Fall 2018 | Volume 4 | No. 2

# Merck Animal Health Equine Respiratory Update

In Collaboration with the University of California, Davis School of Veterinary Medicine

## Putting a Chokehold on Strangles

### *Biosurveillance program sheds light on Streptococcus equi subs. equi prevalence factors*

Misunderstood? Misdiagnosed? Undiagnosed? *Streptococcus equi* subs. *equi* – better known as strangles – has devastated the horse industry with outbreaks and confounded the medical community in our ability to prevent and/or manage it with a high degree of effectiveness. Yet, it is a malady that has impacted horses for hundreds of years, costing the equine industry millions of dollars along the way.

One of the primary challenges presented by strangles is that it can stick around even after the horse has "recovered" – making the horse a seemingly healthy carrier that can infect other horses.

"This becomes important when trying to screen herds or groups of horses to resolve a recurrent strangles problem on a farm or premise," says Fairfield T. Bain, D.V.M., M.B.A., Dipl. ACVIM, Dipl. ACVP, Dipl. ACVECC, Merck Animal Health veterinary professional services, who presented results from the biosurveillance program at a recent Havemeyer Foundation Workshop on strangles.

"Identifying and treating these persistently infected horses is of primary importance for disease management at both national and international levels."

### Strangles Data Trends: March 2008-June 2018[1]

In our ongoing mission to get a better handle on the infectious upper respiratory diseases impacting horses today, strangles has been part of the Merck Animal Health biosurveillance program diagnostic panel for (all) 10 years. During that time, it was the third most diagnosed pathogen, behind equine herpesvirus type 4 and equine influenza, **comprising 7% of all positive samples.** Though traditionally considered a young horse disease, the biosurveillance program revealed that **strangles is the most commonly diagnosed upper respiratory disease in horses 6-10 years of age.**[2]

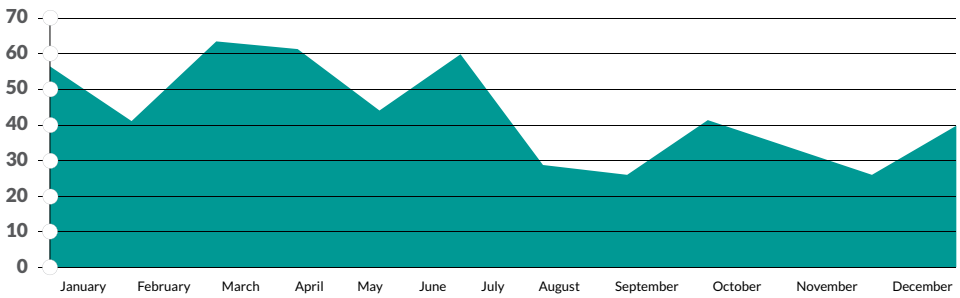
Younger horses seem to exhibit more severe clinical signs with lymph node abscess formation and rupture, whereas older horses are often less severely affected and recover more rapidly. While most horses display classic clinical signs, not every horse presents the same way.[3]

This may explain the surprise factor among practitioners we often hear, "It couldn't be strangles!"

Other findings on strangles from the biosurveillance program include:

- Greater disease incidence in late spring/early summer than other infectious upper respiratory diseases under surveillance (those include EHV-1, EHV-4, EIV, ERAV/ERBV).
  - o The highest incidence occurred in January, March, April and June.
- High frequency in non-traveling pleasure horses, underscoring the importance of preventing exposure from horses traveling on and off the farm and proper biosecurity practices. This may be a result of silent shedders in the herd.
- Higher median age than in horses with other upper respiratory diseases – though all age groups can be affected.
- Clinical signs – other than the classical swollen lymph nodes – include nasal discharge, fever, anorexia and depression strongly associated with positive results for *Streptococcus equi* subsp. *equi*.
- Positive relationship to prior vaccination against *S. equi*.
- Positive relationship with positive qPCR result for EHV-4.

### *S. equi* Disease Incidence Monthly Cumulative 2008-2018




From March 2008 to June 2018, 520 positive cases of *S. equi* have been identified through the biosurveillance program, representing a 7% infection rate.[1] Strangles is the most commonly diagnosed upper respiratory disease in horses 6 to 10 years of age.[2]

"The co-infection with EHV-4 was new information, and the cause of this finding is not yet known," indicated Dr. Bain. "Are both agents circulating together or does infection with one put the horse at risk for infection with the other? Or, does infection with strangles make a horse more likely to recrudesce with and shed EHV-4?"

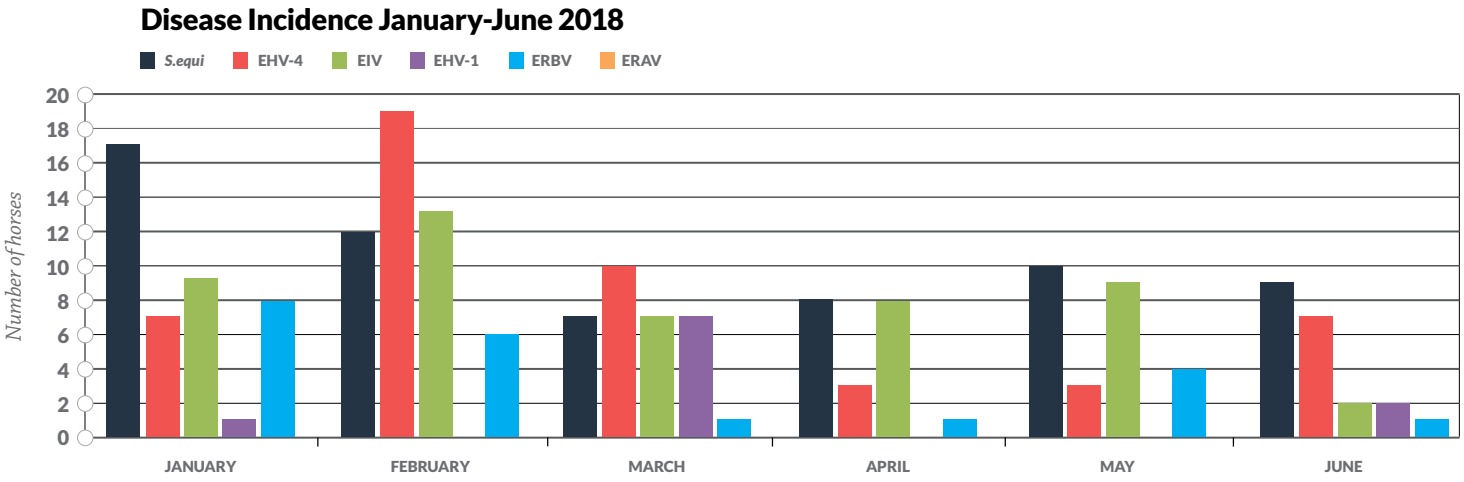
More study is needed to answer these and questions around the positive relationship to prior vaccination. One thing is certain – strangles continues to pose a substantial threat to horses today and we must be vigilant in our efforts to properly diagnose cases of strangles, including horses that may be silent carriers.

The effectiveness of available vaccines is the subject of some discussion within the profession and while vaccination may be helpful in some instances, the importance of proper biosecurity to help prevent disease spread cannot be emphasized enough with owners.

*(Note: Strangles is a ubiquitous disease and these numbers do not indicate all cases of strangles, particularly since it is not a reportable disease in every state. In addition,* ***nasal swabs are not the preferred sampling technique to diagnose the disease. Preferred sampling techniques are guttural pouch lavage, nasal wash or deep nasopharyngeal swab, respectively.)***

## Disease Trends from January to June 2018[1]

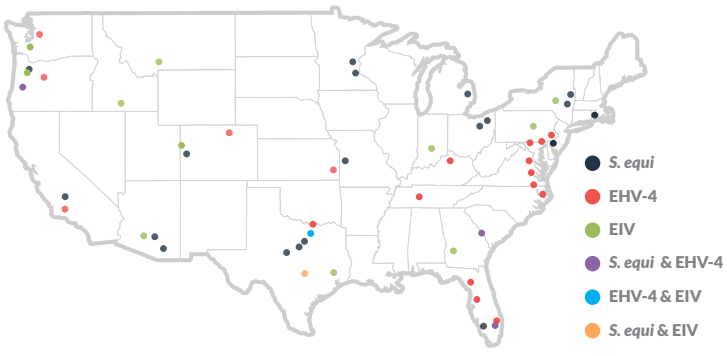
A total of 475 samples were submitted from January to June 2018. Overall, 40% of total samples submitted tested positive for one of the six primary pathogens (*S. equi,* EHV-4, EIV, ERBV, EHV-1, ERAV).




*From January to June 2018, S. equi was the most prevalent infectious upper respiratory disease reported, followed by EHV-4 and EIV.*

| Demographic Summary | ***S. equi*** (63 Cases) | **EHV-4** (49 Cases) | **EIV** (48 Cases) |
|---|---|---|---|
| Median Age | 10 years<br>Range: 2 months – 32 years | 4 years<br>Range: 7 weeks – 28 years | 6 years<br>Range: 8 months – 23 years |
| Predominant Breed(s) | Quarter Horse | Quarter Horse | Quarter Horse |
| Travel | Yes 29%<br>No 67%<br>Unknown 5% | Yes 22%<br>No 69%<br>Unknown 8% | Yes 29%<br>No 69%<br>Unknown 2% |
| Primary Discipline | Pleasure | Show | Pleasure |

*The above table provides a summary of primary demographic parameters for the three major pathogens (January-June 2018).*[1]




*This map shows positive* ***S. equi, EHV-4*** *and* **EIV** *cases from January to June 2018.*[1]


*Samantha with her two favorite four-legged family members*

## *Spotlight: PCR Quality Control with Samantha Barnum*

**Laboratory Manager for the Real-time PCR Research and Diagnostics Core Facility and Dr. Nicola Pusterla's Equine Infectious Disease Laboratory, University of California, Davis**

Inspired by questions from a biosurveillance program participating veterinarian, we recently asked Samantha Barnum how practitioners can ensure proper quality control of the samples submitted for testing.

### Q) What does quality control mean in a PCR lab?

**A)** Sample integrity is of great importance in limiting false negative results and validity of testing and it has a direct impact on pathogen identification. Preserving sample integrity starts with proper packaging. This includes **keeping nasal swabs and blood samples cool from the time of collection through delivery to the laboratory** and submitting to the laboratory as soon as possible. DNA degradation can occur with any sample type, especially if older than four days, or if not kept cold after collection. We test all samples twice for quality control (QC) and use lab specific cut off for passing QC. We will always let the veterinarian know if a sample fails QC, so they can re-sample to ensure confidence in their results.

### Q) What are the most common causes of poor sample quality?

**A)** Use of cotton swabs and not enough contact time. **Rayon swabs should be used** as cotton may contain bleach, pesticide and herbicide residues that act as inhibitors. To ensure proper contact time, remember to **allow swabs to soak for 5 to 10 seconds**, while gently rotating swabs. Sampling from one nostril is sufficient; simply insert two swabs at one time.

### Q) Will dirt or other organic material impact the sample?

**A) Presence of dirt and/or plant material (feces or dirty swabs/washes) will negatively impact the sample**. At the end of the DNA extraction process, we have DNA suspended in an aqueous solution. While not visible to the naked eye, particulate matter originating from the dirt can also find its way into this solution. Particulate matter can inhibit qPCR fluorescence (think of the dirt clouding the qPCR reaction) which leads to a weaker signal (weaker signal means failing quality control). **If the nostril or swab looks dirty, you may need to clean the nostril and collect another sample. Please feel free to throw away the first swab and take a second "clean" sample.**

Plant material, on the other hand, acts a bit differently. The chemicals found in plants can neutralize the lysing of the cells in the first step of the DNA extraction process. If the cells aren't properly lysed, then the DNA can't be extracted efficiently.

### Q) What are some other factors that may affect sample quality?

**A)** Lack of DNA (acellular samples like CSF, urine, joint fluid). Since PCR is a DNA-based technology, if there is a low concentration of DNA in the sample, you will get a weak signal for the housekeeping gene, which is how we determine the sample quality. With any sample type, as soon as a sample is collected, the DNA starts degrading. If the DNA degrades too much during transit, handling, or storage then the effect is a lack of DNA.

### Q) Can an error occur at the laboratory?

**A)** Certainly, if a sample is mishandled, steps are missed during the DNA extraction or PCR process, or if reagents are mixed up or made incorrectly this can play a role in samples failing quality control. Fortunately, this is the easiest error to catch on our end. We have a strict quality assurance protocol which includes running controls at every step of the process. Also, because all samples are batched each day on a semi-automated instrument (to reduce human error) and processed at the same time, if chemicals were mixed up or steps were missed it would affect the whole batch equally, most likely leading to all samples submitted that day failing quality control (which has never happened). Additionally, we run a positive and negative pathogen control on every plate to ensure that the qPCR is working.

## Practice Tips

### The Difference Between Reportable, Actionable and Monitored Diseases

Each state has a different list of reportable, monitored and/or actionable diseases. For example, strangles is a reportable disease in some states but not others. Most diseases fall into "Reportable and Actionable" or "Reportable and Monitored" or "Not Reportable."

- **Reportable diseases:** Attending veterinarians and diagnostic laboratories are required to report any confirmed case to the state veterinarian.
- **Actionable diseases:** The state is required to take action in the event of a case or outbreak of an "actionable" disease.
- **Monitored diseases:** States maintain data on "monitored" diseases.

The requirements for reporting disease differ from state-to-state. Equine infectious anemia is the only uniformly reportable equine disease in the United States.

For a list of reportable diseases by state, visit http://www.equinediseasecc.org/reportable-diseases.

### Equine Disease Communication Center (EDCC) Sharing Biweekly Biosurveillance Program Updates

As a reminder, the EDCC is now sharing biweekly summaries of positive disease cases detected through the Merck Animal Health Biosurveillance Program through its news page, www.equinediseasecc.org/news.

## Quick Tips For Clients

**NEW Strangles Infographic**

We're pleased to offer biosurveillance program participants an equine strangles infographic to support client education. The infographic offers important facts on the disease and proper biosecurity measures. Click to review and share with your clients on your website or through your social media properties.

Also, be sure to ask your Merck Animal Health sales representative about our other disease infographics available to support client education, including:

- Equine Influenza (EIV)
- West Nile Virus (WNV)
- Equine Herpesvirus Type 1 (EHV-1)
- Equine Herpesvirus Type 4 (EHV-4)
- Equine Herpesvirus Myeloencephalopathy (EHM)
- Eastern/Western Equine Encephalomyelitis (EEE/WEE)

*Click to download the infographic*

## About the Newsletter

This bi-annual newsletter is being sent as a value-added service to clinics enrolled in the biosurveillance program. Merck Animal Health is passionate about this program and is providing this newsletter to customer veterinarians to help them stay up-to-date on the latest trends and historical information the study has yielded to date. Technical veterinary advice, interpretation and case management support will be provided by Merck Equine Veterinary Professional Services (Drs. Barnett, Vaala, Gaughan, Craig, Bain and Chappell) and Nicola Pusterla, D.V.M., Department of Medicine and Epidemiology, UC Davis.

If you have questions about the program, please call our professional services team at (866) 349-3497, or email one of the professional services veterinarians at the addresses listed below.

**Dr. D. Craig Barnett**
craig.barnett@merck.com

**Dr. Fairfield Bain**
fairfield.bain@merck.com

**Dr. Wendy Vaala**
wendy.vaala@merck.com

**Dr. Duane Chappell**
duane.chappell@merck.com

**Dr. Earl Gaughan**
earl.gaughan@merck.com

**Dr. Nicola Pusterla**
npusterla@vmth.ucdavis.edu

**Dr. Bryant Craig**
bryant.craig@merck.com

## Relevant Supporting Research

For more information on the latest respiratory disease published research from Merck Animal Health, click on the links below.

1) "Prevalence Factors Associated with EHV-2/5 Among Equines with Signs of Upper Respiratory Infection in the US."
James, K., Vaala, W., Chappell, D., Barnett, D.C., Gaughan, E., Craig, B., Bain, F., Pusteria, N. ACVIM 2017 Abstract.

2) "Prevalence factors associated with equine herpesvirus type 1 infection in equids with upper respiratory tract infection and/or acute onset of neurological signs from 2008 to 2014"
Pusterla, N., Mapes, S., Akana, N., Barnett, D.C., Mackenzie, C., Gaughan, E., Craig, B., Chappell, D., Vaala, W. *Vet Rec.* 2015; doi: 10.1136/vr.103424.

3) "Voluntary Surveillance Program for Equine influenza Virus in the United States from 2010 to 2013"
Pusterla, N., Kass, P.H., Mapes, S., Wademan, C., Akana, N., Barnett, D.C., Mackenzie, C., Vaala, W. J Vet Intern Med 2015; 29:417-422

4) "Surveillance programme for important equine infectious respiratory pathogens in the USA"
Pusterla, N., Kass, P.H., Mapes, S., Johnson, C., Barnett, D.C., Vaala, W., et. al. *Vet Rec.* 2011 July 2;169(1):12. doi: 0.1136/vr.d2157.

5) "Voluntary surveillance program for important equine infectious respiratory pathogens in the United States"
Pusterla, N., Kass, P.H., Mapes, S., Johnson, C., Barnett, D.C., Vaala, W., Gutierrez, C., et. al. AAEP Proceedings 2010.

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

[2] Surveillance programme for important equine infectious respiratory pathogens in the USA. N. Pusterla, P.H. Kass, S. Mapes, C. Johnson, D.C. Barnett, W. Vaala, et al. *Vet Rec* 2011.

[3] Boyle A.G., Timoney J.F., et al. *Streptococcus equi* Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles – Revised Consensus Statement. *J Vet Intern Med* 2018;00:00-00. DOI: 10.1111/jvim.15043

## About the Program

Since March of 2008, Merck Animal Health has been conducting an ongoing, voluntary equine biosurveillance program to study the prevalence and epidemiology of relevant viral and bacterial respiratory pathogens. More than 8,000 samples from U.S. equids of all ages, genders and breeds presenting with fever and signs of acute upper respiratory disease and/or acute neurological disease have been collected since the study began. Samples are submitted by participating Merck Animal Health customer clinics and tested via quantitative PCR at the University of California, Davis School of Veterinary Medicine (UC Davis). **To be eligible for testing, horses must have an unexplained fever (T ≥ 101.5°F) AND one or more of the following signs: Depression, nasal discharge, cough, and/or acute onset of neurologic disease.** The results are then returned to the Merck Animal Health customer within 24 hours and provide invaluable diagnostic and treatment information.

## Four-Fold Purpose:

1) To provide a valuable diagnostic tool to participating Merck Animal Health customers to assist in obtaining an accurate and timely diagnosis during an acute respiratory disease outbreak so they can provide optimal treatment, quarantine and vaccination strategies to their clients and patients.

2) To provide the horse industry with a better understanding of the prevalence and epidemiology of these respiratory pathogens.

3) To identify and monitor the current circulating strains of major equine respiratory pathogens.

4) To evaluate the efficacy of current vaccination protocols.

UCDAVIS VETERINARY MEDICINE

MERCK Animal Health

The Science of Healthier Animals

2 Giralda Farms • Madison, NJ 07940 • merck-animal-health-usa.com • 800-521-5767


MERCK Animal Health