Investigation of the genetic differences between bovine herpesvirus type 1 variants and vaccine strains

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Bovine herpesvirus

- **Bovine herpesvirus-1 (BHV-1)**
  - Pathogen of Infectious Bovine Rhinotracheitis
- **Infectious Bovine Rhinotracheitis (IBR)**
  - Clinical signs: high fever, inflammation of nose, nasal discharge, conjunctivitis, abortion
  - Can predispose animals to secondary bacterial infections, such as shipping fever
  - Spread through nasal secretions, droplets, genital secretions, serum, and fetal fluids
- **Cattle industry: suffers losses of more than $500 million/year**
Molecular basics

- **Family:** *Herpesviridae*
  - **Subfamily:** *Alphaherpesvirinae*
- **Large, double-stranded DNA virus**
  - **Genome size:** 136kb
- **Consists of:**
  - Core containing linear ds DNA
  - Icosahedral capsid
  - Envelope with viral glycoprotein spikes
- **BHV-1:** lifelong latent infection in TG of host
  - Virus reactivation: can infect others in the herd
BHV-1 Vaccine

- Efficacious BHV-1 vaccine used for years
  - MLV or inactivated vaccine
- Recently, BHV-1 vaccine related abortion reported in increasing number of herds
- Pfizer: PregGuard (MLV Vaccines)
  - Abortion after Pfizer BHV-1 Vaccine during pregnancy
    - 2011: Wyoming
    - 2012: UC Davis (California), Texas A&M, and Cornell (New York)
BHV-1 Vaccine-related abortion

- Rise of vaccine-related abortion may be result of several factors:
  1. Emergence of new virulent strain of BHV-1
  2. Genetic recombination between wild type virus and BHV-1 vaccine strains
  3. Combination of environmental factors and reactivation of BHV-1 in latently infected herds
Question?

- Is the abortion caused by the vaccine?
Objectives

1. Sequence BHV-1 isolates from abortion cases and compare them to wild type BHV-1 virus and BHV-1 vaccine DNA genome
2. Establish strain specific nucleotide polymorphism (SSNP) profiles to assist diagnosis of BHV-1 infection
Approaches

• TK, gE, and gG are unique genes in BHV-1 wild type and vaccine strains
• Compare the above genes between vaccine strains and abortion isolates by PCR-DNA sequence
Study Design

1. BEK Cell Cultures
2. Virus Infection
3. Viral DNA Extraction
4. PCR
5. DNA Sequencing
   - Design primers coding for different region
   - No mutation
   - DNA Alignment using Geneious

Options:
- Abortion Isolates
- Fetal tissue from CA abortions
BEK Cell Cultures & Virus Infection

- Bovine Embryonic Kidney Cells: maintained monolayer in flasks
- Infected with virus isolate
- Examined for cytopathic effect (deterioration of monolayer cells)
Study Design

- BEK Cell Cultures
- Virus Infection
- Viral DNA Extraction
- PCR
- DNA Sequencing
- DNA Alignment using Geneious

Abortion Isolates
or
Fetal tissue from CA abortions

Design primers coding for different region
No mutation
Study Design: PCR Primers

- Amplified regions coding for:
  - **Thymidine Kinase (TK)**
    - Involved in viral virulence
  - **gE**
    - Required for direct cell-to-cell cell junctions
  - **gG**
    - Contributes to viral entry and attachment
Study Design: PCR Results

TK: R1

MM: Molecular Marker
1: T739073
2: T754-753
3: E754-753
4: T742-308
5: L6064-914

TK: R2

MM: Molecular Marker
1: T596-042
2: T739-073
3: T754-753
4: E754-753
5: T742-308
Study Design: PCR Results

**gE**

- MM: Molecular Marker
- 1: PG-BHV-1
- 2: E737-501
- 3: T737-501
- 4: E739-051
- 5: T739-051

**gG**

- MM: Molecular Marker
- 1: L675-940
- 2: T519-200
- 3: T739-073
- 4: E739-051
- 5: T739051

400bp
500bp

495bp

461bp
Study Design

BEK Cell Cultures

Virus Infection

Viral DNA Extraction

PCR

Design primers coding for different region

DNA Sequencing

DNA Alignment using Geneious

Abortion Isolates

or

Fetal tissue from CA abortions
Results: TK R1

- Sequenced 35 isolates
- Substitution, Insertion, Deletion
Results: TK R2

- Sequenced 11 isolates
- Deletion, Substitution, Insertion
Results: gE

- Sequenced 31 isolates
- Substitution, Insertion, Deletion
Results: gG

- Sequenced 34 isolates
- Deletion, Insertion
## Results: Wild Type vs. Isolates

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## Results: Isolates vs. Vaccine

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**TK R1 and gG:** most isolates identical to vaccine strains (which were different than the wild type)

**TK R2:** BoviShield Vaccine exhibited 2 mutations found in no other isolates, PregGuard Vaccine, or wild type
Results Summary

- **TK R1**: 100% of isolates different than wild type
  - 27.3% different than vaccine
- **TK R2**: 54.5% of isolates different than wild type
  - 45.5% different than vaccine
- **gE**: 16.1% of isolates different than wild type
  - 16.6% different than vaccine
- **gG**: 100% of isolates different than wild type
  - 12.5% different than vaccine
Conclusion and Discussion

- All the abortion isolates are different from the TK gene of wild types virus and many have similar TK gene sequence as the vaccine strain
- Not all the abortion isolates have the same genetic variation
- The abortion isolates may have been derived from the vaccine strain or other variant strains
- Mutations in TK, gE, and gG may lead these abortion isolates to be more virulent
Future Direction

• Examine the mutations in protein coding region and identify the epitope of the viral protein affected by those mutations discovered in our study

• Design primers to capture variations among different isolates
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