NC STATE
Veterinary Medicine
Population Health and Pathobiology

Immunology, Vaccines, and Prevention of Salmonella

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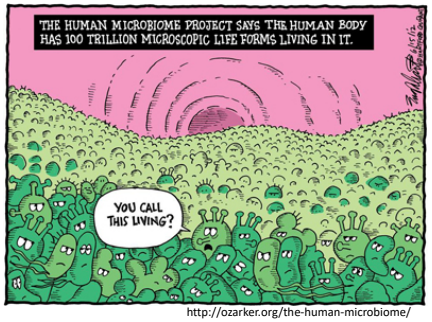
Overview

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

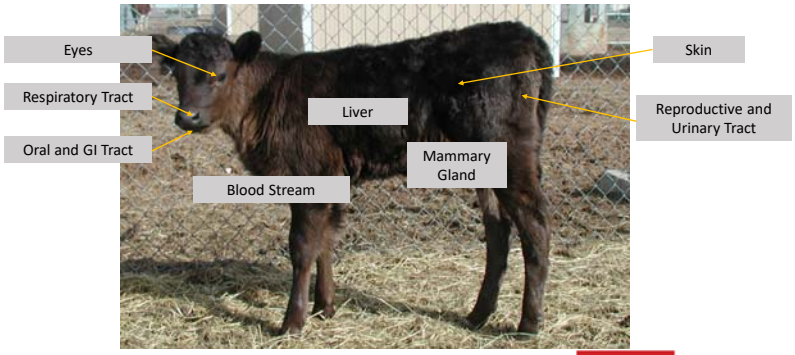
Immune System

- 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



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

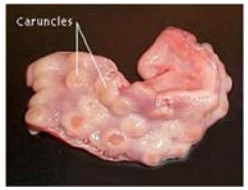
Innate Immune System

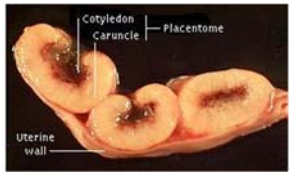
- Barriers
  - Abomasal pH, skin, mucus
- Soluble factors
  - Antimicrobial proteins in mucus and other fluids
- Cells
  - Some reside in tissues
    - Surveillance and defense
  - Some circulate in blood
- "Infantry"
  - Rapid response in almost any body system to any pathogen
  - Amplifies the response 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

Generally the calf's immune response is slower, weaker and less specific

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.

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

Bovine Vaccines

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

Am J Vet Res 1992;53:1895-1899

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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

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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

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Modified Live Vaccines

- More commonly used in calves
- Various methods have been used to make the bacteria weaker in calves

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Modified Live Vaccines

- Calves vaccinated with a ML S. Typimurium 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

Smith BP et al. Am J Vet Res 1984;45:59-66

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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

Smith BP et al. Am J Vet Res 1984;45:59-66

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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

Smith BP et al. Am J Vet Res 1984;45:2231-2235

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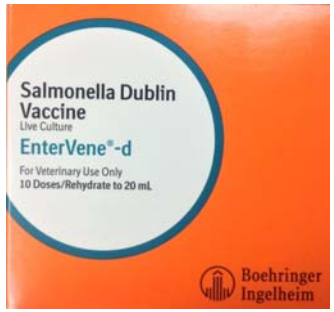
Modified Live Vaccines

- 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)



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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

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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

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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

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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

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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

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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

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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

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SRP Study

	Before Vaccination (Dry-off)	After Vaccination (at calving)
Control Group	0.26 ± 0.03	0.16 ± 0.02
Vaccinated Group	0.20 ± 0.02	0.69 ± 0.03
P Value	0.56	0.01

Smith et al. J Vet Int Med 2014; 28:1602-1605

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SRP Study

	Colostrum	Calves
Control Group	0.66 ± 0.03	0.30 ± 0.02
Vaccinated Group	1.49 ± 0.02	1.04 ± 0.03
P Value	0.011	0.003

Smith et al. J Vet Int Med 2014; 28:1602-1605

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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

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Entervene-d Study

	Before Vaccination	After Vaccination (at calving)
Control Group	-4.2 ± 1.5	-9.4 ± 1.1
Vaccinated Group	-6.9 ± 0.9	40.3 ± 9.1
P Value	0.67	0.008

ELISA results presented as percent positivity

Smith et al. J Dairy Sci 2015; 98:2529-2532

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Entervene-d Study

	Colostrum	Calves (prior to colostrum)	Calves (after colostrum)
Control Group	-17.2 ± 0.4	-12.1 ± 0.4	-3.2 ± 1.2
Vaccinated Group	14.8 ± 7.6	-13.8 ± 0.5	88.5 ± 8.9
P Value	0.04	0.87	<0.001

Smith et al. J Dairy Sci 2015; 98:2529-2532

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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

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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

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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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