Mycoplasma bovis colonization of calves in an endemically-infected herd

Fiona Maunsell[1], Jessica Coan[1], Art Donovan[2], Mary Brown[1]

[1]Department of Infectious Diseases and Pathology
[2]Department of Large Animal Clinical Sciences
University of Florida

Research Summary
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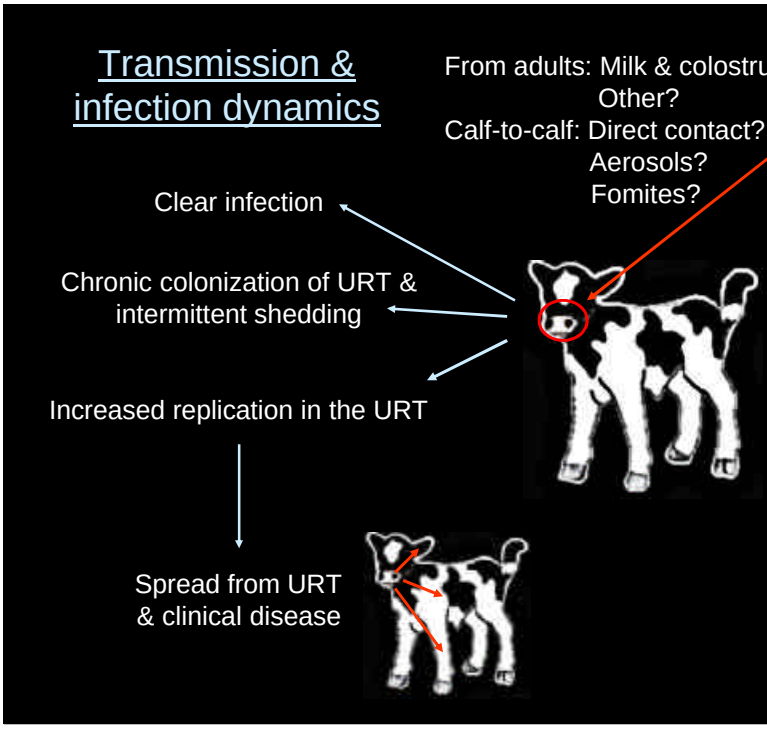
M. bovis-associated disease (*Mb*AD) in dairy calves

- Respiratory disease, otitis media, arthritis, tenosynovitis, meningitis
- 2-12 wk of age
- Poor response to therapy, chronic disease
- Variable disease pattern
  - Endemic, frequent clinical cases
  - Outbreaks

Transmission & infection dynamics



Control of *Mb*AD

- Control measures
  - Feed pasteurized milk, milk replacer
  - Limit other means of exposure
    - Air quality, stocking density
    - Fomites
    - Exposure to sick and older calves
  - Maximize respiratory defenses
    - Control other respiratory pathogens
    - Nutrition, colostrum management, air quality, stocking density, stress
    - Antibiotic metaphylaxis
    - Specific immunity?

But…

- Current control measures often fail to prevent *Mb*AD, especially in endemically-infected facilities
- Better understanding of epidemiology needed

Objective

- Characterize patterns of nasal colonization by mycoplasmas in calves in a facility with endemic *Mb*AD

Study design

- Prospective observational study
  - Randomized blocked clinical trial of an investigational veterinary product (IVP)
    - No association between IVP and mycoplasma colonization (*P*=0.86)
- Study herd
  - 3,700 milking herd
  - All heifers, some bulls raised on site
  - Endemic *Mb*AD, significant contributor to overall calf disease

Study design

- Predetermined sample size=360
- Rolling enrollment
- Healthy heifers enrolled at 4d of age
- Followed until ~23wk (brucellosis vaccination)
- Individual pens until ~8wk
  - Weeks 8-15 in groups of 6
  - Weeks 15-19 in groups of 18
  - Weeks >19 in groups of 36

Study design: calf housing

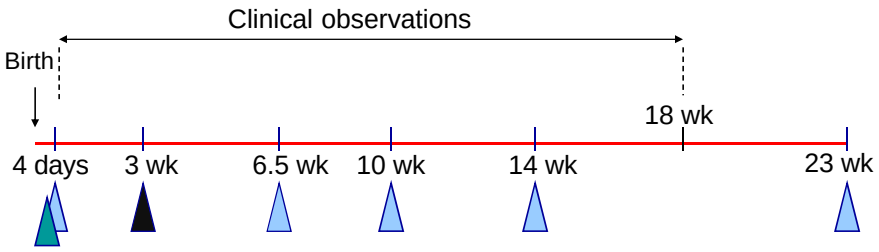
- Main barn
  - 4 rows of 58 pens
- Satellite barns
  - 2 barns, 48 pens each

- Identical pen design, feeding, management in all barns

Study design: calf management

- Reasonable colostrum management (not pasteurized)
- Pasteurized waste-milk
  - SPC, mycoplasma culture on all batches
- Ear notch for BVDV
- Preventives
  - Ecolizer+C (birth)
  - TSV-2 (1 d)
  - Immunoboost-K, MuSe (1 & 5 d)
  - Bovishield Gold 5 (2½, 6, 20 wk)
  - Vision-7 (6, 12, 20 wk)
  - Maxiguard Pinkeye (7, 12, 20 wk)
  - SpiroVac + LA200 (12, 16 wk)
  - Cydectin pour-on (16 wk)

Study design: data collection



- Nasal swabs for mycoplasma culture & PCR
  - PCR for *M. bovis uvr*C gene, if negative followed by PCR for genus-specific 16S rRNA & RE analysis
- Post-colostral serum protein & *M. bovis*-specific serum antibody titers

Study design: clinical *Mb*AD

- Respiratory disease
  - Increased RR &/or effort, with fever + nasal discharge, &/or cough &/or abnormal auscultation
- Otitis media
  - Ear droop &/or ear pain (head shaking, scratching or rubbing ear)
- New cases
  - Nasal and tonsil swabs for mycoplasma culture & PCR
  - Rectal temperature
  - Treated using standardized protocols, all treatments recorded
- Died/euthanized – full necropsy

Study design: data analyses

- Categorical variables
  - Incidence of positive nasal cultures (%)
  - Morbidity (%)
  - IVP group
  - Pre-weaning barn
  - *M. bovis* status of neighboring calves
  - *M. bovis* status of previous stall occupant
- Chi-square, odds ratios
- Multinomial logistic regression

Study design: data analyses

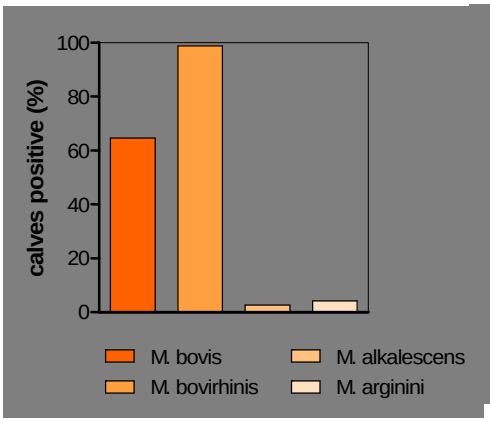
- Continuous variables
  - Age at diagnosis of Res/OM
  - Total serum protein (g/dl)
  - Post-colostral *M. bovis*-specific serum IgG, G_1 or G_2
  - Association with nasal culture or morbidity
    - Ln transformation of end-point titers
    - Student's t-test or ANOVA as appropriate
    - Pearson correlation analysis (age at Dx vs. ELISA data)
  - Effect of sampling time when *M. bovis* was first detected on age at Dx
    - Kruskal-Wallis test

Results

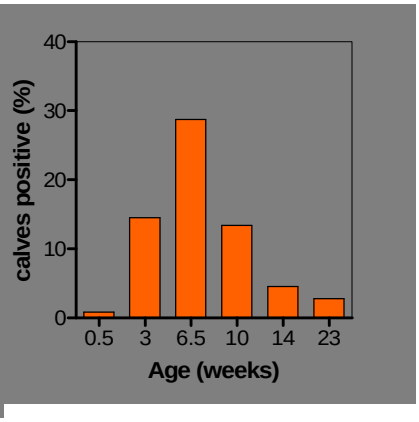
- Calf numbers
  - 359 calves enrolled (Oct – Jan)
  - 17 calves died/lost to follow-up
    - 8 died pre-weaning (2.2%)
    - 5 died post-weaning (1.4%)
    - 4 removed from study
- Calf management
  - Preweaning housing
    - 263 (73%) calves in main barn
    - 96 (27%) calves in satellite barns
  - 4% FTPI (post-colostral TSP <5.5g/dl)
  - No breaks in pasteurization of milk

Nasal culture results

Mycoplasmas isolated from nasal swabs

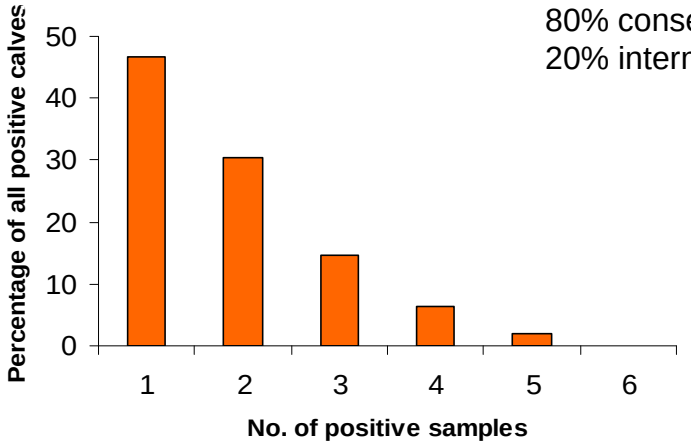


Sampling period when *M. bovis* was first detected



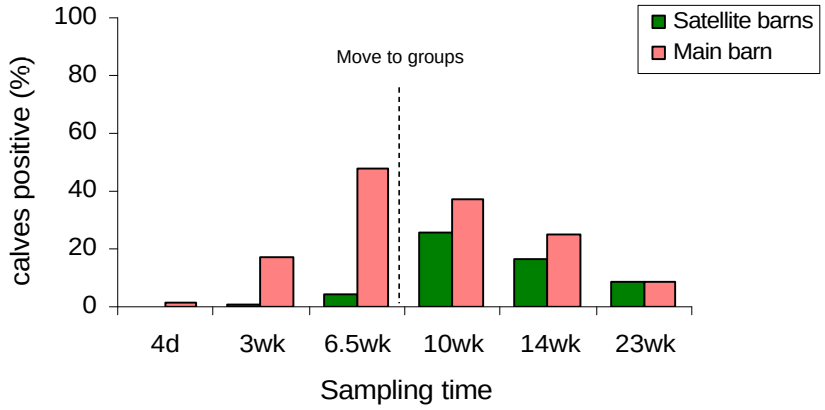
Approximately half the calves had more than one positive *M. bovis* culture

<u>>1 positive sample</u>
80% consecutive
20% intermittent



Risk of detection of *M. bovis* on nasal swabs was influenced by pre-weaning location

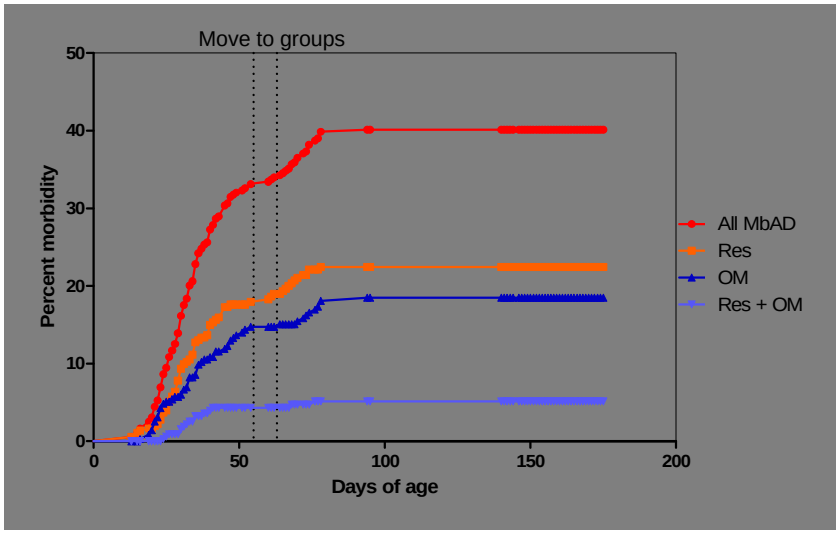
Main barn vs. Satellite barns
OR=15.6 (8.0-30.4, *P*<0.001)



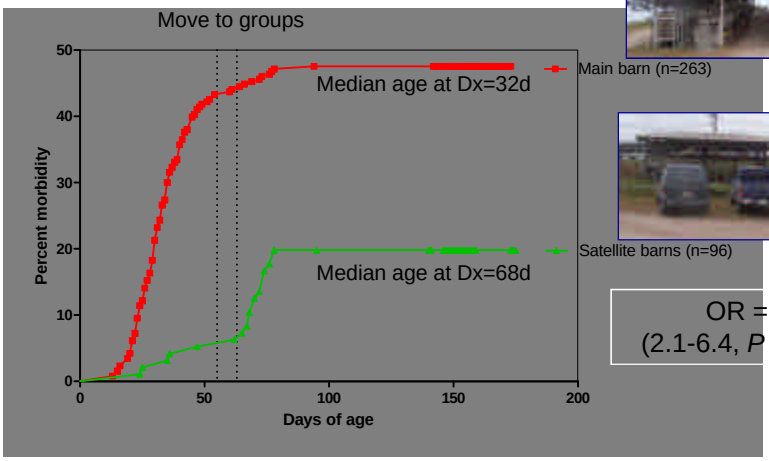
Association of nasal colonization with clinical disease

- Nasal colonization and clinical disease (respiratory disease and/or otitis media)
  - *M. bovis* isolated prior to Dx (yes/no)
  - OR = 2.7 (1.3-5.3, *P*=0.003)
- Age at first colonization and clinical disease
  - Pre-weaning vs. post-weaning
  - OR = 2.4 (1.3-4.3, *P*=0.004)

Cumulative morbidity



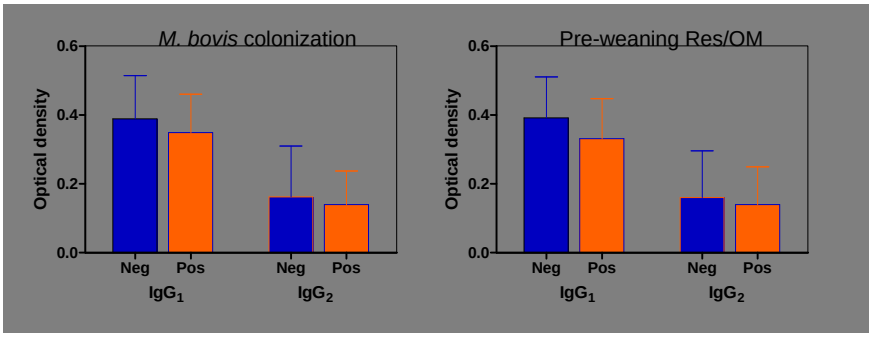
Risk & timing of Res/OM was influenced by pre-weaning housing



OR = 3.7
(2.1-6.4, *P* =0.0002)

Transfer of passive immunity was not associated with *M. bovis* colonization or Res/OM

- No association of serum total protein concentration or post-colostral *M. bovis*-specific IgG_1, IgG_2, or IgG with:
  - *M. bovis* colonization
  - Res/OM (presence, type of disease, age at first treatment)



Risk of being culture positive for *M. bovis* pre-weaning was influenced by other calves

- Previous occupant
  - Paired data for 40 stalls
  - Main barn
  - IR if previous calf was *M. bovis* nasal positive = 0.86
  - IR if previous calf was *M. bovis* nasal negative = 0.44
  - OR = 7.92 (1.7 – 33.6, *P* = 0.007)
- Neighbor(s)
  - All calves
  - IR if adjacent calf was *M. bovis* nasal positive = 0.64
  - IR if adjacent calf was *M. bovis* nasal negative = 0.18
  - OR = 8.3 (5.0 – 13.8, *P* < 0.001)

Conclusions

- Multiple mycoplasma species were isolated from calves in this facility with endemic *M. bovis*-associated disease
- *M. bovis* colonization was detected in 65% of calves
  - Median sampling time when calves were first nasal culture positive was 6.5 weeks
  - A substantial proportion of calves were colonized by *M. bovis* at a young age
    - 0.8% at 4 days
    - 16% by 3 weeks

Conclusions

- Risk of nasal colonization with *M. bovis* was influenced by
  - Location (barn)
  - Infection status of previous pen occupant
  - Infection status of neighbors
- Risk of *Mb*AD was influenced by
  - Location (barn)
  - Infection status of neighbors

➢ Pre-weaning environment had a major influence on nasal colonization rates and incidence of Res/OM

On-farm impact

- Opportunities for intervention in this herd
  - Cleaning protocols between successive calves
  - Maximize distance between calves
  - Maximize distance from older calves
    - Manage main barn in quadrants rather than rows
  - Metaphylaxis to limit post-weaning disease in high-risk animals

Conclusions

- Persistence of *M. bovis* on surfaces may play a significant role in epidemiology
  - Current research
    - Quantify contributors to the load of bacteria calves are exposed to in endemically-infected barn and hutch facilities



Questions