LACTATION-WISE KINETIC STUDIES OF FMD VIRUS TYPE SPECIFIC IGG1, IGG2 AND IGA AND CORRELATION ANALYSIS IN PAIRED MILK AND SERUM SAMPLES OF FMD VACCINATED BUFFALOES

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ABSTRACT

Fifteen randomly selected milch buffaloes at an organized farm were divided into three groups on the basis of lactation to study the foot and mouth disease (FMD) virus type specific antibodies response in milk and serum following FMD vaccination. Milk and serum samples collected before vaccination i.e. 0 day and on 7, 14, 28, 42 and 56 days post vaccination, were analyzed for the detection of FMD virus type specific IgG1, IgG2 and IgA antibody response by indirect double antibody sandwich liquid phase blocking ELISA. Significant FMD virus type specific antibodies (IgG1, IgG2 and IgA) were detected in milk and serum of buffaloes on different days post vaccination, though the levels of antibodies were lower in milk as compared to serum. FMD virus type specific IgG1 was found to be the predominant subclass as compared to IgG2 and IgA both in milk and serum of vaccinated buffaloes. Milk and serum IgG1, IgG2 and IgA antibody titres were positively correlated with values of regression coefficient (R) as 0.506, 0.434 and 0.396, respectively.

Key words: FMD, antibody titre, vaccinated buffaloes

Foot and mouth disease (FMD) is an economically important OIE listed disease of cloven-hoofed animals belonging to more than 33 domestic and wild species. Serological surveillance/diagnosis for FMD has been performed conventionally using the virus neutralization test (VNT; Golding et al., 1976) and/or liquid phase blocking ELISA (LPBE; Hamblin et al., 1986; Kakker and Sharma, 2008). Collection of serum samples requires equipment and training and may cause pain and stress to the animal. Restraining of animal becomes difficult and also infringes socio-religious customs. It is very difficult to persuade owners of the milch animals to collect blood samples for monitoring vaccinal immune response. This necessitates the use of alternative source(s) of antibodies for monitoring immune status of the vaccinated animals. Milk has been shown to be a useful alternative to serum for FMD antibody testing in cattle and sheep (Armstrong, 1997a, b).

MATERIALS AND METHODS

The study was conducted on 15 randomly selected milch buffaloes at an organized farm. The animals were divided on the basis of lactation into three groups: A (1st lactation), B (2nd lactation), and C (3rd lactation). These animals were vaccinated with polyvalent oil adjuvanted FMD virus vaccine (Indian Immunologicals, Hyderabad).

All the animals were kept under same managemental conditions.

Collection of Samples: Paired blood and milk samples were collected before vaccination (0 day) and on 7, 14, 28, 42 and 56 days post vaccination from all the three groups. Sera were separated from blood samples. Serum and milk samples were stored at -20°C until used.

Virus: FMD virus reference serotypes O, A, and Asia-1 were procured from Central Laboratory of Project Directorate on FMD, IVRI, Mukteswar, U.P., India.

Processing of Samples: Serum samples without any further treatment, were used for the assay. Milk samples on arrival in the laboratory were first treated with Arkalone (a flourocarbon compound) for defattening followed by centrifugation at 3000xg for 30 min. at 4°C and then stored at -20°C until used.

Assay Procedure: Milk and serum samples were screened using LPBE essentially as developed by Hamblin et al. (1986). The method for milk LPBE was same as for the serum except that the milk samples were defattened and dilutions of milk samples were made in blocking buffer, composed of 2X MEM and 60 mM HEPES buffer with 1% horse serum, rather than in PBST (as used in serum).

Indirect Double Antibody Sandwich ELISA: The
monoclonal antibodies (Mab; Serotec, Oxford, UK) directed against the Fc portion of bovine IgG1, IgG2 and IgA were used. The dilutions of coating antibodies, FMD virus antigen, monoclonal antibodies and conjugate were optimized by checker board titration.

RESULTS AND DISCUSSION

Humoral and cellular defense mechanisms in the mammary gland play an important role in protection against diseases. Significantly high virus specific IgG1 milk antibody titres were recorded as early as 7 DPV against FMD virus serotypes O, A and Asia 1 and attained peak levels on approximately 28 DPV in all the three groups of buffaloes (Table 1). The milk samples of group B buffaloes showed comparatively higher levels of virus specific IgG1 against all the three serotypes of FMD virus. In the present study a significant positive correlation (R= 0.506; n=162) was recorded between serum IgG1 and milk IgG1 antibody titres against FMD virus serotypes in all the three groups of vaccinated buffaloes.

Virus specific milk IgG1 antibody titres were significantly lower as compared to corresponding serum IgG1 antibody titres (Tables 1 and 2) against FMD virus serotypes in all the three groups of buffaloes. It is surprising that serum IgG1 antibody titres against FMD virus serotype O were significantly low as compared to serotypes A and Asia 1 in all the three groups of buffaloes. Mulcahy et al. (1990) also recorded higher IgG1 antibody response against FMD virus serotype A24 as compared to FMD virus serotype O in cattle immunized with O BFS vaccine and A24 Cruzeiro vaccine, respectively at 21 days post immunization.

Virus specific milk IgG2 antibody response was significantly low as compared to IgG1 antibody titres in all the three groups of buffaloes against FMD virus serotypes O, A and Asia-1 (Table 1). No significant difference was recorded amongst the three groups but group A buffaloes showed comparatively lower antibody titres against FMD virus serotypes O, A and Asia-1. Serum IgG2 antibody titres were lower as compared to serum IgG1 titres but were significantly higher than milk IgG2 antibody titres in all the three groups of buffaloes against FMD virus serotypes O, A and Asia-1. In case of serum IgG2 levels, no significant difference in the antibody titres was recorded amongst the three groups against FMD virus serotypes O, A and Asia-1 (Table 2).

Like serum IgG1, IgG2 antibody response against
Table 2

<table>
<thead>
<tr>
<th>DPV</th>
<th>IgG1 log_{10} antibody titres (Mean±S.E.) against FMD virus serotype</th>
<th>IgG2 log_{10} antibody titres (Mean±S.E.) against FMD virus serotype</th>
<th>IgA log_{10} antibody titres (Mean±S.E.) against FMD virus serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>A</td>
<td>Asia 1</td>
</tr>
<tr>
<td></td>
<td>log_{10}</td>
<td>log_{10}</td>
<td>log_{10}</td>
</tr>
<tr>
<td>0</td>
<td>1.1±</td>
<td>1.1±</td>
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</tr>
<tr>
<td>0.1A</td>
<td>0.1A</td>
<td>0.1A</td>
<td>0.1A</td>
</tr>
<tr>
<td>7</td>
<td>1.6±</td>
<td>1.6±</td>
<td>1.6±</td>
</tr>
<tr>
<td>0.2A</td>
<td>0.1A</td>
<td>0.1A</td>
<td>0.1A</td>
</tr>
<tr>
<td>14</td>
<td>2.0±</td>
<td>1.8±</td>
<td>2.0±</td>
</tr>
<tr>
<td>0.4A</td>
<td>0.1A</td>
<td>0.1A</td>
<td>0.1A</td>
</tr>
<tr>
<td>28</td>
<td>1.8±</td>
<td>1.8±</td>
<td>1.8±</td>
</tr>
<tr>
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<td>0.0A</td>
<td>0.0A</td>
<td>0.0A</td>
</tr>
<tr>
<td>42</td>
<td>1.8±</td>
<td>1.8±</td>
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<tr>
<td>0.0A</td>
<td>0.0A</td>
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<td>0.0A</td>
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<tr>
<td>56</td>
<td>1.8±</td>
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<td>0.0A</td>
<td>0.0A</td>
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</table>

Means with different letters superscript are significantly different (P<0.05).
sparsity of IgA plasma cells in bovine mammary gland suggests that serum transport is quantitatively the more significant route (Guidry, 1995).

Therefore, it may be well concluded that milk could be used as a workable alternative for detection of antibodies against FMD virus. Testing milk in place of sera is useful and practically advantageous in large scale epidemiological studies on milch animals. The present study demonstrated a significant correlation between milk antibody titres (IgG1, IgG2 and IgA) and corresponding serum antibody titres in the three groups of buffaloes vaccinated with polyvalent oil adjuvant FMD vaccine.

REFERENCES


