Immunology, Vaccines, and Prevention of Salmonella

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Overview

- Basic overview of the immune system
- How are calves different?
- How do vaccines work?
- Salmonella and the immune system
- Salmonella vaccines
Immune System

- Constant exposure throughout life to pathogens
  - Viruses
  - Bacteria
  - Fungi
  - Protozoa
  - GI parasites
- Must differentiate the good versus the bad

http://ozarker.org/the-human-microbiome/

Immune System

- Innate immune response
  - Always “on duty”
  - First response
  - Not specific to a pathogen
  - Does not improve with time and exposure

- Acquired immune response
  - Takes days to weeks to be activated
  - Specifically targeted to a pathogen
  - Repeated exposure improves the strength and speed of response
  - Target of vaccines

Innate Immune System

- Barriers
  - Abomasal pH, skin, mucus

- Soluble factors
  - Antimicrobial proteins in mucus and other fluids

- Cells
  - Some reside in tissues
  - Surveillance and defense
  - Some circulate in blood

“Infantry”

- Rapid response in almost any body system to any pathogen
- Amplifies the response to increase the response
- Recruits specific responses from the acquired response
- Can be damaging to the animal
Acquired Immune System

• Soluble factors
  • Immunoglobulins (antibodies)
  • Secreted into the blood stream and other areas of the body (nose, intestine, udder, etc.)
  • Neutralize pathogens and mark them for killings

• Cells
  • “Helper” cells amplify or direct the response
  • “Cytotoxic” cells directly kill infected cells

• “Special forces”
  • Called in by innate immune system to target specific pathogens
  • Take significant training
    • Vaccines
    • Previous exposure
    • Less damaging to the host

How are calves different?

Calf Immunology

• Innate immune system
  • Some barriers are weaker
    • Abomasal pH is high for 3-4 days
  • Can have reduced numbers of cells
  • Cells do not function as well
    • Especially in first week
  • Effect of age vs effect of stress

• Acquired immune system
  • Lack antibodies at birth
  • No specific helper or cytotoxic cells
  • Can get both from colostrum
    • Fresh versus processed colostrum

Maximizing calf immune function

• 1 gallon of high quality colostrum within 4 hours of birth
• Fresh from calf’s dam>fresh from another cow> frozen>powdered replacer
• Must balance with timing, labor, controlling disease, etc.

Generally the calf’s immune response is slower, weaker and less specific
Maximizing calf immune function

- Minimizing exposure to pathogens
  - Reduces chance that response is overwhelmed
  - Sanitation
  - Ventilation
- Minimize stress
  - Appropriate nutrition
  - Adequate shelter
    - Heat and cold stress
  - Processing

How do vaccines affect the immune system?

- Vaccine can include:
  - Killed pathogen
  - Modified live (weakened) pathogen
  - A specific part of a pathogen

- Only target the acquired immune response
  - Response is slow
    - Weeks to achieve maximum protection
  - Requires multiple exposures
    - Booster typically required for best, most persistent response
  - Very specific
    - Depending on pathogen, may be strain specific

How do vaccines affect the immune system?

- Vaccine is recognized by a surveillance cell
  - Carried to a nearby lymph node
- Recruits cells from the acquired immune response
- Specific antibodies are created against the vaccine strain
  - Circulate throughout the body
- A second exposure amplifies this response and creates long-lasting cells capable of producing these antibodies
  - “Memory” cells are created that reside in lymph nodes and tissues around the body
  - Can rapidly produce specific antibodies in the future
Vaccines in calves

- Colostral antibodies can last for 3-4 months with good colostrum management
  - Most vaccines can be delayed until then
- But what if colostrum isn’t enough...
- Calves can respond to vaccines in the first week of life
- Response to vaccines is less than that of juveniles and adults

Vaccines in calves

- Vaccine recognition → inhibited by colostral antibodies, fewer surveillance cells
- Recruitment and antibody production → Decreased number of cells to respond and produce antibodies
- Booster 4-6 weeks later is too late to protect neonates

Improving Immunity in Neonatal Calves

- Dry cow vaccination
  - Provides specific antibodies for pathogens in neonatal calves
  - Best evidence for diarrheal pathogens
    - Can we do this for Salmonella?
  - Must have a good colostrum management program
    - No colostrum replacers
    - Cannot be used in an outbreak

Improving Immunity in Neonatal Calves

- Stimulate immunity at the site of infection instead of everywhere in the calf
  - Intranasal vaccines
  - Oral scours vaccines
- May bypass interference of colostral antibodies
- May stimulate some innate immune responses
- Can be helpful in preventing disease in neonates
So what about Salmonella?

• Causes a significant innate immune response
  • Much of the pathology can be attributed to this "overeager" response

• Evolved mechanisms to survive the immunologic attack
  • Uses some of these cells to spread throughout the body and create long-term carriers

• Can invade oral tonsils or small intestine
  • Through small cuts in skin in feedlot cattle
  • Maybe through respiratory tract too

So what about Salmonella?

• Can cause disease as early as 5 days of age

• Protection appears to require both halves of the acquired immune response
  • Difficult to induce both antibodies and cellular response with vaccines

• Immune response is serotype specific

Vaccination

• Controversy exists over whether or not vaccinating young calves with Salmonella is beneficial

• Complex problem as immunity to different serotypes is quite different

• Most important aspect of control is to limit exposure

• Vaccines available include killed (bacterins), bacterial fractions (subunit) and modified live

Killed Vaccines

• Calves less than 1 year of age generally do not respond well to killed Salmonella bacterins

• There aren’t any killed vaccines on the market in the US now – so these are all autogenous products

• Several studies indicate the use of autogenous or killed vaccines not effective in calves
Killed Vaccines

• Study vaccinated calves between 1 and 19 weeks of age with a killed vaccine as well as a modified live vaccine
• Calves less than 12 weeks of age that received a killed vaccine did not produce any antibodies
• However calves given ML at 1 & 3 weeks of age did have detectable antibodies
• Conclusion – Killed Salmonella vaccines probably not ideal for use in young calves


Killed Vaccines

• Groups of calves were vaccinated with
  • Oral modified live S. Typhimurium (non pathologic)
  • SC with killed S. Typhimurium strain
  • Unvaccinated control group
• Calves orally challenged with S. Typhimurium at 3 weeks of age
• ML oral vaccine gave reasonable protection (other 2 groups developed acute enteritis, pyrexia, etc)
• Less S. Typhimurium recovered from fecal cultures and from tissues at necropsy from ML group

Infect & Immun 1983; 41:742-750

Killed Vaccines

• Even studies in adult cattle with killed vaccines have been underwhelming
• Conclusion – the use of autogenous bacterins in calves is almost certainly to be of no benefit in preventing Salmonella

Modified Live Vaccines

• More commonly used in calves
• Various methods have been used to make the bacteria weaker in calves
Modified Live Vaccines

- Calves vaccinated with a ML S. Typhimurium vaccine either orally or IM at 2-3 weeks of age
- Calves were challenged at 5-8 weeks of age
- All control calves became sick and 14/16 died – all cultured positive at necropsy for Salmonella
- 6/7 calves given 2 doses of vaccine IM lived as well (4/7 did not develop diarrhea)
- All 3 calves given 2 doses of vaccine orally lived


Modified Live Vaccines

- Calves that received only 1 dose of vaccine (either IM or orally) generally died and were not protected after challenge
- No significant side effects of vaccination were noted
- Another ML strain tested was not effective


Modified Live Vaccines

- A follow-up study was done with a ML S. Dublin vaccine
- Calves were vaccinated at 2 and 3 weeks of age – and challenged at 5 weeks of age
- Significant protection was again noted in the vaccinated calves (reduced mortality, diarrhea and culturing of Salmonella)
- In theory – these vaccines are incapable of reverting to virulence


Modified Live Vaccines

- Entervene-d is a ML S. Dublin vaccine approved for use in calves over 2 weeks of age
- Has some cross-protection against Typhimurium (groups B and D similar)
- Use in younger calves has been reported with mixed results
- Anaphylactic reactions are fairly common (likely due to endotoxin)
Oral Vaccination

- 140 calves received ML vaccine orally at 3 and 10 days of age (148 unvaccinated calves)
- No difference in mortality, % of calves that developed diarrhea, % of calves from whom Salmonella was cultured, daily depression scores or growth rates between groups
- >40% of calves in both groups developed diarrhea
- **Conclusion** – oral vaccination not effective

Habing et al, JAVMA 2011; 238:1184-1190

Modified Live Vaccines

- Challenge is to develop a vaccine that provides cross-protection against multiple serovars
- Recent new modified live strains show promise as oral vaccines that may provide cross protection

Conclusions for Modified Live Vaccines

- Modified live vaccines can work very well and offer significant protection against challenge
- Future vaccines show promise for providing broad immunity
- However the ability to protect calves less than 4-5 weeks of age may be limited
- Studies have shown that calves often get exposed to Salmonella within 48 hours of birth

Subunit Vaccines

- Bacterial fractions or extracts (ie surface proteins, flagella, etc)
- Vaccines consist of a single protein – designed to isolate bacterial fragments that will induce an immune response without causing adverse reactions
- Becoming more common in both human and veterinary medicine
Subunit Vaccines

- Consists of extracts of iron transport proteins – common to all Salmonella
- Required for the bacteria to survive
- Approved for use in cattle >6 months
- Attempted use of SRP (S. newport) bacterin in calves produces high rate of adverse reactions
- Possible benefit to using SRP in dry cows

Passive Immunity

- Very little data on the use of Salmonella vaccines in the dry cow and their potential benefit to controlling disease in calves
- A few studies since the 1960s have shown some benefit to calves with dry cow vaccination
  - ML appears better than killed
  - No data with current vaccines

SRP Study

- 30 Holstein cows were vaccinated at dry off and again 4 weeks later (30 cows received saline)
- Colostrum was collected at calving and calves were fed colostrum from their dam within 4 hours of birth
- Calves were bled 24-48 hours later

<table>
<thead>
<tr>
<th></th>
<th>Before Vaccination (Dry-off)</th>
<th>After Vaccination (at calving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>0.26 ± 0.03</td>
<td>0.16 ± 0.02</td>
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<tr>
<td>Vaccinated Group</td>
<td>0.20 ± 0.02</td>
<td>0.69 ± 0.03</td>
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<tr>
<td>P Value</td>
<td>0.56</td>
<td>0.01</td>
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</table>

SRP Study

<table>
<thead>
<tr>
<th></th>
<th>Colostrum</th>
<th>Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>0.66 ± 0.03</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Vaccinated Group</td>
<td>1.49 ± 0.02</td>
<td>1.04 ± 0.03</td>
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<tr>
<td>P Value</td>
<td>0.011</td>
<td>0.003</td>
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Entervene-d Study

- 30 Holstein cows were vaccinated at 3 weeks prior to dry off and again at dry off
- Colostrum was collected at calving and calves were fed Colostrum from their dam within 4 hours of birth
- Calves were bled 24-48 hours later

Entervene-d Study

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<th>After Vaccination (at calving)</th>
</tr>
</thead>
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<tr>
<td>Control Group</td>
<td>-4.2 ± 1.5</td>
<td>-9.4 ± 1.1</td>
</tr>
<tr>
<td>Vaccinated Group</td>
<td>-6.9 ± 0.9</td>
<td>40.3 ± 9.1</td>
</tr>
<tr>
<td>P Value</td>
<td>0.67</td>
<td>0.008</td>
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ELISA results presented as percent positivity

Entervene-d Study

<table>
<thead>
<tr>
<th></th>
<th>Colostrum (prior to Colostrum)</th>
<th>Calves (prior to Colostrum)</th>
<th>Calves (after Colostrum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>-17.2 ± 0.4</td>
<td>-12.1 ± 0.4</td>
<td>-3.2 ± 1.2</td>
</tr>
<tr>
<td>Vaccinated Group</td>
<td>14.8 ± 7.6</td>
<td>-13.8 ± 0.5</td>
<td>88.5 ± 8.9</td>
</tr>
<tr>
<td>P Value</td>
<td>0.04</td>
<td>0.87</td>
<td>&lt;0.001</td>
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Conclusions from dry cow vaccination studies

- Commercially available vaccines can provide antibodies to calves against Salmonella
- More studies needed to determine whether they might be protective
- Antibodies probably of relatively short duration
- Perhaps could be used in combination with ML vaccination at some point

Conclusions

- Both innate and acquired immune responses are critical to calf health
- Calf immune responses are less than adults
  - Colostrum management is critical to calf health
- Vaccines primarily stimulate the acquired immune response
  - Need repeated exposure
  - Slow to develop
  - Very specific

Conclusions

- Immunity to Salmonella is complex
  - Some aspects of the response are harmful
  - Salmonella can hijack some white blood cells
  - Requires both arms of the innate immune response
  - Little cross protection
- Modified live and subunit vaccines provide some protection in older calves and adults
  - Too dangerous for young calves
- Passive immunity through colostrum may provide some protection for the first weeks of life

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