Development of an indirect ELISA for detection of serum antibodies to *leporid herpesvirus*-4

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Leporid herpesvirus-4

- Novel alphaherpesvirus
- 55% morbidity, 20% mortality
- Commercial rabbits (Alaska)
  - Jin et al. (2008) Vet Path. 45(3): 369
- Pet rabbit (N. Ontario)
  - Brash et al. (2010) CVJ. 51(12): 1383
- Clinical signs: lethargy, anorexia, conjunctivitis, fever, abortions
- Pathology: multifocal pulmonary hemorrhage and edema, hemorrhagic dermatitis, splenic necrosis, hepatic necrosis

Syncytia with intranuclear inclusion bodies
Leporid herpesvirus-4

- **Family** Herpesviridae
- **Subfamily** Alphaherpesvirinae
- **Genus** Simplexvirus

- Enveloped, Tegument
- Linear dsDNA – 125 kb
- Aerosol droplet transmission or direct contact
- Rapid growth and cytopathic effect in cell culture

Objective of the study

To develop a **safe, rapid and sensitive** test for screening and diagnosis of LHV4

- Indirect ELISA – Antibody detection
  - Whole virus antigen preparation
  - Sub-unit protein expression in Baculovirus system
Infection trial
Live virus inoculated via intranasal infection

Polyclonal antibody
Inactivated virus injected with adjuvant

Field samples
Collected from 250 commercial and 20 pet rabbits

Sunohara-Neilson et al. Comp Med (accepted for publication, 2013)
Virus Neutralization Assay
Serology Gold Standard

Uninfected CRFK cells

LHV-4 infected CRFK cells

Neutralizing Serum Dilution (1:x)

- Infection Trial
- Polyclonal Antibody
- Commercial & Pet

Rabbits

* <1:8 Toxic effect of serum
Indirect ELISA

Whole virus antigen
• decrease time to diagnosis

Sub-unit protein expression
• increased efficiency
• increased biosafety

• Optimization determined using checkerboard design
• Results compared to VN assay as gold standard
Neutralizing antibodies produced from 11 dpi (VN assay and ELISA)
Whole Virus ELISA

- VN assay seroconversion
- VN assay negative

Mean $+ 2 \text{ SD} = 0.067$
100% Se, 95% Sp
Whole Virus ELISA

- VN assay seroconversion
- VN assay negative

0.100 to 0.300
100% Se, 100% Sp
Herpesvirus glycoproteins

http://www.cixip.com/index.php/page/content/id/942
Immunoreactive protein profiles

A. **Infected Rabbit Serum** (live)
   Representative of test samples

B. **Polyclonal Antibody** (inactive)
   Positive control

120 kDa protein
- Glycoprotein G (suspected)
- Antibodies present in both sera

55 kDa protein
- Glycoprotein D (suspected)
- Antibodies absent in polyclonal antibody preparation
Baculovirus expression system

Glycoprotein G from LHV4

http://www.genscript.com/custom_protein_baculovirus_insect_expression.html
Summary

• *Leporid herpesvirus-4*
  – Novel alphaherpesvirus of rabbits
  – Health concern for the rabbit industry

• Serology – Indirect ELISA
  – Whole virus – sensitive and specific
  – Sub-unit – glycoprotein G and D

• Direct virus detection
  – qPCR assay in development
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