primary cell culture.

* Referred to as subtype A5 when imported as vaccine strain in 1964, but later designated as A10 on recommendation of World Reference Laboratory for FMD, AVRI, Pirbright, UK (7).

**Antisera**

Immune sera against reference viruses were prepared by inoculating sero-negative steers 16-18 months old with monovalent vaccine. The steers were given a booster on the 21st day and bled on the 35th day after vaccination. Sera were pooled before use.

**Animals**

Cross-bred male calves for the experiment were obtained from the Holding Farm of Indian Immunologicals, Hyderabad (India) where unvaccinated calves 1-30 days old were procured from farms which had no outbreak of FMD during the previous two years and were kept as cattle fully susceptible to FMD.

**In vitro examination of antigenic diversity**

The two-dimensional microneutralisation tests were carried out by the method of Rweyemamu et al. (12) using bovine immune sera against the reference viruses.
The antigenic diversity of the field virus from the reference viruses was determined in terms of ‘r’ values obtained as follows:

\[
\frac{\text{serum titre against heterologous virus}}{\text{serum titre against homologous virus}} = r
\]

When ‘r’ values were less than 1 there was antigenic divergence. The significance of this divergence was tested by using an estimated pooled variance of 0.106 (13).

**In vivo examination of antigenic diversity (heterologous challenge)**

Five male calves 16-18 months old were vaccinated with the same dose, route of inoculation and batch of vaccine as used at the farm where the outbreak mentioned above had occurred. The quadrivalent vaccine contained A.22 India 57/79 strain. 45 days after vaccination (DAV) the vaccinated animals were exposed to contact challenge with the field virus. The field virus A KAB 66/84 was inoculated into the tongue of two susceptible animals (nos. 629 and 715) which were then used as a source of virus for contact challenge. Three vaccinated animals (nos. 305, 641 and 687), one unvaccinated control (no. 624) and one FMD virus infected animal (no. 629) in one group and two vaccinated animals (nos. 710 and 711), one control (no. 653) and the other inoculated animal (no. 715) in another group (Table II) were housed in two loose boxes in the high-security premises for large animals at Indian Immunologicals, Hyderabad. All the animals were examined daily for rise in body temperature and development of lesions. Animals showing lesions of the tongue or feet were considered unprotected.

**TABLE II**

*Details of animal groupings for heterologous challenge*

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Tag numbers of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacccinated animals</td>
<td>305,641,687</td>
</tr>
<tr>
<td></td>
<td>710,711</td>
</tr>
<tr>
<td>Animals inoculated with field</td>
<td>629</td>
</tr>
<tr>
<td>virus A KAB 66/84</td>
<td>715</td>
</tr>
<tr>
<td>Controls</td>
<td>624</td>
</tr>
<tr>
<td></td>
<td>653</td>
</tr>
</tbody>
</table>

**Monitoring the anamnestic response after challenge and the development of early convalescent immunity**

The anamnestic response to challenge infection in vaccinated animals and the development of convalescent immunity in unvaccinated animals were monitored by serum antibody assay. The schedule for collecting serum samples for this purpose was as follows:

- The in-contact vaccinated cattle were bled before exposure (45 days after vaccination), 12 hours later and then at 8-hour intervals for 3 days, 12-hour intervals from the 4th to the 7th days and once on days 8, 12 and 16 after exposure.
The inoculated and control animals were bled at 24-hour intervals for seven days and then once 12 days after inoculation or 16 days after exposure (Table III).

**Table III**

*Schedule of serum sampling from contact exposed and inoculated animals*

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Vaccinated contact challenged animals</th>
<th>Unvaccinated inoculated animals</th>
<th>Unvaccinated controls challenged by contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>before exposure (45 DAV)</td>
<td>before inoculation</td>
<td>before exposure</td>
<td></td>
</tr>
<tr>
<td>12 hours after exposure and at 8-hour intervals for 1 to 3 days, then 12-hour intervals from days 4 to 7</td>
<td>24-hour intervals for 7 days</td>
<td>24-hour intervals for 7 days</td>
<td></td>
</tr>
<tr>
<td>once on days 8, 12 and 16</td>
<td>once on days 12 and 16</td>
<td>once on days 12 and 16</td>
<td></td>
</tr>
</tbody>
</table>

**Monitoring the booster response**

To monitor the response to booster vaccination a group of five animals was revaccinated 21 days after primary vaccination. These animals were sampled before the booster vaccination, then at 8-hour intervals for 3 days, and 12-hour intervals on the 4th and 5th days, and finally 35, 60 and 90 days after primary vaccination. The anamnestic response was monitored by serum antibody assay against all the four valencies incorporated in the vaccine used for primary and booster vaccination.

**Serum antibody assay**

Serum antibody assay was done by the microneutralisation cytopathic effect test. Serial two-fold dilutions of serum were reacted with 100 TCID$_{50}$ of virus, and residual infectivity was detected by employing IBRS2 cell lines. Serum neutralisation end points were expressed as the log$_{10}$ value of the reciprocal of highest serum dilution which neutralised 100 TCID$_{50}$ of virus. Sera from vaccinated contact-challenged animals were assayed for antibodies against type A virus while those from unvaccinated and boosted animals were tested against O, A, C and Asia 1 serotypes of FMD virus. The sera were tested in replicate, and mean values of the serum titres were employed for further analysis.

**Homologous and heterologous neutralisation by anamnestic response sera**

The peak antibody levels obtained in the anamnestic response to booster vaccination and contact challenge were examined for homologous virus neutralisation characteristics by the two-dimensional microneutralisation test as described previously (12). One serum sample from a boosted animal and two sera from vaccinated contact-challenged animals (nos. 305 and 710) were employed for comparison.

**RESULTS**

**Serological relationship**

The 'r' values from two-dimensional neutralisation tests are presented in Table IV.
Antigenic relationship (‘r’ value) of field virus A KAB 66/84 with reference virus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Antiserum</th>
<th>A&lt;sub&gt;22&lt;/sub&gt; India 57/79</th>
<th>A Holland 7/82</th>
<th>A India</th>
</tr>
</thead>
<tbody>
<tr>
<td>A KAB 66/84</td>
<td>0.01*</td>
<td>0.1*</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

* ‘r’ significantly <1 at p = 0.01

Heterologous challenge

All the vaccinated and control animals exposed to contact challenge developed FMD with various degrees of clinical signs. The animals inoculated with the virus showed a rise in body temperature and vesicle formation at the site of inoculation 24 hours later; the disease generalised 84 to 108 hours after inoculation. Two of the vaccinated animals and one control animal did not show a rise in body temperature. The remaining animals, one control and three vaccinated, showed a rise in body temperature 84 to 132 hours (3 to 5 days) after the challenge exposure. While the disease in all the animals exposed to contact challenge became generalised 5 to 7 days after exposure, only the control animals had tongue lesions and no vaccinated animal developed vesicles on the tongue. It was observed that the disease in inoculated animals became generalised 60 to 72 hours after the rise in body temperature, whereas ‘in-contact’ animals developed the generalised disease within 24 hours of the rise in temperature (Table V).

| TABLE V |

| TABLE IV |

Development of the anamnestic response to challenge and convalescent immunity

The results obtained from serum antibody assay are presented in Fig. 1, showing antibody profiles of vaccinated animals and their group mean. These give an indication of the development of anamnestic response against type A virus in animals.
primed with quadrivalent vaccine and stimulated with type A heterologous challenge. On challenge (45th day) 4 of 5 animals had high serum titres (log$_{10}$SN$_{50} > 2.0 < 3.0$) while one had lower titre (log$_{10}$ SN$_{50} > 1.0 < 2.0$) for type A homologous virus strain. These levels remained more or less the same until the 7th day after exposure when a rising trend was observed which continued to rise until day 16 (sampling limit). Fig. 2 shows the development of convalescent immunity in inoculated and contact-exposed unvaccinated control animals. Neutralising antibodies appeared in the inoculated (unvaccinated) animals after 5-6 days and did not become type-specific until the 12th day. By the 16th day heterotypic neutralisation appeared to be on the decline while type-specific antibodies (against type A) continued to rise. In the unvaccinated control animals, neutralising antibodies were detected from 6 to 8 days after exposure. Heterotypic and type-specific neutralisation characteristics were similar to those in the inoculated animals.

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

**FIG. 1**

Serum antibody profiles of contact-challenged vaccinated animals and their group mean against type A virus

**Anamnestic response after booster vaccination**

The mean serum antibody profiles against four vaccine valencies from a group of five animals are presented in Fig. 3. It can be seen that upon revaccination 21 days after primary vaccination, titres in these animals were too high for a clear appreciation of a anamnestic response. However, no significant rise in antibody levels occurred before 5 days after revaccination for any virus type. There was then an increase in antibody level which peaked at the 5th day for valencies O and C, on the 14th day for A and Asia 1 and declined thereafter as sampled 14, 39 and 69 days after revaccination.
FIG. 2
Serum antibody profiles of unvaccinated, inoculated and contact-exposed animals and their respective group means against type A virus
Comparison of heterologous and homologous virus neutralisation by serum obtained after challenge and after revaccination

The homologous and heterologous neutralisation titres obtained in the two-dimensional microneutralisation test are presented in Table VI. It was observed that the homologous neutralisation titre increased after booster dose but the heterologous neutralisation titre remained unaffected. In contrast, higher heterologous neutralisation titres were obtained with 16-day serum than with pre-challenge serum from “vaccinated convalescent” animals.

**TABLE VI**

*Comparison of field virus A KAB 66/84 with vaccine virus using serum samples obtained before and after vaccination (A) or challenge (B)*

<table>
<thead>
<tr>
<th>Animal identification</th>
<th>Serum</th>
<th>$\log_{10} SN_{50}$ for vaccine virus</th>
<th>$\log_{10} SN_{50}$ for field virus</th>
<th>Animal identification</th>
<th>Sera</th>
<th>$\log_{10} SN_{50}$ for vaccine virus</th>
<th>$\log_{10} SN_{50}$ for field virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>659</td>
<td>0 DAR</td>
<td>1.94</td>
<td>&lt;1.2</td>
<td>305</td>
<td>0 DAC</td>
<td>1.31</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 DAC</td>
<td>4.91</td>
<td>3.3</td>
</tr>
<tr>
<td>5 DAR</td>
<td>3.02</td>
<td>&lt;1.2</td>
<td></td>
<td>710</td>
<td>0 DAC</td>
<td>2.18</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 DAC</td>
<td>6.07</td>
<td>3.58</td>
</tr>
</tbody>
</table>

DAR = days after revaccination. DAC = days after challenge.
DISCUSSION

Antigenic variation among type A viruses has been recorded by many authors, including Traub and Mohlmann (16) for European strains, Brooksby et al. (5) for Mexican, Arrowsmith (2) for Middle Eastern, Anderson et al. (1) and Ndeti et al. (8) for Kenyan, and Rwemymamu et al. (14) for Asian strains. To delineate the antigenic relationship among Indian type A strains, we compared (3) a range of type A viruses employing representative strains from different geographical regions. The complex interrelationship among Indian type A viruses indicated the occurrence of more than one distinct antigenic group within the type. Viruses prevalent in northern and north-western territories could be placed in one group while viruses from southern Indian regions could be placed in another. The magnitude of diversity was suggestive of a possible lack of cross-protection. The present study demonstrates that the virus responsible for breakdown of immunity in a southern Indian territory was serologically close to a north-western type A strain (A India 7/82) and divergent from the vaccine strain (A22 India 57/79) which is of south India origin. This substantiates an argument proposed in our previous report (3) that the emergence of a variant strain in another region could endanger the vaccinal immunity afforded by a representative strain from that region.

The difference in time interval for a rise in body temperature and generalisation between inoculated and contact-exposed animals (60 to 72 hours versus 24 hours) might be due to the different route of entry of virus. However, the results in vitro and in vivo indicate that the field virus A KAB 66/84 differs antigenically from vaccine virus A22 India 57/79 (r<1 at 1% significance level) and was able to break through the vaccinal immunity afforded by the latter.

Analogous to the practice of establishing a serodiagnosis by examining seroconversions in “paired” serum samples, obtained 3 weeks apart, in the case of infectious diseases, we examined “paired” sera from FMD-affected animals and observed (unpublished data) that in the animals primed with vaccination, even the serum collected on the same day as tongue lesion samples contained very high antibody levels against the homologous serotype which had not changed 3 weeks later. This suggests that an anamnestic response occurred concurrent with the development of clinical symptoms. The present study suggests that, although the anamnestic peaks were obtained one or two days after development of clinical symptoms (Fig. 1), it is difficult to draw any definite conclusion owing to the absence of mouth lesions in the animals. In the case of high antibody titres in the first of “paired” serum samples from a field case, mouth lesions are invariably observed.

The observation that anamnestic peaks obtained after booster vaccination give high homologous titres, while heterologous neutralisation remains more or less unchanged, supports the views put forth by Rwemymamu (11) and Pay (10). The former argued that outbreaks caused by viruses giving r<1 (0.05 > p > 0.01) may be restrained by booster vaccination, but the presence of virus exhibiting higher antigenic diversity (r < 1, p = 0.01) warrants a change of vaccine virus in situations where the emergent variant has established itself endemically. Pay (10) observed that, in situations where r < 0.01, the immunity provided by the booster dose may be effective for only 1 to 2 weeks. In the present situation with a quadrivalent vaccine, it was demonstrated that, although the homologous neutralisation titres increase to a high level after booster vaccination, there is not even a brief gain in heterologous neutralisation owing to the magnitude of antigenic diversity of the field virus.
According to Brooksby (4), heterotypic neutralisation by early convalescent sera was demonstrated at Pirbright in the 1930's. Skinner (15) also reported similar results and Brown and Graves (6) investigated the classes of immunoglobulins finding that Ig M, which was dominant in early convalescence, could be responsible for the lack of strain specificity. In our study the sera were non-specific to four serotypes of FMD virus until 16 days after inoculation (sampling limit). However, from the 12th day onwards the type-specific neutralisation titres increased, whereas heterotypic neutralisation titres seemed to decline. From the present observations and cited work it seems probable that the lack of strain specificity in early convalescent bovine serum extends to its inability to distinguish between the distinct serotypes. Since the establishment of serotype identity involves demonstration of complete lack of cross-protection in a convalescent animal, determination of the time scale at which virus neutralising activity of such serum becomes type-specific is important, because heterotypic neutralisation is likely to mask the true serotype identity of an atypical isolate.

* * *


Résumé : Une souche de virus aphteux de type A, isolée dans un foyer de fièvre aphteuse sur un troupeau vacciné, a été comparée avec le virus vaccinal par un test de microséronutralisation à deux dimensions et par une épreuve d'immunité croisée sur bovins.

L'hétérogénéité antigénique du virus sauvage par rapport au virus vaccinal a été révélée par l'analyse sérologique et confirmée par l'absence de protection croisée sur animaux primovaccinés. La réponse anamnestique consécutive à l'infection a été comparée à celle obtenue par une vaccination de rappel en ce qui concerne le taux des anticorps et l'immunité hétérologue. On a observé que la réponse anamnestique avait peu d'effet sur la séronutralisation hétérologue en raison de la grande différence antigénique entre ces deux virus.


* * *


Resumen: Una cepa de virus aftoso de tipo A aislada en un rebaño vacunado situado en un foco de fiebre aftosa fue comparada con el virus vacunal
mediante un test de microseroneutralización en dos dimensiones y una prueba de inmunidad cruzada en bovinos.

El análisis serológico puso en evidencia la heterogeneidad antigénica del virus salvaje respecto del virus vacunal, hecho que se vio confirmado por la ausencia de protección cruzada en los animales primovacunados. La tasa de anticuerpos y la inmunidad heteróloga obtenida mediante la respuesta de anamnesis posterior a la infección fue comparada con la obtenida por medio de la revacunación. Se constató que, debido a la gran diferencia antigénica entre ambos virus, la respuesta de anamnesis no tenía mayor efecto sobre la seroneutralización heteróloga.

PALABRAS CLAVE: Enfermedades de bovinos - India - Serotipos - Técnicas serológicas - Vacunación - Virus aftoso.

REFERENCES


