Zweig

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A report from the Harry M. Zweig Memorial Fund for Equine Research at the College of Veterinary Medicine at Cornell University

What Horses Can Tell Us Now about the Human Influenza Pandemic to Come

In 1973, Zweig-funded researcher, Dorothy Holmes, DVM, PhD, obtained samples of equine influenza virus from an outbreak at a racetrack in Florida. She named it A/equine/Cornell/74 and for many years studied its properties and developed nasal spray vaccines. Then suddenly, as influenza viruses alone can do, the H7 serotype disappeared from the environment while a new one emerged; designated as the H3 serotype, it's what causes equine influenza today.

Fifteen years later, another Zweig-funded researcher, Judith Appleton, PhD, studied the properties of this and related H7 viruses. Although the reagents she used (virus stocks, monoclonal antibodies, and other by-products of the research) seemed no longer useful, Appleton (now the Alfred H. Caspary Professor of Immunology) nevertheless stored them in liquid nitrogen in a freezer at the Baker Institute for Animal Health, where this live archive remained until virologist Gary Whittaker, PhD, came long.

And a good thing, too, because Whittaker is among a host of scientists—from the U.S. Centers for Disease Control and Prevention and elsewhere in the human medicine community—who are betting that the dreaded worldwide influenza pandemic to come will be an H7 serotype.

For H7, it seems, outperforms all other serotypes in its lethal powers.

"Influenza H7 is unique in its capability to invade not only the lungs but other parts of the host's body, including the brain, and this is why it's so dangerous," explains Whittaker, an associate professor of virology in the Department of Microbiology and Immunology.

Here at Cornell, Whittaker has found combined resources to study this virus unavailable at any other single insti-

Alexis Wenski-Roberts

Whittaker believes that the next influenza pandemic is a lot more likely to be an H7 serotype rather than an H5, which has been circulating in the human population for 8 to 10 years now and never acquired the ability to take hold. He wants to understand what happened in horses in the 1950s to get ready for when H7 returns to infect horses and perhaps, this time, humans, too.

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Gary Whittaker

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tution in the world. First there is the invaluable store of equine H7 reagents and the Zweig funds that have allowed him to scrutinize them. Too, there are the facilities and staff at the Cornell Center for Advanced Computing (CAC) who help analyze the molecular structure of the H7 virus.

Now into the second year of a two-year study titled "Emergence and Establishment of Influenza in Equines," Whittaker is using a three-dimensional model of the molecular structure of the H7 influenza virus coat protein (hemagglutinin or HA, for short) generated for him at the CAC and derived from the RNA of Holmes' and Appleton's virus to see why H7 has the capacity to be so distinctly deadly.

What he's zeroing in on can be seen in figure 3.

"It's that stretch of amino acids tinted pink on the model that has me fascinated," Whittaker says. "No other influenza ever in any species has this sequence apart from equine H7 virus."

It's the exposure of this sequence when the HA is clipped that Whittaker believes controls the virus's ability to invade the tissues of many regions of the body rather than being confined to just the lungs as the equine H3 serotype (as well as most human influenza) currently is. The goal of his research is to understand exactly what goes on when this happens.

There's another, equally compelling reason, for Whittaker's focus on the surface proteins of the equine influenza virus.

"Mutations occurring at the entry site are also very often what allows host switching, that is the virus's ability to jump from one species to another—say from birds to horses or from birds to people," Whittaker points out. "So the larger question I'm pursuing is how these viruses change themselves in order to move between species."

The H7 serotype—the first documented equine influenza virus—was isolated from an outbreak of respiratory disease in Czechoslovakia in 1956 that spread around the world. Subsequently it was shown that the virus was a very highly pathogenic H7 virus in chickens or birds that moved into horses causing the outbreak. While H7 is no longer seen among horses, it is prevalent in birds today, a fact that gives public health officials great pause.

Whittaker thinks there's a significant probability that the characteristics of H7 as it infected horses in the 1950s would be similar to the characteristics of virus behavior when the next virus pandemic occurs in horses and in humans; hence the importance of studying equine H7, a virus that's never been studied in any kind of molecular detail before.

As a result of Whittaker's preliminary findings generated with the Zweig monies, he has been given a $3,000,000 seven-year award from the National Institutes of Health (NIH) to expand his studies of equine influenza H7 HA as co-investigator in NIH's Centers for Excellence for Influenza Research and Surveillance (CEIRS) located at the University of Rochester, in Rochester, N.Y. NIH is spending approximately $23 million per year for seven years on these centers. Their goal is to provide the federal government with research-based information upon which to build public health strategies for controlling and lessening the impact of seasonal influenza as well as an influenza pandemic.

"The horse," Whittaker says, "can give incredibly valuable information for our global understanding of influenza." ■

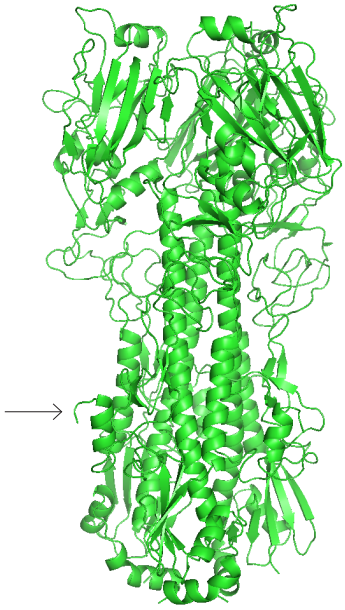

Figure 1. A computer-generated three-dimensional model of the molecular structure of the H7 influenza virus coat protein (hemagglutinin or HA, for short), the molecule responsible for enabling the influenza virus to recognize the host's cell and invade it. The names of all the amino acids that make up this protein are listed across the top of Whittaker's computer screen above this model. He can put his cursor on a group of amino acids of interest and their location will be highlighted on the model. Note the single strand hanging loose on the left side about one-third of the way from the bottom.

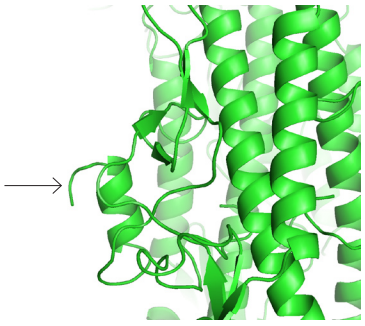

Figure 2. A closer view of this region allows Whittaker to identify it as the "cleavage site" where a stretch of amino acids is clipped by proteases released from cells in the lining of the horses' lungs. If the HA isn't cleaved, the virus remains totally non-infectious because can't invade the host's cells.

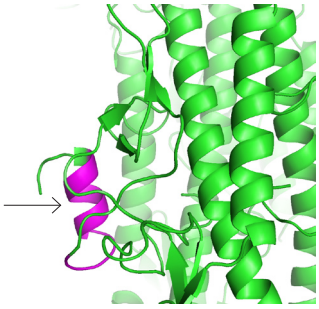

Figure 3. The spiral strand in pink is adjacent to a polybasic stretch of amino acids that become exposed at the cleavage site, making it possible for a respiratory virus to infect any tissue in the body, hence becoming a systemic lethal infection.

"With this model we can look inside the structure of the virus and make a really good prediction of what's going on. Without it we'd be shooting completely in the dark."
Gary Whittaker

Behind the Scenes—Graduate Student Laura Goodman Solves a Riddle of Equine Herpesvirus Type 1

It's been a long-standing question in stables around the world: Why does equine herpesvirus type 1 (EHV-1)—a highly contagious viral infection—cause mild flu-like symptoms with a speedy recovery during one outbreak, while in another a neurological disease so severe that nearly one-third of the horses that develop it end up dead?

"There had been some evidence that a genetic component—a difference in the virus strain itself—was involved but that had never been proven," says Laura Goodman, PhD, who chose to investigate this further as a project for her doctoral dissertation. To do so Goodman spent three years conducting intensive studies in molecular virology as a member of the research group of Klaus Osterrieder, DVM, DVM Habilitation [equivalent to PhD]. Osterrieder, a professor of virology in the Department of Microbiology and Immunology, receives Zweig support for his work in studying the many aspects of virulence and vaccine efficiency of EHV-1.

In the end Goodman showed that a single amino acid variation in the DNA copying enzyme of EHV-1 is enough to create a different virus type, one much more capable of causing neurological disorders. She presented her findings to the scientific community as lead author of the paper "A Point Mutation in a Herpesvirus Polymerase Determines Neuropathogenicity." It appeared in the November 2007 issue of *PLoS Pathogens*, published by the Public Library of Science.

"There are apparently two distinct pathotypes of the EHV-1 out there, and one is more likely than the other to cause the neurological disease. This study provides the ultimate proof," says Klaus Osterrieder, the paper's senior author and an authority on viral pathogenesis (how viruses cause disease in animals) and particularly the role that genetics might play in the mechanisms of disease.

Goodman says of finding and studying the D752 variant among the 150,000 nucleotides residing in the virus's 80 genes that the process was much like finding a needle in a haystack.

Alexis Wenski-Roberts

After solving one of the most puzzling questions about equine herpes, virus type 1, Laura Goodman received a PhD in the field of comparative biomedical sciences. She now works at the Baker Institute for Animal Health, and is shown here in the Parrish Lab, which overlooks the pastures where Cornell's research horses graze.

"As with herpesviruses of humans, EHV-1 contains all the genes that it needs for its own replication," Goodman explains. "In addition, many of its components interact with the horse's own genes, so it is very difficult but also very interesting to study."

> "There are apparently two distinct pathotypes of the EHV-1 out there and one is more likely than the other to cause the neurological disease. This study provides the ultimate proof."
>
> *Klaus Osterrieder*

Goodman first cloned the genome of the virus obtained from a mare that had both lost a fetus—another often-seen consequence of the EHV-1 infection—and developed neurological signs. Then she altered one amino acid in the viral enzyme known as DNA polymerase and that rendered the virus unable to cause neurological disease. The amino acid change reduced levels of the virus in the horse's bloodstream, and lower levels of the virus reached the central nervous system. The reduced replication and levels of virus in the blood may be why one form of the virus does not cause neurological disorders. The mutation also made the virus more susceptible to antiviral drugs.

However, Osterrieder issues a caution: "The two pathotypes replicate to similar levels in the horse's nose and spread to other horses with similar efficiency, so interventions should be equally rigorous for all infections."

Goodman's interest in how viruses cause disease began when she was an undergraduate at the University of Michigan, where she conducted research on the effects of global climate change on the spread of disease. She found that areas of the world where malaria can be transmitted are shifting; tropical diseases are appearing in places where they never were before.

"Recognizing that this was happening had a big effect on me," Goodman

Laura Goodman
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"Being on this project was a wonderful pairing of the two of us," Gillian Perkins says. While Perkins provided an expert assessment of neurological signs of horses with EHV-1, Goodman acclimated Perkins to working in a laboratory by collaborating together on creating a diagnostic test called quantitative real time PCR, which detects how much EHV-1 is present in a horse's blood, nasal secretions, and spinal fluid. This year, Perkins will continue her research in EHV-1 with a Zweig grant of her own.

recalls. "I began to ask myself why: Is it just an environmental component or are changes also taking place in the disease agent? Or in the vector?"

Goodman was keen to explore all three components: the epidemiology, the pathogen, and the vector, but settled on the pathogen for her PhD work. She came to the Vet college because of the rare opportunity to study the behavior of a virus in its primary host. Much of the time viruses must be studied in hosts other than their natural ones, such as using a mouse model for humans, but, as Goodman points out, a human virus in a mouse will behave differently than it will in a person. By contrast, EHV-1 is a virus that spreads rapidly from horse to horse and is of great economic consequence as healthy horses die in their prime and abortions can devastate breeding herds.

Goodman also found here a wealth of related resources, among them clones of horse chromosomes from the Equine Genomics Center and clinical consultation with large animal and neurological specialists.

"Most of them are now or have been recipients of Zweig grants, so it was a very supportive and coordinated effort from a large number of people," Goodman says. Cornell faculty members who participated in this study include Assistant Professor of Immunology Bettina Wagner, DVM, DVM Habilitation (equivalent to PhD); Assistant Professor of Biomedical Sciences Beth Buckles, DVM, PhD, and Tracy Stokol, PhD; an assistant professor in the Department of Population Medicine and Diagnostic Sciences.

Gillian Perkins, DVM, Dip ACVIM, who is the section chief of Large Animal Medicine in the Cornell University Hospital for Animals, gave expert opinion on whether the horses developed neurological signs—something only a highly trained veterinarian could do. Goodman also videotaped Perkins's exams of the horses and sent the images to experts in the field for an independent assessment, thus increasing the study's validity. Professor of Medicine Thomas Divers, DVM, Dip ACVIM, Dip ACVECC, also consulted on these exams.

"Laura was always 150 percent devoted to the project; we made a good team," Perkins says.

In addition to receiving consultative support from faculty here, Goodman spent a month at the Animal Health Trust in Newmarket, England, collaborating with experts there.

While a member of Osterrieder's lab, Goodman also conducted a study as part of the Zweig-supported effort to find effective vaccines against the neurological disease caused by EHV-1. Goodman compared two commercially available vaccines and published her finding that the modified live vaccine is superior to the combination vaccine in the paper, "Comparison of the Efficacy of Inactivated Combination and Modified-live Virus Vaccines Against Challenge Infection with Neuropathogenic Equine Herpresvirus Type 1 (EHV-1)," in the January 2006 issue of the journal *Vaccine*. This finding advances the lab's larger effort of preparing the next generation of live vaccines that are optimized for generating a targeted immune response against EVH neurologic disease.

In August of 2007 Goodman completed her PhD and has gone on to accept a postdoctoral fellowship in virology in the lab of Colin Parrish, PhD, the John M. Olin Professor Virology at the Baker Institute for Animal Health. She's studying canine parvovirus antibody neutralization and receptor binding. Canine parvovirus is a model for how viruses can change their host species range.

Since she's now working on diseases of dogs, Goodman is looking forward to enjoying her life long love of horses in other ways. During her undergraduate days, she volunteered with the North American Riding for the Handicapped Association. Now that she has completed her degree, Goodman would like to get back into that again.

What's ahead for her professionally? An academic life, perhaps, as she enjoys research so much.

"But with my background and interest in epidemiology I'm also drawn to public health," Goodman says. ■

Harry M. Zweig Memorial Fund for Equine Research 2008 Research Awards

$55,697 to Dr. Dorothy Ainsworth for "Modeling Equine Pulmonary Disorders in vitro: Epithelial-Derived Proteins and Inflammatory Airway Diseases"

$11,540 to Dr. Sylvia Bedford-Guaus for "Characterization of Equine Phospholipase C Zeta (PLC Zeta) as it Relates to Stallion Fertility"

$20,000 to Dr. Yung-Fu Chang for "Vaccination Against Equine Leptospirosis"

$60,000 to Drs. Norm Ducharme and Jeremy Rawlinson for "Factors Affecting Airway Stability of Horse Exercise: A Combined Neuroanatomical, Clinical, and Engineering Methodology"

$62,191 to Dr. Julia Flaminio for "The Phagocyte Response Against *R. equi* in Foals"

$53,506 to Dr. Lisa Fortier for "Characteristics of Stem Cells Derived from Bone Marrow Aspirates, Adipose Tissue, and Muscles"

$9,840 to Dr. Susan Fubini for "Indices of Intra-Abdominal Fibrilolysis in Colic Foals: Pathogenic and Prognostic Markers"

$87,275 to Dr. Alan Nixon for "Pro-Inflammatory Cytokine Targets in Joint Disease and Check-Points for Gene Inhibition"

$56,059 to Dr. Alan Nixon for "Genomic Profiling of Osteochondritis Dissecans Using an Equine Whole Transcript Exon Array"

$60,205 to Dr. Gillian Perkins for "Therapy and Prevention of Equine Herpesvirus-1 (EHV-1)–Induced Disease"

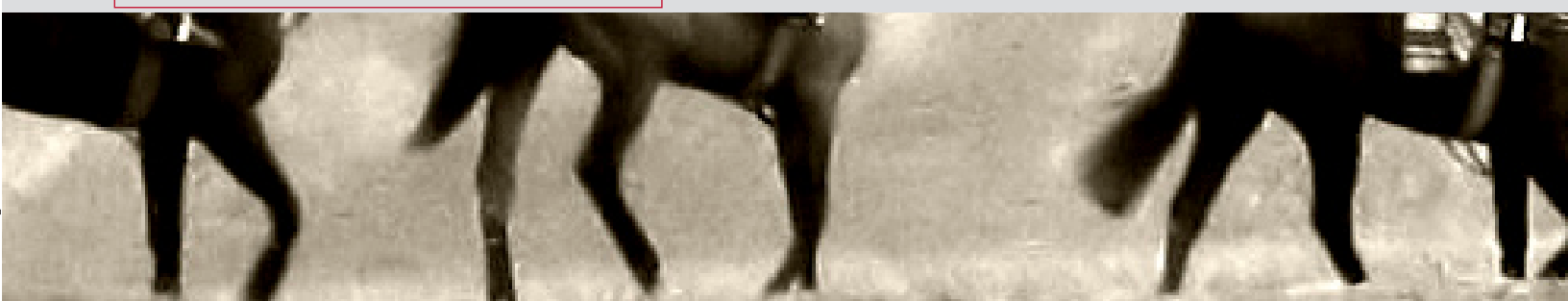
$36,378 to Dr. Gary Whittaker for "Emergency and Establishment of Influenza Virus in Equines"

Have You Visited Our Web Site Lately?

This site (www.vet.cornell.edu/public/research/zweig/index.htm) provides information on the projects and publications that have resulted from funding by the Zweig Memorial Fund. It also demonstrates the objectives of the Zweig Memorial Fund in promoting equine health with regard to the racing industry.

The Zweig News Capsule is published twice a year and can be downloaded in pdf format by the click of a mouse!

Please encourage other equine enthusiasts to visit this site. If you would like to receive a published copy or have a change of address, contact the secretary of the Harry M. Zweig Memorial Fund for Equine Research, whose e-mail address appears at the bottom of the web page.



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The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State legislature created the Harry M. Zweig Memorial Fund to promote equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

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