Immune response of newborn calves to vaccination with foot-and-mouth disease vaccine

H. SHANKAR* and P.K. UPPAL**

Summary: The immune response of calves (1-90 days old) born to FMD vaccinated and unvaccinated cows, after vaccination with monovalent saponified gel type A FMD vaccine, was studied. Most of the prevaccination sera of calves (born to FMD vaccinated cows) showed varying levels of maternal antibody with the SN indices ranging from 0.5 to 3.2, while those born to unvaccinated cows showed negligible levels. Calves of both groups showed significant rise in SN antibody titres at 21 days post-vaccination. However, this rise was more appreciable in calves (born to vaccinated cows) having log SN index less than 1.0 before vaccination and in those which were born to unvaccinated cows. The second inoculation of other half of the adult dose at 21 days after primary vaccination resulted in boosting up the antibody response in all calves. The calves vaccinated twice withstood challenge infection with homologous virulent virus. These findings indicate that vaccination of calves with FMD vaccine given in split doses at 21 days intervals may be taken up successfully even within a week after birth.

Foot-and-mouth disease (FMD) is an important animal health problem. It inflicts heavy mortality in newborn calves where the virus generally acquires myotropic propensity (Geiger and Garbe, 1959; Brooksby, 1967). Our earlier study of a cross section of population of colostrum fed calves (1-90 days old) born to FMD vaccinated cows, revealed that many calves were deficient in colostral antibodies (Uppal et al., 1975; Shankar and Uppal, 1981). In view of these facts and the endemicity of the disease in India, it was felt necessary to protect these calves from birth. Uppal et al. (1975) suggested that calves could be vaccinated at birth. The present paper records further investigations on the immune response of calves (1-90 days old) to vaccination with monovalent saponified gel type A FMD vaccine.

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MATERIALS AND METHODS

1. Virus.

FMD virus type A (subtype A5) was obtained as infected cattle tongue epithelium from the FMD Laboratory, Indian Veterinary Research Institute, Mukteswar-Kumaon (U.P.). Goat kidney cell culture adapted strain was used for preparation of the vaccine, while the strains maintained in cattle were used for propagation in calf kidney cell culture and challenge infection in calves or hill bulls.

2. Vaccine.

Type A (subtype A5) FMD vaccine was prepared using infected goat kidney cell monolayers, as described by Uppal et al. (1972). The composition of the vaccine included 10% aluminium hydroxide gel, 80% virus suspension (with titer $10^{-6}$ TCID$_{50}$ per ml), 2% phosphate buffer, 1% glycerol, 0.06% formalin. The sterility, safety and potency testing of the vaccine was by the standard procedures. The adult dose of this vaccine was 5.00 ml.

3. Experimental design.

Calves (1-90 days old) for vaccination experiments were selected randomly from the vaccinated and unvaccinated cow herds. The cows in the vaccinated herds received several annual vaccinations of tetravalent (O, A, C and Asia 1) alhydrogel FMD vaccine, while the unvaccinated cow herd comprised those which were neither vaccinated nor had known history of an FMD outbreak during the past 6-8 years. Sera from these calves were collected before vaccination. The calves were then divided into two groups each by randomization with a minimum of four animals in each group. Experiments on calves born to FMD vaccinated and unvaccinated cows were carried out separately.

One group of animals was vaccinated with a single full adult dose (5.00 ml) by subcutaneous route in the dewlap region. Another group of calves was vaccinated with half of the adult dose (2.5 ml) which was followed by the inoculation of the other half dose at 21 days after primary vaccination.

Sera samples from vaccinated calves were collected at 21, 50 and 80 days after primary vaccination. These were centrifuged and stored at $-20^\circ$C without preservative till used. Seroconversions were estimated for each individual calf.

4. Serum neutralization test.

Constant serum and variable virus dilution method was followed. Tenfold serial dilutions of the virus were made in Hank's maintenance medium. To this an equal volume of 1:10 dilution of the test serum was added. The serum neutralization (SN) results were calculated by the method of 50% end
point virus neutralization dose according to Reed and Muench (1938) formula and expressed as log SN index (SNI).

5. **Challenge infection.**

Some of the vaccinated calves (born to vaccinated cows) were challenged by inoculation of 10,000 cattle ID 50 per ml of virulent virus (homologous strain) at each of the 10 sites on the dorsal mucosa of the tongue, along with unvaccinated control calves (Henderson, 1952). These animals were observed for a period of ten days after challenge infection for the development of primary and secondary lesions.

6. **Statistical tests.**

The experimental data were subjected to statistical analysis using standard methods (Snedecor and Cochran, 1967). The significance of values was estimated by analysis of variance, « t » test and Duncan’s multiple range test.

**RESULTS**

1. **Calves born to FMD vaccinated cows.**

The prevaccination sera of these calves had varying levels of maternal antibody with the SN indices ranging from 0.5 to 3.2. The results are presented in Table I.

Analysis of variance of collected data of neutralizing antibody response in respect of calves of both groups revealed that there was a significant rise in SN antibody titres at 21 days post-vaccination in spite of varying levels of SN antibody in their prevaccination sera (Table III). It was noted that the calves having a log SN less than 1.0 before vaccination, showed a higher rise in titre after vaccination (Table I).

In the group of calves which received a single vaccination, the mean log SNI values were recorded to be 1.70, 3.54, 3.20 and 2.83 respectively at 0, 21, 50 and 80 days post-vaccination. In the calves which were vaccinated twice with half of the adult dose at 21 days interval, the mean log SNI values were 2.00, 3.00, 4.64 and 4.1 at 0, 21, 50 and 80 days after primary vaccination respectively.

Mean log SNI values of the calves in both groups were compared by applying Duncan’s multiple range test. In the calves which received a single adult dose, the mean SNI values at 21 and 50 days post-vaccination were significantly higher than the prevaccination values. However, the difference in values at 21 and 50 days post-vaccination were not significant (Table I). In the sera of calves of the second group, receiving half of the adult dose, the values were significantly higher at 21 days post-inoculation than pre-inoculation values. When the other half dose was given at 21 days after pri-
TABLE I

*Immune response in calves (born to vaccinated cows)*
to vaccination with monovalent saponified gel type A FMD vaccine

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vaccination</th>
<th>No. of calves</th>
<th>Age at 1st vaccination (mean in days)</th>
<th>Dose of vaccine</th>
<th>Mean log SNI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primary vaccination</td>
<td>Secondary vaccination</td>
</tr>
<tr>
<td>I</td>
<td>Single</td>
<td>5</td>
<td>19.8</td>
<td>5 ml</td>
<td>1.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.50</td>
<td>±0.23</td>
</tr>
<tr>
<td>II</td>
<td>Double</td>
<td>5</td>
<td>13.6</td>
<td>2.5 ml 2.5 ml</td>
<td>2.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.28</td>
<td>±0.29</td>
</tr>
</tbody>
</table>

TABLE II

*Immune response in calves (born to unvaccinated cows)*
to vaccination with monovalent saponified gel type A FMD vaccine

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vaccination</th>
<th>No. of calves</th>
<th>Age at 1st vaccination (mean in days)</th>
<th>Dose of vaccine</th>
<th>Mean log SNI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primary vaccination</td>
<td>Secondary vaccination</td>
</tr>
<tr>
<td>I</td>
<td>Single</td>
<td>4</td>
<td>56.2</td>
<td>5 ml</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.12</td>
<td>±0.21</td>
</tr>
<tr>
<td>II</td>
<td>Double</td>
<td>4</td>
<td>41.5</td>
<td>2.5 ml 2.5 ml</td>
<td>0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.18</td>
<td>±0.13</td>
</tr>
</tbody>
</table>

* Second vaccination was carried out on 21st day of primary vaccination.
### Table III

*Analysis of variance for immunological response of calves to vaccination with type A saponified gel vaccine*

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Calves born to vaccinated cows</th>
<th>Calves born to unvaccinated cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>S.S.</td>
</tr>
<tr>
<td>Between groups</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Between days</td>
<td>2</td>
<td>22.418</td>
</tr>
<tr>
<td>Between groups × days</td>
<td>2</td>
<td>4.938</td>
</tr>
<tr>
<td>Between animals/groups</td>
<td>8</td>
<td>8.43</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>4.814</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>4.814</td>
</tr>
</tbody>
</table>

* Significant at 5% level of significance.
mary vaccination, the mean SNI values were significantly higher at 50 days than those observed either before inoculation or at 21 days after primary vaccination.

The SNI values of the calves which received single and double vaccinations were compared at 0, 21 and 50 days post-vaccination. No significant differences in the mean SNI values before vaccination (0 day) of the calves of both groups was evident (Table I). In calves bled at 21 days post-immunization with a single full adult dose, the mean log SNI values were higher than the values at 21 days after primary vaccination in the calves with half of the adult dose, but the differences were not significant. The calves which received the other half dose of the vaccine at 21 days after primary vaccination showed significantly higher SNI values at 50 days after primary vaccination than at 21 and 50 days post-vaccination in the calves receiving a single full dose of the vaccine.

Measurement of the SNI values at 21, 50 and 80 days post-vaccination of the calves vaccinated with a full adult dose of vaccine revealed peak antibody titre at 21 days post-vaccination followed by a gradual decline in titre at 50 and 80 days post-vaccination (Fig. 1). Similarly, the calves which received half of the adult dose also showed a rise in the mean SNI values at 21 days post-inoculation but it was less than the calves in the single dose group. The second inoculation at 21 days resulted in boosting up the antibody response at 50 days after primary vaccination, which was higher than that of the calves of the first group. Thereafter the decline in titre was observed at the 80th day.

![Immune response of calves to vaccination with saponified gel type A FMD vaccine](image)
2. Calves born to unvaccinated cows.

The prevaccination sera of calves showed negligible level of SN antibodies and their log SNI values ranged from 0.00 to 1.0 (Table II). Analysis of variance of collected data of antibody response of calves of both groups revealed that there was a significant rise in SN antibody titres at 21 days post-vaccination (Table III).

In calves which were inoculated with a single full adult dose of vaccine, the mean log SNI values were recorded to be 0.25, 3.30, 3.00 and 2.53 respectively at 0, 21, 50 and 80 days after primary vaccination. In calves vaccinated twice the respective values were 2.00, 3.00, 4.64 and 4.10 (Table II).

In general, the pattern of antibody response in these calves was more or less similar to that observed in calves born to vaccinated cows (Tables II and III).

3. Comparison of antibody response of calves born to vaccinated and unvaccinated cows.

It was observed that the increase in antibody titres at 21 days post-vaccination in calves born to unvaccinated cows with negligible levels of antibodies before vaccination was significantly higher than in calves of vaccinated cows having varying levels of antibodies prior to vaccination (Table IV).

4. Challenge infection.

Two calves immunized with a single and two calves with a double dose of vaccine were challenged at 50 days post-vaccination and 50 days after primary vaccination respectively. The calves receiving a single vaccination dose showed mild lesions on the tongue and very mild vesicles on gums and one of them showed very mild vesicles on the lips also. One showed a small vesicle in the right fore and the other in the right hind foot. The calves vaccinated twice showed moderate to marked lesions on the tongue only. No secondary lesions were seen in these two calves (Table V).

Control animals reacted severely showing both primary and secondary lesions (Table V). One of the three calves died on the 7th day of challenge infection showing typical lesions on heart and rumen pillars. Virus was recovered, in unweaned mice, from heart muscle of this calf.

It is evident from these studies that calves vaccinated twice were resistant to challenge, whereas control calves showing severe challenge reactions possessed poor SNI values ranging from 1.2 to 2.0 at the time of challenge.

DISCUSSION

Since maternal antibodies have been known to persist in calves up to 2-6 months of age (Gagliardi, 1974; Kalmer, 1874; Van Bekkum, 1974), the question of optimal age at which FMD vaccination should be started has
<table>
<thead>
<tr>
<th>Groups</th>
<th>Test animals</th>
<th>Mean of the increase in SNI values at 21 DPV (+)</th>
<th>Difference of means</th>
<th>t</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>I*</td>
<td>Calves born to unvaccinated cows</td>
<td>3.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calves born to vaccinated cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II**</td>
<td>Calves born to unvaccinated cows</td>
<td>1.84</td>
<td>1.21</td>
<td>2.872</td>
<td>7</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Calves born to vaccinated cows</td>
<td>2.375</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(+ ) DPV = Days post-vaccination.
* Single vaccination.
** Double vaccination.
# Challenge infection in relation to SNI in calves (born to vaccinated cows) after vaccination with monovalent saponified gel FMD vaccine

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calves Nos.</th>
<th>Age at 1st vaccination (in days)</th>
<th>Log SNI after primary vaccination at</th>
<th>Log SNI before vaccination at</th>
<th>Results of challenge infection</th>
<th>Age at Log SNI at challenge</th>
<th>Unvaccinated controls</th>
</tr>
</thead>
</table>
| I      | 184         | 21 days                         | 0.5                                 | 2.8                           | 3.0 ± ± ± ± ± + + + + + + + + + + + + + | 21 days | 3
| II     | 673         | 50 days                         | 1.0                                 | 3.2                           | 3.0 + + + + + + + + + + + + + + + + + + + | 50 days | 60
|        | 218         | 21 days                         | 2.0                                 | 2.0                           | 2.0 + + + + + + + + + + + + + + + + + + + | 21 days | 11
|        | 197         | 20 days                         | 2.0                                 | 3.2                           | 3.0 + + + + + + + + + + + + + + + + + + + | 20 days | 20

Died after 7 days of inoculation.

± = Diffused reaction on tongue or vesicle formation in feet.

= Reaction at the site of inoculation or very mild vesicle.

T = Tongue; G = Gums; L = Lips; LFF = Left fore foot; LHF = Left hind foot; RFF = Right fore foot; RHF = Right hind foot.
been raised in countries where FMD is endemic and vaccination of cattle is the policy. Following his observations that traces of maternal antibody could partially or completely destroy the ability of FMD vaccine to evoke an immune response and in some instances a second dose also elicited only a very limited immune reaction, Van Bekkum (1974) suggested that immunization of cattle be started from the age of 6 months onward. In the Netherlands, vaccination of cattle against FMD is carried out over the age of 4 months (Van Bekkum et al., 1963).

Our findings in the present investigation suggest that the higher maternal antibody titres in the calves (born to FMD vaccinated cows), might have interfered with the immune response in these calves on primary vaccination either with half or full adult dose of the vaccine. On the other hand, the response of calves (born to unvaccinated cows) to primary vaccination was appreciable. The satisfactory response of such calves has also been reported by various other workers (Graves, 1963; Wisniewski and Jankowska, 1972, and Radisich et al., 1976).

The calves receiving vaccine in two half doses showed significant rise in titre irrespective of their pre-vaccination titres, indicating that double vaccination resulted in effective and lasting immunity in calves vaccinated at early age. The booster effect of revaccination has also been observed by others (Muntiu et al., 1972; Gagliardi and Zoletto, 1972; Prudovsky, 1973, and Uppal et al., 1975).

There was no effect of the dose size of FMD vaccine on immune response of calves after primary vaccination. However, Prudovsky (1973) found that the dose size may affect immune response to primary vaccination in calves born to unvaccinated cows, but the dose effect was insignificant following revaccination.

Our observations suggest that colostral (maternal) antibodies might to a certain extent interfere with the immune response of calves born to FMD vaccinated cows, yet not block it completely. Furthermore, such interference seems limited to the response to primary vaccination. The second vaccination carried out at 21 days after primary vaccination resulted in a typical response, which might not have occurred had there been no previous sensitization. Therefore, it seems that first vaccination primed the immune competent cells whereas the second one boosted up the immune response. It was suggested by Prudovsky (1973) that the role of colostral antibodies seems comparable to that of antitoxin inoculated simultaneously with a first dose of toxoid in diphteria and tetanus.

These findings suggest that vaccination of calves with FMD vaccine given in split doses at 21 days interval may be successful even at a very early age i.e. within a week after birth.
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REFERENCES