Pentavalent Inactivated Adjuvanted Vaccines for Bovine Leptospirosis

G. Balakrishnan, Parimal Roy and T.V. Meenambigai

Department of Veterinary Microbiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 600 007, India.

(Received: 10 November 2014; accepted: 09 January 2015)

Leptospiral serovars namely australis, ballum, hardjo, hebdomadis and pomona were selected as seed bacteria for the preparation of Leptospira vaccines based on the seroprevalence studies conducted using 3605 bovine serum samples of Tamil Nadu, Andhra Pradesh and Gujarat states. By incorporating the above serovars two different kind of adjuvanted pentavalent vaccines were prepared. Vaccine I was adjuvanted with Montanide ISA 206 and vaccine II was adjuvanted with Aluminium hydroxide gel. The two vaccines were found to be safe and potent, hence considered as better vaccines.

Key words: Bovines, Leptospirosis, Vaccines.

Leptospirosis is an important disease hindering the development of livestock industry in India. It causes enormous losses due to death of animals, decreased milk production, abortion, stillbirth and infertility. All the vaccines used for the control of bovine leptospirosis (Samina et al., 1997) do not confer protection against the serovars not incorporated in the vaccine composition but are prevalent in the field. Hence the present study has been undertaken to develop a vaccine for bovine leptospirosis.

MATERIALS AND METHODS

Leptospira bacterins were prepared as per the production outline of British Pharmacopoeia (1985). The leptospiral reference strains namely L. australis Ballico, L. ballum Mus127, L. hardjo Hardjoprajitno, L. hebdomadis Hebdomadis and L. pomona obtained from Regional Medical Research Centre (RMRC), Indian council of Medical Research (ICMR) were used for the preparation of vaccines. Low protein media containing 0.2 per cent of Bovine serum albumin (BSA) was prepared following the protocol described by Regional Medical Research Centre (2007) and were used for growing the leptospires. Leptospira vaccines were prepared as per the protocol described by Balakrishnan (2009). The 10 to 11 day old cultures were harvested and their concentrations were adjusted to 2 X 10⁹ cell per ml. Formalin at the concentration of 0.4 per cent was used to inactivate the leptospires and two types of vaccines, vaccine I adjuvanted with Montanide ISA 206 and vaccine II adjuvanted with Aluminium hydroxide gel were prepared. Tests recommended by the British Pharmacopoeia (loc cit) and Monograph of the European Pharmacopoeia for Leptospira Veterinary vaccine preparation were followed for the standardization of the vaccines as described by Balakrishnan (loc cit). Both the vaccines were subjected to sterility test (Balakrishnan, loc cit), Safety test (British pharmacopoeia, loc cit and Balakrishnan, loc cit), Potency test in guinea pigs (OIE, 2008; and
RESULTS AND DISCUSSION

Immunity to leptospires is serovar specific (Chen, 1986). Serovars present in the field but not incorporated in vaccines pose a problem (OIE, loc cit). Hence serovars namely L. australis, L. ballum, L. hardjo, L. hebdomadis and L. pomona were selected as seed bacteria, based on the seroprevalence study conducted using the 3605 bovine serum samples of Tamil Nadu, Andhra Pradesh and Gujarat states. By incorporating the above serovars two different kind of adjuvanted pentavalent vaccines were prepared. Vaccine I was adjuvanted with Montanide ISA 206 and Vaccine II was adjuvanted with Aluminium Hydroxide gel. In inactivation tests, the tubes containing EMJH media inoculated with the inactivated suspension of the 5 serovars which were subcultured 5 times did not show any leptospiral growth even after 14 days of incubation. In the control test for inactivation, the tubes containing EMJH medium inoculated with live cultures of L. australis, L. ballum, L. hardjo, L. hebdomadis and L. pomona showed growth of leptospires when observed by dark field microscopy on day 14 after inoculation.

Table 1. Results of the potency test in guinea pigs vaccinated with Leptospira vaccines

<table>
<thead>
<tr>
<th>Test</th>
<th>Serovars</th>
<th>Sample</th>
<th>Vaccine I</th>
<th>Vaccine II</th>
<th>Control Vaccine I</th>
<th>Control Vaccine II</th>
<th>Control Vaccine I</th>
<th>Control Vaccine II</th>
<th>Control Vaccine I</th>
<th>Control Vaccine II</th>
<th>Control Vaccine I</th>
<th>Control Vaccine II</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT (GMT in Log 2) Serum (On day 14)</td>
<td>L. australis</td>
<td>8.24</td>
<td>7.04</td>
<td>-</td>
<td>7.24</td>
<td>6.24</td>
<td>-</td>
<td>7.24</td>
<td>6.64</td>
<td>-</td>
<td>8.04</td>
<td>6.64</td>
</tr>
<tr>
<td>DFM Liver</td>
<td>L. ballum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>7.44</td>
<td>7.04</td>
<td>-</td>
<td>8.04</td>
<td>7.40</td>
<td>7.94</td>
<td>8.44</td>
<td>7.54</td>
</tr>
<tr>
<td>PCR Kidney</td>
<td>L. hebdomadis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PCR Liver</td>
<td>L. pomona</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: DFM – Dark Field Microscopical examination
MAT – Microscopic Agglutination Test
PCR – Polymerase Chain Reaction
Vaccine I – Vaccine adjuvanted with Montanide ISA 206
Vaccine II – Vaccine adjuvanted with Aluminium Hydroxide gel
requirements of the USDA (1984). The viscosity of the both vaccines was found to be below the recommended flow time of eight seconds (Palya, loc cit). For vaccine I (Montanide based), it was four seconds and for vaccine II (Aluminium hydroxide based), it was two seconds.

In the potency test, there was 100 per cent protection of the vaccinated guinea pigs as against 100 per cent mortality (Table - 1) in the controls after challenge with *australis, ballum, and pomona*, following OIE, (loc cit). For the vaccines to pass the test, at least 80 per cent of the vaccinated animals should remain in good health for 14 days after the death of the controls (OIE, loc cit). But challenge with serovars *hardjo* and *hebdomadis* did not cause death of the unvaccinated controls but induced antibody titre (GMT in Log.) of 7.94 and 8.04, respectively on day 35 of observation period. For the serovars that are not lethal to hamster or guinea pig, such as *hardjo*, potency is measured by induction of a suitable antibody titre (OIE, loc cit). It is not uncommon that some strains may be virulent in host animals but not in the experimental model as in the case for serovar *hardjo* (Bulach et al., 2006). The vaccinated (on day 1) and challenged (on day 15) guinea pigs were sacrificed humanely on day 35 and collected liver, kidney, serum for DFM, PCR and MAT analysis. All the vaccinated animals (vaccine I and vaccine II) were found negative for *Leptospira* organisms by DFM and PCR, but MAT titre (GMT in Log.) of 8.24 to 8.84, 7.24 to 7.44, 7.24 to 8.04, 8.04 to 8.44 and 8.24 to 9.14 were observed for vaccine I on day 14 to day 35 of the experiment against *australis, ballum, hardjo, hebdomadis* and *pomona*, respectively. In contrast, the MAT titre (GMT in Log.) of 7.04 to 8.24, 6.24 to 7.04, 6.64 to 7.04, 7.4 to 7.54, and 7.54 to 8.24 were observed for vaccine II against *australis, ballum, hardjo, hebdomadis* and *pomona*, respectively on day 14 to day 35. The control animals challenged with *australis, ballum and pomona* on day 15 died between day 22 and day 25 of experiment. Liver and kidney samples collected from dead animals were found positive for *Leptospira* serovars *australis, ballum* and *pomona*, based on DFM and PCR. The control animals challenged with *hardjo* and *hebdomadis* showed seroconversion, but no mortality. When they were sacrificed on day 35, liver, kidney and serum samples were analysed. *Leptospira* serovars *hardjo* and *hebdomadis* were seen in liver and kidney. Serum antibody titre was found to be 7.94 and 8.04, respectively on day 35. Thus both the vaccines, vaccine I (Montanide based) and vaccine II (Aluminium hydroxide based) fulfilled the criteria stated by OIE (loc cit).

The stability of the vaccine I and vaccine II was assessed for only six months period following OIE (loc cit) standards. The potency, viscosity and safety of the vaccine I and vaccine II were found to be retained at the storage temperature of 4°C for six months. The deterioration of both vaccines started from day 5 at 37°C and at room temperature. The potency of the vaccine is to be retained for one to two years (OIE, loc cit), but in the present study for a limited duration of six months, stability of the both the experimental vaccines were found to be satisfactory.

**Summary**

By incorporating the serovars *australis, ballum, hardjo, hebdomadis* and *pomona*, two different kind of adjuvanted pentavalent vaccines were prepared in the present study. Vaccine I was adjuvanted with Montanide ISA 206 and vaccine II was adjuvanted with Aluminium hydroxide gel. The two vaccines were found to be safe and potent, thus considered as better vaccines.

**REFERENCES**

1. Balakrishnan, G., PhD., Thesis submitted to Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai - 51, 2009