A COMPARATIVE STUDY BETWEEN MICROSCOPIC AGGLUTINATION TEST AND DIFFERENT PROTEIN ANTIGENS BASED ENZYMELINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF LEPTOSPIROSIS IN BOVINE

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ABSTRACT
The present study compared the efficacy of Microscopic Agglutination Test (MAT) with LipL32 and LipL41 recombinant antigen based Enzyme-linked immunosorbent assay (ELISA) for the diagnosis of bovine leptospirosis. The LipL32 and 41 genes were amplified using specific primers and the polymerase chain reaction products (PCR) were cloned. The gene inserts from positive recombinant clones were inserted into expression vector and transformed in *Escherichia coli* DH5α cells. The recombinant LipL32 and 41 proteins were expressed, purified and standardized. The optimum antigen concentration of 100ng of each anti. en was used for the screening of serum samples from cattle suspected of leptospirosis. It was found that recombinant antigen ELISA detected more positive cases of illness than MAT. Between the two recombinant antigens ELISA, LipL32 ELISA was able to detect more positives.

Key words: Microscopic agglutination test (MAT), LipL32, LipL41, ELISA, Leptospirosis.

INTRODUCTION
Leptospirosis has assumed considerable significance now a days as the disease is increasingly being involved in cases of abortions, repeat breeding and other reproductive problems in livestock resulting in huge economic losses. The isolation of the organism is difficult to achieve due to its fastidious nature. The serological tests have now become the corner stone for diagnosis of leptospirosis. The universally accepted test, Microscopic agglutination test is hazardous to perform, as live *Leptospira* culture is used as antigen and the test is serovar specific. Moreover it is tedious and time consuming (Theirmann, 1989; Cousin et al., 1985, Bolin et al., 1989). Enzyme–linked immunosorbent test has been found to be a more sensitive serological test than the conventional methods for diagnosis of leptospirosis (Pappas et al., 1985; Vijayachari et al., 2001). Recombinant antigen based ELISA is a suitable and safe tool for the examination of a large number of sera as it involves an immunodominant antigen and lacks the non-specific moieties present in whole cell preparations (Flannery et al., 2001).

MATERIAL AND METHODS

Maintenance of *Leptospira* strain
The *Leptospira* strains were maintained in liquid and semi-solid Ellinghausen and McCullough (EM) medium with regular subculture.

Production of recombinant proteins
The LipL32 and 41 genes were amplified using primers designed from previously reported sequences of *Leptospira interrogans* serovar Grippotyphosa and Canicola (accession number 6909321 for LipL32 and 642287 for LipL41). The PCR amplified products were cloned in pGEMT vector (Promega). The cloned products were inserted into pPROEX HTb expression vector (Life technologies, USA) and then transformed into *Escherichia coli* DH5α cells. The protein expression
was induced with 1mM isopropyl-beta-D-thiogalactopyranoside. The proteins were purified and dialyzed, and these proteins were used as antigens in ELISA.

**Microscopic agglutination test**

MAT was performed using a micro titration procedure as described by Faine, (1982) for detecting antibodies using live *Leptospira* serovars. Highest serum dilution exhibiting agglutination of 50% or more *Leptospira* cells was considered as the antibody titre.

**Enzyme-linked immunosorbent assay**

ELISA was performed following the method of Engwall and Perlmann, (1971) to compare different antigens based ELISA. The dilution of serum and conjugates that were found optimum for the test were 1:100 and 1:5000 respectively. The LipL32 and LipL41 antigen concentration of 100ng/well used in the test was standardized in *Leptospira* laboratory of Indian Veterinary Research Institute, Izatnagar.

**Determination of ELISA cut-off value**

The Mean Percent Positivity values (PP) of the negative control group were calculated as follows:

\[
\text{Mean OD value of known negative group / Mean OD value of the positive group} \times 100
\]

The serum samples showing a PP value double or more of this value was taken as the cut off PP value and considered to be positive for leptospirosis as described by Wright et al. (1993).

**Evaluation of MAT and ELISA**

The relative sensitivity, specificity and accuracy of different antigen based ELISA were evaluated in comparison to MAT as described in Veterinary Epidemiology (Thrusfield, 1995).

Sensitivity = \( \frac{a}{a+c} \times 100 \) where 'a' is the number of sera positive by MAT and ELISA, 'c' the number of sera positive by MAT but negative by ELISA.

Specificity = \( \frac{d}{b+d} \times 100 \), where 'd' is the number of sera negative by ELISA and MAT, 'b' the number of sera negative by MAT but positive by ELISA.

Accuracy = \( \frac{a+d}{a+b+c+d} \times 100 \)

# RESULTS AND DISCUSSION

The currently used test for the diagnosis of leptospirosis is MAT, but it is serovar specific, hazardous and laborious to perform. The ELISA test has several advantages over MAT, such as; it is safer to conduct and requires a little amount of serum. Furthermore, the recombinant antigen based ELISA provides genus and serovar specificity. So the present study was carried out to compare the MAT and two recombinant antigens based ELISA for the diagnosis of bovine leptospirosis. The LipL32 and 41 proteins chosen in the study appear to be expressed constitutively by all pathogenic *Leptospira* serovars. A total of 500 bovine serum samples were tested with 100ng concentration of the recombinant antigens. With MAT, 128 sera samples came positive, with LipL32 ELISA, 164 came positive and with LipL41 ELISA, 154 came positive. The sensitivity of recombinant antigen based ELISA as compared to MAT was found to be 100% suggesting that it was as efficient as MAT in identifying true positive animals. However, the specificity was analyzed as 90.32% for LipL32 ELISA and 93% for LipL41 ELISA, which reflects the inability of ELISA to detect all the MAT negative animals as ELISA negative too. This could be explained on the basis that all those

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**Table 1: Distribution of Percent Positivity values (PP) obtained with bovine serum samples using different protein antigens based ELISA**

<table>
<thead>
<tr>
<th>Range of PP values</th>
<th>*No of samples</th>
<th>Results</th>
<th>Range of PP values</th>
<th>*No of samples</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-16</td>
<td>215</td>
<td>Negative</td>
<td>0-16</td>
<td>223</td>
<td>Negative</td>
</tr>
<tr>
<td>17-32</td>
<td>121</td>
<td>Negative</td>
<td>17-35</td>
<td>123</td>
<td>Negative</td>
</tr>
<tr>
<td>33-50**</td>
<td>38</td>
<td>Positive</td>
<td>36-50**</td>
<td>32</td>
<td>Positive</td>
</tr>
<tr>
<td>51-67</td>
<td>68</td>
<td>Positive</td>
<td>51-67</td>
<td>67</td>
<td>Positive</td>
</tr>
<tr>
<td>68-84</td>
<td>47</td>
<td>Positive</td>
<td>68-84</td>
<td>38</td>
<td>Positive</td>
</tr>
<tr>
<td>85-101</td>
<td>11</td>
<td>Positive</td>
<td>85-101</td>
<td>17</td>
<td>Positive</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>Total</td>
<td>500</td>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

* Same sera (n=500) were used for evaluation of various antigens
** Cut off PP value
Table 2: Evaluation of different protein antigens based ELISA to detect anti-leptospiral antibodies in bovine sera as compared to MAT

<table>
<thead>
<tr>
<th></th>
<th>Recombinant LipL32 ELISA</th>
<th>Recombinant LipL41 ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAT Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>128</td>
<td>36</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>336</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>372</td>
</tr>
</tbody>
</table>

* Same sera (n=500) were used for evaluation of various antigens

LipL32 ELISA: Sensitivity = 100%; Specificity = 90.32%; Accuracy = 92.8%

LipL41 ELISA: Sensitivity = 100%; Specificity = 93%; Accuracy = 94.8%

MAT negative sera may not be actually negative as only 11 Leptospira serovars used as antigens in the test. Moreover, ELISA being a more sensitive test, detected more number of positive cases than MAT. It was concluded that the use of recombinant antigen ELISA detected more positive cases than MAT and between the two recombinant antigens ELISA, LipL32 ELISA detected more cases of illness than LipL41 ELISA. LipL32 ELISA could be a putative candidate antigen for diagnosis of bovine leptospirosis.

ACKNOWLEDGEMENTS
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REFERENCES