Correlation analysis between milk and serum LPBE FMD virus type specific antibody titres in buffaloes vaccinated with polyvalent oil adjuvant FMD vaccine

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ABSTRACT
The present investigation was carried out on 15 randomly selected milch buffaloes divided into three groups on the basis of lactation of an organized farm, to study the Foot and Mouth Disease Virus (FMDV) type specific antibodies 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 antibodies by liquid phase blocking ELISA (LPBE). Milk LPBE antibody titres against different FMD virus types were significantly different showing higher antibody titres against FMD virus type O followed by A and Asia 1. Serum LPBE antibody titres against FMD virus type O and Asia 1 were not significantly different, except for type A. Milk and serum LPBE antibody titres were positively correlated with value of regression coefficient (R) as 0.6376 and correlation was highly significant (P<0.01). Testing milk in place of sera is useful and practically advantageous in large scale epidemiological studies on milch animals after demonstration of a significant correlation between milk antibody titres and corresponding serum antibody titres in the study.

Key words: Foot and Mouth disease, LPB-ELISA, Milk and serum antibody, Specific isotype antibody response.

INTRODUCTION
Foot and mouth disease (FMD) is an economically important OIE ‘list A’ disease of cloven-hoofed animals belonging to more than 33 domestic and wild species. Clinical signs are essentially similar in all species, but with considerable variation in severity. The typical signs are pyrexia followed by vesicle formation in mouth and on feet as well as on snout and teats. FMD is one of the most important animal diseases affecting international trade in livestock, meat and other animal products.

While clinical disease is confirmed by virus isolation and typing, serological surveillance has also been widely used to confirm absence of infection. Serological surveillance for FMD has been performed conventionally by using the virus neutralization test (VNT; Golding et al., 1976) and/or liquid phase blocking ELISA (LPBE) (Hamblin et al., 1986). Collection of serum samples requires equipment and training and may cause pain and stress to the animal. It is very difficult to persuade owners of the milch animals to take 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. In situations, milk samples can afford certain advantages over blood samples as source of antibodies. Collection of milk is non-invasive and its testing would thus be easier and more acceptable than blood sampling for monitoring vaccinal immune response. Milk is collected routinely on daily basis and may present a convenient method of measuring immunity at a minimal cost.

Studies have been conducted using milk for detection of antibodies against various viral diseases. Niskanen et al. (1991) used ELISA to demonstrate responses against bovine viral diarrhoea in both individual and bulk tank milk samples. While working with bovine respiratory syncytial virus, Elevander et al. (1995) found that there was a strong correlation between milk and serum antibody titres in ELISA but the levels of antibodies were lower in milk. Dhennin et al. (1997) showed that neutralizing antibodies in milk could be detected throughout the year in milk from cows vaccinated annually against FMD. Milk has been shown to be a useful alternative to serum for FMD antibody testing in cattle and sheep (Armstrong, 1997a,b). A strong correlation was demonstrated between milk antibody titres, and those in serum as measured by liquid phase blocking ELISA (Armstrong et al., 2000). In addition to testing individual

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milk samples it has become possible to predict herd protection against FMD in bulk tank milk samples (Armstrong and Mathew, 2001).

MATERIALS AND METHODS

The study was conducted on 15 randomly selected milch buffaloes in an organized farm. The animals were divided on the basis of lactation into three groups: A (1\textsuperscript{st} lactation), B (2\textsuperscript{nd} lactation), and C (3\textsuperscript{rd} lactation). These animals were vaccinated with polyvalent oil adjuvant FMD virus vaccine (Indian Immunological, Hyderabad). All the animals were kept under the same managemental conditions.

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

Virus: FMD virus reference serotypes O, A, and Asia 1 were procured from Central Laboratory of Project Directorate on FMD, IVRI, Mukteshwar-Kumaon 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 and collected after centrifugation at 3000×g for 30 min. at 4°C and stores at -20°C until use.

Assay procedure: Milk and serum samples were screened using liquid-phase blocking ELISA (LPBE) essentially as developed by Hamblin et al. (1986). Dilutions of serum sample were made in PBS containing 0.05 per cent (v/v) Tween-20 (PBST). In order to counteract acidity of milk; dilutions were made in blocking buffer composed of 2X MEM and 60 mM HEPES buffer. Protease activity was neutralized by addition of 1% horse serum in MEM. The dilutions of the coating antibodies, the FMD virus antigen, the tracing antibodies and conjugate were optimized by checker board titration.

Liquid phase sandwich blocking ELISA: The liquid phase sandwich blocking ELISA was performed as described by Hamblin et al. (1986).

RESULTS AND DISCUSSION

The LPBE was successfully modified to counteract the acidity of milk and apparent protease activity, probably caused by bacterial contamination. Significantly higher antibody titres were obtained in serum as compared to milk in all the three lactation wise categories of buffaloes against FMD virus types on different days post vaccination (Table 1 & 2). In the present study, significant titres were detectable against FMD virus type O, A and Asia 1 as early as 7 DPV in milk samples collected from all the three lactation wise categories of buffaloes (Table 1). The antibody titres against FMD virus type O were significantly higher as compared to type A and Asia 1 in all the three lactation wise categories of buffaloes. The present study could not provide evidence to explain why antibodies against FMD virus type O were higher as compared to type A and Asia 1 as the quantum of antigen pay-load of different serotypes in vaccine had not been mentioned by the manufacturer. However, comparatively low antibody titres against FMD virus type A had also been recorded in a previous study on vaccine trial in rural cohort (Anonymos, 2002). In this study, no significant difference was recorded in the milk antibody levels against FMD virus type O, A and Asia 1 among the three lactation wise categories of buffaloes except that the third lactation buffaloes showed significantly lower milk antibody titres on 42 and 56 DPV.

TABLE 1: FMD virus type specific antibody titres in milk of buffaloes vaccinated with polyvalent oil adjuvant FMD vaccine

<table>
<thead>
<tr>
<th>Days post vaccination</th>
<th>O 1\textsuperscript{st} lactation</th>
<th>O 2\textsuperscript{nd} lactation</th>
<th>O 3\textsuperscript{rd} lactation</th>
<th>A 1\textsuperscript{st} lactation</th>
<th>A 2\textsuperscript{nd} lactation</th>
<th>A 3\textsuperscript{rd} lactation</th>
<th>Asia 1 1\textsuperscript{st} lactation</th>
<th>Asia 1 2\textsuperscript{nd} lactation</th>
<th>Asia 1 3\textsuperscript{rd} lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.5 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
<td>0.6 ± 0\textsuperscript{A}</td>
</tr>
<tr>
<td>7</td>
<td>1.4 ± 0.05\textsuperscript{A}</td>
<td>1.2 ± 0.05\textsuperscript{A}</td>
<td>1.0 ± 0.13\textsuperscript{A}</td>
<td>1.2 ± 0.05\textsuperscript{A}</td>
<td>1.2 ± 0.05\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.2 ± 0.05\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.2 ± 0.05\textsuperscript{A}</td>
</tr>
<tr>
<td>14</td>
<td>1.5 ± 0.05\textsuperscript{A}</td>
<td>1.6 ± 0.05\textsuperscript{A}</td>
<td>1.7 ± 0.15\textsuperscript{A}</td>
<td>1.25 ± 0.05\textsuperscript{A}</td>
<td>1.25 ± 0.05\textsuperscript{A}</td>
<td>1.25 ± 0.05\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
</tr>
<tr>
<td>28</td>
<td>1.9 ± 0.05\textsuperscript{A}</td>
<td>1.7 ± 0.05\textsuperscript{A}</td>
<td>1.6 ± 0.05\textsuperscript{A}</td>
<td>1.25 ± 0.05\textsuperscript{A}</td>
<td>1.25 ± 0.05\textsuperscript{A}</td>
<td>1.25 ± 0.05\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
</tr>
<tr>
<td>42</td>
<td>1.9 ± 0.05\textsuperscript{A}</td>
<td>1.6 ± 0.05\textsuperscript{A}</td>
<td>1.6 ± 0.05\textsuperscript{A}</td>
<td>1.45 ± 0.05\textsuperscript{A}</td>
<td>1.45 ± 0.05\textsuperscript{A}</td>
<td>1.45 ± 0.05\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
</tr>
<tr>
<td>56</td>
<td>2.35 ± 0.05\textsuperscript{A}</td>
<td>1.65 ± 0.05\textsuperscript{A}</td>
<td>1.65 ± 0.05\textsuperscript{A}</td>
<td>1.65 ± 0.05\textsuperscript{A}</td>
<td>1.65 ± 0.05\textsuperscript{A}</td>
<td>1.65 ± 0.05\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
<td>1.275 ± 0.074\textsuperscript{A}</td>
</tr>
</tbody>
</table>

Means with same letters superscript are not significantly different but means with different letters superscript are significantly different (P<0.05)
against FMD virus type O and significantly high antibody levels in first lactation buffaloes against FMD virus type A on 28 DPV. This finding supported the earlier observation of Armstrong and Mathew (2001), that no significant difference were found between milk SIA antibody titres against FMD virus type O in the colostrum and milk of first and second lactation cattle immunized against FMD.

Further, serum antibody titres against FMD virus type O and Asia 1 were higher as compared to those recorded against FMD virus type A (Table 2). No significant difference was recorded in serum antibody titres against FMD virus type O in three different lactations. Second and third lactation buffaloes showed higher serum antibody titres against FMD virus type A and Asia 1 as compared to buffaloes in the first lactation. Armstrong and Mathew (2001) showed higher titres in serum LPBE antibodies against FMD virus type O in second lactation as compared to first lactation cattle.

The correlation analysis between milk and serum LPBE antibody titres is shown in Fig.1. Milk and serum LPBE antibody titres were positively correlated with value of regression coefficient (R) as 0.6376 and correlation was highly significant (P<0.01). The regression equation was

\[ y = 0.6707x + 1.7864 \]

A highly significant correlation with regression coefficient (R) of 0.6376 (P<0.01) was recorded between serum and milk LPBE antibody titres in three lactation wise categories of buffalo. However, there was considerable scatter about the regression line for values of individual buffaloes. It appears that milk LPBE is suitable for use in the animals having higher serum antibody levels.

### TABLE 2: FMD virus type specific antibody titres in serum of buffaloes vaccinated with polyvalent oil adjuvant FMD vaccine

<table>
<thead>
<tr>
<th>Days post vaccination</th>
<th>O 1st lactation</th>
<th>O 2nd lactation</th>
<th>O 3rd lactation</th>
<th>A 1st lactation</th>
<th>A 2nd lactation</th>
<th>Asia 1 1st lactation</th>
<th>Asia 1 2nd lactation</th>
<th>Asia 1 3rd lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.9 ± 0.1 A</td>
<td>1.8 ± 0.1 A</td>
<td>2.0 ± 0.1 A</td>
<td>1.5 ± 0.1 A</td>
<td>1.7 ± 0.1 A</td>
<td>1.5 ± 0.1 A</td>
<td>1.8 ± 0.1 A</td>
<td>1.4 ± 0.1 B</td>
</tr>
<tr>
<td>7</td>
<td>2.95 ± 0.05 A</td>
<td>2.66 ± 0.166 A</td>
<td>2.78 ± 0.147 A</td>
<td>2.21 ± 0.202 A</td>
<td>2.6 ± 0.199 A</td>
<td>2.8 ± 0.199 A</td>
<td>3.0 ± 0.1 A</td>
<td>3.1 ± 0.1 A</td>
</tr>
<tr>
<td>14</td>
<td>3.2 ± 0.1 A</td>
<td>2.8 ± 0.172 A</td>
<td>3.2 ± 0.1 A</td>
<td>2.45 ± 0.1 A</td>
<td>2.8 ± 0.199 A</td>
<td>3.2 ± 0.199 A</td>
<td>3.5 ± 0.1 A</td>
<td>3.5 ± 0.1 B</td>
</tr>
<tr>
<td>28</td>
<td>3.4 ± 0.1 A</td>
<td>3.15 ± 0.226 A</td>
<td>3.2 ± 0.18 A</td>
<td>2.5 ± 0.05 A</td>
<td>3.4 ± 0.199 A</td>
<td>3.5 ± 0.199 A</td>
<td>3.5 ± 0.1 A</td>
<td>3.6 ± 0.1 B</td>
</tr>
<tr>
<td>42</td>
<td>3.5 ± 0.1 A</td>
<td>3.25 ± 0.217 A</td>
<td>3.4 ± 0.1 A</td>
<td>2.55 ± 0.05 A</td>
<td>3.3 ± 0.173 A</td>
<td>3.2 ± 0.173 A</td>
<td>3.2 ± 0.149 A</td>
<td>3.2 ± 0.1 B</td>
</tr>
<tr>
<td>56</td>
<td>3.1 ± 0.1 A</td>
<td>2.9 ± 0.233 A</td>
<td>3.4 ± 0.1 A</td>
<td>2.41 ± 0.083 A</td>
<td>3.0 ± 0.18 A</td>
<td>3.0 ± 0.18 A</td>
<td>3.0 ± 0.194 A</td>
<td>3.2 ± 0.1 B</td>
</tr>
</tbody>
</table>

Means with same letters superscript are not significantly different but means with different letters superscript are significantly different (P<0.05)

A serum titre of 1:100, which is considered protective, correlated with the milk LPBE titres of approximately 1:2. Armstrong et al. (2000) also recorded significant correlation between serum LPBE antibody titres and milk SIA in cattle immunized against FMD. Their findings revealed that the serum LPBE titre of 1:100 correlated with milk SIA titre of approximately 1:4.8. However, these workers did not compare serum LPBE with milk LPBE titre in FMD immunized cattle. It may be well concluded from the present study 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 after demonstration of a significant correlation between milk antibody titres and corresponding serum antibody titres in the study. Inspite of the limitation of milk for detection of antibodies against FMD virus types that it can only be used in milch animals, we can not undermine the importance of present study. In country like India milch animals are more important and kept in large number than draught animals.
REFERENCES