



# An Influenza Strain by Any Other Name Is Just Not the Same

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Tracking the ever-changing flu virus demonstrates that influenza vaccines and related practices must evolve with the strain.

There's much ado about flu of late and with good reason. The incidence of equine influenza virus (EIV) has been trending upward since 2008, showing considerable spikes in recent years.<sup>1</sup> Equine influenza was the most common infectious upper respiratory disease of the horse in 2019, with significant increases noted in February, March and again in June.<sup>1,2</sup>

Adding to the flu intrigue is the high number of cases occurring in vaccinated horses. Of the horses with known vaccination status, 61 percent of positive EIV cases occurred in horses vaccinated against EIV.<sup>2</sup>

The impact from this growing flu threat is playing out in barns across the country. Its effects range from medical challenges to economic loss and time away from performance and production. Veterinarians tasked with managing cases face the daunting assignment of mitigating spread from this highly contagious disease.

So, what's driving this conundrum of increased incidence and apparent vaccine failure? The answers lie in understanding the virus's evolution, and a critical assessment of when and how we are vaccinating.

## Tracking an evolving target

To find a plausible cause for the EIV vaccine failure that's been ramping up since 2013, researchers at the University of California-Davis School of Veterinary Medicine compared the heterogeneity of circulating wildtype EIV strains in the United States with Ohio '03 (OIE recommended clade 1 influenza strain) to determine whether vaccine failure was due to the introduction of foreign EIV strains or the natural selection of EIV strains (antigenic drift).

Their conclusion: Foreign EIV strains have not been circulating in the United States. Only EIV Florida clade 1 strains have been circulating and have evolved separately from clade 2 strains.<sup>3</sup>

"EIV strains are mutating through selection—antigenic drift—in order to escape the immune system," Kyuyoung Lee, DVM, MPVM, PhD Candidate and lead study author said. "Antigenic drift of U.S. EIV is the most plausible cause

for the high rates of vaccine failure, underscoring the need to update vaccine strains.”

Dr. Lee goes on to explain the role of sequencing to understand the significance of this antigenic drift. “While genetic analysis compares various strains and determines their homology, the number of single amino acid changes is not what’s relevant. Instead, the important focus is the position of the amino acid changes as they pertain to immunodominant sites, which is determined through sequencing.”

Different strains of EIV can be compared by sequencing the hemagglutinin (HA) gene. HA contains the receptor binding sites that enable the influenza virus to attach to host cells. If antigenic drift decreases binding of vaccine antibodies with the HA glycoprotein at these “key sites,” it may cause reduced vaccine protection or a total lack of protection.

## Learning from outbreaks

Ongoing EIV sequencing through a process called *genetic characterization* (comparing genetic sequences of influenza isolates) is critical to making informed decisions regarding:

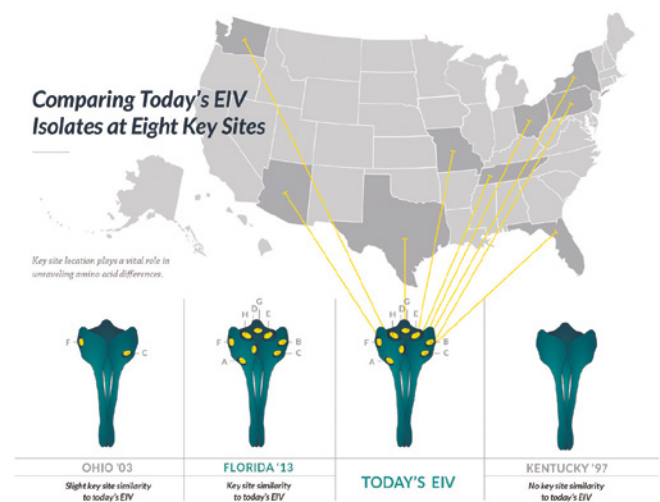
- How equine influenza viruses are ‘related’ to one another
- How equine influenza viruses are evolving
- The genetic variations (mutations) that appear when viruses begin spreading more easily or causing more-severe diseases
- How well an equine influenza vaccine might protect against a particular strain of circulating influenza virus

Recent equine influenza outbreaks bring this idea into focus. Consider the 2013 outbreak that occurred in southern Florida in a large number of well-vaccinated horses.

By analyzing samples submitted to the Equine Respiratory Biosurveillance Program, researchers identified and isolated a new influenza strain, Florida ‘13. Ongoing sequencing through the time of this article reflects that Florida ‘13 is representative of current circulating U.S. field strains. Florida ‘13 differs from Ohio ‘03 (OIE clade 1 isolate recommended for EIV vaccine production) by six amino acid changes, and all six of those mutations occur on the surface of the HA glycoprotein and are in or near regions associated with antibody binding or receptor binding sites<sup>4</sup>—making them critically significant.

In response to these findings, Merck Animal Health added in 2018 the Florida ‘13 influenza strain to the PRESTIGE<sup>®</sup> inactivated influenza-containing vaccines.

“Merck Animal Health is the leading industry partner that has realized the need to not only surveil respiratory viruses but also determine their evolution,” said Nicola Pusterla, DVM, Dipl. ACVIM, who leads the UC Davis Equine



Similarity of isolates from recent (2018-2019) EIV outbreaks and key antigenic sites of the hemagglutinin (HA) glycoprotein. (Courtesy Merck Animal Health.)

## Homology

**Definition:** The existence of shared ancestry between a pair of structures or genes.

**Purpose:** Identifies overall similarities between two samples to establish the comparison of like with like, such as comparing EIV with EIV vaccine strains versus comparing EIV with a rabies vaccine virus.

## Genome sequencing

**Definition:** The process that determines the order or sequence of the RNA nucleotides (e.g., A, C, G, U) in each of the genes present in the virus’s genome.

**Purpose:** Focused on the hemagglutinin (HA) and neuraminidase (NA) glycoproteins found on the virus surface, sequencing identifies the difference in key locations on the virus to determine how they impact disease spread or vaccine protection.

## Impact of EIV by the Numbers

**7 days.** Amount of rest a horse needs for every day of fever.

**50 yards.** Distance a coughing horse can propel equine influenza virus.

**7 to 10 days.** Length of time horses can shed virus following infection.

**72 hours.** Length of time EIV can survive on wet surfaces.

**48 hours.** Length of time EIV can survive on dry surfaces.

**29%.** Incidence of equine influenza.<sup>1</sup>

Infectious Disease Research Laboratory where Biosurveillance Program samples are submitted and analyzed. “Overall, we are seeing greater numbers of EIV-positive horses, not only in young unvaccinated horses but also in adult vaccinated horses. Unless EIV isolates are continually collected and analyzed, there will be no way to determine how different vaccine strains are from contemporary EIV strains.”

### New vaccine timing for ‘flu season’

Recent data from the program compels a critical new question: Are we vaccinating at the right time of the year and are the recommended guidelines of biannual vaccination providing optimal influenza protection?

In addition to keeping vigil over mutating flu strains, Merck Animal Health tracks infection rates through the Equine Respiratory Biosurveillance Program. Every year since the inception of the study, data shows increased flu incidence beginning in December and running through April, which is typically prior to when at-risk horses are receiving their bi-annual EIV vaccinations.<sup>2</sup> Following that logic, an EIV booster in late November or early December may be more advantageous than late summer or early fall.

With data clearly pointing to an equine flu season, veterinarians may benefit from critically evaluating the optimal time to administer biannual EIV vaccines. Even a one- to two-month adjustment in EIV vaccination timing could make a significant difference for the horse.

Additionally, ongoing (since 2008) Biosurveillance Program data indicates many horses are under-vaccinated—receiving just one EIV vaccination per year (or less), further complicating our efforts to contain this highly infectious virus.

### The take-home message

Ultimately, that’s the point of all this flu talk—doing what we can as individual practitioners and as an industry to keep horses as healthy as possible. Regardless of the disease, we must do our best to provide optimal protection through properly timed and administered vaccinations. The recent rise of EIV infections demonstrates the need for vigilant tracking and sequencing to keep up with antigenic drift. Data shows the virus is evolving and our vaccinations should evolve along with it. Florida ‘13 is an example of this important concept and has demonstrated through both homology and sequencing work that it is the closest influenza strain to what is currently circulating in our horses.

<sup>1</sup> Merck Animal Health and University of California-Davis School of Veterinary Medicine (Nicola Pusterla). Infectious Upper Respiratory Disease Surveillance Program. Ongoing research 2008-present.

<sup>2</sup> Vaala W, Barnett DC, James K, Chappell D, Craig B, Gaughan E, Bain F, Barnum SM, Pusterla N. Prevalence Factors Associated with Equine Influenza Virus Infection in Equids with Upper Respiratory Tract Infection from 2008 to 2019. *AAEP Proceedings*. 2019 Vol 65.

<sup>3</sup> Lee K, Pusterla N, Barnum S, Martinez-Lopez B. Is Current Vaccine Failure of Equine Influenza Virus Due to Evolution of Endemic Strains or Introduction of Foreign Strains? *AAEP Proceedings*. 2019 Vol 65.

<sup>4</sup> Merck Animal Health Technical Bulletin, December 2019.

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Duane E. Chappell, DVM, received his doctorate of veterinary medicine from Purdue University. He joined the Merck Animal Health equine veterinary professional services team in 2014. Chappell began practicing in the Midwest at mixed animal clinics. Throughout his career, Chappell has owned and managed solo and group mixed animal practices and was a resident veterinarian at Richland Ranch Quarter Horse breeding farm. He also served as research monitor, participant and leader in trials involving *R. equi*, equine influenza, exercise cytokines and plasma antibodies while in practice. Chappell was most recently an assistant professor at Morehead State University in Kentucky.

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