Expansion of in vitro potency testing: Case Study with Serovar Hardjo

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• Challenges with Serovar Hardjo identification
• Challenges with and current status of Serovar Hardjo potency testing
• Current efforts to develop a challenge model in advance of an in vitro ELISA potency test
Serovar Hardjo identification

- Classification based on serologic reactivity
- *Leptospira interrogans* Serovar Hardjo
  - Type strain isolated in 1938 from human in Sumatra named Hardjoprajetno
BRENDA or REA

- 80’s Marshall, Robinson, Thiermann, Ellis and others
  - Differentiated field strains from type strain
  - Split Hardjo into types
    - Hardjo-bovis isolated around the world
    - Hardjo type prajitno UK, Africa and Mexico
BRENDA or REA

“The degree of difference between hardjo field strains on the one hand and the Hardjoprajitno strain on the other is of the same order as that between different leptospiral reference serovars.”

Robinson et al 1982
DNA Probes

- Late ‘80s early ’90s
- Specific DNA probes to hardjobovis and hardjoprajitno
  - LeFebvre, Van Eys, Zuerner and Ramadass
Genetic Relatedness and Classification

- 1987 Yasuda *et al.*
- 1992 Ramadass *et al.*
- 1999 Brenner *et al.*
- Reclassification resulting in:
  - *Leptospira interrogans* Serovar Hardjo
  - *Leptospira borgpetersenii* Serovar Hardjo
Reason for Serologic similarity

- MAbs are unable to differentiate
- 1999, 2000, 2001 Moctezuma, Bulach, Kalambaheti and Adler
  - Characterized and described highly similar rfb LPS biosynthetic loci in these two subtypes
Identification

- 16S rRNA gene sequencing will yield differences
- Currently when testing unknowns
  - *L. interrogans*-IS1500 PCR
  - *L. borgpetersenii*-IS1533 PCR
  - *L. kirschneri*-flagella using B64-I and B64-II
Effective immune response to leptospiral infection

- Humoral Immunity
  - Protective Ab, serovar specific
  - Ab to LPS sufficient for protection
  - MAbs to LPS
    - Yan et al 1999, MAb to *L. borgpetersenii* serovar Hardjo LPS protective in hamster
- Vaccine potency/efficacy easily measured
Clinical Signs: Why we are interested in Serovar Hardjo

- Chronic or persistent infection
- Late-term abortions, stillbirths, weak calves
- Persistently infected, normal calves
- Retained placenta, interstitial nephritis
- Infertility
- Zoonotic potential
Bovine-Serovar Hardjo Host Relationship

- Cattle humoral response-natural infection
  - MAT titers often low
  - Low or no detectable titers can resist infection
- Host adaptation *L. borgpetersenii*
Dr. Bolin’s Work at NADC

  - Commercial 5-way vaccine (hardjoprajitno)
  - 1 or 2 doses
  - Challenge conjunctival during pregnancy
  - 5/5 controls 13/15 vaccinates infected
  - Stillbirths, abortions, healthy infected calves
Idea for Vaccine Improvement

- Hardjo-bovis in vaccines?
- More frequent vaccination?
- Monovalent hardjo vaccine?
- Increase antigenic mass?
- Change adjuvant?
- Change in antigen preparation?
Previous Work at NADC

• Trial 2 Bolin *et al* 1989.
  • Hardjo-bovis in 5 way vaccine
  • 1 or 2 doses of vaccine
  • Challenge 6 months
  • 14/14 animals infected after challenge
Idea for Vaccine Improvement

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Previous Work at NADC

• Trial 3, Bolin et al 1991.
  • Hardjo-bovis monovalent
  • High dose vs low dose
  • Challenge 2, 3 or 4 months
  • 18/18 infected after challenge
Conclusions

• Cattle with abundant anti-LPS antibody are not protected from serovar Hardjo infection
• Not all infected animals produce anti-LPS antibody, yet they resist reinfection
• Anti-LPS antibody is not sufficient for protection in all host-serovar systems
• Vaccine efficacy/potency not straightforward
Additional Work at NADC

- Evaluation of a commercial product
    - *L. borgpetersenii* Hardjo-bovis only
      - (Commercial vs. US Std)
      - US Std-NVSL protocol
    - Two doses 4 weeks apart, challenge 16 weeks later
Additional Work at NADC

- Challenge IP
  - Control 4/4
  - US Std 4/4
  - Commercial 0/4
- Challenge conjunctival
  - Control 4/4
  - US Std 4/4
  - Commercial 0/4
Additional Work at NADC

• Commercial Trial 2
  • 12 vaccinates; 12 controls
    • Two different challenge strains
    • 12/12 controls; 0/12 vaccinates
    • Repro tract colonized in controls, not in vaccinates
Other studies

• Ellis et al 2000. Commercial product
  • *L. interrogans* hardjoprajitno based
  • (0/8) vaccinates-6 months, (1/8)-12 months
Cell Mediated Immune Response

- Both products
  - Cell mediated immune response
- Vaccination associated TH1 response
  - Antigen-specific IFN-gamma production
Conclusions: Hamsters and whole cell vaccines

- Not valid for Hardjo whole-cell products
  - LPS based protection
- Potential
  - Evaluation of alternative vaccines
  - Subunit or recombinant antigen based
Hamsters and Hardjo

- Hamsters commonly used in research
  - Disease pathogenesis
  - Evaluation of vaccines
- Limitations with Hardjo
  - Few strains result in acute disease
  - Those described, published prior to genetic classification
Bovine Infection

- Clinical signs
  - Usually only observed in relation to reproduction
  - Development of chronic shedding
    - Source of exposure to herd
    - Source of zoonotic exposure
Development of acute/chronic hamster models

- Zuerner et al, 2011.
  - Strain 203
    - Used as bovine infectious challenge
  - Strain JB197
    - DNA sequence available
    - Also used as bovine infectious challenge
Hamster Chronic Model

- Strain 203 via IP route
  - No LD50 determined, $1 \times 10^9$ IP did not result in lethal infection
  - ID50 similar to strain resulting in acute disease, $\sim 1.5 \times 10^2$
  - Found in renal tubules by 4 DPI
  - Necropsy 30 DPI, no overt clinical signs
Hamster Acute Model

- Strain JB197
- Acute disease, LD50 calculated $3.6 \times 10^4$
- Dose dependent
  - $10^7$ and above clinical signs in 4-5 DPI
  - $10^6$ or lower variable onset of signs
Hamster Acute Model

- Observable clinical signs
  - External hemorrhage
  - Tissue distribution studied in $10^2$ or $10^3$
    - Survival until 12 DPI
  - Detected in pancreas and kidneys 3 DPI
    - Broad tissue dissemination
Benefits of Hamster Models

- Study of disease pathogenesis
  - Difference in clinical course
    - (acute vs. chronic)
  - Genetically closely related strains
- Preliminary evaluation of vaccine candidates
  - Potential alternative to initial trials in cattle
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Questions?