



THE UNIVERSITY OF  
MELBOURNE



# The Future of Veterinary Vaccines

Glenn Browning

Asia-Pacific Centre for Animal Health

Faculty of Veterinary Science

Poultry Cooperative Research Centre

The University of Melbourne

# A Personal Perspective

RESOURCES

- Where will the vaccines of the future come from?
- How should we test efficacy and what are the challenges?
- What safety risks are we not assessing?
- What are the risks of the next generation of vaccines and how might we mitigate these risks?
- What are the benefits and challenges of the bioinformatics age for veterinary vaccinology?

# Traditional Vaccines

PRESENTATION

- Major contribution to animal welfare and productivity
- Current vaccines based on methods unchanged in 100 years
- Bacterins and inactivated viruses
- Toxoids
- Live attenuated vaccines
  - Naturally occurring mutants
  - Selection by culture in unusual conditions

# What Do We Need?

PRESENTATION

- **Better “inactivated” vaccines**
  - Immune response against most protective antigens
  - Greater antigenic load
  - Cheaper production
  - Delivery to mucosal surfaces
- **Better attenuated vaccines**
  - More reliable and more graduated attenuation
  - Greater protective response
  - Differentiation of vaccine and wild type strains

# How Can We Achieve This?

PRODUCTION

- Inactivated and Subunit Vaccines
  - Subunit vaccines containing major protective antigens
  - Improved *in vitro* growth conditions for production
  - Better strains for production
  - Vectored delivery

# How Can We Achieve This?

PRESENTATION

- Attenuated Vaccines
  - Identification of optimal parental strains
  - Determining best targets for attenuation
  - Generation of “hyperimmunogenic” strains
  - Deleting genes for serological markers

# First Generation Biotechnology

PRODUCTION

- Temperature sensitive mutants
  - Random mutagenesis
  - Selection of phenotypically different mutants
- Bacterins from culture in nutrient-restricted media
  - Upregulation of expression of critical protective antigens

# First Generation Examples

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- Temperature sensitive mycoplasma vaccines
  - Grow in upper, but not lower, respiratory tract
- Inactivated *Pasteurella* vaccines grown in iron-restricted media
  - Produce key protective outer membrane proteins

# Second Generation Biotechnology

PRESENTATION

- Rational and targeted attenuation
  - Auxotrophic mutants unable to grow *in vivo*
  - Infect, but very limited growth
  - Can generate heterotypic immunity, but generate very low antibody responses
  - Currently all based on *aro* gene deletions

# Second Generation Examples

PROBIOGEN

- *aroA* deleted *Salmonella* Typhimurium
- *aroA* deleted *Escherichia coli*
- *aroA* deleted *Pasteurella multocida*
  - Infect but can't synthesise aromatic amino acids

# Third Generation Biotechnology

PRESENTATION

- Subunit and inactivated vaccines
  - Identify key novel antigens
  - Optimise production and purification
  - Increase expression *in vitro*
- Attenuated Vaccines
  - Titration of attenuation
  - Rational improvement of efficacy
  - Identify genes for serological markers

# Vectored Vaccines

1/25/2017

- Poxvirus and herpesvirus vectors
- Variation in performance of different vectors still not fully understood
- Is efficacy dependent on absence of prior exposure to the vector?
- Potential risks from recombination between gene for antigen and wild type pathogen?
- Bacterial vectors likely in the future

# New Biotechnological Tools

PRESENTATION

- Finding virulence genes
  - *In Vivo* Expression Technology
  - Signature Tagged Mutagenesis
- Finding protective antigens
  - Reverse Vaccinology
- Tools for the future
  - High Throughout Sequencing
  - Gene Synthesis
  - Genome Mining/Bioinformatics

# *In Vivo* Expression of Virulence Factors

QUESTION

- Bacterin immunity usually serotype specific
- Immunity after infection often broader
- Many key virulence factors poorly expressed *in vitro*
- Bacteria detect *in vivo* environment
- Upregulation of expression at specific times during infection
- Limited period of expression and thus reduced immune response
- These factors often less antigenically diverse

ANSWER

# Generating Better Vaccines

PRESENTATION

- *In Vivo* Expressed Virulence Factors
  - More cross reactive subunit vaccines
  - Targets for rational attenuation
- *In Vivo* Regulatory Systems
  - Target to upregulate expression of protective antigens
    - Improve efficacy of inactivated and attenuated vaccines
  - Blockage of upregulation may lead to attenuation
- Intrinsic Adjuvantation
  - Expression of immunomodulatory genes by vaccine strains (or deletion of immunosuppressive genes)

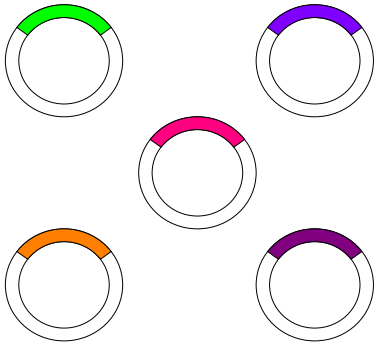
# *In Vivo* Expression Technology

PROSODY

- Used to detect genes only expressed *in vivo*
- Gene required *in vivo* included in vector without promoter
- Random fragments from bacterial genome placed in front of gene
- Vector introduced into pathogen
- Infect animal with transformed strains
- Transformants recovered from animals include promoter of gene only expressed *in vivo*

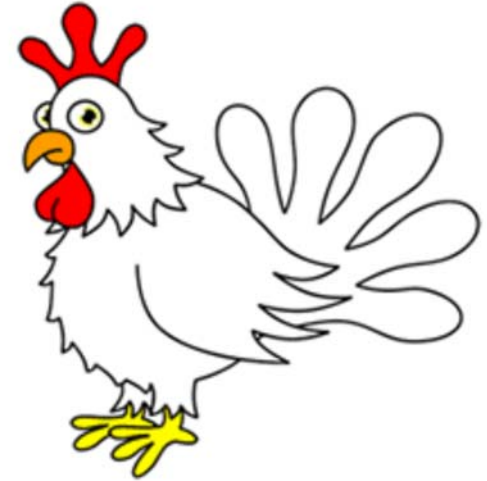
# In Vivo Expression Technology

RESOURCES

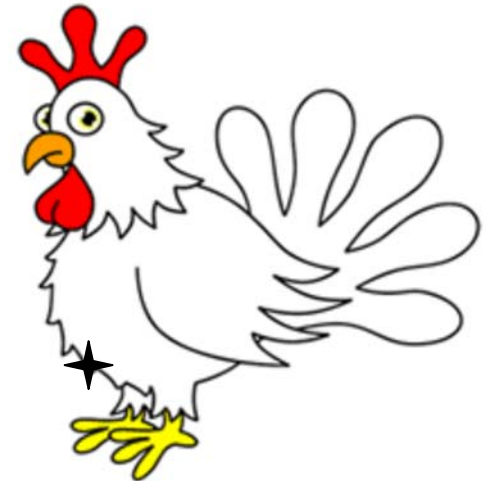


Inoculation Pool

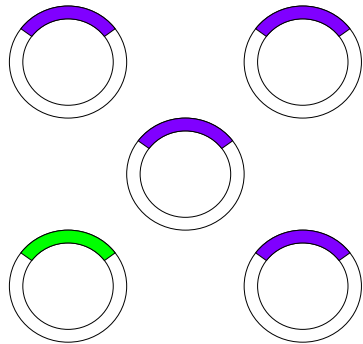
Infect



Reisolate



Recovered Pool



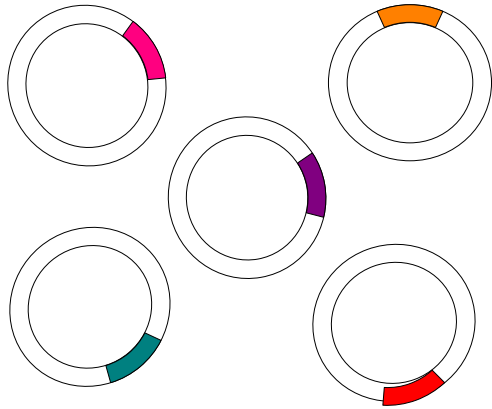
# Signature Tagged Mutagenesis

PROBIOLOGY

- Used to identify genes required for survival *in vivo*
- Transposons carrying different specific DNA tags used to create mutants
- Pools of mutants carrying different tags used to infect animals
- Mutants in input pool but not recoverable from animals contain mutations in critical genes

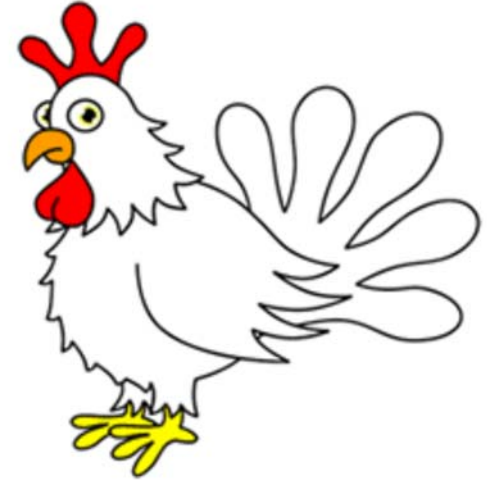
# Signature Tagged Mutagenesis

PLASMID

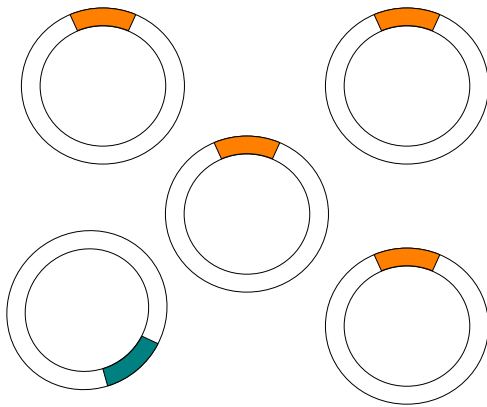


Inoculation Pool

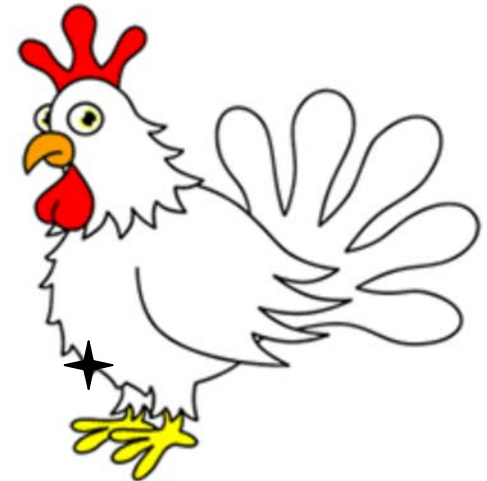
Infect



Reisolate



Recovered Pool



# Defined mutants as vaccines

FRANÇOIS

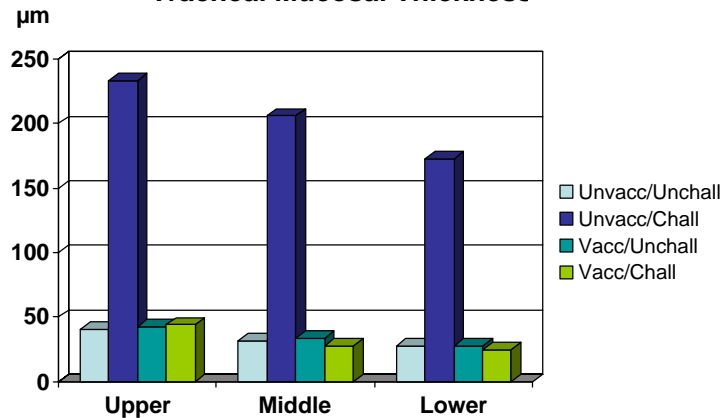
- Library of signature tagged mutants of *M. gallisepticum*
- Screened by infection of chickens with pools of mutants
- Selected mutants that could colonise but not compete as well
- Examined these for safety and efficacy
- Mutant with insertion in *oppD* gene showed most potential

# Protective Efficacy of *oppD* mutant

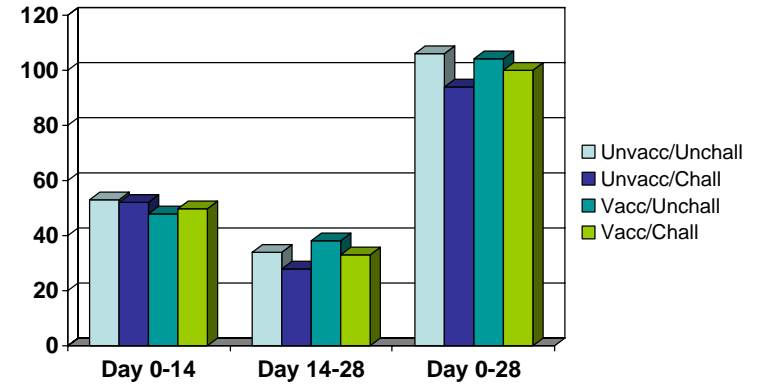
RESULTS

## *Mycoplasma gallisepticum*

Tracheal Mucosal Thickness

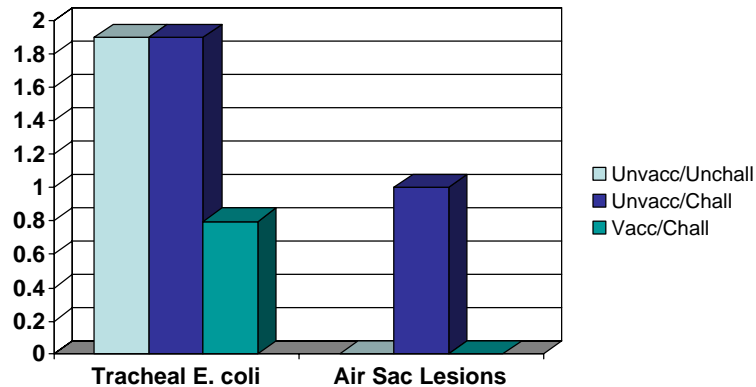


Weight Gain

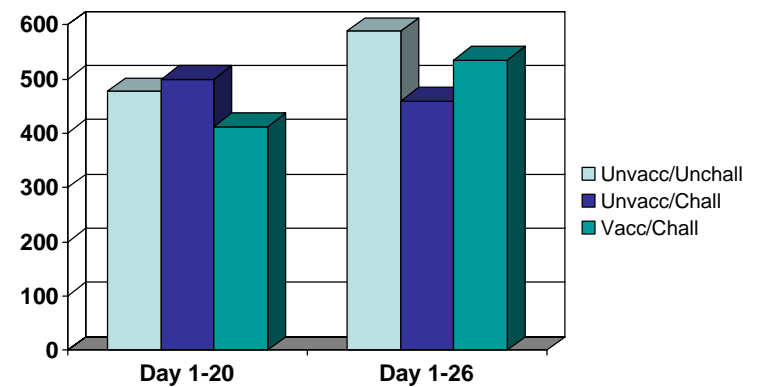


## *Escherichia coli*

Reisolation & Lesion Scores



Weight Gain



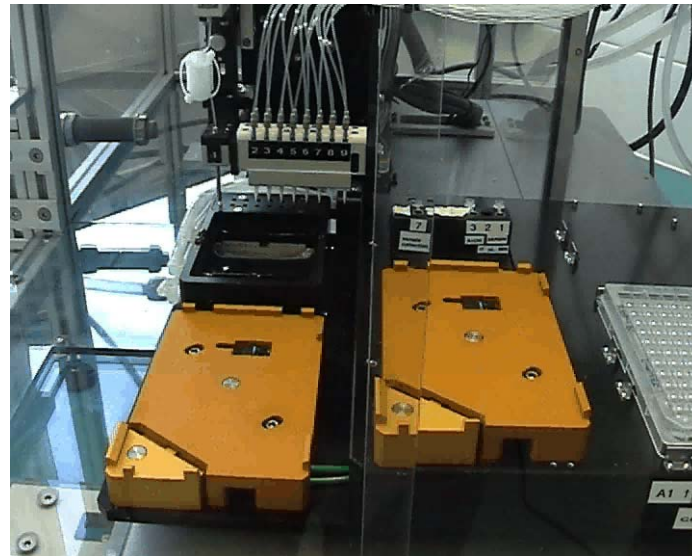
# Reverse Vaccinology

Reverse Vaccinology

- High throughput screening of recombinantly expressed genes for protective potential
- *In vitro* gene synthesis
- Optimise codon usage for expression system
- Purify proteins and use to vaccinate animals
- Challenge to determine whether protection induced

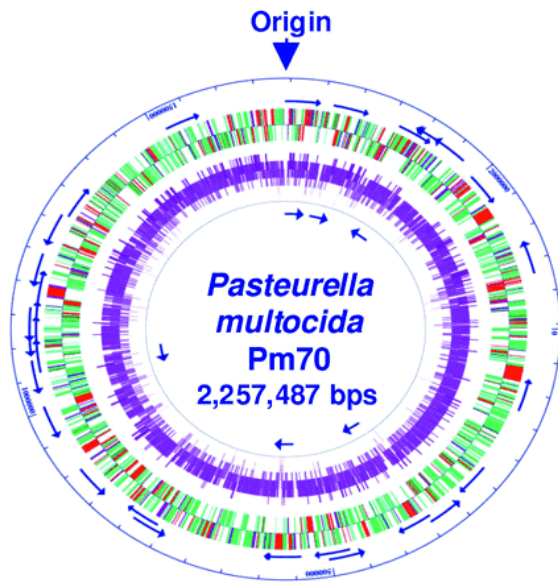
# Reverse Vaccinology

PRODUCTION



# Reverse Vaccinology

*Pasteurella multocida*



Microbial



Pipeline

115 vaccine antigens  
for testing



# Genome Mining

PRESENTATION

- Use of bioinformatics to identify likely targets
- Prediction of function, or only location or regulatory group
- Cell surface and secreted proteins of particular interest
- Genes with specific promoters or members of clusters of virulence genes

# Limitations of IVET and STM

1/25/2017

- Mainly identify genes only expressed or essential *in vivo*
- Deletion of these genes results in mutants unable to establish infection
- Infection and growth needed to induce immune response
- Can look at signature tagged mutants with decreased, rather than no capacity to grow *in vivo*

# Limitations of Reverse Vaccinology

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- Solubility of recombinant proteins
- Only suitable for investigating humoral and parenteral immunity
- Useful for systemic extracellular pathogens and possibly for *in ovo* inoculation
- Will need delivery vector for economical administration to intensively raised livestock

# Limitations of Genome Mining

1/23/2013

- Limited capacity to predict function
- High proportion of genes in bacterial pathogens still hypothetical
- Partial understanding of regulatory elements, particularly complex interactions
- Need experimental exploration of degree of effect on virulence using targeted mutagenesis

# Most Promising Approach

PROS/CONS

- High throughput sequencing
- Bioinformatic analysis to identify likely targets for attenuation
  - Predict role based on
    - Clustering with other virulence genes
    - Promoter characteristics
    - Cell surface location or secretion
    - Functional sequence motifs
- Targeted mutagenesis to assess extent of effect of likely candidates on virulence and growth *in vivo*

# Most Promising Approach

PROS/CONS

- Titration of attenuation and extent of growth
- Combine with upregulation of expression of key protective antigens
- Result in attenuated strains that induce higher levels of protection
- Likely to be suitable as vectors for delivery of other antigens
- Inclusion of cytokine genes as intrinsic adjuvants

# Intrinsic adjuvantation

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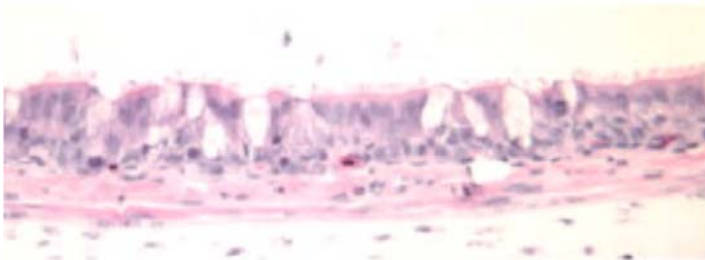
- Include immunomodulatory genes in vaccine strain
- Chemokines are small and active without glycosylation
- Potential to overcome immunosuppressive effects of vaccine
- Enhance specific aspects of immune response to vaccine

# Response to IFN- $\gamma$ plus *M. gallisepticum*

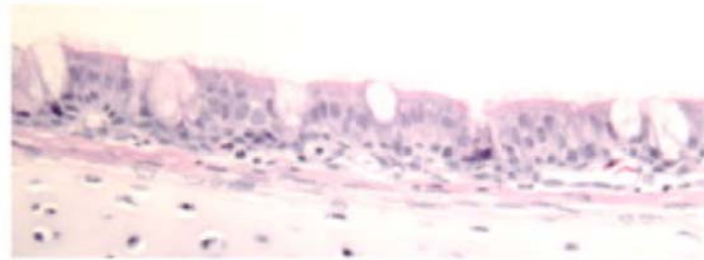
FIGURE 11.11

- Unvaccinated birds, birds vaccinated with vaccine strain, and birds vaccinated with strain expressing IFN- $\gamma$
- Weight gains and mucosal thickness similar
- Greater heterophil infiltration (20 fold) with IFN- $\gamma$  expression

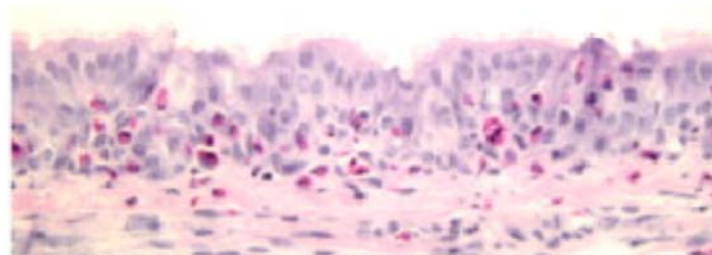
(1) Group 1



(2) Group 2



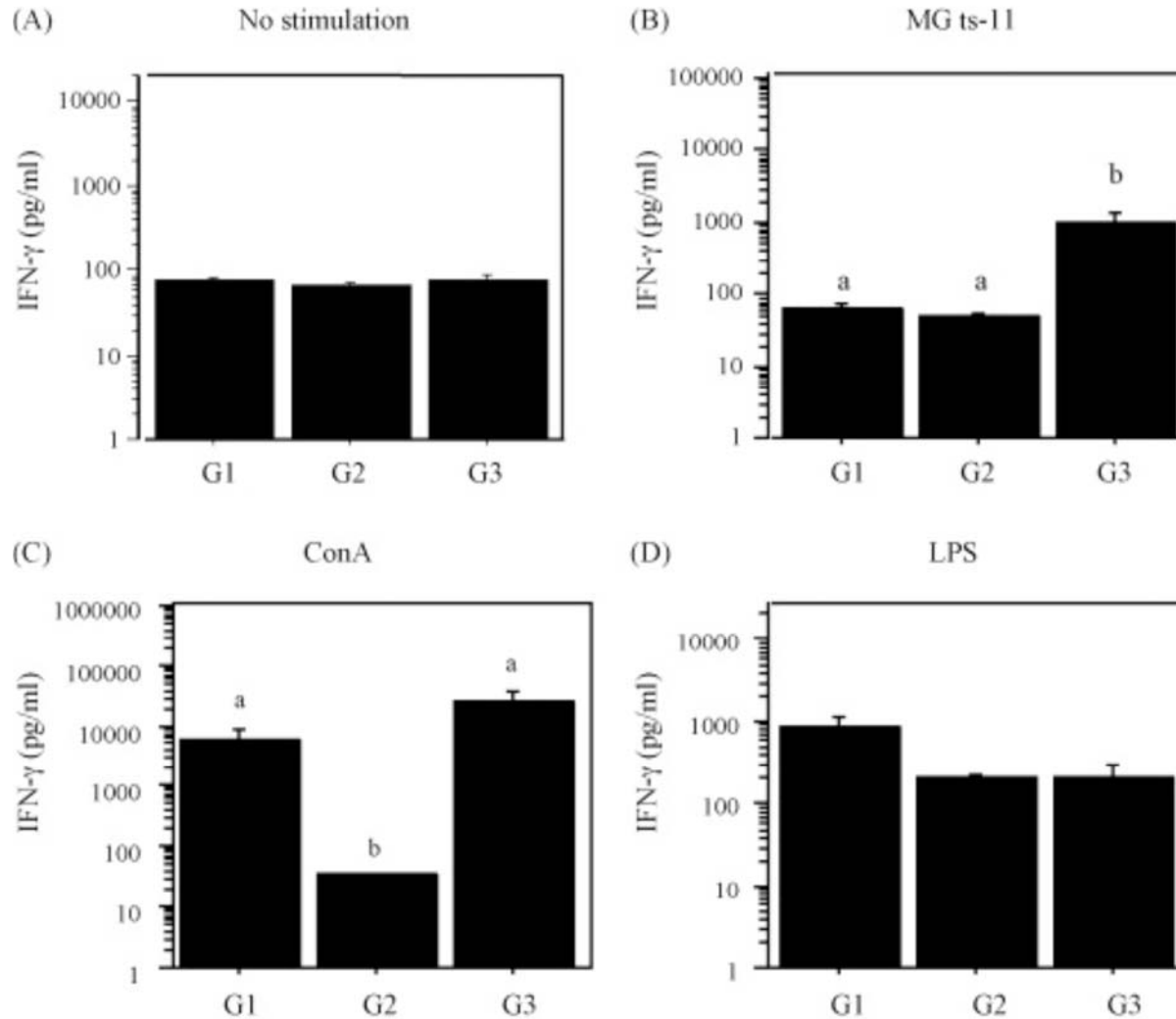
(3) Group 3



x 400

# IFN- $\gamma$ Reverses Immunosuppression

IP: 129.20.255.130



# Successes with New Techniques

PRESENTATION

- STM for identifying attenuating mutations in mycoplasmas and other bacteria
- Reverse vaccinology used to identify protective antigens in *Pasteurella multocida*
- Genome mining for detection of novel *C. perfringens* toxin



# Current Challenges and Opportunities

PRESENTATION

- Duration of immunity
- Efficacy testing
- Reversion to virulence testing
- Assessing risks of recombination
- Role and effects of high throughput sequencing and genome synthesis

# Duration of Immunity

18/03/2017

- Some consumer pressure to minimise revaccination in companion animals
- Very difficult to assess maximal safe revaccination intervals experimentally
- In human medicine these intervals based on epidemiological data
- Implies a need for much better collection of epidemiological data after vaccines released

# Efficacy Testing

1/25/2017

- In the past sometimes based on predetermined defined measures of disease (fever, clinical scores, lesion scores, microbiological assessment)
- Many of these measures have little relevance to the consumer
- Should efficacy testing focus on what the consumer wants protection from?
- Is control of experimentally reproduced disease an appropriate test for diseases with a complex aetiology?

# Defining Efficacy for Absent Pathogens

QUESTION

- How do we establish the need for a specific vaccine?
  - Evidence of the presence of the infectious organism?
  - Evidence of disease?
  - Evidence of significance (social or economic) of the disease?
- If routine vaccination against exotic pathogens is allowed how can requirements for testing efficacy be met?

ANSWER

# Efficacy against Complex Diseases

1/25/2017

- Models for testing efficacy generally assume a straightforward single aetiology
- Efficacy of a vaccine against a disease complex may be influenced by multiple factors
- Are simple experimental assessments applicable, or can efficacy only be assessed in the field?
- How do we assess the suitability of a specific field site?

# Safety Testing

FAO/WHO

- Reversion to virulence testing based on five or so back passages through small numbers of animals
- In large herds and flocks incomplete vaccination may be common, with much greater potential for vaccine cycling
- Particularly a risk when vaccines are mass administered using less reliable methods or at lower doses

# Recombination

RECOMBINATION

- Deletion mutant vaccines
  - Significant advantages
    - Eliminate risk of reversion
    - May enable differentiation of vaccinated from infected animals
    - Basis of attenuation fully defined and easily confirmed
  - But still a risk from recombination in the field if parent strain was more virulent than field strains
  - Not only a risk of deletion mutants, but single locus of attenuation may make this more likely

# High Throughput Sequencing

1/25/2011

- New techniques enable generation of very large volumes of data rapidly and cheaply
- Can compare large numbers of viral and bacterial genomes simultaneously
- Can sequence seedlots and look for variation or for extraneous sequences (but what might be their significance?)

# Gene Synthesis

PRESENTATION

- Capacity to chemically synthesise infectious viral genomes already
- Synthesis of bacterial genomes may be close
- Significant advantages in deriving master seeds free of adventitious agents

# What Else is Coming?

1/25/2014

Identification of novel pathogens has always been driven by development of new detection techniques

- Clinical signs
  - Microscopy
  - Culture - bacteriological media, eggs, then cell culture
  - Electron microscopy
  - Serology
  - Nucleic acid based techniques
- New burst of discovery already developing from current sequencing revolution
- Many of these pathogens may not be cultivable and will require unconventional approaches to vaccine development and testing

