Mannheimia haemolytica Immunity

Are We There Yet?

Anthony W. Confer, DVM, MS, PhD, Diplomate ACVP

Regents Professor & Sitlington Chair

Dept. Veterinary Pathobiology

Oklahoma State University

Center for Veterinary Health Sciences

“Vaccine history: The past as prelude to the future.” *Vaccine* 2012

History informs us about progress in vaccine development

1. **Progress is made incrementally**
2. **Progress often requires “game-changing” event or events**
3. **Progress is closely tied to development of improved technologies from other fields**
4. **Progress will occur through application of novel science-based technologies and strategies**

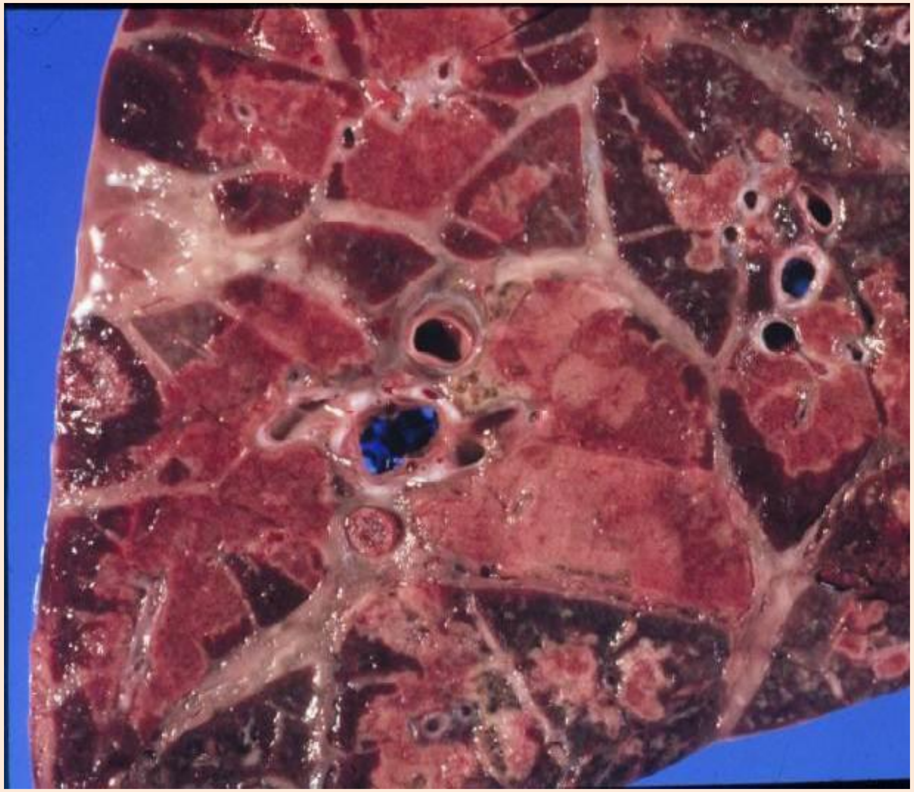
Objectives of the presentation

- **Review several historically important findings in *M. haemolytica* pathogenesis & immunity**
- **Provide overview of experimental approaches in improving *M. haemolytica* vaccines**

M. haemolytica is associated with severe bovine bacterial pneumonia

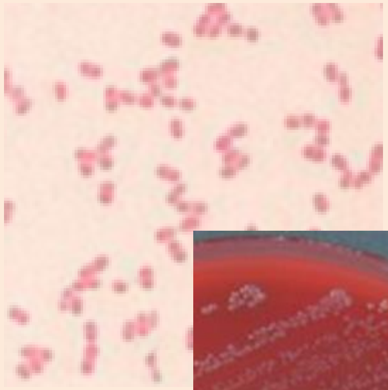
Acute fibrinous pleuropneumonia.

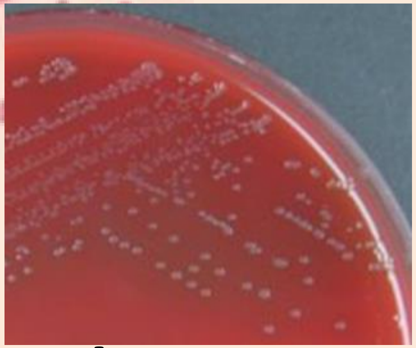
- **Shipping fever – beef cattle**
- **Enzootic Pneumonia – dairy calves**



"Failure of innate immunity!" Robert Fulton

Mannheimia haemolytica





Gram negative coccobacillus

Previous names:

- ***Bacillus bovisepticus***
- ***Pasteurella haemolytica***
  - **Biotype A – Arabinose fermenters**
  - **Biotype T – Trehalose fermenters**
  - **Divided among serotypes based on capsular antigens**

As of 1999: *Mannheimia haemolytica*

- **11 Biotype A serotypes - 1,2,5,6,7,8,9,12, 13, 14, and 16** (Angen et al. *IJSEM* 49:67086, 1999)
- **Serotype 1 (S1) is responsible for 60% or more of pneumonia cases**

Critical findings 1970s & 1980s

Pathogenesis: Changes in the nasopharyngeal flora of stressed or viral infected calves.

- In stressed calves, *M. haemolytica* proliferate and are in increased concentrations in the tracheal air (Grey & Thomson, CJCM, 1971)
- Serotype 1 is in low nasal concentrations until stressed or viral infected; then S1 is readily isolated (Frank and Smith, AJVR, 1983; 1986)

Pathogenesis: Discovery of leukotoxin

- *M. haemolytica* secretes a leukotoxin (then called "cytotoxin") that kills leukocytes from ruminants (Shewen & Wilkie, I & I, 1982)

R.G. Thomson

G.H. Frank & R.E. Briggs

B.N. Wilkie & P.E. Shewen

Critical findings 1970s & 1980s

Immunity

- **At feedlot entry, cattle with higher anti-*M. haemolytica* antibodies have less respiratory disease than those with low antibodies (Thomson et al., CJCM, 1975)**
- **Bacterins do not protect and may enhance disease (Friend et al., CJCM 1977; Wilkie et al., AJVR 1980)**
- **Cattle dying of shipping fever had lower LKT neutralizing titers than did those that died of other causes (Shewen & Wilkie, CJCM 1983)**
- **Direct correlation of LKT neutralizing antibody titers and resistance to *M. haemolytica* challenge (Gentry et al., VI & I, 1985)**
- **Immunity requires antibodies to leukotoxin and to surface antigens (Shewen & Wilkie, CJVR, 1988)**
- **Major surface antigens are not LPS or capsule but OMPs (Mosier et al., I & I,1989; Confer et al., AJVR 1986; Confer et al., AJVR 1989)**

Central Dogma of Vaccine-induced Immunity to *M. haemolytica*

- Immunity is serum antibody-mediated
- Antibodies MUST neutralize leukotoxin
- Antibodies against surface antigens (OMPs) must stimulate complement-mediated killing and/or phagocytosis & killing
- When given properly, vaccines that stimulate antibodies to surface antigens and to leukotoxin SHOULD reduce colonization of the lower respiratory tract & protect cattle

Commercial *M. haemolytica* Vaccines currently available or available in the past

- **Bacterin ("antigens from chemically inactivated cultures")**
- **Bacterin – leukotoxoid combination**
- **Leukotoxin-rich culture supernatant**
- **Recombinant leukotoxin-outer membrane combination**
- **Live streptomycin-dependent mutant vaccine (parenteral or intranasal delivery)**
- **Other avirulent(?) live cultures***
- **Autogenous vaccines**

***no longer marketed**

Commercial *M. haemolytica* vaccines: Do they work?

- **18 *M. haemolytica or M. haemolytica + P. multocida* vaccine field trials**
  - **3/18 significant reduction in BRD morbidity**
  - **4/18 increased BRD morbidity**
  - **11/18 decreased morbidity but not statistically significant**
- **"the published body of evidence does not provide a consistent estimate of the direction and magnitude of effectiveness in feedlot cattle vaccination against *Mannheimia haemolytica, Pasteurella multocida*, or *Histophilus somni*." (Larson & Step, *Vet Clin N Amer Food An Prac* 2012)**

What are approaches to potentially improve *M. haemolytica* vaccines?

Potential modern approaches to bacterial vaccines

- Recombinant protein subunit vaccines
- Chimeric protein vaccines
- Genetically modified bacterial vaccines
- Live recombinant organisms
- DNA vaccines
- Bacterial ghosts
- Bacterial vesicles
- Alternative delivery methods
- Immunostimulants

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Recombinant protein vaccines: Addition of Immunogenic Recombinant *M. haemolytica* Proteins to Commercial Vaccines: Recombinant LKT (rLKT)

Conlon et al. (*Infect. & Immun*. 1991)

- **Vaccination with rLKT alone did NOT stimulate protection**
- **Addition of rLKT increased efficacy of a culture supernatant vaccine (Presponse) against experimental challenge with *M. haemolytica* with reduced clinical signs and lesions.**

Recombinant protein vaccines: Addition of Immunogenic Recombinant *M. haemolytica* Proteins to Commercial Vaccines: Recombinant Sialoglycoprotease (rSGP)

Shewen et al. (*Vaccine* 2003) –– SGP, a protease in culture supernatant.

- **Addition of rSGP fusion protein (Gcp-F) & rLKT increased efficacy of a culture supernatant vaccine (Presponse) against experimental challenge with *M. haemolytica* with lower mean clinical scores, but the differences were not significant.**

Recombinant protein vaccines: Addition of Recombinant *M. haemolytica* Proteins to Commercial Vaccines: Recombinant OMP PlpE (rPlpE)

PlpE: a major surface-exposed 45 kDa outer membrane lipoprotein of *M. haemolytica* with sequence homology between serotypes 1 & 6. (Ayalew et al., *Vet Microbiol* 2006; Confer et al., *Vaccine* 2003 & 2006; Pandher et al., *I & I* 1998)

- **Addition of 100 µg of rPlpE increased efficacy of a culture supernatant vaccine (Presponse) or bacterin toxoid (One Shot) against experimental challenge with *M. haemolytica* S1 or S6.**

Addition of rPlpE to Presponse®: Mean Lung Lesion Scores ± SD after challenge with *M. haemolytica* Serotype 1

Group	Lesion score (% reduction)
Control	7.75 ± 3.58
Presponse®	3.00 ± 1.26 (67.9%)
Presponse/PlpE	1.08 ± 0.92 (95.3%)

Addition of PlpE improved resistance by 27.4%

Addition of rPlpE to Presponse® followed by Serotype 6 challenge

Vaccine	No. of cattle	Mean lesion ± SD (% reduction)
Control – adjuvant only	6	8.1 ± 2.2
100 μg PlpE + adjuvant	8	4.4 ± 4.7 (45.1%)
Presponse®	8	4.8 ± 2.2 (41.2%)
Presponse® + 100 μg PlpE	8	2.0 ± 1.2 (75.3%)

Addition of PlpE improved resistance by 34.1%

Other potential recombinant *M. haemolytica* OMPs for vaccine consideration

Serotype-specific antigen-1

- **Highly conserved between S1 & S2 (Gonzalez et al., *Infect & Immun* 1995)**
- **Highly immunogenic mice and cattle (Ayalew et al., *CVI* 2011, Lo et al. *Infect & Immun* 1991)**
- **Addition of rSSA-1 to other recombinant proteins enhanced responses to those proteins (Ayalew et al., *CVI* 2011)**

GS60 – Surface-exposed outer membrane lipoprotein (Weldon et al., *Vet Microbiol* 1994; Lo & Mellors, *Vet Microbiol* 1996).

- **Conserved among all *M. haemolytica* serotypes**
- **Correlation between antibodies to Gs60 and resistance to challenge (Orouji et al., *CJVR* 2012)**

Other potential recombinant *M. haemolytica* OMPs for vaccine consideration

OmpA – Conserved OMP with adhesin properties (Kisiela & Czuprynski, I & I 2009; Lo & Sorensen, FEMS Microbiol Lett. 2007)

- **High antibodies correlate with resistance against experimental challenge (Mahasreshti et al., Infect & Immun, 1997)**
- **Highly immunogenic (Ayalew et al., CVI 2011)**
- **Anti-OmpA antibodies stimulate complement-mediated killing**
- **Addition of rOmpA to other recombinant OMP may reduce responses (Ayalew et al., CVI 2011, Zeng et al., PhD dissertation, 1999)**

PlpF – outer membrane lipoprotein (Ayalew et al., Vaccine 2011)

- **Conserved among S1, S2, & S6 with variations in repeats regions**
- **Highly immunogenic in mice and cattle**
- **Stimulates high titers of C'-mediated bactericidal antibodies**
- **Protection studies not done**

Chimeric (fusion) protein vaccines

Recombinant proteins derived from the spliced genes for multiple proteins.

Experimental *Bordetella bronchiseptica* fimbrial protein-*M. haemolytica* LKT Chimeric Protein Vaccine: Recombinant genes expressing a fusion protein composed of combinations of

- **C-terminus-neutralizing region of *lktA***
- **Fimbrial protein (*fim N* gene)**
- **Glutathione-*S*-transferase (GST)**

- **Vaccination of mice resulted in anti-LKT antibodies**

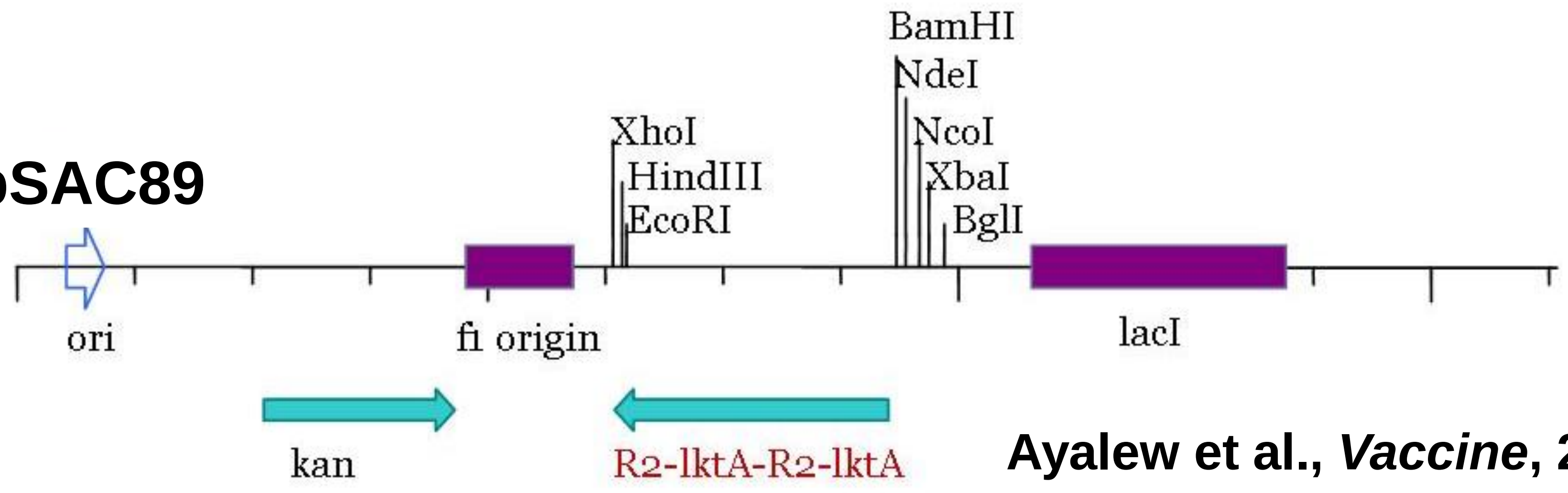
Rajeev et al., *Vaccine*, 2001

Chimeric protein vaccines: PlpE-LKT Chimeric Protein Vaccine

- **Plasmids developed that expressed several chimeric genes (pSAC86-89, pSAC91) composed of various combinations of:**
  - **C-terminus-neutralizing region of *lktA* (NLKT)**
  - **N-terminus – major surface epitope (R2) of *PlpE***

SAC89 protein: R2-NLKT-R2-NLKT

pSAC89



Ayalew et al., *Vaccine*, 2008

Vaccination of cattle with 100 µg SAC89 + bacterin: *M. haemolytica* challenge

Group	Lesion score (% reduction)
SAC89 + adjuvant	7.1 ± 6.3 (39.6%)
SAC89 + Bacterin + adjuvant	3.1 ± 1.2 (73.7%)
Bacterin + adjuvant	7.6 ± 6.8 (34.7%)
PBS + adjuvant	11.7 ± 9.7

Addition of PlpE/LKT chimeric protein enhanced protection of a bacterin by 39%

Confer et al., *Vaccine*, 2009

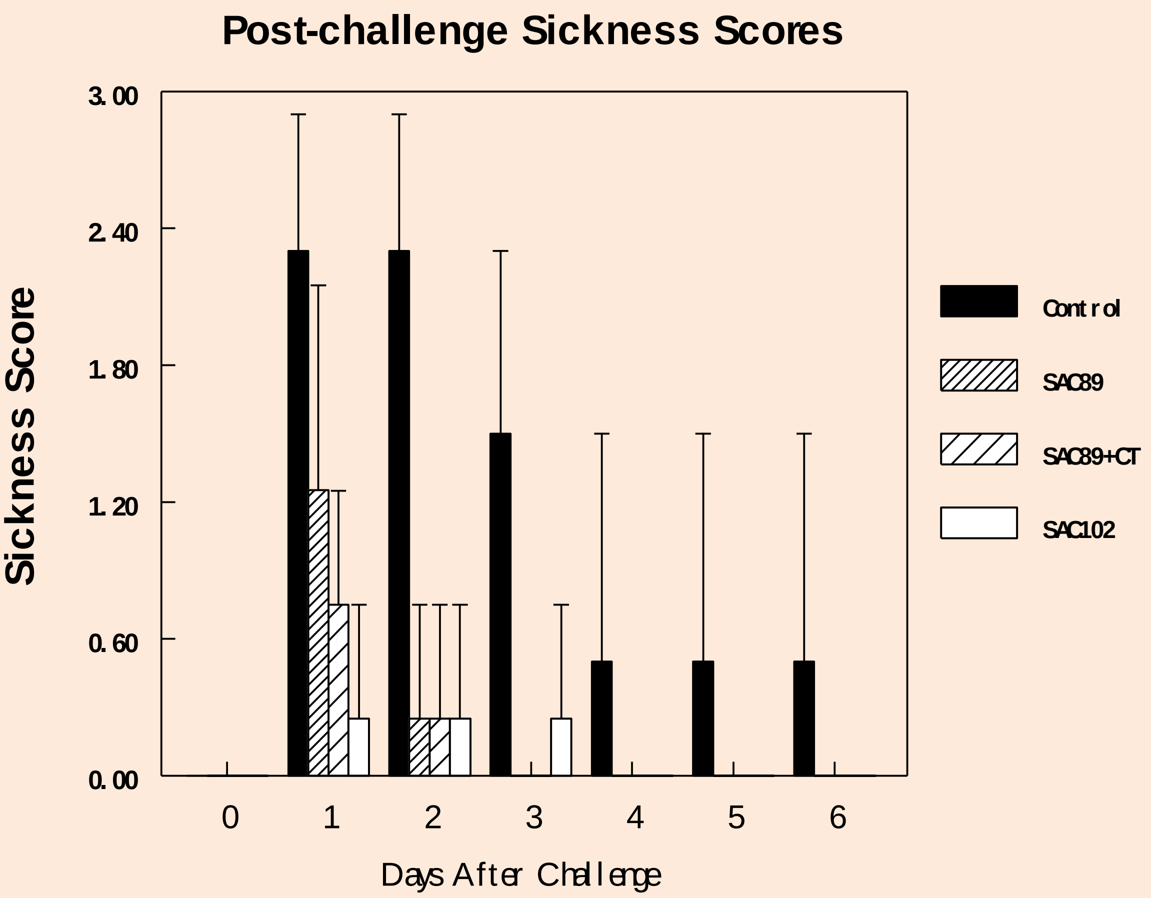
Intranasal CTB-R2-NLKT Chimeric Protein Vaccine in Cattle

- **Cholera toxin is one of best mucosal adjuvants**
- **Because of potential hazard in using cholera toxin, SAC102 was developed: Protein derived from a chimeric gene for Cholera Toxin Subunit B (CTB)-major epitope of PlpE (R2)-neutralizing epitope of leukotoxin (NLKT).**
- **In IN vaccinated calves, SAC102 stimulated serum antibodies against formalin-killed *M. haemolytica,* PlpE, and LKT**

Ayalew et al., *Vet Immunol & Immunopathol*, 2009

Clinical responses of SAC102 vaccinates after intrabronchial challenge with *M. haemolytica*

- **Clinical responses to challenge evaluated using 0-4 scale criteria (DART™). Significantly less clinical disease with SAC102 vaccinates.**



Ayalew et al., *Vet Immunol & Immunopathol*, 2009

M. haemolytica chimeric vaccine – Bighorn sheep

- Vaccination of mice with mammalian cell culture-expressed LKT/PlpE chimeric protein stimulated antibodies to LKT and PlpE Batra et al., *Vet Immunol Immunopathol* 2016
- Bighorn sheep vaccinated intranasally with recombinant BHV-1 vectored vaccine encoding LKT neutralizing epitope and surface-dominant epitope of PlpE. Batra et al., *Vaccine* 2017
- Sheep developed antibodies but were not protected against *M. haemolytica* challenge.

So, where are we with commercial *M. haemolytica* vaccines "spiked" with recombinant proteins?

- **Under experimental conditions, supernatant and bacterin-toxoid vaccines can be enhanced by adding recombinant antigens.**
- **Chimeric vaccines alone may not induce complete protection**
- **Understandably, animal health companies have been reluctant to add recombinant proteins to their current vaccines due to increasing cost of production and cost to producers.**
- **One vaccine, NUPLURA™ PH by Elanco, contains rLKT and "extracted and purified outer membrane proteins".**

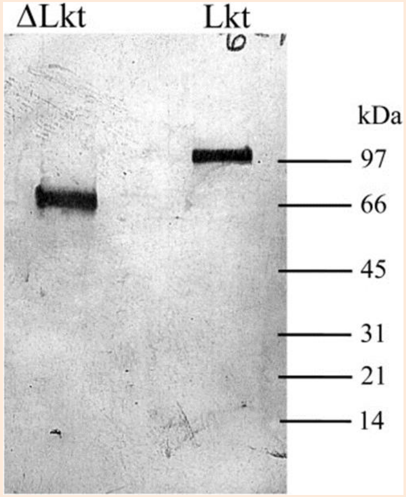
Genetically modified *M. haemolytica* vaccines

- **Streptomycin-dependent mutant *M. haemolytica***
  - Induced by N-Methyl-N'-nitro-N-nitrosoguanidine (Chengappa & Carter, *AJVR* 1979)
  - Streptomycin-dependent *Pasteurella multocida* (type A:3) and *M. haemolytica* (type 1) vaccination improved performance in a field trial (Kadel et al., *AJVR* 1985)
  - Commercial vaccine ONCE PMH ®
- **AroA deletion mutants (AroA required for synthesis of aromatic amino acids)**
  - Homchampa et al. (*Vet Microbiol* 1994) reported generation of *M. haemolytica* aroA mutant. Mutant highly attenuated in a mouse challenge model and mice immunized with the mutant were protected against challenge.

Genetically modified *M. haemolytica* vaccines

LKT mutants

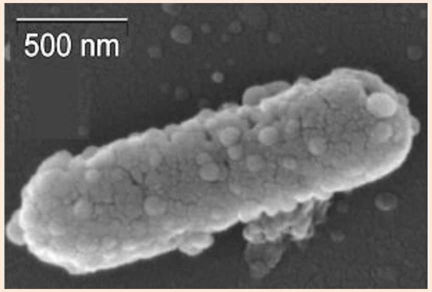
- Multiple isogenic *lkt* $^{-}$ -mutants *M. haemolytica* studied mainly related to pathogenesis
- Briggs et al. (*Microb Pathog* 2012) reported *lktA* deletion mutant that is a non-hemolytic truncated form of LKT (ΔLKT) that stimulates anti-LKT but is not leukotoxic.
  - Subcutaneous and oral vaccination of the MLV – ΔLKT *M. haemolytica* had significantly reduced lung lesions following challenge than did controls.



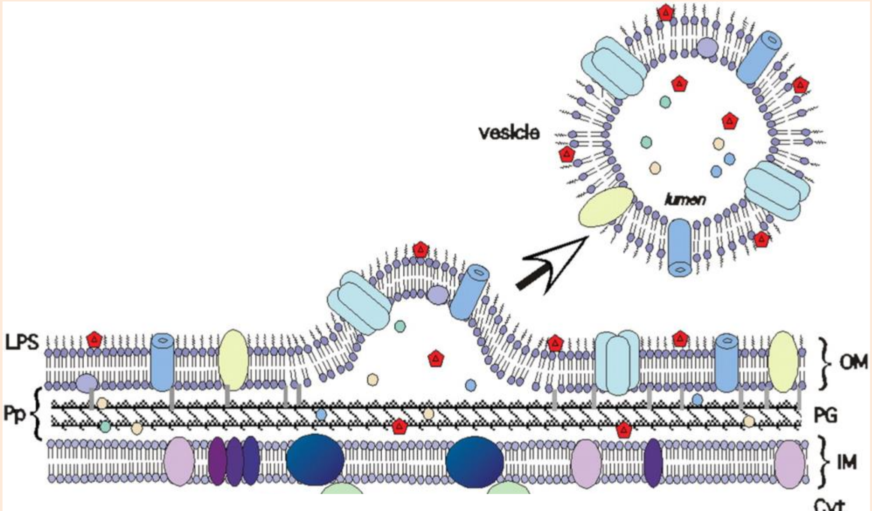
Group	% Lung lesions (% reduction)
IM vaccinates	7.0 ± 7.3 (78%)
Oral vaccinates	4.4 ± 4.5 (86%)
Controls	32.0 ± 13.4

Bacterial vesicle vaccines

- **Rapidly growing bacteria produce outer membrane "blebs" that detach as vesicles (outer membrane vesicles or OMV).**
- **Vesicles contain full complement of membrane proteins and secreted proteins, such as toxins.**
- **Highly immunogenic and do not require bactericidal treatments that can damage immunogenicity of proteins.**
- **In some cases serve as their own adjuvant**



Ellis & Kuehn, Microbiol Mol Biol Rev 2010



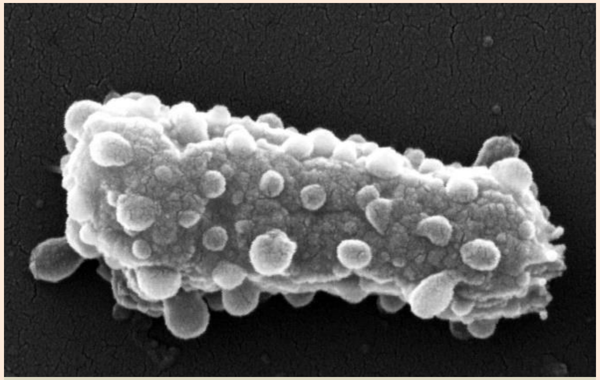
Kuehn & Kesty, Genes & Devel 2005

M. haemolytica vesicle (MHV) vaccines

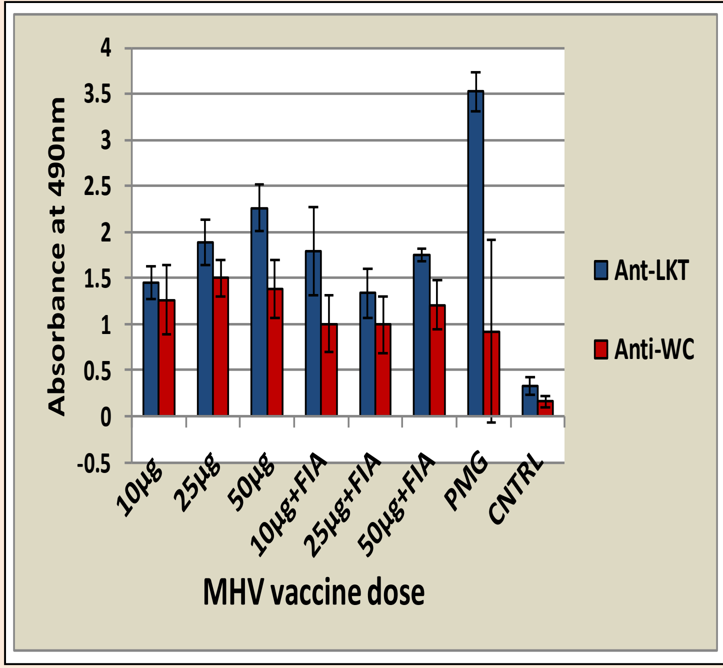
Ayalew et al., *CVI* 2013 - Proteomic analyses – MHV full complement of OMPs + many secreted proteins including LKT

- **Vaccination with MHV stimulated high anti-whole cell and anti-LKT antibodies in mice and calves.**
- **After challenge, MHV-vaccinated calves compared to controls**
  - **44.2% lower clinical scores ($p < 0.05$)**
  - **62.8% less severe pneumonia ($p < 0.05$)**

Roier et al., *Int J Med Microbiol* 2013 – Similarly demonstrated *M. haemolytica* vesicle vaccination of mice stimulated antibody responses.



***M. haemolytica* – SEM photo by Dr. K. Kocan**



Additional approaches

Alternative routes

- **Intranasal**
  - **One commercial vaccine for IN delivery**
  - **Recently, we found and characterized two *M. haemolytica* IgA proteases, which may assist the bacterium to evade mucosal immunity. IgA proteases are potential intranasal vaccine targets.** (Ayalew et al., *Vet Microbiol* 2017)
- **Oral**
  - **Attempts by R. Lo & Shewen to make an edible vaccine using transgenic alfalfa. Seems to be off the table**

Immunostimulants

- **Probiotics**
  - **Diaz et al., *Benef microbes,* 2018 –Mice vaccinated with *M. haemolytica/P. multocida* vaccine and given intragastric *Enterococcus faecalis* CECT7121 had enhanced antibody response, antibody avidity, and higher interferon-γ than with vaccine alone.**
- **Unmethylated CpG DNA dinucleotides**
  - **Stimulate innate and adaptive immunity through TLR9. Role in enhancing *M. haemolytica* vaccine?**
  - **Addition of CpG to *Bordetella pertussis* antigens enhanced production of IFN-γ in mice following vaccination.** **(Bakhshaei et al., *J IFN Cytokine Res,* 2018)**


ARE WE THERE YET !?!
MATT GROENING

Relative to the four points made on vaccine history (*Vaccine* 2012)

1. **Progress is made incrementally**
   - **Incremental progress made since 1980s by incorporating LKT into vaccines and understanding the role of surface antigens.**
2. **Progress often requires "game-changing" event or events**
   - **Discovery of LKT was A MAJOR game changer. What are the next ones?**
3. **Progress is closely tied to development of improved technologies from other fields**
   - **Through molecular biology and genomics, we better understand the antigens and epitopes involved in immunity and starting to better understand the respiratory microbiome.**
4. **Progress will occur through application of novel science-based technologies and strategies**
   - **Future, more efficacious vaccines will apply molecular techniques, improved novel production techniques, immunostimulants, and/or better vaccine delivery methods.**

A. W. Confer – Official retirement date, July 1, 2019

"Regrets, I've had a few. But then again, too few to mention." Frank Sinatra - *I did it my way*

My *M. haemolytica* regrets that I WILL mention

- Our lab: Focused too much on the organism and serum antibody and not enough on *M. haemolytica/*host interactions & innate immunity.
- *M. haemolytica* research community: Focused too much on LKT, not enough on other virulence factors, or the bacterial/host interaction.
- Animal health companies: *M. haemolytica* vaccines not improved beyond those from early 1990s.

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The OSU BRD project was started by R.J. Panciera & R.E. Corstvet ~1970. Many good faculty and staff have kept it alive. Most recently:

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Thank you.

Are there any Questions?