

New assay improves diagnostic detection of strangles disease in horses

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The Texas A&M Veterinary Medical Diagnostic Laboratory now has an improved assay to diagnose strangles in horses. Credit: A&M AgriLife photo by Laura McKenzie

Research initiated at the Equine Infectious Disease Laboratory, EIDL, at

Texas A&M University to explore the genomic components of strangles has now led to an improved assay at the Texas A&M Veterinary Medical Diagnostic Laboratory, TVMDL.

Strangles is a highly contagious disease that affects the upper respiratory system and lymph nodes of horses. The disease is caused by the bacterium *Streptococcus equi* subspecies *equi*, *S. equi*. Though [clinical signs](#) may be present, diagnostic detection is the only definitive method of identifying strangles.

The ancestor of *S. equi*, *Streptococcus equi* subspecies *zooepidemicus*, *S. zoo*, is considered a commensal organism and may cause pneumonia, endometriosis and abortion in horses. Together, both organisms contribute to high morbidity and variable mortality among horses.

Ellen Ruth Alexander Morris, Ph.D., began working on the project early in 2020 as a graduate research assistant with Noah Cohen, VMD, Ph.D., director of the EIDL, which is located in the Department of Large Animal Clinical Sciences in the School of Veterinary Medicine and Biomedical Sciences.

"There was no initial plan to develop a new assay," Alexander Morris said. "The idea evolved from another ongoing project, where we were examining the genome-wide differences between 50 strains of *S. equi* and 50 strains of *S. zoo* using whole genome sequencing."

Their evaluation of the different strains led to the development of additional primers, which are DNA sequences that can be used to detect both *S. equi* and *S. zoo*.

Current detection methods

Classically, strangles was detected by culturing *S. equi* and *S. zoo* in a

slow and not very sensitive method. Over time, multiple tests using [polymerase chain reaction](#), PCR, technology have been developed to detect *S. equi* and *S. zoo* individually.

Although quicker and more sensitive than bacterial culture, PCR testing for strangles is still somewhat limited. PCR testing uses primers to target specific DNA sequences to determine an organism's presence. Because of genetic similarities between *S. equi* and *S. zoo*, some tests may not be able to differentiate between the two organisms and may lead to false positives.

Conversely, in some samples the typically targeted sequence of *S. equi* has been truncated or deleted, and therefore testing leads to a false negative. Other PCR tests, such as the one previously offered at TVMDL, cannot differentiate coinfection with *S. equi* and *S. zoo* due to the organisms' multiple genetic similarities.

Improved PCR testing

The new primers were designed from *S. equi* and *S. zoo* strains collected from clinical samples of Texas horses and using publicly available *S. equi* and *S. zoo* strains from across the world. TVMDL's molecular diagnostics section performed validation testing using the new primers and determined they could be used to detect and differentiate between *S. equi* and *S. zoo*. This assay also includes an internal control that serves as a monitor for PCR efficiency and sample inhibition. Following validation, TVMDL can now use these primers for routine diagnostic testing.

"Our hope is these new PCR targets will aid in the diagnosis of strangles, identify cases of concurrent infection of *S. equi* and *S. zoo*, or improve differentiation between the two organisms," said Alexander Morris, who is now a postdoctoral research assistant at TVMDL.

Provided by Texas A&M University

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