INTRODUCTION

Thirty-seven animals aged over 2 years in a paratuberculosis endemic herd were selected to study the usefulness of ELISA and gamma interferon assay in bovine paratuberculosis diagnosis. All the animals were screened by a comparative single intradermal test for tuberculosis and paratuberculosis. Of the 37 animals tested by gamma interferon assay and ELISA, 21 animals were positive by $\gamma$-IFN and nine animals were positive by ELISA. This study showed that selection of a diagnostic test should be based on the stage of infection of the animal. For early detection and paratuberculosis control programmes, gamma interferon assay can be used since it detects subclinical animals, and ELISA is useful in shedders.

Paratuberculosis in cattle is a chronic and progressive infection caused by Mycobacterium avium subsp. paratuberculosis (MAP) (Chiodini et al., 1984). Infections with MAP are characterized by long incubation periods, which may be of variable length.

In paratuberculosis, cell mediated immunity occurs in the early part of infection and can be assessed by antigen specific delayed type IV hypersensitivity reactions, particularly by estimation of gamma interferon production (Wood et al., 1990; Wood and Rothel, 1994). After infection, cell-mediated immune responses are followed by humoral immune responses (Stabel, 2000). Enzyme-linked immunosorbent assays for serum antibodies against MAP have been used extensively for purposes of monitoring and control of paratuberculosis in cattle because they are inexpensive and widely available, provide results rapidly, and have sufficient sensitivity and specificity for use as a screening tool (Roussel et al., 2007).

MATERIALS AND METHODS

Thirty-seven animals aged over 2 years in a paratuberculosis endemic herd (26 cross bred cattle and 11 graded Murrah) were selected to find the usefulness of ELISA and gamma interferon assay in bovine paratuberculosis diagnosis. All the animals were screened by a comparative single intradermal test for tuberculosis and paratuberculosis as described in standard protocols.

All the graded Murrah animals were clinically healthy, had no diarrhea and one animal was pregnant. In the cross-breds, six animals showed rough hair coat, reduced milk yield and intermittent diarrhoea and four animals were pregnant. The pregnant animals were at about 4 to 5 months of gestation.

From each animal, 5 ml of blood in a
heparinized vial and 5 ml of blood without anticoagulant were collected by jugular venipuncture. For the γ- IFN assay, the heparinized blood samples were transported to the laboratory at 37°C within 6 h of collection. For ELISA, serum was separated from clotted blood samples.

Each heparinized blood sample was evenly mixed and aliquoted into 1.5 ml quantities in duplicate. One well was filled with 100 μl of PBS (nil antigen control), and another well was filled with 100 μl Johnin PPD (IVRI, Izat nagar). The antigens were mixed thoroughly with aliquoted 1.5 ml heparinized blood and incubated at 37°C in a humidified chamber for 24 h. After incubation, the plasma from each well was collected and used for gamma interferon assay. For gamma interferon enzyme linked immunosorbent assay, a BOVIGAM kit (Biocor animal health Inc., USA) was employed. In γ- IFN EIA, the required wells were filled with 50 μl of plasma diluent (0.01% thimerosal) followed by 50 μl of control and test samples in appropriate wells. The samples were mixed well followed by incubation at room temperature for 60 minutes. After incubation the wells were washed 6 times followed by 100 μl of freshly prepared conjugate (HRP labeled anti-bovine γ- IFN) and incubated. After washing 6 times, 100 μl of enzyme substrate (TMB in DMSO₄, H₂O₂) solution was added and incubated for 30 minutes, followed by 50 μl of stopping solution (0.5 M H₂SO₄) and a reading was taken using 450 nm filter with 650 nm reference filter. The results were interpreted as positive and negative if the difference between the antigen stimulated plasma OD and the nil antigen control OD was ≤ 0.1, and ≥ 0.1 respectively.

M. phlei absorbed cattle type MAP antibody ELISA (SVANOVA, Germany) was performed using serum as described by the manufacturer’s protocol.

RESULTS AND DISCUSSION

All the animals tested gave negative for tuberculosis, and few animals gave positive for paratuberculosis.

Of the 37 animals (26 cross bred cattle and 11 graded Murrah) tested by gamma interferon assay and ELISA, 21 animals (11 cross breds and 10 graded Murrah) were positive by γ- IFN and nine animals (six cross bred cattle and three graded Murrah) were positive by ELISA. Out of four pregnant cross breds and one graded Murrah, two pregnant animals were positive by gamma interferon assay and not by ELISA; the other pregnant animals were not positive by either of the tests.

The diagnostic sensitivity and specificity of ELISA over γ- IFN was 28.5%, 53.57%, respectively. The concordance between these two tests was 62.12. Kappa statistics were used to find the agreement between these two tests, and a value 16.4 indicated that there was low agreement (Table 1).The association between the OD value of gamma interferon assay and ELISA OD is shown in Figure 1.

Nielsen et al. (2002) observed a sensitivity of ELISA between 27-86% and specificity between 55-98% in cattle while Sockett et al. (1992) reported a sensitivity between 47 and 65% in cattle, the lower sensitivity was mainly because of sub clinically infected animals. Sweeny et al. (1995) reported a sensitivity between 15 and 87% in cattle, and Whitlock et al. (2000) observed the sensitivity to be between 15 and 75%.

Gasteiner et al. (2000) used two different types of ELISA in clinical and sub clinical paratuberculosis animals and found the sensitivity
Table 1. Diagnostic evaluation of ELISA test over gamma interferon assay.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>$\gamma$- IFN positive</th>
<th>$\gamma$- IFN Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Positive</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>ELISA negative</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
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(Sensitivity - 28.5%; Specificity - 81.25 %; Kappa statistics - 16.4; Positive predictive value - 66.66; negative predictive value - 46.42; apparent prevalence - 24.32; true prevalence -56.75).

Figure 1. Relationship between the ELISA OD value and $\gamma$- IFN OD value.
to be between 70% and 74% and specificity between 98% and 82%; however, the ELISAs could not detect all culture positive animals.

Billman-Jacobe et al. (1992) evaluated the sensitivity of an IFN-γ bioassay, an IFN-γ ELISA and a *M. paratuberculosis* specific ELISA test in six cattle herds with a known history of the disease, and results demonstrated that the IFN-γ ELISA test was more sensitive for detection of sub-clinically infected cattle than other two assays.

Cell-mediated immune responses to mycobacterial infections are the first to develop and strongest host response; hence, CMI tests may be useful for early detection of MAP (Collins, 1996). The CMI response can be measured by intradermal skin test and gamma interferon (γ IFN) assay (Wood *et al*., 1989). The IFN-γ assay determines CMI response by measuring interferon release by sensitized lymphocytes. Measuring this release of interferon is considered to be a very sensitive diagnostic tool for detection of tuberculosis and paratuberculosis. IFN-γ assay had good sensitivity and specificity in detecting animals irrespective of age of the disease, and two or three tests at 6 month intervals would be effective in identifying most of the infected animals (Jacobe, 1992). Culling of CMI test-positives could be a cost-effective means of removing infected animals before they actually start faecal shedding. (Whipple *et al*., 2001)

The animals which were positive to γ- IFN and negative to ELISA assay might be in sub clinical stage, elicited by strong cell mediated immune response and less humoral response. The animals which were positive by both the tests might be in transition between the subclinical and clinical stage. During clinical stage of the disease the immune response shift towards humoral and was better detected by ELISA.

From this study, it is observed that selection of diagnostic test should be based on the stage of infection of the animal. For early detection and paratuberculosis control programmes, gamma interferon assay can be used since it detects subclinical animals, and ELISA is useful in shedders.

**REFERENCES**


