Systems Biology and Unconventional Animal Models as Tools in Basic and Applied Research within a One Health Theme

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Larry Hanson, Kari Lunsford, Camilo Bulla and Stephen Pruett.

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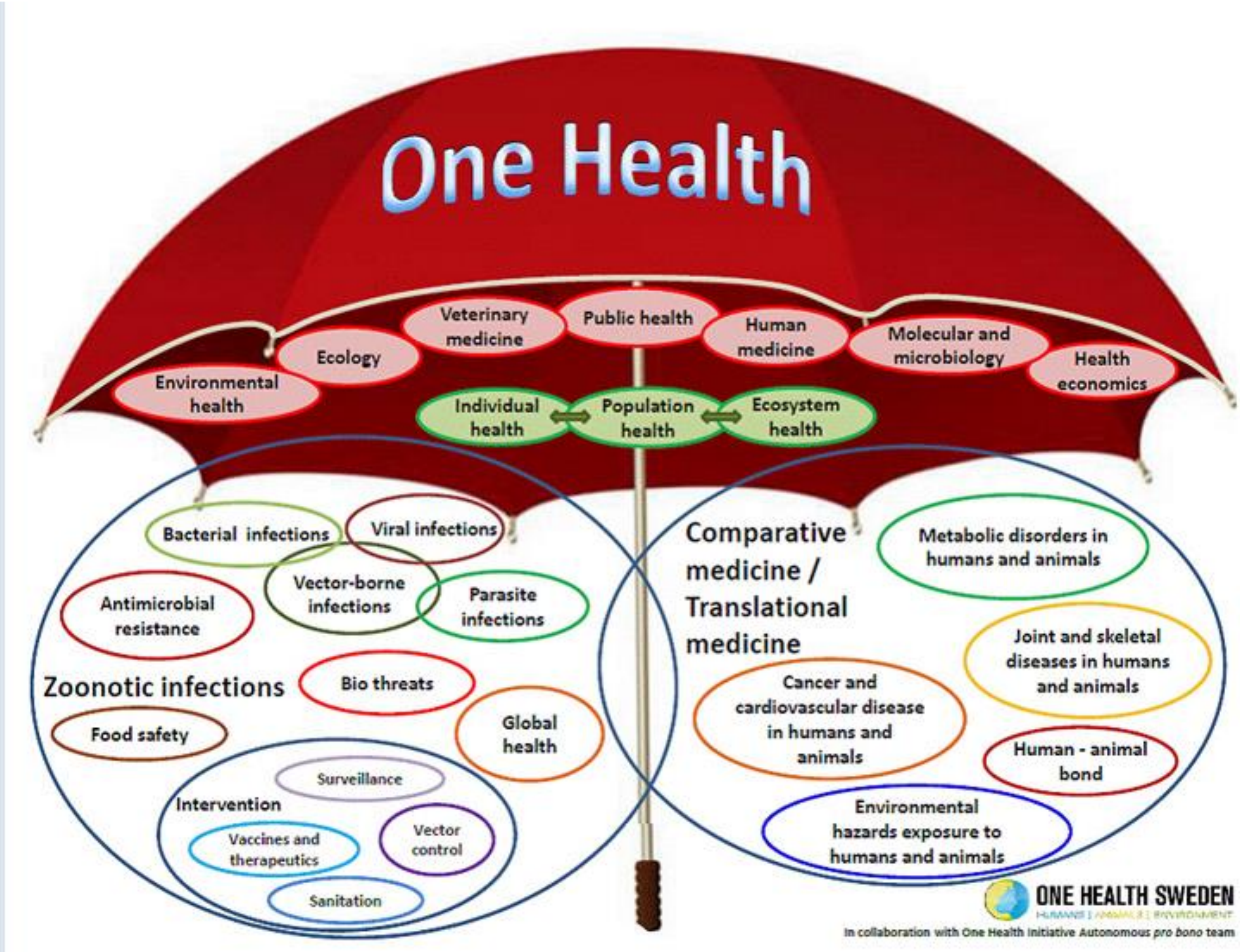

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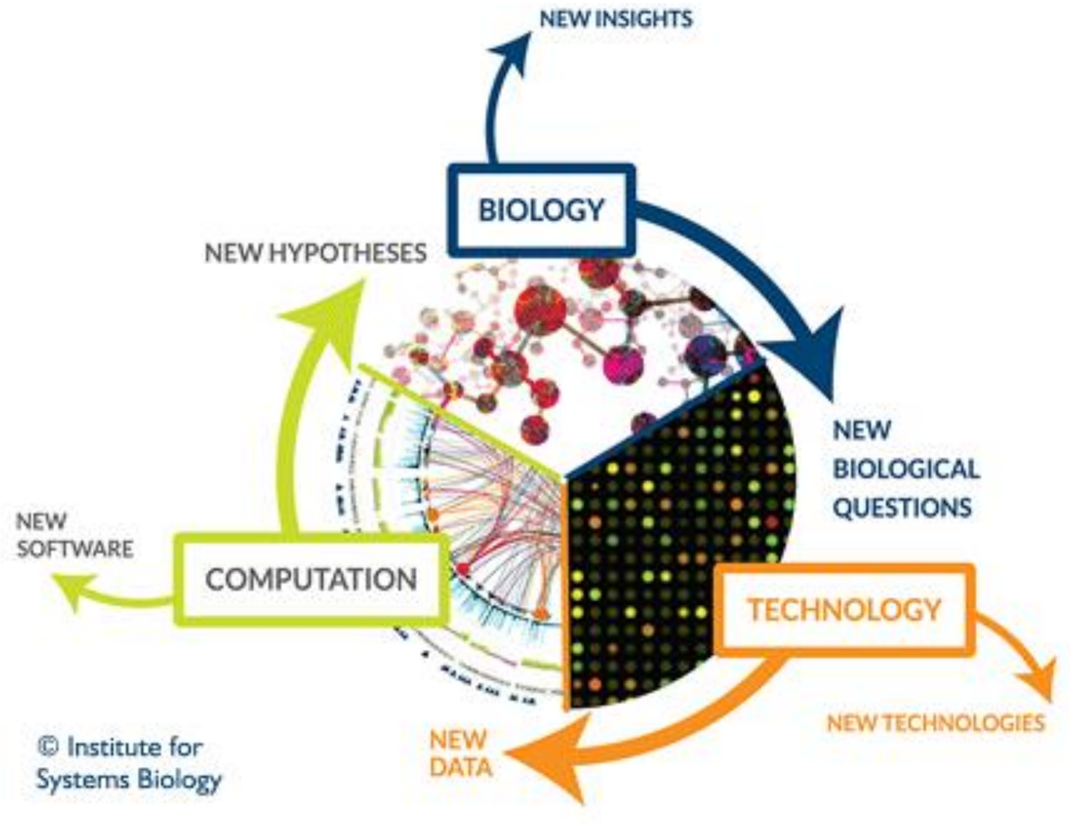
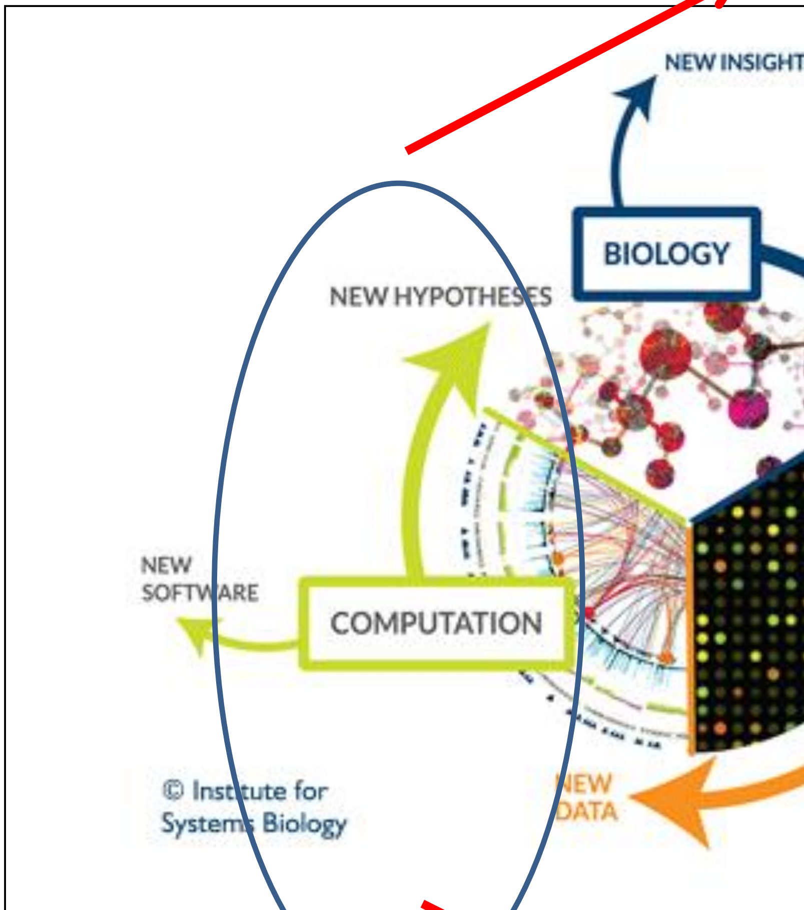
Nanduri, Bindu

Systems Biology



http://www.systemsbiology.org/about-systems-biology



http://www.agbase.msstate.edu/

Nanduri, Bindu

http://sysbio.cvm.msstate.edu/software.php

Risk Assessment of H6N6 swine influenza viruses using systems biology approaches

*Hailiang Sun, Bryan S. Kaplan, Guihong Zhang, Jianqiang Ye, Li-Ping Long, Sherry Blackmon, Jim Cooley, Dave Smith, Ming Liao Richard Webby, and Xiu-Feng Wan**

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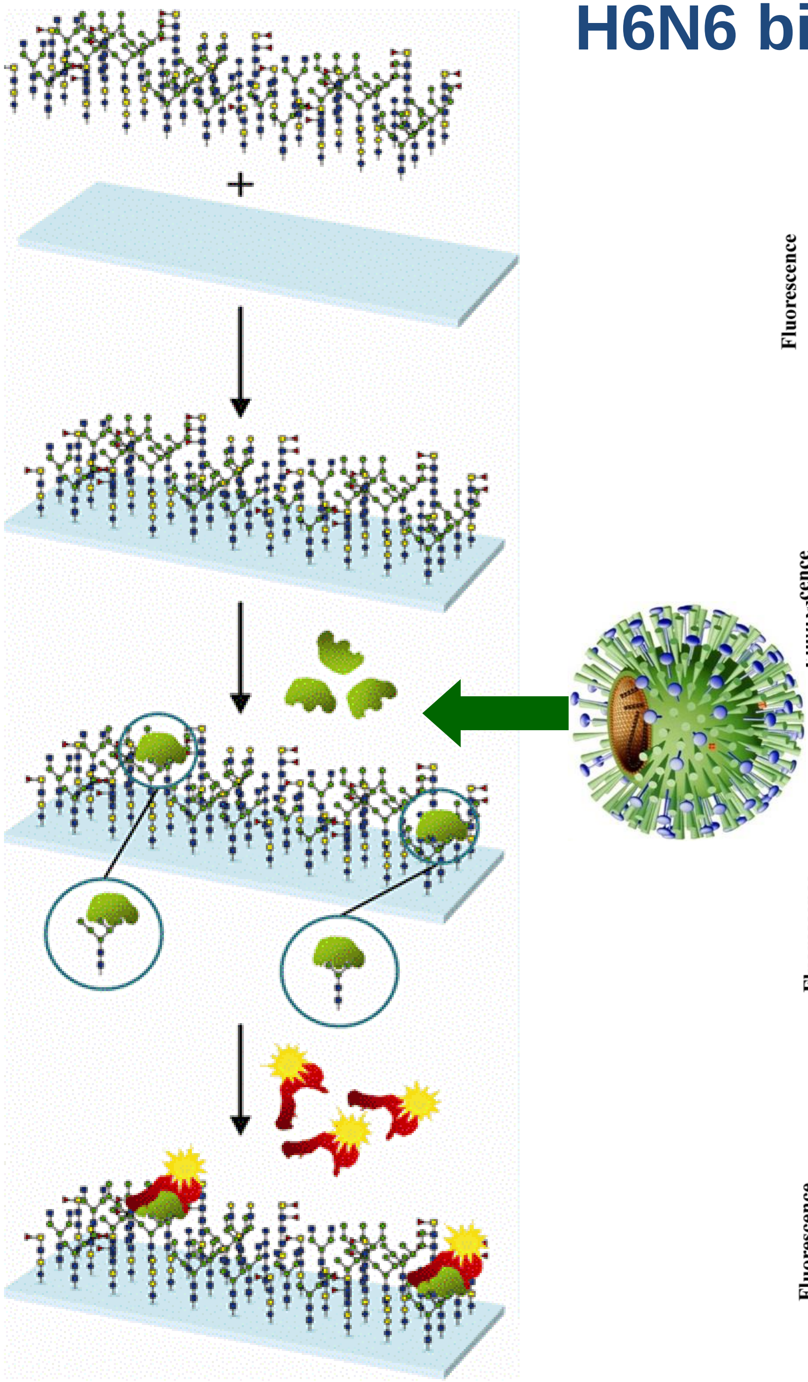
Introduction

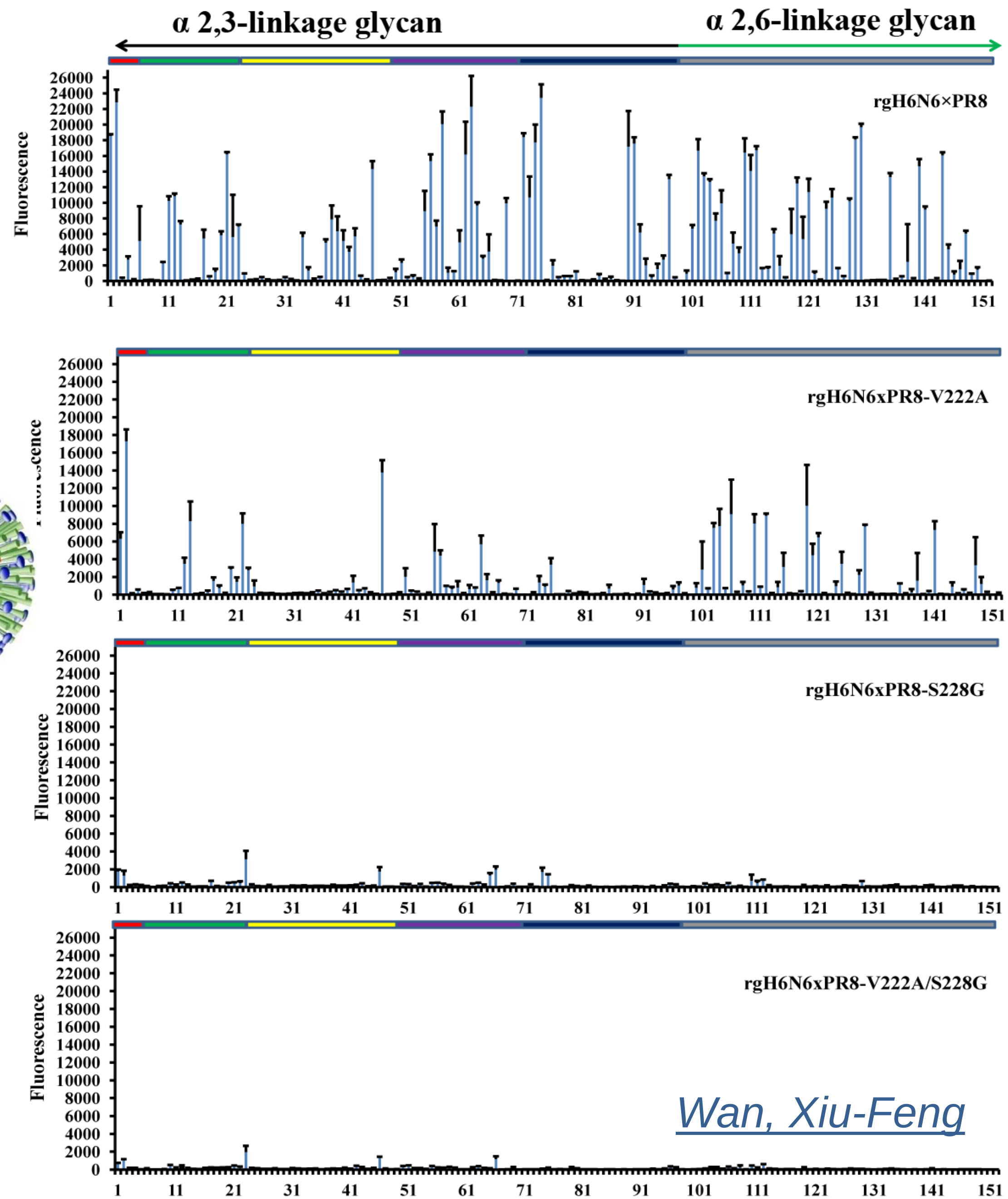
- H6 subtype of influenza A virus has been identified widely in migratory waterfowl as well as the resident birds such as chicken, quail, pheasant, domestic geese and ducks.
- Experimental infection demonstrated that H6 avian influenza virus (AIV) can replicate in the upper respiratory track and cause mild clinical symptoms in humans.
- Serological surveillance suggested that H6 AIVs could infect veterinarians exposed to domestic birds. This subtype caused also an outbreak in chicken farms in South Africa.
- We identified an emerging H6N6 swine influenza virus from south China.

Objectives

- To evaluate the potential threats of the emerging H6N6 swine influenza viruses to public health
- To study the mechanisms of H6N6 AIVs from avian to swine, especially the roles of two mutations at receptor binding sites: A222V and G228S

H6N6 binds to alpha 2,6-linkage glycans

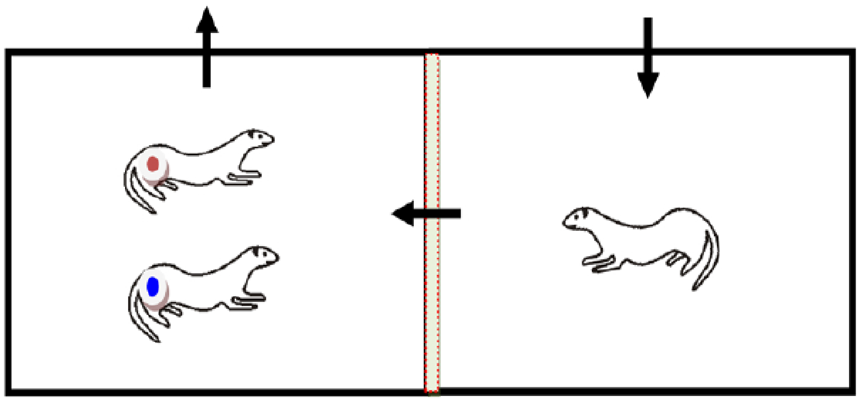


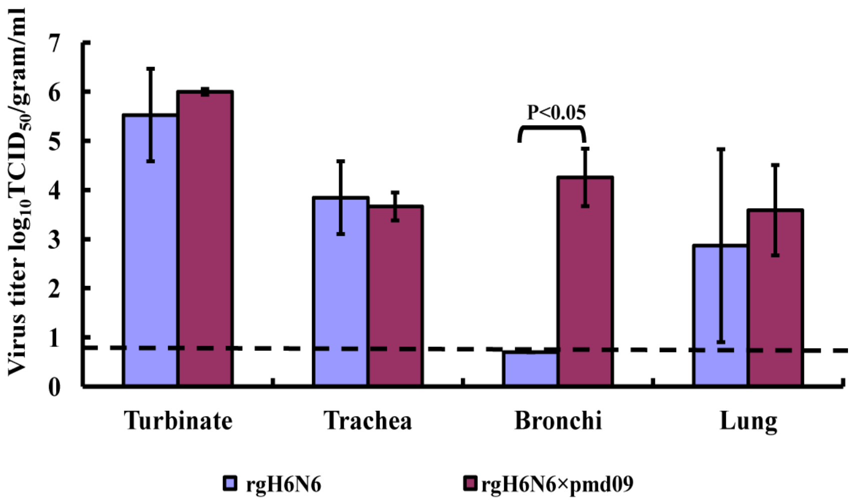


Wan, Xiu-Feng

Wan, Xiu-Feng

H6N6 causes aerosol transmission in ferrets





Viruses	Experimental Group			HI titer Ferret #1	HI titer Ferret#2
H6N6 wt	Aerosol	Donor	21 DPI	640	320
		Exposed	21 DPI	320	<10
	Contact	Exposed	21 DPI	<10	640
rgH6N6xpdm09	Aerosol	Donor	21 DPI	640	640
		Exposed	21 DPI	<10	<10
	Contact	Exposed	21 DPI	640	640

Viruses	DPI	Virus titers ($log10\ EID_{50}/ml$)[c] Ferret #1	Virus titers ($log10\ EID_{50}/ml$)[c] Ferret#2
H6N6 wt	2	ND	ND
	4	2.75	ND
	6	3.75	ND
	8	3.5	ND
rgH6N6xpmd09	2	ND	ND
	4	ND	ND
	6	1.00	ND
	8	1.00	ND

Summary

- Our glycan array profiling showed H6N6 swine influenza virus had higher binding affinity to both alpha 2,6 and 2,3 linked salic acids.
- Virus tissue binding assay further demonstrated H6N6 viruses showed strong binding affinity to swine upper respiratory tracheal tissues.
- H6N6 swine influenza virus caused both aerosol and direct transmission between ferrets
- The internal genes of the 2009 H1N1 virus, which is prevalent in the swine population, increases the replication efficiency of the H6N6 virus in the lower respiratory tract of ferrets ,but not the transmission ability of the H6N6 virus between ferrets.
- These findings suggest that the H6N6 virus has undergone some adaptation to the mammalian host, thus its evolution and spread should be closely monitored.

Acknowledgments

- The authors also acknowledge the **Consortium for Functional Glycomics** funded by the National Institute of General Medical Science GM62116 and GM98791 for services provided by the Glycan Array Synthesis Core (The Scripps Research Institute, La Jolla, CA) that produced the mammalian glycan microarray and the Protein–Glycan Interaction Core (Emory University School of Medicine, Atlanta, GA) that assisted with analysis of samples on the array. This study was supported by **NIH P20RR032694.**

Translational Modeling of Respiratory Disease in Horses

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[*]College of Veterinary Medicine, Mississippi State University
[†]College of Agriculture and Life Sciences, University of Arizona

Horses Spontaneously Develop an Asthma-like Disease

- Recurrent Airway Obstruction (aka Heaves, COPD)
  - Horses housed in barns during winter in temperate regions
    - Pasture associated during humid summers in southeastern US
- Winter disease is a recognized asthma model*
- ***Increasing prevalence of adult asthma in southeastern states***

*M LeClere et al. Heaves, an asthma-like disease of horses. Respirology, 2011 16(7):1027

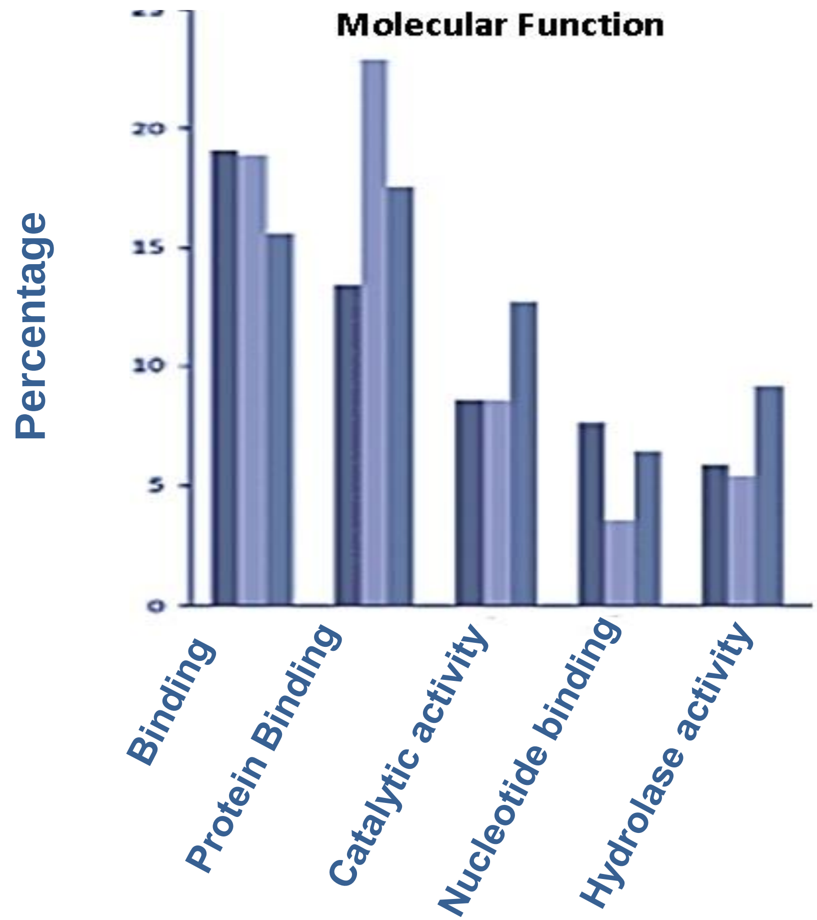
ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi:10.1111/j.1365-2052.2010.02158.x

Functional modelling of an equine bronchoalveolar lavage fluid proteome provides experimental confirmation and functional annotation of equine genome sequences

L. A. Bright*,†, N. Mujahid*, B. Nanduri†,‡, F. M. McCarthy†,‡, L. R. R. Costa§, S. C. Burgess†,‡,¶ and C. E. Swiderski*,†

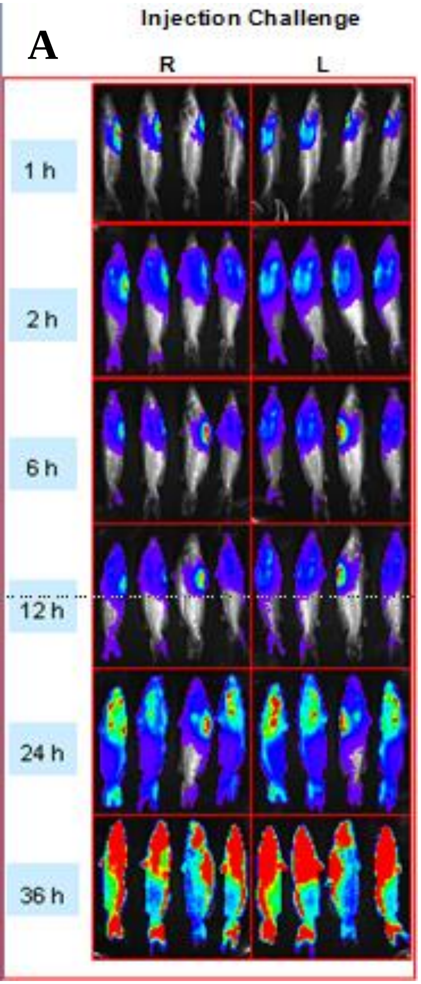
- 582 proteins identified in lung fluid from normal horses
- Functions compared to proteins identified in normal human lung fluid
- Horse proteome contained 91 of the 92 human ontology categories
- 8 of the top 10 molecular function categories are conserved between horse and man
- Conserved biological function supports the comparative utility of horse as a model within the respiratory system

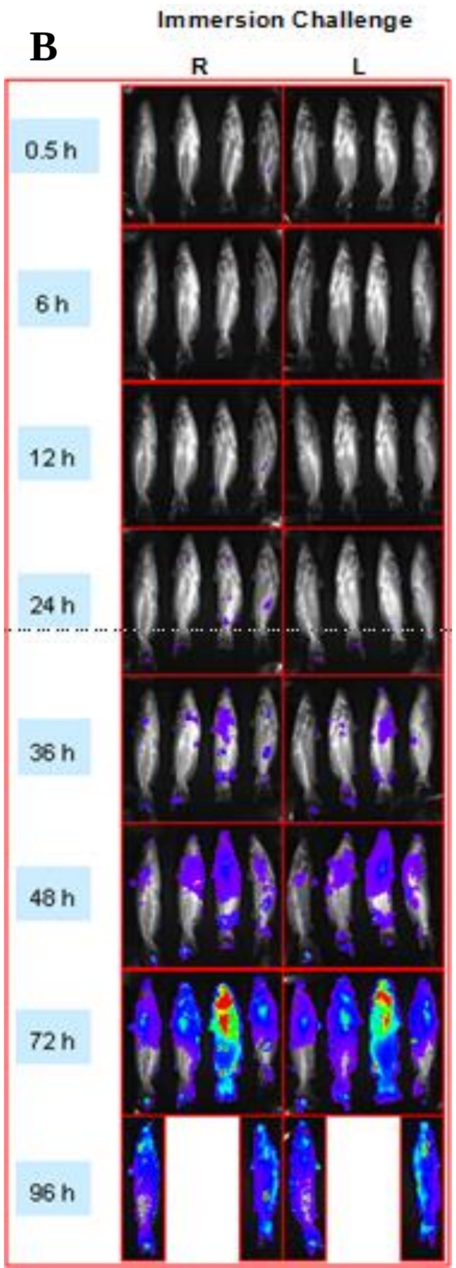


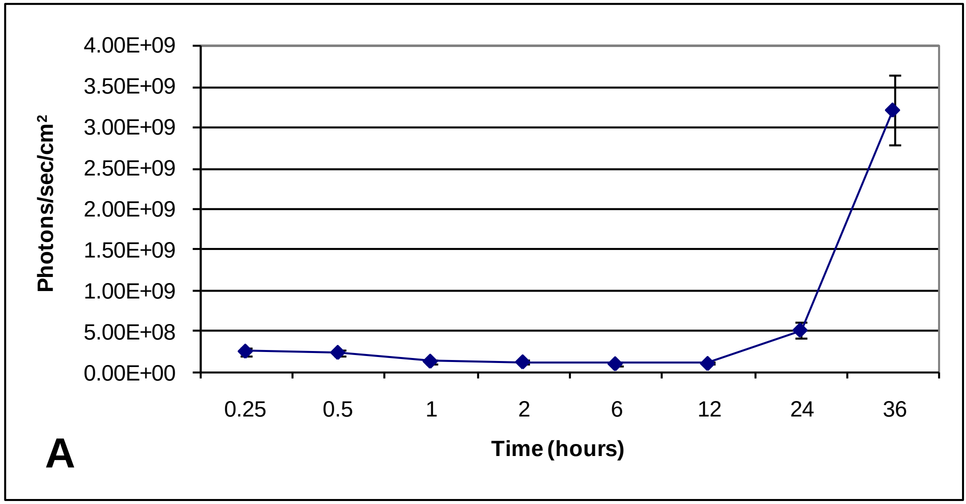
Swiderski, Cyprianna

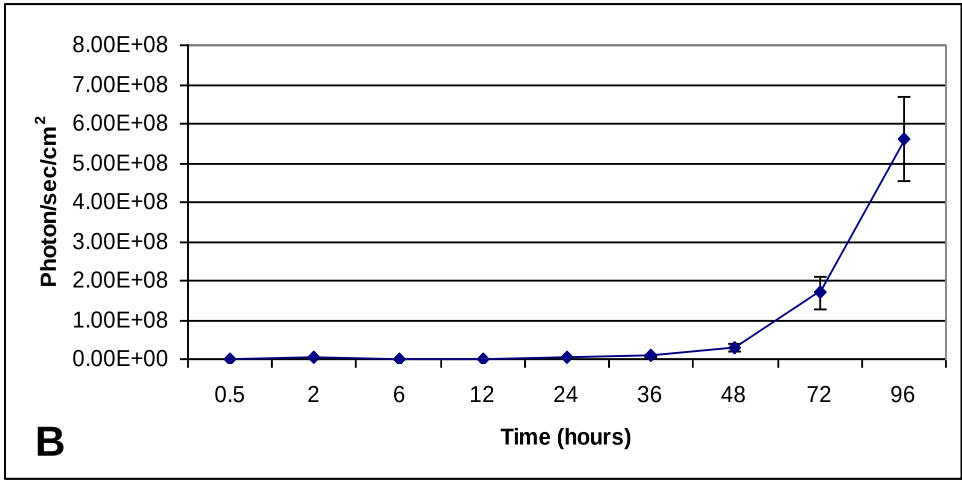
PATHOGEN TRACKING

Karsi, Attila



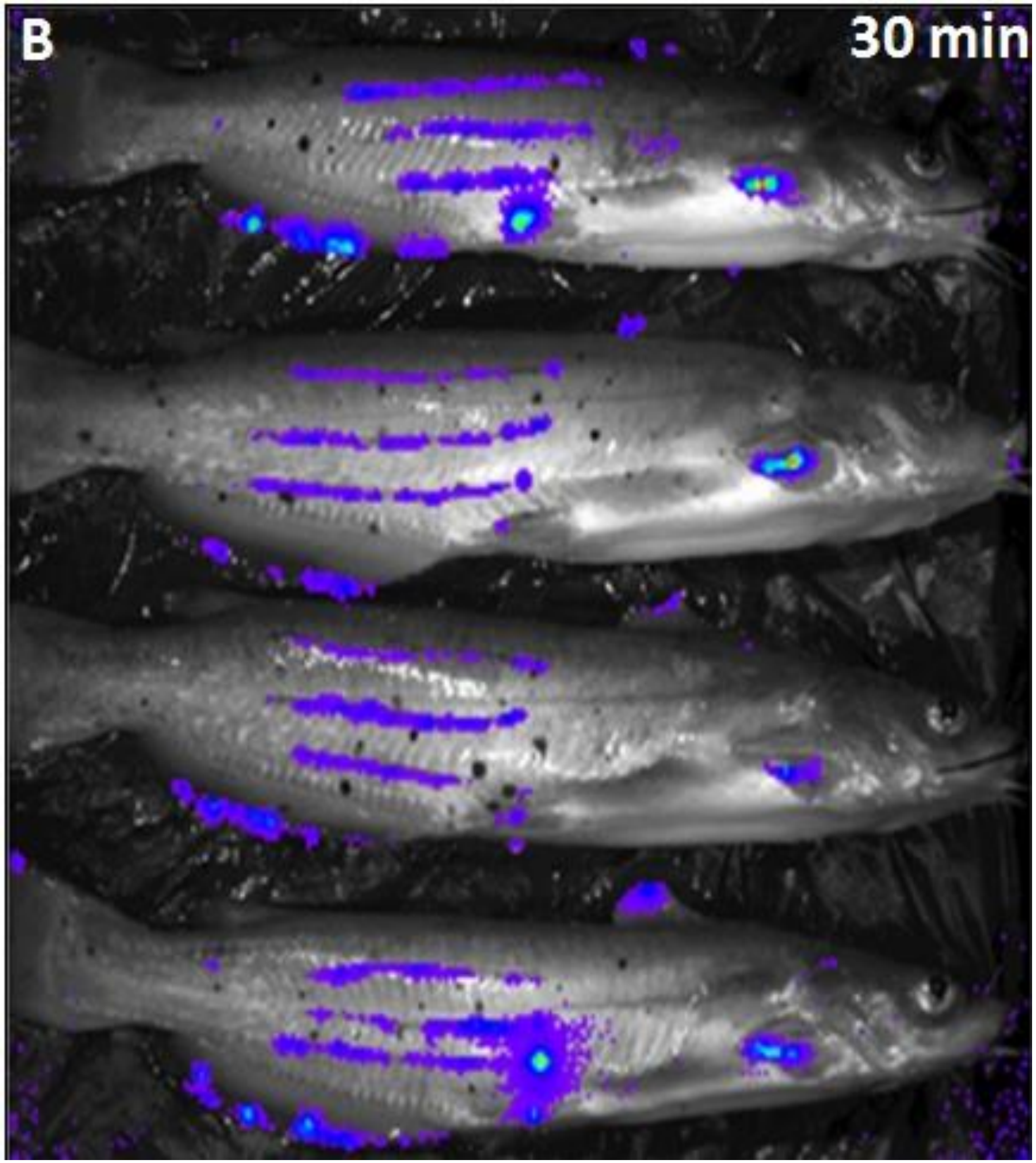






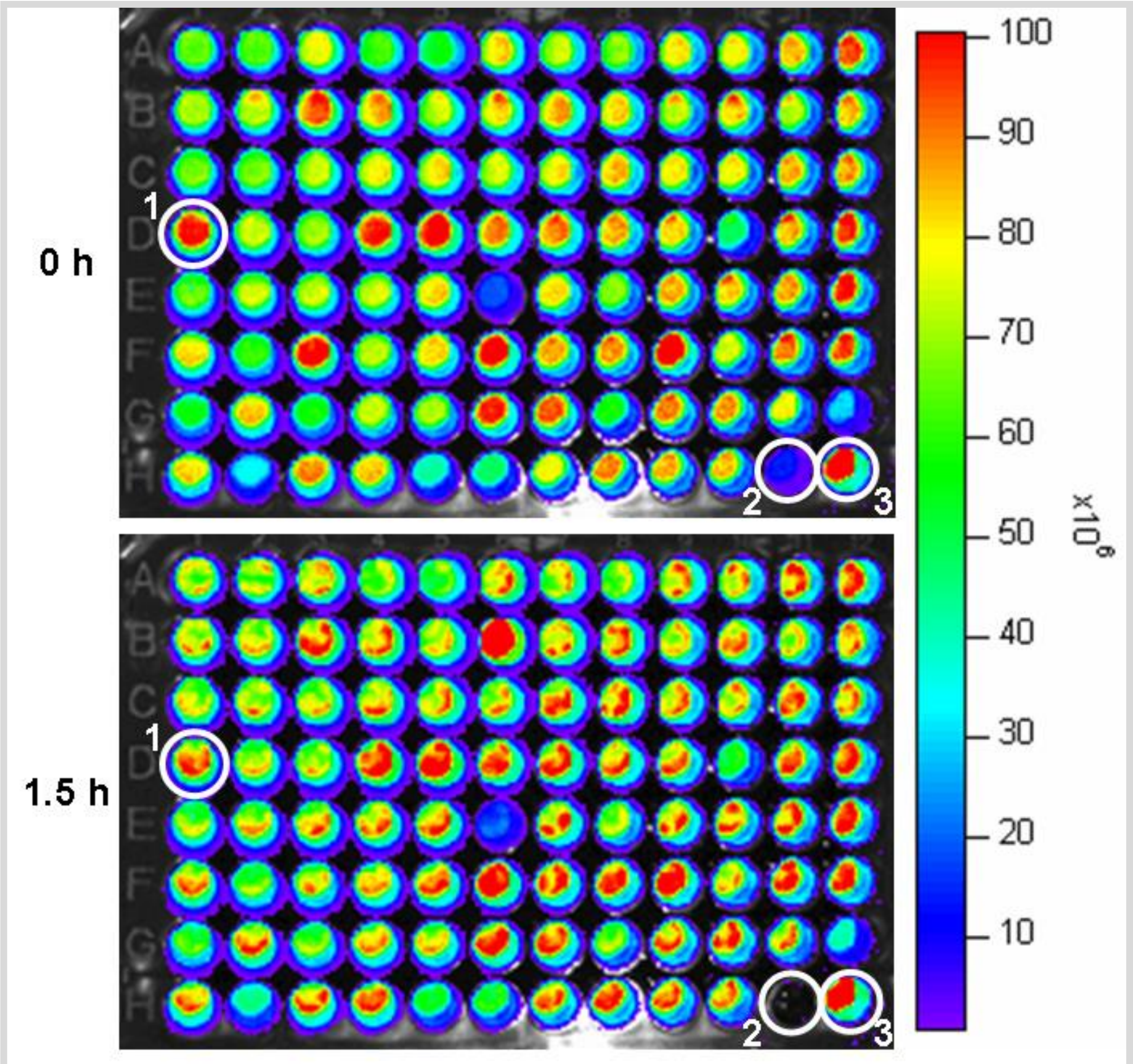
Karsi, Attila

ABRASION MODEL

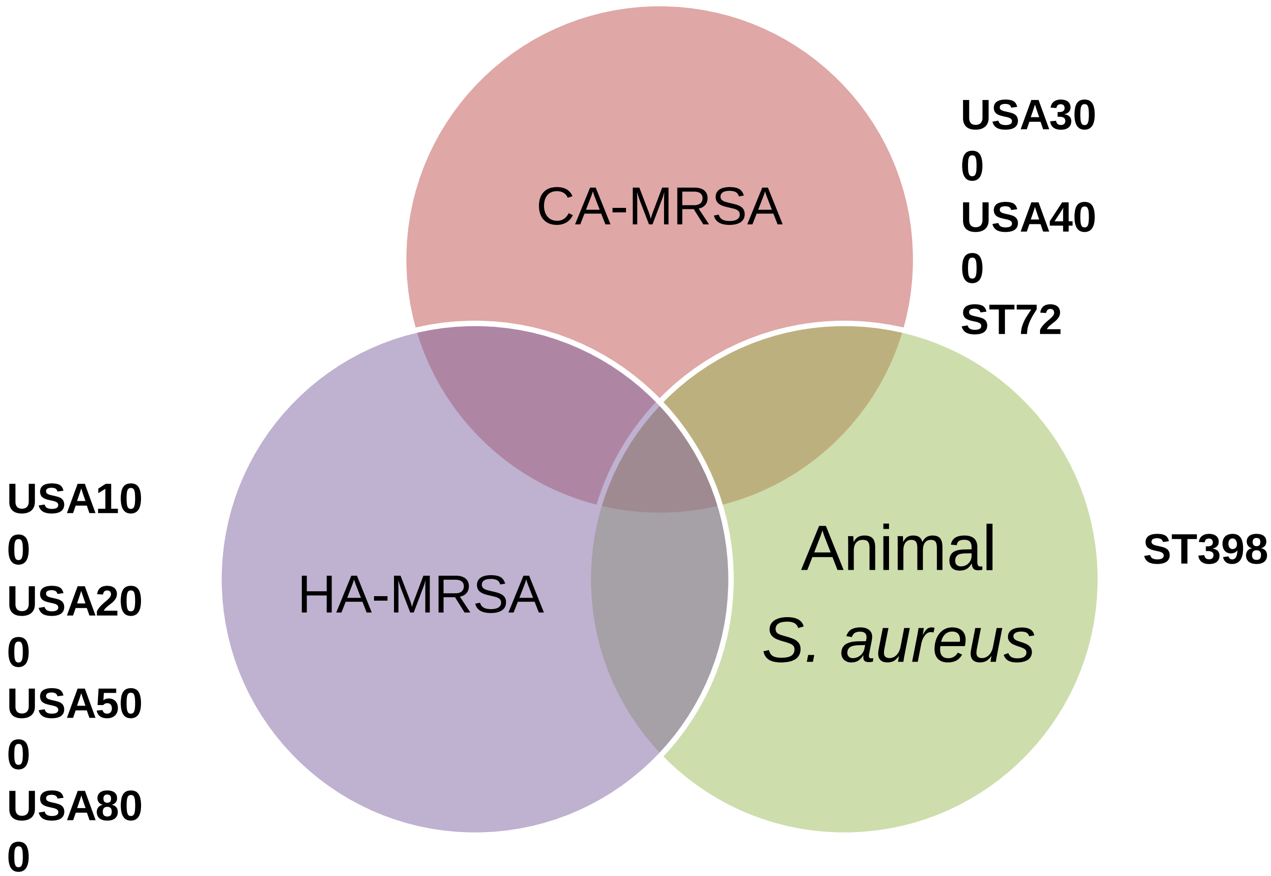


Karsi, Attila

MUTANT SCREENING USING BLI



Re-emerging infectious disease



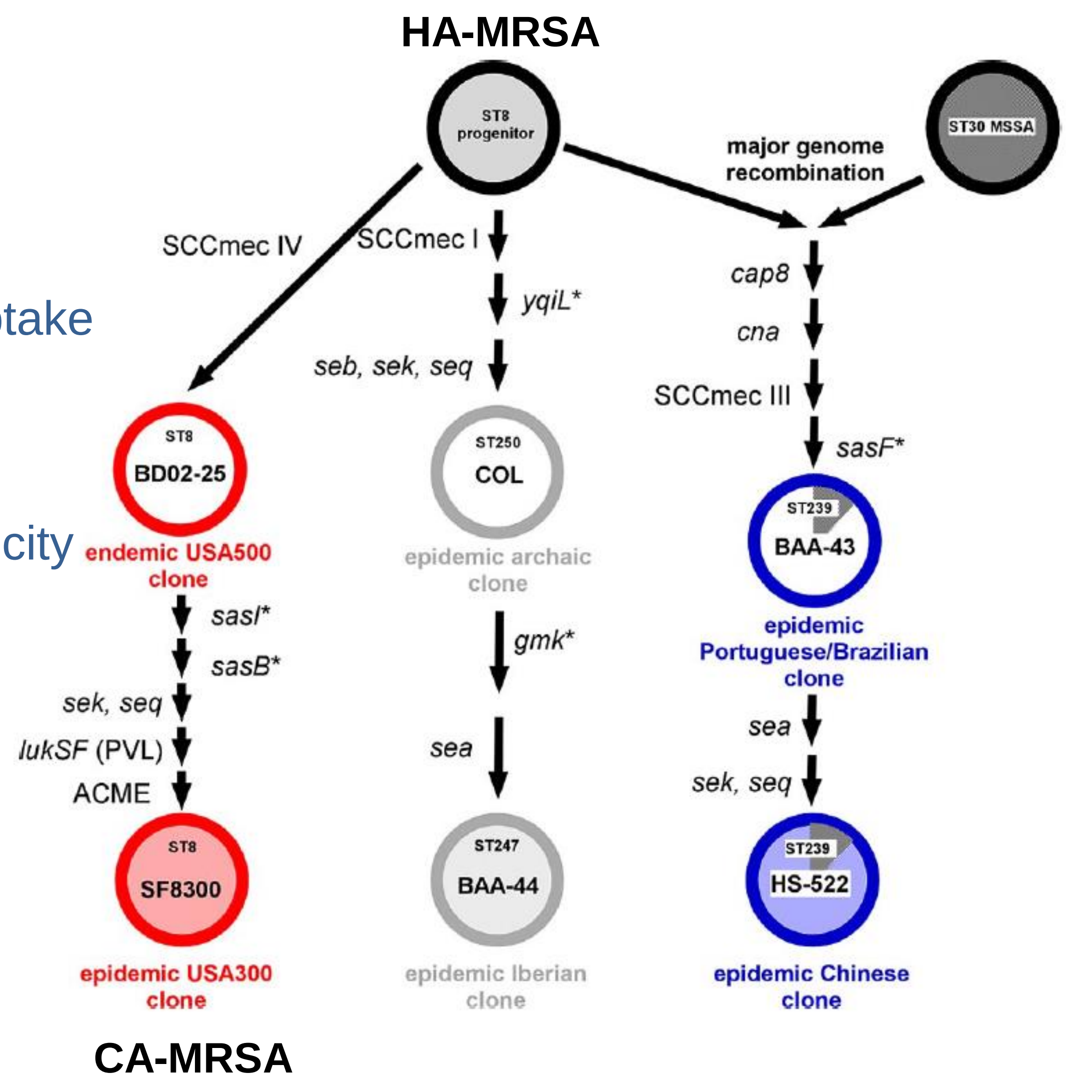
Seo, Keum Seok

Evolution of CA-MRSA USA300

Series of mobile genetic elements uptake

What is mobile genetic elements?

- Chromosomal cassette
- Staphylococcus aureus Pathogenicity Islands (SaPI)
- Plasmids
- Bacteriophages



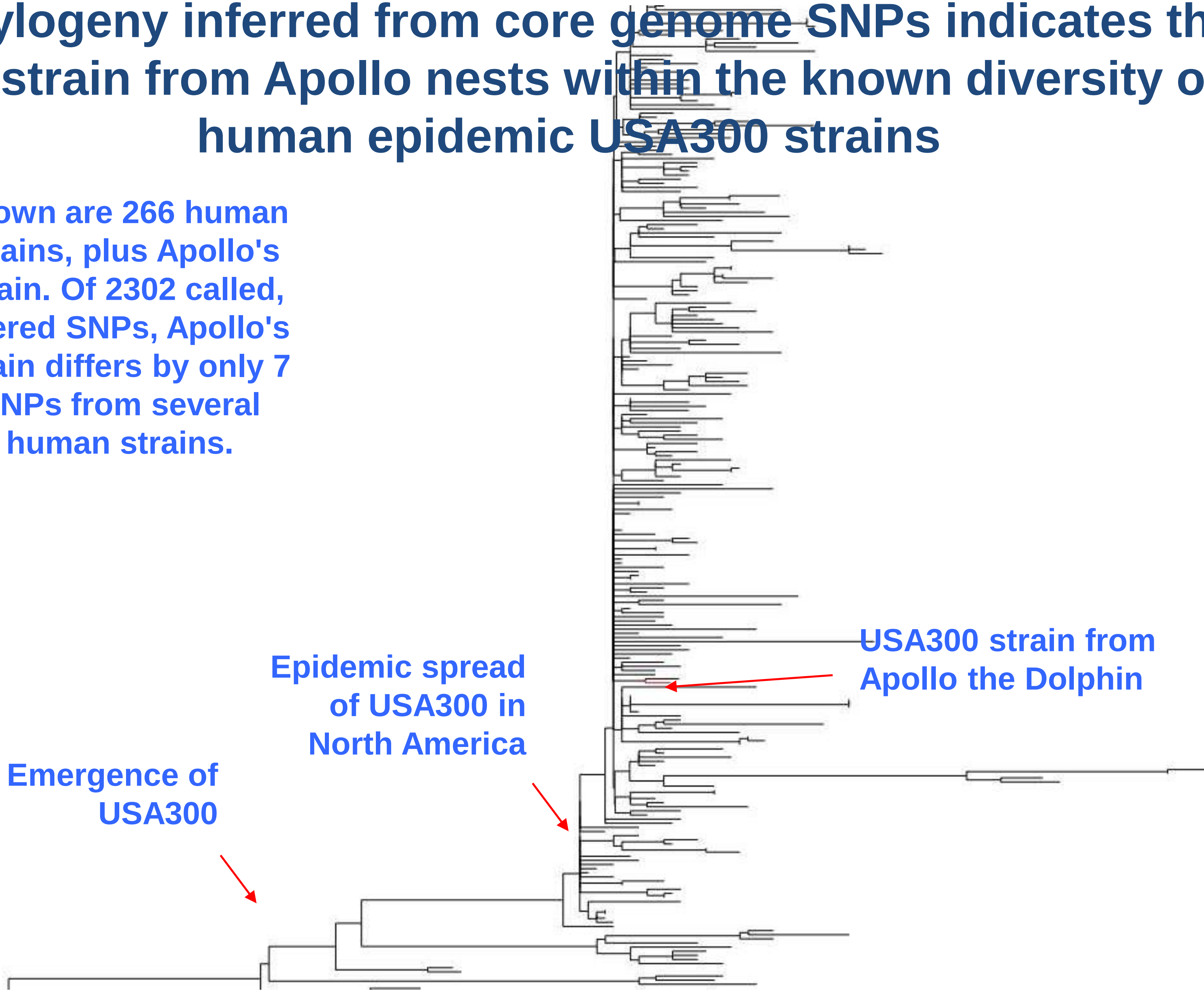
Li et al, PNAS, 2009

Case Involving a Bottlenose Dolphin

- A juvenile male bottlenose dolphin suffers from skin infection
- Our microbiology group responses to the case and isolates Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Molecular typing reveals it is MRSA epidemic in humans in USA (USA300)
- First case of CA-MRSA (USA300) isolation in marine mammals

A phylogeny inferred from core genome SNPs indicates that the strain from Apollo nests within the known diversity of human epidemic USA300 strains

Shown are 266 human strains, plus Apollo's strain. Of 2302 called, filtered SNPs, Apollo's strain differs by only 7 SNPs from several human strains.

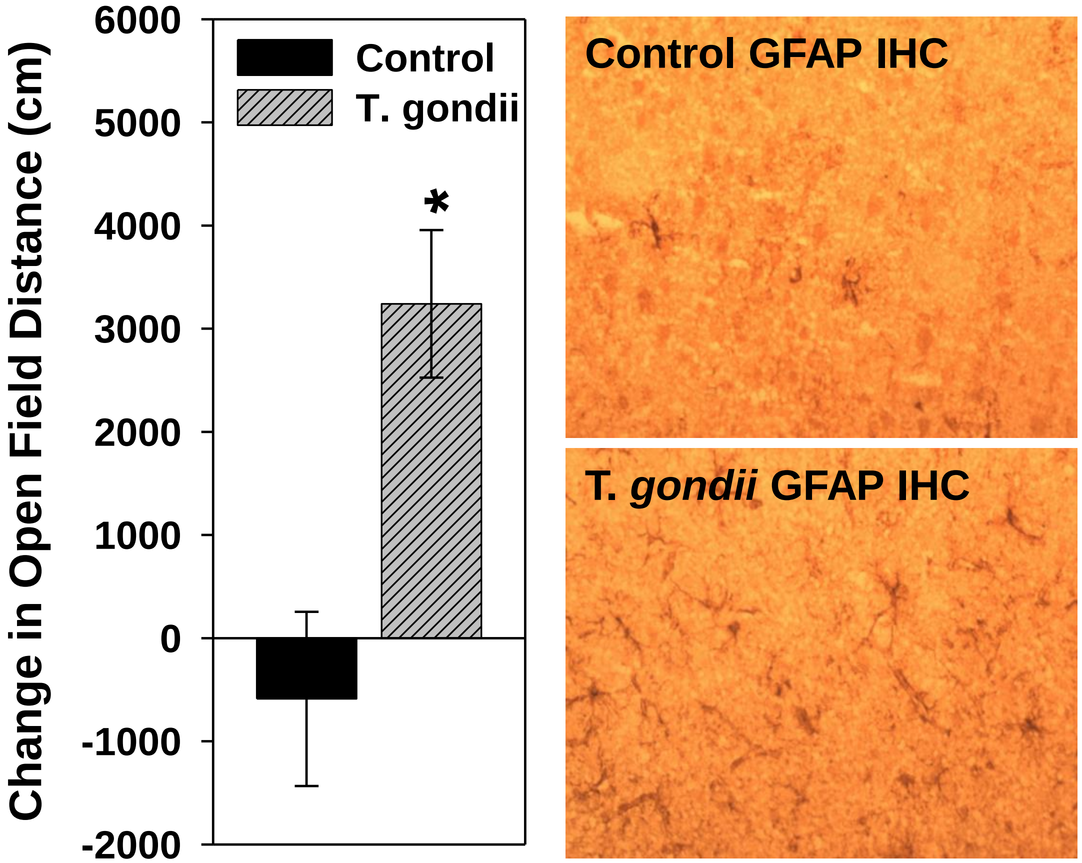


Seo, Keum Seok

Toxoplasma gondii

- An intracellular protozoan parasite that infects most warm-blooded animals where it forms cysts in muscle and brain, but only goes through sexual reproduction in cats.
- Approximately 30 % of humans have been exposed as determined by antibodies to the parasite.
- Individuals with antibodies to *T. gondii* are at increased risk of accidents, demonstrate different personality traits, and have an increased risk of schizophrenia, suicide and other mental illnesses.

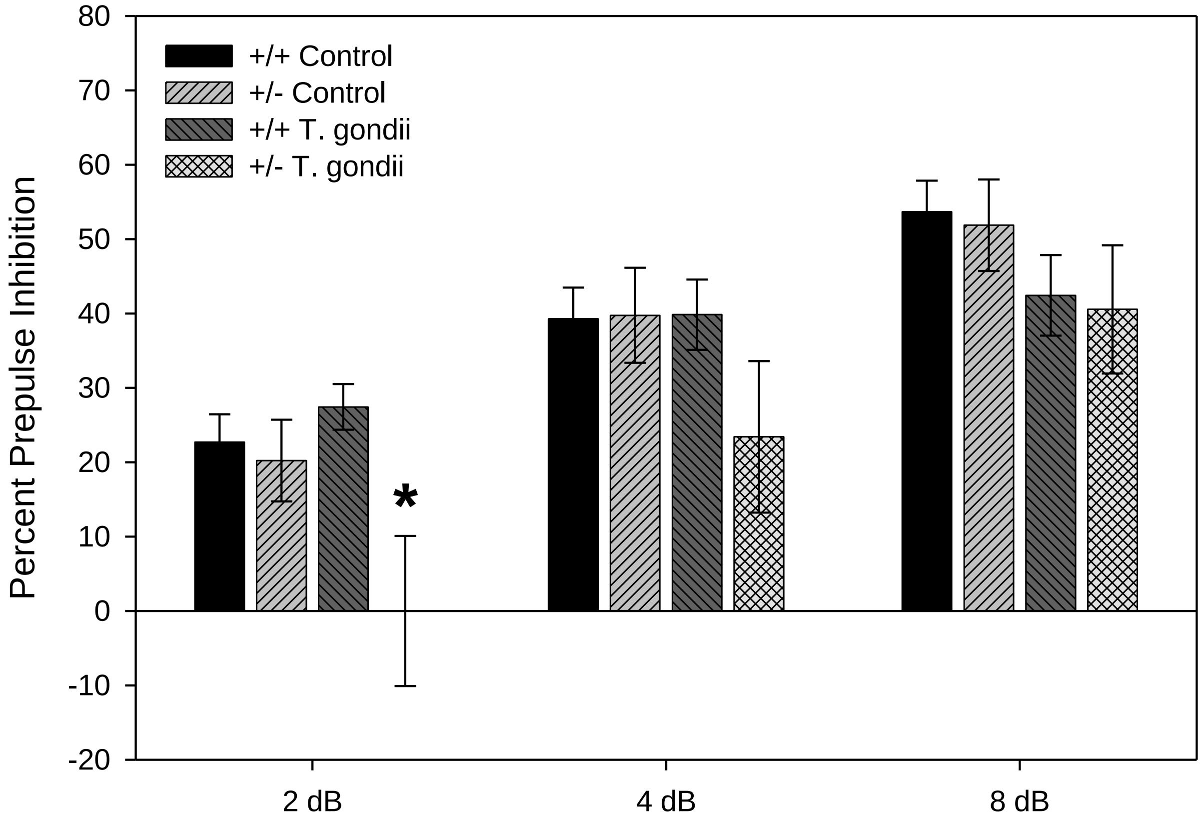
Eells, Jeffrey and Stokes, Andrea Varella



Infection with the parasite *T. gondii* and high antibody titers to this parasite has been shown to increase the incidence (~2.7 fold-increase in risk) and severity of schizophrenia.

Mice infected with *T. gondii* and with high antibody titers to the parasite show elevated locomotor activity and an increase in cerebral inflammation as compared to uninfected controls.

Eells, Jeffrey and Stokes, Andrea Varella



Genetically suceptible mice (Nurr1-null heterozygous mice) infected with T. gondii have disrupted prepulse inhibition, a behavior altered in schizophrenia and which correlates with symptom severity.

The goal off this research is to understand the mechanisms of how *T. gondii* infection alters behavior in mice in order to understand how it alters behaviors in humans and contributes to mental illnesses such as schizophrenia.

T and B cell deficient zebrafish (RAG1-/- mutant)

- The immune system of fish provides important information about conserved processes of the mammalian immune system
- Fish are an excellent model for studying innate immunity since their innate immune components are homologous to mammals
- Innate immune responses may be more critical in fish (lower vertebrate) immunity
- Dr. Petrie-Hanson has developed a strain of T and B cell deficient zebrafish (*RAG1*$^{-/-}$ mutant)
- Primary immune response is not impaired in the mutant zebrafish

T and B cell deficient zebrafish (RAG1-/- mutant)

- Lack T cell receptor (-> lack mature T cells)
- Lack immunoglobulin (->lack mature B cells)
- Have Natural Killer cells (Petrie-Hanson et al 2009)
- The mouse and zebrafish rag mutants are the only animal models available for investigating immune responses in the absence of lymphocytes
- It has recently been demonstrated that Natural killer cells can mount antigen-specific immunological memory

T and B cell deficient zebrafish (RAG1-/- mutant)

- Adaptive immune responses to chemical haptens and cytomegalovirus has been shown in RAG deficient mice
- Protective secondary responses against a bacterial pathogen was observed in RAG1$^{-/-}$mutant zebrafish (Hohn and Petrie-Hanson 2012)
- This was the first report of any RAG1$^{-/-}$ mutant vertebrate mounting a protective secondary immune response to a bacterial pathogen
- NK cell memory may have an enormous impact on mammalian and fish vaccinology

Hanson, Larry

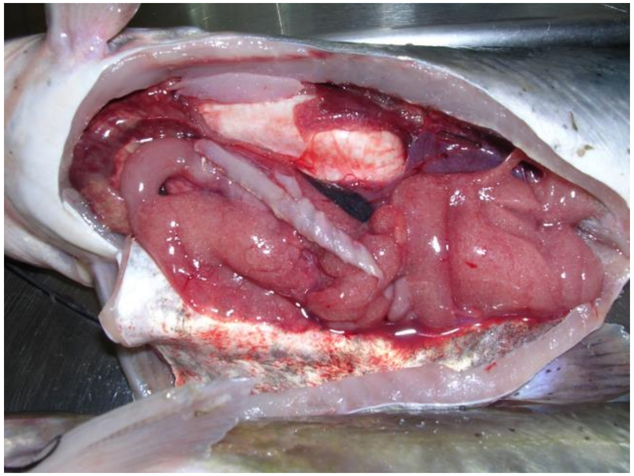
Botulinum

- Collaboration with (Dr. Gaunt)
- Studying intervention strategies for botulism type E toxicosis in catfish
  - Diagnosis- Zebrafish effective bioassay organism
  - Vaccines- using recombinant CCV expressing neurotoxin heavy chain
  - Studying genic modifications of SNAP25 target to render fish resistant (zebrafish model)
- One health- these methods applicable to animals and humans

<u>Hanson, Larry</u>

Environmental factors associated with virulent Aeromonas hydrophila caused disease

- Collaboration with Drs. Griffin, Cunningham Mischke and Wan.
- Studying how a virulent strain of *Aeromonas hydrophila* (VAH) is maintained in the catfish pond environment and transmitted between ponds.
  - Have shown that fish eating birds that consume VAH infected fish can shed high levels of culturable VAH in their feces over 24 hours later.
  - Plan to look at invertebrates as potential reservoirs
- One health- *Aeromonas hydrophila* and other similar bacteria are important pathogens in fish, birds, and mammals (including humans). Understanding environmental factors will allow development of practical control methods



Bulla, Camilo; Lunsford, Kari

Trichler *et al. BMC Veterinary Research* 2013, **9**:144
http://www.biomedcentral.com/1746-6148/9/144

BMC Veterinary Research

METHODOLOGY ARTICLE Open Access

Ultra-pure platelet isolation from canine whole blood

Shauna A Trichler[1], Sandra C Bulla[1], John Thomason[2], Kari V Lunsford[2] and Camilo Bulla[1*]

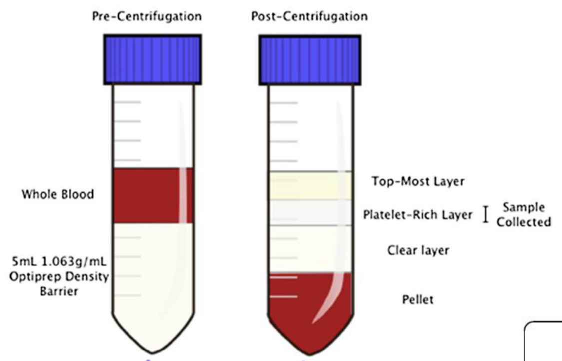


Figure 1 Density barrier layering before and after centrifugation with whole blood.

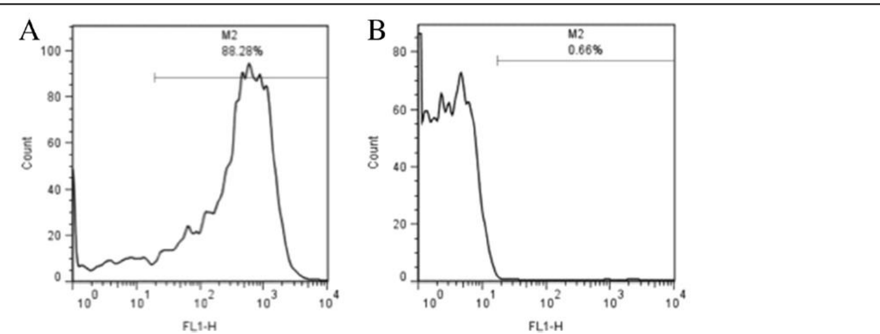
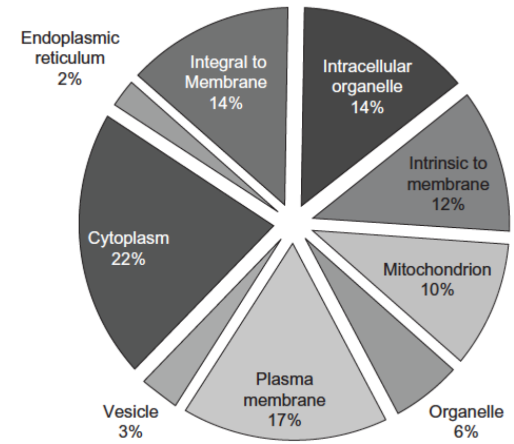


Figure 4 Flow cytometric analyses of platelet activation by Annexin V labeling. (A) Collagen activated platelets. **(B)** Platelet sample after density barrier centrifugation.

Bulla, Camilo; Lunsford, Kari

Identification of canine platelet proteins separated by differential detergent fractionation for nonelectrophoretic proteomics analyzed by Gene Ontology and pathways analysis



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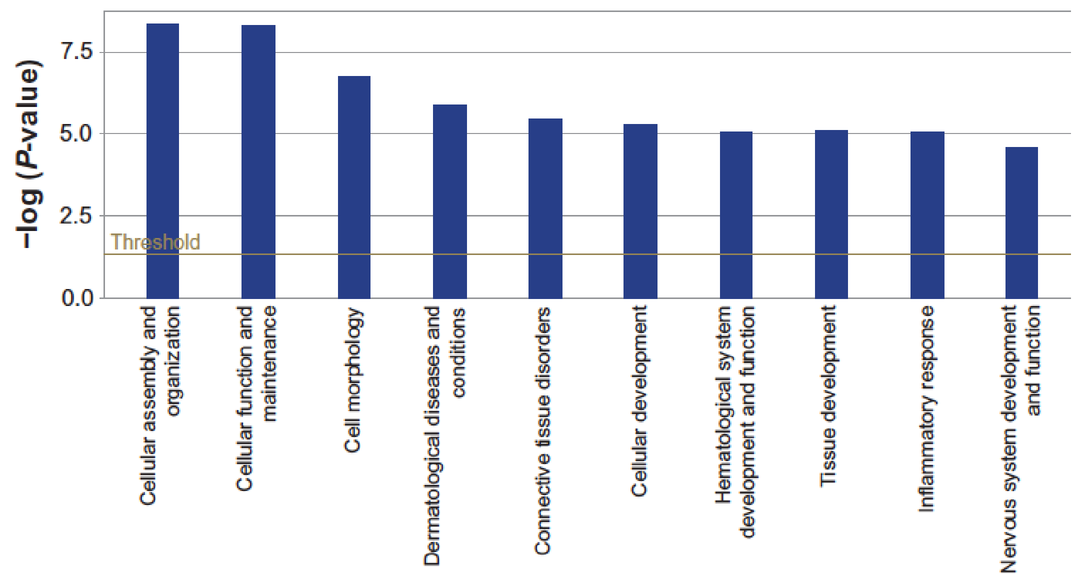
*These authors contributed equally to this work



Questions?