Use of a novel adjuvant in a Leptospira borgpetersenii Hardjo vaccine to induce a cell-mediated immune response

Jayne Wiarda¹ and Jennifer Wilson-Welder²

Department of Animal Science, Iowa State University, Ames, Iowa 50011
Infectious Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, USDA, Ames, Iowa 50010

Abstract:
Infection of cattle with Leptospira borgpetersenii serovar Hardjo can have negative impacts on the cattle industry and pose the risk of transmission to other mammals, including humans. Current commercial vaccines used to protect against Leptospira serovar Hardjo are ineffective at providing long-term immunity, and a more cell-mediated immune response is thought to be necessary for lasting protection. IgG and IgG₃ levels can be indicative of what type of T cell response predominates; in a cell-mediated type 1 (Th1) immune response, interferon-γ (IFN-γ) secreted from helper T cells will stimulate B cells to class-switch to manufacture and secrete IgG. In a humoral type 2 (Th2) immune response, helper T cells secrete interleukin-4 (IL-4) that stimulates B cells to class-switch to manufacture and secrete antibodies of the IgG₁ isotype. In this study, a novel vaccine formulated with an oil-adjuvant was shown to induce a stronger and more balanced cell-mediated response in comparison with current commercial serovar Hardjo vaccines. This was demonstrated by analyzing antibody levels in response to vaccination and challenge with Leptospira serovar Hardjo.

Background:
• Leptospirosis is thought to be the most widespread zoonotic disease in the world.
• Chronic infection by Leptospira serovar Hardjo in cattle results in reproductive problems that can have detrimental effects on the cattle industry.
• Infected cattle also pose the risk of transmitting the disease to other mammals, including humans.
• Current commercial monovalent vaccines against Leptospira serovar Hardjo are inadequate at preventing chronic infection in cattle.
• Current monovalent vaccines contain alum adjuvant that induces a primarily humoral Th2 response.
• An oil-based adjuvant, Montanide ISA 201 VG, is thought to induce a more cell-mediated Th1 type response when used in vaccines.
• It is thought that a stronger and more balanced humoral and cell-mediated responses is needed to provide long-term immunity.
• In a cell-mediated Th1 response, IFN-γ secreted from helper T cells stimulates B cell class-switching to secrete IgG. In this way, IgG₃ antibody levels can be indicative of a Th1 response.
• In a humoral Th2 response, IL-4 secreted from helper T cells stimulates B cell class-switching to secrete IgG₁. In this way, IgG₁ antibody levels can be indicative of a Th2 response.

Experimental Design:
<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Blood Collection</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Week 4</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>Week 8</td>
<td>32</td>
<td>4</td>
</tr>
</tbody>
</table>

Vaccines:
- Seppic Bacterin
  - Novel vaccine
  - 5x10⁶ form viable L. borgpetersenii Hardjo 203 (in mL)
  - 1/1 in Montanide ISA 201 VG
- Spravaro (Pfizer, New York, NY)
  - Commercial monovalent vaccine
  - Inactivated L. borgpetersenii Hardjo in alum adjuvant
- Lechypet adjuvant
  - Commercial monovalent vaccine
  - Inactivated L. interrogans Hardjo B:44 in alum adjuvant
- Adjuvant Only
  - Control group
  - 1/1 alum adjuvant (Alhydrogel, InvivoGen, San Diego, CA) and Montanide ISA 201 VG adjuvant

- 24 mixed breed heifers ~18 months of age were divided into 4 vaccine groups (n=6/group).
- Animals were vaccinated subcutaneously with 2 mL of their respective vaccine. An identical booster dose was administered 4 weeks later.
- Blood was collected throughout the study via jugular venipuncture. Blood sera were separated via centrifugation and stored at -20°C.
- Animals were challenged over 3 consecutive days. 5x10⁶ L. borgpetersenii Hardjo 203 in 1 mL PBS was administered to each respective (eye and intranasally) per day.
- ELISA was used to detect sera antibody levels. Sera was serially diluted and tested for L. borgpetersenii Hardjo 203-specific antibodies of IgG, IgM, IgA, and IgA.
- Optical density (OD) values were used to determine titers. Titers are expressed as the reciprocal of the highest dilution with an OD value greater than or equal to the determined background level.
- Two-way analysis of variance (ANOVA) was performed using time and vaccine group as factors along with Tukey pair-wise comparison post-tests (GraphPad Software, La Jolla, CA).

Comparison of IgG Isotypes:

Preliminary Conclusions:
• The novel vaccine made using Montanide ISA 201 VG oil-based adjuvant induced a stronger and more balanced cell-mediated Th1 response in comparison to the two commercial monovalent vaccines.
• Commercial vaccines showed no indication of successfully stimulating an immune response.
• An expected increase in IgG response to vaccination and challenge was not observed.
• A possible correlation may exist between non-vaccination and increased levels of IgA in response to Leptospira infection.

Discussion:
• Characterization of cell populations, especially IFN-γ and IL-4 producing cells, would be beneficial in better determining the exact mechanisms needed to induce a balanced response.
• Duration of immunity studies should be conducted to determine potential long-term efficacy of the novel vaccine.
• Lack of a well characterized IgG response may be attributed to sample collection intervals being too widely dispersed, or it could be an indication that T cell activation to form helper T cells was adequate since large amounts of IgM do not require helper T cells.
• Results indicating higher IgA titers in nonvaccinated animals are currently being investigated as a potential diagnostic marker for Leptospira infection.
• High IgA titers in nonvaccinated animals may be due to kidney damage from infection that causes local IgA to leak into the bloodstream, or it may be due to vaccinated animals being able to class-switch to IgA because of previous irreversible switching to produce IgG in response to vaccination.

Acknowledgments:
Thanks to Dena All, Richard Honkatyr, and Ana Frank for providing laboratory assistance, culture microscopy images, and sample collection in the NADC anatomic care cell for excellent internal care; and Judy Blaken and Francois Debeije for project supervision.

Serum Antibody Evaluation by ELISA:

Serum collected throughout the study was evaluated using sheep anti-bovine IgG. Significant increases (P < 0.05) in IgG were observed in the Seppic Bacterin group in comparison with Adjuvant Only control group. There were no significant differences in the Seppic Bacterin group when compared with the controls. There were no significant differences in the Seppic Bacterin group to the respective control vaccine group. Significant levels of IgG were observed in the Seppic Bacterin group compared to the control vaccine group. Significant increases (P < 0.05) in IgG were observed in the Seppic Bacterin group when compared with the control vaccine group. Significant increases (P < 0.05) in IgG were observed in the Seppic Bacterin group when compared with the control vaccine group. Significant levels of IgG were observed in the Seppic Bacterin group compared to the control vaccine group.

Preliminary Conclusions:
• The novel vaccine made using Montanide ISA 201 VG oil-based adjuvant induced a stronger and more balanced cell-mediated Th1 response in comparison to the two commercial monovalent vaccines.
• Commercial vaccines showed no indication of successfully stimulating an immune response.
• An expected increase in IgG response to vaccination and challenge was not observed.
• A possible correlation may exist between non-vaccination and increased levels of IgA in response to Leptospira infection.

Discussion:
• Characterization of cell populations, especially IFN-γ and IL-4 producing cells, would be beneficial in better determining the exact mechanisms needed to induce a balanced response.
• Duration of immunity studies should be conducted to determine potential long-term efficacy of the novel vaccine.
• Lack of a well characterized IgG response may be attributed to sample collection intervals being too widely dispersed, or it could be an indication that T cell activation to form helper T cells was adequate since large amounts of IgM do not require helper T cells.
• Results indicating higher IgA titers in nonvaccinated animals are currently being investigated as a potential diagnostic marker for Leptospira infection.
• High IgA titers in nonvaccinated animals may be due to kidney damage from infection that causes local IgA to leak into the bloodstream, or it may be due to vaccinated animals being able to class-switch to IgA because of previous irreversible switching to produce IgG in response to vaccination.

Acknowledgments:
Thanks to Dena All, Richard Honkatyr, and Ana Frank for providing laboratory assistance, culture microscopy images, and sample collection in the NADC anatomic care cell for excellent internal care; and Judy Blaken and Francois Debeije for project supervision.