CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

CIHMID

# SUMMER SYMPOSIUM 2019

## Wednesday July 31, 2019

9:15 am – 4:00 pm

*coffee at 8:30, closing reception to follow*

Stocking Hall

Conference Center & PepsiCo Auditorium

Cornell University

## Schedule of Events

**8:30 – 9:15** **Coffee and Welcome Reception, poster setup**

**9:15 – 9:25** Opening Remarks (Brian Lazzaro, CIHMID Director)

**9:25 – 9:50** Steve Ellner (Ecology and Evolutionary Biology, CAS)
*"Individual specialization and multi-host epidemics: Disease spread in plant-pollinator networks"*

**9:50 – 10:10** Jon Sanders (CIHMID Postdoc)
*"The evolution of the tetrapod gut microbiome"*

**10:10 – 10:30** Gabrielle Lê-Bury (CIHMID Postdoc)
*"Transcriptional Profiling of HIV-1-Infected Human Alveolar Macrophages from Human Volunteers by Single Cell Sequencing"*

**10:30 – 10:50** Jingjing Fu (CIHMID Postdoc)
*"Gut commensal-produced metabolites mediate colonization resistance to* Salmonella typhimurium*"*

**10:50 – 11:20** **Coffee Break**

**11:20 – 11:35** Weishan Huang (Microbiology and Immunology, CVM)
*"Heterogeneity of regulatory T cells in the mouse airway during influenza infection"*

**11:35 – 11:50** Brian Wasik (Baker Institute, CVM)
*"Mutational dynamics of three influenza strains during mouse passage"*

**11:50 – 12:15** Hector Aguilar-Carreno (Microbiology and Immunology, CVM)
*"Deadly Viral Glycoprotein Team Burglary: How do we Stop It?"*

**12:15 – 12:30** Brian Lazzaro (CIHMID Director)
*CIHMID State of the Union, 2.5 years after inception*

**12:30 – 2:00** **Lunch and Poster Viewing**

**2:00 – 2:25** Chris Smart (Plant Pathology and Plant Microbe Biology, CALS)
*"Hemp-microbe interactions – who's in control of the substance?"*

**2:25 – 2:40** Melanie Smee (Microbiology, CALS)
*"Insects as phyllosphere microbiome engineers for plant pathogens"*

**2:40 – 2:55** Chase Mayers (Plant Pathology and Plant Microbe Biology, CALS)
*"Endosymbiotic bacterial diversity across arbuscular mycorrhizal fungi"*

**2:55 – 3:10** Shannon Murphy (Microbiology, CALS)
*"*Vibrio cholerae *encodes a cell wall hydrolase adapted for zinc-starved environments"*

**3:10 – 3:25** Lydia Baker (Microbiology, CALS)
*"Diverse deep-sea anglerfishes share a genetically reduced luminous symbiont that is acquired from the environment"*

**3:25 – 3:50** Corrie Moreau (Entomology, CALS)
*"Symbiosis and the Evolution of the Ants: Macroevolution to Microbiomes"*

**4:00 – 6:00** **Closing Reception, poster takedown**

## SUBMITTED ABSTRACTS

## Talks

### Diverse deep-sea anglerfishes share a genetically reduced luminous symbiont that is acquired from the environment

Lydia J. Baker[a1], Lindsay L. Freed[b], Cole G. Easson[b,c], Jose V. Lopez[b], Danté Fenolio[d], Tracey T. Sutton[b], Spencer V. Nyholm[e], Tory A. Hendry[a1]

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Deep-sea anglerfishes are relatively abundant and diverse, but their luminescent bacterial symbionts remain enigmatic. The genomes of two symbiont species have qualities common to vertically transmitted, host-dependent bacteria. However, a number of traits suggest that these symbionts may be environmentally acquired. To determine how anglerfish symbionts are transmitted, we analyzed bacteria-host codivergence across six diverse anglerfish genera. Most of the anglerfish species surveyed shared a common species of symbiont. Only one other symbiont species was found, which had a specific relationship with one anglerfish species, Cryptopsaras couesii. Host and symbiont phylogenies lacked congruence, and there was no statistical support for codivergence broadly. We also recovered symbiont-specific gene sequences from water collected near hosts, suggesting environmental persistence of symbionts. Based on these results we conclude that diverse anglerfishes share symbionts that are acquired from the environment, and that these bacteria have undergone extreme genome reduction although they are not vertically transmitted.

## Heterogeneity of regulatory T cells in the mouse airway during influenza infection

Weishan Huang[1,2,3], Kaixiong Ye[4], Michael C. McGee[1], Natalie F. Nidetz[1,2], Sabrina Soluoki[3], Candice B. Limper[3], and Avery August[2,3]

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Influenza (flu) infection is the leading cause of respiratory illness worldwide. While flu vaccines are effective at reducing morbidity and mortality, viral clearance relies on the development of a strong immune response. Virus-specific antibodies and T cells are required for killing of flu-infected cells and clearance of the virus. Although strong T cell responses are desired, the cytotoxic effector function of the innate and adaptive immune responses, can lead to the development of pulmonary immunopathology, which together with recurrent bacterial infections is a major cause of death during flu pandemic. While T cells produce inflammatory cytokines during flu infection, they can also produce the immunosuppressive cytokine IL-10, which is critical for limiting the immunopathology caused by the excessive immune responses. However, the composition of this population and their molecular signatures are unclear. Using mouse models that report the production of IL-10 by GFP and expression of conventional regulatory T cell marker Foxp3 by RFP, a mouse adapted model of influenza infection, transcriptomic analyses at the population and single cell levels, and transgenic mouse models that are impaired in T cell-derived IL-10 production, we found that, during flu infection, regulatory T cells in the mouse airway are mainly comprised of CD4+ Foxp3+, CD4+ Foxp3- and CD8+ subsets, and they differ from IL-10-producing T cells under pneumonitis or asthmatic conditions. Furthermore, within each subset, IL-10-producing T cells exhibit significant molecular heterogeneity. Information gained from this dataset provides insights into the T cell subset heterogeneity and signature markers, and shed light for future strategic design for therapeutic development utilizing the immunomodulatory features of T cells for the treatment of pulmonary immunopathology.

## *Endosymbiotic bacterial diversity across arbuscular mycorrhizal fungi*

Chase G. Mayers[1], Matthew T. Kasson[2], William Wheeler[2], Madiha Saeed[1,3], Teresa E. Pawlowska[1]

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[3] Center for Interdisciplinary Research in Basic Sciences, International Islamic University, Islamabad, Pakistan

All three lineages of the early-diverging Mucoromycota harbor transient, obligately intracellular endosymbiotic bacteria (EB). This pattern is particularly pronounced among the arbuscular mycorrhizal fungi (AMF) of Glomeromycotina, whose EB may play a role in their own obligate symbioses with nearly all land plants. The AMF-EB symbiosis has persisted for 400 million years or more, yet the role of these EB remains elusive, compounded by gaps in the understanding of their diversity and patterns of association among AMF taxa. Previous studies sampled a limited taxonomic scope of AMF or used bacterial primers specific for the two known AMF EB, namely Candidatus Glomeribacter gigasporarum (related to Burkholderia, Betaproteobacteria) and Candidatus Moeniiplasma glomeromycotorum (related to Mycoplasma, Mollicutes). Evidence, however, suggested that cryptic EB diversity existed outside of these two species. We leveraged the collections of the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University, the largest collection of living AMF cultures in the world, to perform the most taxonomically-comprehensive study of AMF EB. For 39 AMF species in 17 genera, representing 8 families in all 4 orders of Glomeromycotina, we performed single-cell whole-genome amplification of surface-sterilized spores and amplified both fungal and bacterial DNA using a complement of universal primers. The precise function of AMF EB remains unclear, but this study serves as a foundation for further exploration of their significance.

## *V. cholerae* encodes a cell wall hydrolapse adapted for zinc-starved environments

Shannon Murphy[1, 2], Laura Alvarez[3], Myfanwy Adams[4], Shuning Liu[1,2], Brianna Johnson[1,2], Joshua Chappie[4], Felipe Cava[3], Tobias Dörr[1,2]

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The cell wall provides a bacterium with structural integrity and has served as a powerful and long-standing target for antibiotic development. The wall is comprised of a strong, yet flexible, meshwork of peptidoglycan (PG) that must be continuously remodeled to accommodate a growing cell. This task requires a delicate interplay between PG synthesis and degradation, as well as the ability to adapt these processes to harsh and fluctuating environments. *Vibrio cholerae*, the causative agent of diarrheal cholera disease, encodes a group of three nearly identical endopeptidases (EPs) that are predicted to hydrolyze PG to facilitate cell growth. Two of these (ShyA and ShyC) are conditionally essential housekeeping EPs, while the third (ShyB) is not expressed under standard laboratory conditions. To investigate the role of ShyB, we conducted a transposon screen to identify mutations that activate *shyB* transcription. We found that *shyB* is part of the Zur regulon and is induced by zinc starvation, a stress frequently encountered within the human host. *In vivo*, ShyB alone was sufficient to sustain cell growth in low-zinc environments. *In vitro*, ShyB retained its PG hydrolytic activity in the presence of the metal chelator EDTA. This insensitivity to metal chelation is likely what enables ShyB to substitute for other EPs during zinc starvation. This mode of zinc-dependent regulation has not been previously reported for cell wall lytic enzymes; however, our survey of transcriptomic data identified candidate Zur-regulated EPs in diverse bacterial lineages. These findings suggest that many Gram-negative bacteria employ a cell wall hydrolase adapted for zinc-starved conditions. Since anti-infective therapies commonly target the bacterial cell wall, an improved understanding of how cell wall homeostasis adapts to host-induced zinc starvation could lead to new antibiotic development.

**Insects as phyllosphere microbiome engineers for plant pathogens.**

Melanie R. Smee*, Tory Hendry

*Microbiology Department, Cornell University

The phyllosphere, or the above-ground parts of plants, hosts many interacting organisms including diverse bacteria. Insect herbivores are also common in the phyllosphere and encounter plant-associated bacteria, yet how these organisms interact and influence ecological communities remains largely unknown. Strains of the diverse bacterium Pseudomonas syringae grow well epiphytically and have been shown to infect and kill some hemipteran insects like the pea aphid, Acyrthosiphon pisum. Aphids are hypothesized to be an alternative host for these epiphytic bacteria but it is unclear if aphids provide fitness benefits to bacterial pathogens. To determine if epiphytic bacteria could be adapted for infecting aphids, we characterized 21 strains of P. syringae for epiphytic ability and virulence to pea aphids and found that the two traits were positively correlated. Eight strains were further selected to determine if the bacteria derived a fitness benefit from the presence of aphids. Some strains benefited significantly from aphid presence, with epiphytic titers 18.3% higher than without aphids. However, further investigation found that honeydew, the sugary waste product of aphids, and not growth in aphids, increased P. syringae growth on leaves. This suggests that aphids may be important microbiome engineers in the phyllosphere, but evolutionarily dead-end hosts for epiphytic bacteria.

# Mutational dynamics of three influenza strains during mouse passage.

Brian R. Wasik[1], Ian E.H. Voorhees[1], Karen N. Barnard[1], Brynn K. Lawrence[1], Wendy S. Weichert[1], Grace Hood[1,2], Aitor Nogales[3], Luis Martinez-Sobrido[3], Edward C. Holmes[4], Colin R. Parrish[1]

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Host ranges of animal viruses are generally well established, and adaptation of a virus to a new host is a complex process that involves overcoming many different barriers. Tracking the dynamics of mutations during host passage would inform the mechanisms underlying virus host range, including the nature and context of mutations that arise. Laboratory mouse passage has long been used during studies of viruses, including for Influenza A virus (IAV). While mouse-adapted IAV mutations have been characterized in multiple studies, there is little consistency in results found, and little information about the virus population dynamics during the process of host passage that would disentangle the roles of mutational selection or drift that occurs in the viral sequences. Here we performed serial passage of three IAV strains in mice, collecting and deep sequencing the virus populations in the lungs of the mice at each passage. We also investigated whether mutational dynamics differed when passaged in a host receptor genetic variant. Mice contain high percentages of the sialic acid (Sia) variant N-glycolylneuraminic acid (Neu5Gc) in addition to N-acetylneuraminic acid (Neu5Ac), so we compared viruses in CMAH-/- mice that lack Neu5Gc, to identify any role of this receptor variant. Serial passage was performed with human H1N1 (pandemic) virus, human H3N2 (seasonal) virus and the canine (H3N2) virus derived from reverse genetic plasmids. Those were inoculated into C57BL/6 mice, or CMAH-/- variants. The seasonal H3N2 human influenza virus failed to establish infection in mice, while the pdmH1N1 virus gave moderate pathogenesis with weight loss in both strains of mice. In contrast, canine H3N2 influenza virus replicated well within both strains of mice without weight loss. The pdmH1N1 virus populations generated several low-frequency mutational variants without fixation, while canine H3N2 populations showed the emergence and fixation of mutations in the NA, PA, and PB2 segments.

## POSTERS (**CIHMID Undergraduate Research Experience)

### Life history trade-offs in Drosophila melanogaster

**Ashlyn Amsden, Katie Gordon, Brian Lazzaro

Department of Entomology, Cornell University

Female Drosophila melanogaster are more susceptible to bacterial infection after mating. Previous studies have shown that the male seminal fluid protein, Sex Peptide, suppresses the female immune response by stimulating production of Juvenile Hormone (JH). JH is required for the incorporation of yolk proteins into complete oogenesis. We hypothesize that limiting investment in reproduction would allow females to mount a stronger immune response to infection. This hypothesis is supported by the observation that females who do not produce eggs survive infection better than those that produce eggs, regardless of mating status. To test this hypothesis, we are subjecting different oogenesis mutants, with various levels of reproductive investment, to a systemic infection with the gram-negative bacterium Providencia rettgeri. The mutant yolkless, which is a mutant that fails to secrete yolk proteins, is currently being used within my experiments. The results of this experiment will answer the question as to whether there is a stage of oogenesis that is most costly to the immune response in D. melanogaster. I predict that oogenesis mutants that arrest early prior to large investments via yolk protein incorporation will survive infection like unmated females while oogenesis mutants that arrest late will be more susceptible to infection. This will allow us to determine the stage of oogenesis where investment in egg production is most costly later immune performance. Alternatively, if the suppressive effect of JH via Sex Peptide is independent of egg production, all mated oogenesis mutants will be more susceptible to infection than unmated females.

## The search for secondary symbionts in diverse aphid species

Catalina Zuluaga Arias, Melanie R. Smee, and Tory A. Hendry

Department of Microbiology, Cornell University

Symbionts are present in an extensive range of organisms and have crucial roles in the ecology and evolution of animals, especially within insects. Among these, aphids engage in a wide variety of symbiotic relationships with bacteria which makes them an outstanding example to study the importance and effects of this interaction. They rely on an obligate bacterial symbiont Buchnera for their survival. Aphids also interact with mutualistic facultative symbionts who can protect them from parasitism, heat stress, and provide additional dietary requirements in some instances. However, many species of aphid have not yet been surveyed for the presence of facultative symbionts. The aim of the present study is to identify if seven species of aphids, across 4 different genres, contain facultative symbionts and subsequently identify the symbiont species. A phylogenetic analysis of the aphid host species and the facultative symbionts will be used to elucidate relationships between the aphid species and the symbionts. Identifying which facultative symbiont is present in these species, which are usually considered as pests for important agricultural crops, is essential to develop any bio-control applications because they can protect the aphid, as is the case of the common facultative symbiont, *Serratia symbiotica.*

# EnvISiON: Building an Agent-based model to monitor disease spread in healthcare facilities using IoT-based Bluetooth tracking of human movements

Cecil Wilfried Barnett-Neefs, Alison E. Stout, Mike Philips, Renata Ivanek

Department of Population Medicine & Diagnostic Sciences, Cornell University

Agent-based models (ABM) are a key tool in animal and public health for understanding and predicting disease spread within a healthcare facility as well as identifying sites at most risk for patients' exposure to pathogens. These models are built on data gathered through biological samples, population observation and mathematical rules. Movements of healthcare personnel is a known risk factor for pathogen spread but is difficult to measure for parameterization in ABMs. To this end we developed a Bluetooth-based proximity sensor using the Raspberry Pi 3, programmed to detect participant's mobile phones when nearby, allowing for passive observation of healthcare personnel that would not disrupt a participant's normal behavior. As a proof of concept the sensor is implemented in a large animal veterinary hospital to allow parameterization of an ABM that evaluates the spread of a nosocomial pathogen, Salmonella. To track and process data on traffic trajectory patterns from individuals back into the ABM we developed EnvISiON ("Environmental Internet-connected Simulation and Observation Network") that bridges the gap between active data gathering and modelling. The information gathered through EnvISiON can then be collated to establish movement trends through the facility and refine the model's predictive functionality. Furthermore, this network is designed to be adaptable and capable of being rapidly redeployed into new facilities with minimal difficulty, using a central cloud server to collect data from sensors for analysis.

## Metabolic costs of a defensive symbiont for its insect host

Frances Blow, Nana Y. Ankrah, and Angela E. Douglas

Department of Entomology, Cornell University

Many animals bear defensive symbionts, i.e. microorganisms that antagonize natural enemies of their host. Symbiont-mediated protection can be mediated by interference (e.g. by toxins), competition for resources, and activation of host immune function against the enemy. Defensive symbionts are generally costly to the host under enemy-free conditions, independent of their mechanism of protection. The nature of these costs is largely unknown, beyond the expectation that they consume nutrients which would otherwise be allocated to host growth and reproduction.

Here, we test this expectation by investigating the metabolic consequences of a defensive bacterial symbiont Hamiltonella defensa, which protects its pea aphid host from parasitic wasps likely via phage-encoded toxins. Hamiltonella is auxotrophic for several of the ten essential amino acids (EAAs) that are overproduced by the obligate bacterial symbiont Buchnera aphidicola in the aphid. Comparisons between the metabolome of aphids bearing and lacking Hamiltonella revealed differences in B-vitamins, TCA cycle metabolites, and EAA biosynthetic intermediates. These metabolic effects are recapitulated by metabolic models. Specifically, expanding the two-member model of the aphid host and Buchnera to a three-member model in which Hamiltonella consumes Buchnera-derived EAAs reduces net protein growth of the aphid by up to 20%. These metabolic interactions likely contribute to the negative impact of Hamiltonella on aphid growth and reproduction, but may also impair parasite development by depleting host pools of essential nutrients. In other words, our data suggest that nutrient consumption by defensive symbionts may have complex fitness consequences for the host: although costly to host growth and reproduction, it may contribute to host protection against natural enemies.

# Identification of host factors involved in Clostridium difficile TcdB induced cytotoxicity

Yingxue Li[1,2], Yutian Ren[1, 2], Elvis Hungchi Cheung[1], Chih-Jung Kuo[2], Yung-Fu Chang[2], Linfeng Huang[1]

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**Background**: C. difficile infection (CDI) is the leading cause of antibiotic-associated intestinal disease. Antibiotic treatment increases patients' susceptibility to CDI by disrupting normal gut microbiota. The clinical outcomes of CDI can range from asymptomatic carrier status to diarrhea and pseudomembranous colitis. The main virulent factors of C. difficile are toxins TcdA and TcdB, and CDI is often regarded as a toxin-mediated disease. Toxins cause cytopathic effects characterized by 'cell rounding' and cytotoxic effects including induction of different types of cell death and activation of inflammasome, resulting in destruction of the intestinal epithelium barrier. Nevertheless, as a global healthcare problem, the mechanism mediated by toxins remains unclear. Therefore, in this study, we aims to unravel the network of host genes involved in toxins' cytotoxic effects using our home made library screen system. In addition, targeting host genes necessary for toxin-induced cell death could be a new strategy for treating C. difficile infections.

**Methods**: To systemically investigate host genes involved in C. difficile toxins' cytotoxic functions, we carried a transcriptome-wide RNAi screen using a unique RNAi technology. Cell line based caspase activity and cell viability assay systems for testing toxins' effects have been explored in Caco-2 cell line with purified recombinant TcdB. Chemically synthesized siRNAs were used to validate screened out candidates. The top hit gene, HMGB1, was further investigated by a small HMGB1 inhibitor called glycyrrhizic acid. We first tested its protection from TcdB induced damage on an experimental cell line, and then performed mice colon ligated loop and C.difficile infection experiments.

**Results**: Seven candidate genes involved in TcdB-induced cell death have been screened out and validated. As the top hit in the screening, knockdown of HMGB1 gene expression rescued TcdB induced cell death and reduced caspase 3/7 activation, demonstrating the important role of HMGB1 in the interaction between TcdB and host cells. Glycyrrhizic acid, as a known inhibitor of HMGB1, alleviated TcdB induced cytotoxicity and reduced caspase activation compared with groups pretreated with control buffer. Also, glycyrrhizic acid pretreatment delayed the onset of TcdB-induced cell rounding. In mice ligated loop experiment, glycyrrhizic acid contributed to protection against TcdB-mediated disruption of colon epithelium. Furthermore, glycyrrhizic acid treatment potentially increased mice survival rates after challenge with C.difficile spores as compared to control groups.

**Conclusion**: Our screening result supports our home-made, cell-specific pro-siRNA library are qualified for screening investigation. This study presents further evidence that HMGB1 plays a part in TcdB induced cytotoxicity, which provide another approach to the therapeutics for C. difficile infection.

# Investigating hyphal fusion as a mechanism for horizontal transmission of fungal endosymbiotic bacteria

**Thomas Chu, Teresa Pawlowska, and Chase Mayers

Plant Pathology & Plant Microbe Biology, Cornell University

Heritable endobacteria of fungi challenge the traditional models of bacterial evolution developed from observations of insect endosymbionts. Candidatus Moeniiplasma glomeromycotorum (CaMg) is a heritable endosymbiont of arbuscular mycorrhizal fungi (AMF). Though horizontal transmission of CaMg has never been observed in AMF, it has been inferred through comparative phylogenetic analyses. These analyses suggest that CaMg transmission is predominantly vertical, in common with insect endosymbionts. However, there is also evidence for occasional host-switching events and bacterial recombination, which is indicative of horizontal transmission. If CaMg is transferred horizontally, even occasionally, this could help explain how CaMg combats the genetic degradation suffered by the strictly vertically-transferred symbionts of insects. One possible mechanism for such horizontal transmission is the hyphal fusion (anastomosis) of germ tubes from different AMF spores, through which CaMg populations could travel and intermix. This project will yield important insights into a possible mechanism for the cryptic horizontal transmission of CaMg in nature.

# Regulation of post-mating immune response in female Drosophila melanogaster

Kathleen E. Gordon[1], Mariana F. Wolfner[1], Brian P. Lazzaro[2]

[1]Department of Molecular Biology and Genetics, Cornell University, and [2]Department of Entomology, Department of Ecology and Evolutionary Biology, and Cornell Institute of Host-Microbe Interactions and Disease, Cornell University

In *D. melanogaster* and many other species, female reproductive investment comes at a cost to immunity and resistance to infection. Within hours of mating, *D. melanogaster* females become more susceptible to bacterial infection. Previous studies showed that females were less resistant to bacterial infection at 2.5 and 26.5 hours after mating, but did not test whether a mated female would eventually recover virgin levels of immunity. We tested whether mated females could recover virgin levels of immunity when infected at 2, 4, 7, or 10 days after mating. We observed no recovery of immune capacity in mated females over time. We conclude that mating has a permanent suppressive effect on the female immune system. Knowing that females mate multiply, we tested whether a second mating further affected immune performance. We hypothesized that females who mated twice might become more susceptible to infection than females mated once. Instead, we found that females mated either once or twice before infection survived at equal proportions and both significantly lower than virgin females. This indicates that effects of a single mating are sufficient to suppress the immune response and a second mating does not compound the effect. During mating, the male transfers seminal fluid proteins, like Sex Peptide, that change female physiology and behavior. Sex Peptide induces the female to produce Juvenile Hormone (JH), which promotes egg development. We and others have previously shown that JH is immunosuppressive and decreases resistance to bacterial infection. We thus hypothesize that JH signaling might control resource allocation between reproduction and immunity. Future experiments will seek to understand whether JH titers in mated and virgin females correlate with our understanding of the dynamics of the post-mating immune response and whether limiting investment in reproduction can improve immune capacity.

## Cellular heterogeneity underlying poly-functional Drosophila fat body tissue

Vanika Gupta[1], Brian P. Lazzaro[2]

[1]Department of Entomology, Cornell University, and [2]Department of Entomology, Department of Ecology and Evolutionary Biology, and Cornell Institute of Host-Microbe Interactions and Disease, Cornell University

The insect fat body is a multifunctional tissue that can serve as a generic model for how poly-functional organs achieve diversified tasks, including management of immune response to infection. Fat body functions span those of at least three vertebrate organs: immune system, adipose tissue, and liver. The fat body is the primary systemic immune organ in insects, but also serves as the metabolic control organ responsible for storage and release of lipids and other nutrients and produces most of the yolk used to provision developing eggs. This is analogous to vertebrate adipose tissue, which is a cellularly heterogeneous tissue that stores lipids and also produces cytokines and inflammatory reactions in response to infection. We hypothesize that cellular heterogeneity in the fat body allows subsets of cells to specialize in each function, collectively resulting in a tissue with highly varied capabilities. We further hypothesize that stimuli such as bacterial infection alter either the number or identity of sub-functionalized cells, resulting in quantitatively dynamic responses at the tissue level. We are using single-cell RNA sequencing (scRNAseq) on the 10X platform to test the hypothesis of cellular heterogeneity in Drosophila melanogaster (fruit fly) fat body. Using tissue-specific drivers, we have tagged adult fat body cells with GFP which are then sorted using FACS to obtain well-dissociated fat body cells. We are using flies which remain either challenged or unchallenged with a gram-negative bacteria Providencia rettgeri, both while actively engaged in egg development and in the absence of reproductive investment. We seek to identify cells subpopulations within the fat body which are responsible for specific functions. We further want to identify plasticity in the fat body tissue under different physiological environments. We will use the data to understand how poly-functional tissues balance competing physiological functions, providing mechanistic understanding for the classical life history tradeoff between immunity and reproduction.

# Toxoplasmal meningoencephalitis in a 14 week-old shelter cat with presumed feline infectious peritonitis

M. Erin Henry[*1,2], Nicole M. André[*3], Andrew D. Miller[4], Emma Davies[5], Manigandan Lejeune[1], Elizabeth A. Berliner[1,2], and Gary R. Whittaker[3,6]

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***Case Series Summary***

This report describes a cat that was initially surrendered at 6 weeks of age to an animal shelter along with her mother and four littermates. The cat had no significant abnormalities and was adopted, along with a sibling/littermate. At 10 weeks of age the cat was returned to the shelter, at which time the cat displayed upper respiratory signs and was underweight with an elevated temperature. The cat was placed into foster care for support but continued to have waxing and waning fever and lethargy, and to be underweight on recheck appointments. At 14 weeks of age. bloodwork revealed elevated globulins, elevated liver enzymes and elevated bilirubin, and clinical signs were considered to be consistent with feline infectious peritonitis (FIP). Other differentials included systemic toxoplasmosis or a portosystemic shunt. The cat was euthanized based on poor prognosis. Despite the presumptive diagnosis, no histologic lesions consistent with FIP were present in tissues examined, and molecular testing for feline coronavirus gave negative results. However, histopathology of the brain revealed disseminated necrotizing meningoencephalitis with bradyzoites and tachyzoites consistent with Toxoplasma gondii, which was confirmed by molecular testing.

***Relevance and Novel Information***

This case report describes the presentation of a 14 week-old FIP-suspect cat with toxoplasmal meningoencephalitis, with genotyping of the parasite from brain tissue.

## Identification of insect genes involved in baculovirus AcMNPV entry into insect cells

Jeffrey J. Hodgson[1], Nicolas Buchon[2], and Gary W. Blissard[1]

[1]Boyce Thompson Institute at Cornell University, and [2]Department of Entomology, Cornell University

The baculovirus *Autographa californica* nucleopolyhedrovirus (AcMNPV) is a model enveloped DNA virus that infects and replicates in lepidopteran insect cells, and can efficiently enter a wide variety of non-host cells. Budded virions of AcMNPV enter cells by endocytosis and traffic to the nucleus where the virus initiates gene expression and genome replication. While trafficking of nucleocapsids by actin propulsion has been studied in detail, other important components of trafficking during entry remain poorly understood. We used a recombinant AcMNPV virus expressing an EGFP reporter in combination with an RNAi screen in Drosophila DL1 cells, to identify host proteins involved in AcMNPV entry. The RNAi screen targeted 86 genes involved in vesicular trafficking, including genes coding for VPS and ESCRT proteins, Rab GTPases, Exocyst proteins, and Clathrin adaptor proteins. We identified 24 genes required for efficient virus entry and reporter expression, and 4 genes that appear to restrict virus entry.

## Identification of insect genes involved in baculovirus AcMNPV entry into insect cells

** Brianna Johnson, Shannon Murphy, Tobias Dörr

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The cell wall is a structure found in all free-living bacteria that provides shape and stability to the organism. Maintenance of this structure is vital to survival, yet it is incompletely understood. This project seeks to identify the role of a gene, aroK, in cell wall maintenance. AroK is known to serve as a synthesizer of aromatic amino acids in Gram-negative bacteria. However, aroK has unexpectedly been implicated in cell wall homeostasis. Loss of aroK in Vibrio cholerae increases sensitivity to mecillinam, a beta lactam antibiotic that inhibits cell wall synthesis. In order to untangle the two potential functions of aroK, we will construct mutations to determine which residues are responsible for the two suspected functions. Interestingly, in Escherichia coli, another Gram-negative pathogen, an aroK deletion shows the converse phenotype when plated on mecillinam; it confers resistance. To distinguish between aroK functionality in V. cholerae and E. coli, we will create complementation mutants with gene homologs from the two species and identify genetic and environmental factors that favor aroK expression using transcriptional reporters. Understanding the role of aroK in cell wall homeostasis is a small piece of the larger puzzle that is the bacterial cell wall. Given the role of the cell wall as a powerful antibiotic target, gaining a more complete picture of cell wall maintenance will lead to a better understanding of how to combat multidrug resistance in human pathogens.

# The effect of gut microbiota on avian developmental temperature priming

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Gut microbiota may play a role in temperature priming during development by mobilizing and diverting energy to tissues involved in thermoregulation. In mammals, gut microbiota increases thermogenic capacity by promoting the browning of white adipose tissue. However, the effects of gut microbiota on the thermogenic capacity of birds have yet to be studied. In this study, we raised wild Tree Swallow (Tachycineta bicolor) chicks for 12 days at two temperatures (31, 35°C) under controlled laboratory conditions and gave the chicks either an antibiotic orally on days 6-9 to dysregulate their gut microbiomes or a water control. We measured lipid metabolism (plasma beta hydroxybutyrate levels) and thermogenic capacity (PEC mass and cold-induced metabolic rate). We predict that under cold 31°C conditions, birds treated with antibiotics will exhibit decreased metabolic rates exhibited by decreased plasma BOH levels, and decreased thermogenic capacity exhibited by decreased PEC mass and cold-induced metabolic rate. Investigating the mechanisms behind developmental temperature priming is significant in light of the increased prevalence of climate change. Our findings may provide novel conclusions to aid conservation efforts toward declining species.

# Sex, seminal fluids, and swarms: determinants of harmonic convergence in Aedes aegypti and Anopheles gambiae mosquitoes

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Prior to mating, mosquito males and females pair up to harmonize their flight tones in a phenomenon known as harmonic convergence (HC). This behavior is an important indicator of reproductive fitness and as such, understanding the behavioral and physiological determinants of HC is biologically interesting as well as critical for optimizing mass rearing for genetically modified or Wolbachia-based mosquito control strategies. We recorded the pre-copulatory acoustic interactions of the arbovirus vector Aedes aegypti to investigate how mating status and male accessory gland (MAG) fluids affected the likelihood of HC. We found that mating status and MAG fluids influence the likelihood of HC, as both mated and MAG extract-injected female Ae. aegypti mosquitoes converged less frequently with males than their virgin female counterparts. We further demonstrated that this behavioral shift was dependent upon female antennal function. In parallel studies in the malaria vector Anopheles gambiae, we tested whether depletion of the hormone 20-hydroxyecdysone (20E) in MAGs or swarming behavior regulate HC. We found that 20E-depleted male and wild type female pairs harmonized less frequently than homogeneous wild type pairs, showing that MAG hormones can influence acoustic interactions. In a further study focusing on swarming behavior in An. gambiae, we found that HC is strongly linked to swarming periods, when male flight tones experience a transient increase in frequency. Together, these studies show that a variety of physiological and behavioral inputs combine to determine HC outcomes and suggest that these factors should be considered when assessing mosquito reproductive fitness.

# Assessing the error rate of mammalian orthoreovirus across strains

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The introduction of random mutations during the copying of an organism's genetic material is key to its evolution. Viruses have high mutation rates in comparison to cellular organisms, and this aspect of their life cycle helps to generate a genetically diverse progeny. Here, we used high-throughput sequencing to compare the mutation rate of two strains of mammalian orthoreovirus, a dsRNA virus with a segmented genome. We hypothesize that the strain Type 3 Dearing (T3D) will have a higher mutation rate than Type 1 Lang (T1L) because of a known temperature-sensitive folding defect in the viral protein mu2. The mu2 protein is incorporated into viral core particles and is believed to act as a polymerase co-factor, and so has the potential to affect polymerase fidelity. To assess differences in mutation rate between strains, we compared the average level of genetic diversity in the genomic dsRNA and the viral mRNAs resulting from one round of replication. Genomic dsRNA was recovered from purified virus particles and viral mRNA from infected cells, and selected reovirus gene segments were amplified by RT-PCR. The resulting cDNAs were subjected to deep sequencing. We have found that as expected, the mRNA samples show a higher level of genetic variability than the dsRNA, indicating that errors are introduced as viral mRNAs are transcribed. Furthermore, initial data suggests that T3D has a slightly larger increase in diversity from one round of replication than T1L at an incubation temperature of 35 □C. We are currently awaiting sequencing data that will allow us to compare diversity of the T3D and T1L RNAs at a higher incubation temperature of 39 □C. Here we expect T3D's mutation rate to be significantly higher than that of T1L, given that its mu2 protein is temperature-sensitive.

# Transmission dynamics of highly pathogenic avian influenza among multiple waterfowl species and poultry

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Annual outbreaks of highly pathogenic avian influenza (HPAI) in wild birds and poultry have caused concern regarding the increased likelihood of an epidemic comparable to the 2016/17 spread of H5N8 across Europe, affecting wild bird populations, the poultry industry and posing a risk to human health. The objective of this study was to analyse the transmission dynamics of HPAI among migratory and non-migratory waterfowl and poultry, using as model systems three wetland regions in Croatia previously assessed as high, medium and low risk zones for HPAI. The choice of these study locations was motivated by the occurrence of H5N1 and H5N8 outbreaks in Croatia in 2005 and 2016/17, respectively. Under a simplifying assumption of homogenous mixing, we developed a deterministic SIR compartmental model that can account for both the direct and indirect modes of pathogen transmission. The model includes a compartment for backyard poultry and a compartment for Mute Swans (Cygnus olor), a sentinel species for HPAI outbreaks. Additionally, we modeled Mallards (Anas platyrhynchos), an asymptomatic carrier of HPAI, represented with two compartments corresponding to the migratory and non-migratory (resident) birds. The uncertainty and variability in model predictions will be analyzed using Monte Carlo simulations. The preliminary results support that introduction of HPAI via migratory waterfowl into a disease-free area may result in large mortalities in susceptible avian species and that there is a risk of an outbreak in the subsequent year from subclinically infected resident mallards.

# Immune response effects of sex peptide receptor expression in female *Drosophila*

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Previous studies have shown that reproductive efforts in female Drosophila melanogaster can weaken their immune response. Mated females are more likely to die from infection, have higher bacterial loads, and produce fewer AMPs than virgins. During mating, male flies transfer seminal fluid proteins that have dramatic effects on female behavior and physiology. The seminal fluid Sex Peptide induces the production of Juvenile Hormone, which is suppressive to the immune response. I hypothesize that the receptor of SP (SPR) is necessary to induce suppression of the female post-mating immune response. We used RNAi to perform a full body knockdown of expression of SPR in both virgin and mated females. Virgin and mated SPR knockdown and control females were challenged with a systemic infection via pinprick with the gram-negative bacteria, Providencia rettgeri and survivorship was recorded for five days. Preliminary data from three rounds of infection have shown a clear mated-virgin difference between experimental groups of flies, suggesting that the SPR is not required for post-mating immune suppression. However genetic control groups containing a balancer chromosome have shown an overall decrease in survivorship when compared to the knockdown experimental groups. Additionally, mated females are dying from infection more quickly and in higher quantities than virgins. To control for this discrepancy, future rounds of infection will be performed with a different genetic control, by crossing the genetic background (KK) of the RNAi line to our whole body GAL4 line. Future experiments will assess the immune response of SPR knockdown females by quantifying the production of antimicrobial peptides and measuring bacterial loads.

# Potential off target effect of GSK219 implicated in TLR7 and TLR9 signaling pathways in macrophages

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Toll-like receptors initiate host protective as well as pathological inflammation as an immunological response to various foreign stimuli. Dysregulation of TLR signaling and associated cytokine secretion in macrophages promotes chronic inflammatory and autoimmune disease. Recently, transient receptor potential vanillin (TRPV) 4, as well as other TRP channels, have been implicated in TLR regulation. TRPV4 is a non-selective Ca2+ permeable cation channel activated by mechanical and chemical stimuli. During previous studies, we discovered discordance between the TLR signaling in the presence of the TRPV4 inhibitor GSK 2193874 (GSK219, $C_{37}H_{38}BrF_3N_4O$) and in TRPV4 knockout mice. GSK219 treatment reduced TLR signaling in macrophages derived from both wild type and TrpV4 deficient mice. These data support the idea that GSK219 has an off target effect, unrelated to TrpV4.

While this non-TRPV4 target of GSK219 is unknown, our data suggest that GSK219 may target a secondary mediator in the cytokine signaling pathway of TLR7 and TLR9. Treating TrpV4-deficient murine bone marrow-derived macrophages with GSK219 decreased IFN and TNFα secretion levels from both TLR 7 and 9. UNC93b1 is a critical chaperone required for the correct folding and trafficking of both TLR7 and TLR9. My studies show that in the DMSO control treatment of both WT and TrpV4 deficient macrophages UNC93b1 is present as a cleaved, 28kDa fragment. However, treatment of both WT and TRPV4 deficient macrophages with GSK219 failed to generate the 28kDa fragment of UNC93b1. In my future studies, I plan to identify non-TRPV4 GSK219 protein targets using chemically modified GSK219 to capture interacting proteins. My studies may lead to novel mechanisms for a well-studied drug and suggest we may need to reinterpret findings implicating TRPV4 in processes and diseases that have been made using GSK219. Overall, my studies are important for understanding the mechanism of TLR-induced cytokine regulation in macrophages because of the role these receptors play in host defense and in the development of autoimmune disease.

# Understanding morphology and phylogeny in Epulopiscium spp. C and J morphotypes

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*Epulopiscium* spp., and related bacteria known as 'epulos', are large, highly polyploid heterotrophs that are morphologically diverse. Additionally, epulos are gut symbionts of surgeonfish and likely influence the digestion of their surgeonfish hosts. Certain surgeonfish host distinct groups of these giant bacteria, and while some *Epulopiscium* spp. are well-characterized (e.g., the A and B morphotypes), there are several morphologies that we can better understand. For instance, the Cs and Js are fascinating because of their sporulation abilities, reproductive strategies, and morphological diversity; however, the C and J morphotypes are challenging to collect and, thus, little data has been generated on their morphology or genetic information. This research seeks to better understand the diversity of the C and J morphotypes found in surgeonfish hosts *Naso lituratus* and *Naso unicornis*. The objectives of this research are twofold: (1) to characterize populations of the C and J morphotypes from *N. lituratus* and *N. unicornis* samples from Australia through 16S rRNA gene survey data, and (2) to further investigate the distribution of these morphotypes (and phylogenetic subtypes) in surgeonfish using 16S rRNA probes. We have found that *N. lituratus* and *N. unicornis* host different epulo populations. Additionally, epulo phylogeny is determined by morphotype, their host, and the location the host was collected. Overall, the knowledge obtained through this project will help broaden our understanding of epulo morphologies among related fish, to understand the global distribution of epulos, and to provide insight into complex host-microbe systems.

# Human matriptase proteolytically cleaves H7N9 hemagglutinin and facilitates the activation of influenza A/Shanghai/2/2013 in cell culture

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Influenza A is a zoonotic disease that infects millions of people each year including hundred thousands of casualties and which can cause devastating pandemics. There are several subtypes of influenza A viruses (IAVs) but only a few seem to be able to adapt to humans and to cause disease. In 2013 a H7N9 IAV subtype emerged in China that does not cause clinical symptoms in its chicken host but leads to severe infections when transmitted into humans. Since 2013 there have been 6 epidemic waves with 1567 laboratory-confirmed human infections and 615 deaths. A crucial feature contributing to the virulence of IAVs is the activation of their hemagglutinin (HA) fusion protein by host proteases that leads to the internalization of the virus into the host cell and subsequent virus propagation. H7N9 HA is proteolytically cleaved by the TMPRSS2 proteases in mice but little is known about its activation in humans. Here we show that human matriptase, a trypsin-like serine protease that is involved in the activation of a number of other IAVs, is able to cleave H7N9 HA. Cleavage of H7N9 HA expressed in cell culture results in fusogenic HA and syncytia formation. We observed in infection studies with viral pseudo particles carrying H7N9 HA a high infectivity of cells comparable to the positive control. Finally, human matriptase also activated H7N9 live virus which resulted in high infectivity. Our data demonstrates that human matriptase is a likely candidate protease which promotes H7N9 propagation in human infections.

# Using RNA in situ hybridization as a more highly sensitive method for pathology-based diagnosis of feline infectious peritonitis as compared to immunohistochemistry

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Feline infectious peritonitis (FIP) is a fatal disease in cats caused by a mutated form of feline coronavirus. Two serotypes of FIPV exist: type 1 viruses constitute 85% to 95% of FIP cases, while type 2 viruses are responsible for the remaining 5% to 15% of infections. Immunohistochemistry (IHC) currently serves as the gold standard for diagnosis of FIPV within tissue. However, IHC has some limitations, such as relatively low specificity and a wide variation in sensitivity. In situ hybridization (ISH) targeting viral RNA has an established foothold in infectious disease diagnostics and presents a potentially improved method for detection of FIPV. This study sought to evaluate the efficacy of RNA ISH probes targeted to FIPV, as compared to IHC using monoclonal antibody FIP 3-70. Formalin-fixed paraffin-embedded tissues from FIP-positive cats were used for ISH, with RNA presence determined colormetrically. ISH tissue slides were then compared to their IHC counterparts, with efficacy determined based on metrics including staining intensity and abundance of stained cells. Positive ISH staining on tissue was found to be more intense and abundant than for IHC—suggesting that ISH serves as a more sensitive method for the detection of FIPV in comparison to IHC.

# Exploring protein-protein interactions in the Mycobacterial periplasm

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The current genetic approach to characterize protein-protein interactions in Mycobacteria uses a split dihydrofolate reductase protein system which confers trimethoprim resistance when cytosolic bait and prey proteins interact. Unfortunately, this methodology cannot be applied to study secreted proteins; the current approaches to study secreted proteins in the Mycobacterial periplasm are limited to crosslinking, mass spectroscopy, and pull-down assays. Therefore, we are developing an alternative genetic approach to characterize protein-protein interactions in the periplasm of Mycobacteria using a split β-lactamase system. Mycobacteria express a native β-lactamase and are intrinsically resistant to β-lactam antibiotics. We have now constructed a Mycobacterial strain lacking the β-lactamase enzyme (M. smegmatis ΔBlaS1) and confirmed this strain is sensitive to β-lactam antibiotics. We are now genetically engineering fusions of the two separated domains of an Ambler class A β-lactamase to bait and prey proteins. These fusions also contain native secretion signal sequences necessary for these proteins to be exported to and interact in the Mycobacterial periplasm. We anticipate that co-expressing these proteins in M. smegmatis ΔBlaS1 will produce a functional β-lactamase enzyme which will confers resistance to β-lactam antibiotics.

# What Factors Structure the Bacterial Communities in Turtle Ants?

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Ants are everywhere - they thrive in forests, fields, deserts, and cities. In addition, many ants have symbiotic interactions with other organisms. One of the symbiotic interactions in which ants engage are with mutualistic gut bacteria. Turtle ants (genus Cephalotes), have dense bacterial communities in their digestive tracts that contribute nitrogen to the host ant. The goal of this project is to understand the factors structuring the diversity of bacteria associated with turtle ants. Our hypotheses are 1) turtle ants acquire their bacterial communities from their local environment; 2) bacteria can be transferred between coexisting species of turtle ants; and 3) the bacteria are stable and co-evolve with their hosts. For the experimental design, we sampled 68 turtle ants from 12 species from several Neotropic countries. Ant species living alone in a tree were sampled, while also ant species coexisting in the same tree, and this sampling strategy was duplicated for all the species included. To test our hypotheses, we extracted total DNA and sequenced mtDNA from the turtle ants and amplicon sequenced bacterial 16S rRNA. We reconstructed the phylogeny of the turtle ants and for the bacterial data bioinformatics was done using QIIME2 and R. Our results show that local environment and host phylogeny structure gut bacterial communities, but we did not find evidence for horizontal sharing between species. These results provide a better understanding of the association between bacteria and ants, showing that turtle ants have a stable microbiome that is not impacted by host species interactions.

# Establishing Essentiality of Lytic Transglycosylases in Vibrio cholera

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The bacterial cell wall is a structure required by almost all bacteria in order to survive. A key component of the cell wall is the peptidoglycan layer, which consists of cross-linked repeating molecules that provide structural support to the bacteria. In order for a cell to grow, move, infect, and divide, different enzymes must be able to act on this dynamic peptidoglycan layer. One class of such enzymes is called lytic transglycosylases (LTGs). Though they vary in structure and size, LTGs serve similar biochemical functions. The purpose of this project is to understand which LTGs are essential to life and which are redundant under various conditions in Vibrio cholerae. To do so, we use a key, cutting-edge method called multiplex genome editing by natural transformation (MuGENT), which allows us to delete multiple LTG genes at a time to more efficiently screen for essential combinations.

# Leptospirosis in Horses: Experimental infection of Leptospira interrogans serovar Bratislava in horses, immunological analysis and a better understanding of serological response.

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Little information is available about experimental induction of leptospirosis in horses and the pathogenicity of Leptospira interrogans serovar Bratislava in this host. This strains has been postulated to be a host-adapted strain in horses, although it has never been isolated before and genetically sequenced. In this work, we determined the serologic, clinical, pathologic and hematologic responses of horses to L. interrogans serovar Bratislava strain PigK151 in ten female foals (7-8 months old) seronegative for Leptospira (MAT titre ≤ 50). This study was composed by 2 groups: Control (4 animals) and Challenged (6 animals). The challenged group was challenged with 1 x 109 Leptospires, divided equally between topical ocular and intraperitoneal injections. Temperature, blood and urine samples were collected at intervals (day -1, 0, 3, 5, 7, and then weekly) until euthanasia 30 days after challenge. No pyrexia was noted during the challenge. PCR and RT-PCR were negative from all plasma and urine samples. We were not able to recover Leptospires from either plasma or urine culture. All 6 challenged foals developed antibodies against Leptospira serovar Bratislava as determined by microscopic agglutination test (MAT) beginning on day 3 until the last day of the study. Cross-reacting titers to heterologous strains developed but declined earlier than the titers to the challenge strain. This is the first report of a challenge in horses using L. interrogans serovar Bratislava and thus provides some important insights about its pathogenicity and the significance of agglutination antibodies found in many equine leptospirosis cases. L. interrogans serovar Bratislava did not cause disease in challenged horses. The gold-standard serologic test (MAT) allowed the recognition of the infecting serovar but it also provided cross-reactive results, which are explained by the genomic analysis. Around 97% of the L. interrogans serovar Bratislava strain PigK151 genome is covered by the six L. interrogans serovars used in the Pangenomic analysis. Results from the detection methods (PCR, RT-PCR and culture) showed that infected foals cleared the bacteria within 3 days post challenge from blood and urine; thus, this strain seemed unable to colonize the host. Based on the findings presented in this work, we suggest that L. interrogans serovar Bratislava is not pathogenic for horses and it is not a host-adapted strain, although these results might have varied if another strain from the same serovar had been used instead.