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Streptococcus equi/ Strangles Culture and PCR Testing

General Information

Diagnostic testing to detect shedding of the bacteria which causes Strangles in horses, *Streptococcus equi*, currently includes bacterial isolation by aerobic culture and subsequent biochemical identification, and bacterial DNA detection by the polymerase chain reaction (PCR) test.

See references at the end of this fact sheet for additional information.

Bacterial culture---Live *Strep equi* organisms must be recovered by a swab or wash of the nasopharynx, nasal passages, or draining abscesses in order to get a positive culture result. Samples with large amounts of mucopurulent discharge have been found to be negative on culture even if positive for *Strep equi*. If clinical signs suggest strangles, a repeat test should be done. Our normal turnaround time for culture results is 2-3 days.

Additionally, the modified live vaccine strain of *Strep equi* (currently used in the only licensed intranasal strangles vaccine) will grow in culture, and cannot be differentiated from the field/wild pathogenic strains by routinely employed microbiological techniques. Sequencing techniques at the University of Kentucky Gluck Equine Research Center will allow for differentiating the wild strain from the vaccine strain.

A bacterial culture will be reported positive if a beta-hemolytic *Streptococcus* species is grown and identified specifically as the Strep equi (formal name is *Streptococcus equi* subspecies *equi*). A culture which does not grow a beta-hemolytic *Streptococcus* sp. within 48 hours on appropriate media is reported as negative for *Strep equi*. A culture which grows a beta-hemolytic *Streptococcus* sp, will be subjected to appropriate procedures to further identify the isolate, requiring at least an additional 24 hours of time to grow the organism, and sometimes longer, depending on the growth characteristics of the isolate

PCR--- (Polymerase Chain Reaction) has been extensively used for detection of *Strep equi* since the species specific M protein (SeM) sequence in this organism was reported by Timoney *et al* (1997). *Strep equi* PCR targeting this specific M protein has been proven to be quite specific.

The Animal Health Diagnostic Center (AHDC) at Cornell University employs *Strep equi* PCR with primers targeting this M protein sequence. *Strep equi* PCR is also sensitive and rapid. *Strep equi* PCR is more sensitive than culture, and due to its fast processing, results could be available on the same day that samples are submitted if STAT is requested

Our normal PCR turn-around time is 2-3 working days. STAT testing (same day results) for strangles PCR is available at the AHDC, but prior arrangements must be made with the laboratory. A STAT fee will be charged and a special weekend fee applies if results are needed

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on the weekend. Contact the laboratory or see the website at: http://ahdc.vet.cornell.edu/ for a description of these charges.

Sample Collection Techniques for PCR or Bacterial Culture

ALL SAMPLES SHOULD BE TAKEN WITH DISPOSABLE GLOVES USING CAREFUL TECHNIQUES TO PREVENT CROSS CONTAMINATION. THE OUTSIDE OF THE CONTAINERS SHOULD BE CAREFULLY DISINFECTED TO PREVENT CROSS CONTAMINATION.

Nasopharyngeal wash: see link to AAEP guidelines and article from The Horse http://www.aaep.org/pdfs/control_guidelines/Streptococcus%20equi%20var.pdf and https://s3.amazonaws.com/assets.prod.vetlearn.com/ca/9442c0b24011e087120050568d3693/file/PV0311_Boyle_CE.pdf. This procedure covers a large area of the nasal passages thus increasing the chance of recovery of the organism if present.

Nasal swabs or draining lymph nodes: swabs or material obtained from draining abscesses can be cultured for bacterial growth and/ or for PCR testing. When obtaining nasal swabs, it is acceptable to use routine length culture swabs. Two swabs can be introduced into the nares at the same time and kept in contact with the mucosa for a minimum of 20 seconds to insure good contact. One swab can be used for bacterial culture and the other for PCR.

Guttural pouch washes: Endoscopic examination and washes of both guttural pouches should be performed on any horse that has had positive PCR or culture. Three negative consecutive guttural pouch washes should be done 7 days apart for a period of 21 days to confirm negative status. Some veterinarians can submit samples from blind guttural pouch washes for testing, but to rule out carrier status visualization of the pouches should be done along with PCR as per the recommendations of the ACVIM. See link in reference section.

PCR Samples--- Handling and Shipping

Guttural pouch washes and/nasopharyngeal wash samples should be submitted in a sterile plain red top tube or in a leak proof container. These samples must be shipped chilled and for overnight delivery.

PCR samples on swab---swabs should be placed in a sterile red top blood tube or a container that contains 1-2 drops of sterile saline to keep swab slightly moist. **Bacterial transport media is not acceptable under any circumstance**. These samples should be shipped chilled and for overnight delivery.

Bacterial Culture---Handling and Shipping

The same sample types obtained for PCR testing can be used for culturing, but swabs should be put in bacterial transport media such as Amies Media. Swabs or liquids shipped without a bacterial transport media may be cultured, but must be shipped chilled for overnight delivery. If more than 24 hours will lapse before arrival to laboratory, washes or swabs should be put into

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a transport media to assist in recovery of bacteria. A sterile swab should be saturated with the wash and placed into transport media. The bacteria may die without transport media after 24 hours; thus, causing a false negative.

Endoscopic/guttural pouch wash Bacterial Culture Sample Handling: If bacterial culture is requested from the guttural pouch wash, the liquid may be sent for direct culture if received chilled in a leak proof container within 24 hours post collection. If sample will not arrive within 24 hours, a sterile swab should be saturated with the wash material and placed in a suitable bacterial transport media such as Amies transport media or Stuart's media

Interpretation of Results

Positive results by *Strep equi* PCR means that *Strep equi* DNA was detected in the sample. DNA can be detected from live, dead or modified live vaccine origin bacteria. Therefore, scheduling *Strep equi* PCR testing after administration of antibiotic treatments or intranasal vaccination should be carefully planned. The 9ÖÌÛÐÚ‰ÍÖÙÔÌÙÓà‰/ÍÐáÌÙÀ drug company, manufacturers of Pinnacle IN (the intranasal strangles vaccine), is currently telling equine veterinarians that the *Strep equi* culture may be positive on routine culture up to 36 hours post administration of intranasal vaccine. PCR tests after vaccination with the intranasal vaccine, Pinnacle, may be positive for up to 30 days. If a determination of whether the *Strep equi* isolated by culture is the wild or vaccine strain is required, sequencing can be done. If this is needed, the laboratory needs to be informed so as to keep the isolated *Strep* for further testing.

PCR testing may result in a **SUSPECT** result. This indicates that a positive signal was detected from the sample in a range near the limit of detection for the assay. While the signal detected in this range is most often specific, it can sometimes be the result of non-specific amplification. Any animal with exposure risk that has a **SUSPECT** result on PCR should be considered positive until proven otherwise.

References

A thorough review and discussion of *Strep equi* infection, transmission and control is available through the American College of Veterinary Internal Medicine
http://www.acvim.org/uploadedfiles/Consensus_Statements/Strangles.pdf

"*Streptococcus equi* infections in Horses: Guidelines for Treatment, Control and Prevention Strategies" by Corinne Sweeney, John Timoney, Richard Newton, and Melissa Hines. J Vet Intern Med 2005;19:123-134.

AAEP website link for strangles information:
http://www.aaep.org/pdfs/control_guidelines/Streptococcus%20equi%20var.pdf.

Excellent article from The Horse on procedures and pictures of strangles:
https://s3.amazonaws.com/assets.prod.vetlearn.com/ca/9442c0b24011e087120050568d3693/file/PV0311_Boyle_CE.pdf.

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Short Communications

Detection of *Streptococcus equi* in equine nasal swabs and washes by DNA amplification

J. F. Timoney, S. C. Artiushin

Veterinary Record (1997) **141**, 446-447

Streptococcus equi, the causative agent of equine strangles, is scce in nasal discharges and pus from lymph nodes of affected animals Routine laboratory detection of the bacterium involves culture of nasal swabs, nasal washes and pus from abscesses and is often difficult because of background contamination, small numbers of the organism, or the presence of *S zooepidemicus* and other β-haemolytic streptococci. Completion of culture and identification usually takes two to three days, an excessively lengthy interval given the highly contagious nature of strangles and the need to quickly identify shedding horses so that they may be isolated in the early stages of an outbreak. The recent elucidation of the DNA sequence of *SeM*, the gene for the protective, type-specific M-like protein unique to *S equi* has provided information for the development of a test for *S equi* DNA (Timoney and others 1997) based on the polymerase chain reaction (PCR). PCR takes advantage of the ability of DNA polymerase to synthesise many copies of as few as one DNA template molecule by repetitive cycles of strand separation, primer extension and subsequent strand annealing. Primers are short pieces of DNA made from the upper end of the coding strand and from the lower end of the non-coding strand of the DNA of interest. The resulting amplified fragment has a characteristic size. This short communication describes the application of PCR to the detection of *S equi* in nasal swabs and washes from horses from five different outbreaks of strangles in horses in Kentucky in the spring of 1996.

Nasal swabs were collected from affected and exposed in contact horses one to five days after clinical diagnosis of strangles on farms A, B, C and D. Some horses were sampled more than once over the following three weeks. Nasal washes were also collected from 13 horses on a UK farm, 15 and 35 days after exposure to two horses with clinical strangles. All 13 horses developed strangles within 17 days of this exposure. Nasal washes were collected by instilling 50 ml phosphate buffered saline (pH 7.3) via an 8 mm diameter latex tube (Fisher Scientific) inserted 15 cm into the nostril and collecting the fluid that drained out. The fluid was centrifuged at 3000 *g* and the pelleted debris separated for culture and PCR.

Swabs and nasal wash pellets were cultured on Columbia CNA horse blood agar (Difco Laboratories) and incubated for 18 hours at 37°C. β-haemolytic colonies were subcultured and their fermentation behaviour tested in lactose, sorbitol and trehalose broths. Mucoid β haemolytic colonies that did not ferment any of these sugars were identified as *S equi*. DNA for PCR from the nasal swabs and washes was prepared as follows. Swab tips were placed in 200

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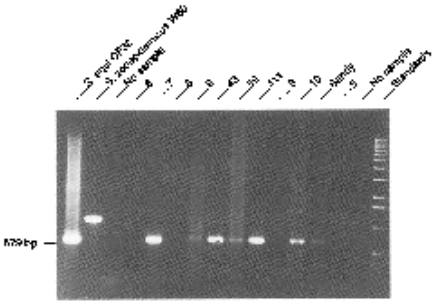

FIG. 1: Agarose gel electrophoresis of PCR products following amplification of DNA in nasal washes from a group of horses 15 days following exposure to a horse with strangles. Numbers 9 and 10 were culture positive

µl of sterile water, vortexed, the tips removed and the fluid centrifuged at 14,000 *g* for 10 minutes. The sediments were resuspended in 20 µl of K buffer (1X Gen Amp Buffer II [Perkin Elmer], 0·5 per cent Tween 20, 100 µg/ml proteinase K). Nasal wash pellets were suspended in an equal volume of K-buffer. The suspensions were incubated for 30 minutes at 55°C, boiled for five minutes and then centrifuged for five minutes at 14,000 *g*. The reaction mix for PCR in a 100µl volume of 30 µl was prepared in Gen Amp Buffer II and contained 2 mM magnesium chloride, 0·2 mM dNTP, 0·5 units Taq polymerase, 0·25 µM SeM6 and SeM7 primers, and 5 µl of sample. The primer sequences were 5'TGCATAAAGGAAGTTTCCTTTC (SeM7 forward) and 5'GATTCGTTAAGAGCTGAGG (SeM6 reverse) representing nucleotide sequences −102 to −83 and 558 to 577 from *SeM* (Timoney and others 1997). Mineral oil (30 µl) was added to seal the reaction mix. Cycling was performed as follows: 92°C for two minutes, 92°C for one minute, 58°C for one minute, 72°C for one minute; repeated 30 times; then 72°C for five minutes and then 4°C final. The PCR products were separated by ethidium bromide agarose gel electrophoresis. The *SeM* fragment amplified with primers SeM6 and 7 is 679 bp (Fig 1).

The results of culture and PCR assays are summarised in Table 1 The PCR detected a 679 bp DNA fragment in 37 specimens including 14 of 15 that were positive by culture. Thus the sensitivity of the PCR appears to be much greater than culture for nasal specimens. Laboratory experimentation has demonstrated a detection sensitivity of 10 or fewer colony forming units (S. C. Artiushin, J. F. Timoney, unpublished data). Although culture is the gold standard, it provides only a minimal estimate of the occurrence of *S equi* in the nose because of inadequate numbers and failure of transfer from swab to culture medium or overgrowth by contaminants. The putative greater sensitivity of the PCR compared to culture is also explained by the fact that the entire swab is extracted for PCR, whereas only a portion of the swab surface is cultured. Despite its putative lack of sensitivity relative to PCR, culture is of value because it definitively establishes infection and may conveniently be performed on the same specimens used for PCR. Since PCR can be completed in four to five