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ETIOPATHOGENESIS

Strangles is a highly contagious disease of equids caused by the gram-positive bacterium, *Streptococcus equi* subspecies *equi*. Although *S. equi* is thought to have evolved from ancestral *S. equi* subspecies *zooepidemicus*, it has more in common with the human pathogen, *S. pyogenes*. Both organisms are host-restricted; both share identical surface proteins; both have acquired genes that encode for toxins and both produce similar disease—lymphadenitis in horses and tonsillitis in humans.1

*Strep equi*, which is not a normal inhabitant of the equine oropharynx, is spread to susceptible horses by either direct contact with infected horses or by indirect contact, through fomites on contaminated halters, lead ropes, buckets, brushes, handlers or flies. Most outbreaks in a herd are associated with the introduction of a horse or another equid that is shedding the organism. Such horses may show the obvious clinical signs of the infection or they may be asymptomatic carriers that maintain the organism in their guttural pouches. Intermittent shedding of *Strep equi* from the guttural pouches of otherwise normal horses contributes to maintenance of interepizootics.1

Limited experimental data suggest that the organism does not survive for an extended period of time in the outside environment.2 Weese and colleagues (2009) examined survival of saline or mucus-based inoculums of *S. equi* on wood, rubber and metal surfaces exposed to outdoor conditions. The study was conducted in Ontario, Canada between July and September when weather conditions varied from sunny to cloudy and partly-cloudy. They found that mucus-based inoculums of *S. equi* persisted longer than saline-based inoculums and that rain, daily temperatures or surface type did not affect the persistence of *S. equi*. When exposed to sunny weather, none of the samples yielded organisms after 24 hours whereas approximately 20% of the samples exposed to partly cloudy or cloudy weather conditions remained positive for *S. equi*. The investigators cautioned against extrapolating their results to other environmental conditions (stalls inside barns, shady outdoor areas, soil or grass surfaces, water or feed). There are anecdotal reports that *S. equi* can survive in the water for 4-6 weeks but objective data to support this are lacking. Many consider drinking water as a major source of infection.

Following ingestion or inhalation of the organism, there is an incubation period of 2-7 days, the length of which depends upon host and pathogen factors (virulence, numbers of organisms, susceptibility). Experimental studies of intranasal inoculation of *S. equi* provide insight into the kinetics of the infection.3 Following inhalation or ingestion, there is a very rapid entry into the oro- and nasopharyngeal tonsillar tissue by the bacteria: Within 3 hours of exposure, small numbers of organisms are detected in the tonsillar crypts and in the subepithelial follicular tissue. By 48 hours post-inoculation, large numbers of cocci are found in the tonsils and then in the lymph nodes. It is thought that tonsillar macrophages or monocytes with intracellular bacteria transport the organisms to the mandibular or retropharyngeal lymph nodes.4 At the onset of
fever, mandibular and retropharyngeal lymph nodes are heavily infiltrated with neutrophils and organisms but the nodes may not yet show evidence of lymphadenopathy. **Nasal shedding of S. equi** is usually evident 2 to 3 days after the onset of fever and often persists for 2 to 4 weeks.

**THE “TYPICAL” CASE OF STRANGLES**

**Clinical signs.** In “typical” cases of strangles, fever and inappetence precede the development of a purulent pharyngitis and lymphadenitis of the mandibular and retropharyngeal lymph nodes. Affected horses initially exhibit a serous nasal discharge that becomes mucopurulent within a few days. In naïve, infected horses the nasal and ocular discharge is often copious while in older horses, a mild short-lasting disease with or without mandibular lymph node abscessation, may occur. This mild clinical course is thought to reflect partial immunity to the organism or infection with a low-virulence strain of *S. equi*.

Retropharyngeal lymphadenopathy (7-10 days post-infection) can narrow the upper airway and produce inspiratory stridor either by direct mechanical obstruction of the airway or indirectly, by involvement of the recurrent laryngeal nerves (cervical lymph nodes). Dysphagia may also develop in affected horses if the pharyngeal plexus on the ventral floor of the gullet pouches becomes involved. Laboratory data in “typical” cases reflects anemia (chronic inflammation); a neutrophilic leukocytosis, usually without a left shift; an increase in plasma fibrinogen and globulin concentrations and a mild decrease in serum albumin. Although cases classically present as an infection of the upper respiratory tract, any anatomical site can be involved, accounting for the myriad of signs and secondary complications (see below). In farm or stable outbreaks, morbidity rates reach 90%, but case fatalities are usually low (8%). Horses of all ages are susceptible but infections typically occur in horses < 5 years of age. Foals < 4 months old may initially be protected by colostral passive immunity.

**Diagnosis.** Samples are obtained by a variety of techniques including nasal or lymph node swabs, nasopharyngeal swabs, nasopharyngeal washes and gullet pouch (GP) lavages. Samples are typically placed in Ames media for microbial culture or submitted in sterile tubes for PCR technology. Although **culture of nasopharyngeal swabs** has traditionally been regarded as the “gold standard” for diagnosing *S. equi*, some investigators have found that during strangles outbreaks, a higher percentage (88%) of “culture positives” are obtained from GP lavages as compared to nasopharyngeal swabs (45% culture positives). The lower microbial yield from nasopharyngeal swabs may be the result of (1) swabbing too early in the course of the disease; (2) retrieving insufficient amount of secretions or (3) having overgrowth of other organisms such as *Strep zooepidemicus* which may secrete an inhibitory protein (equibactin).

The advantage of obtaining nasal, mandibular lymph node (draining) or nasopharyngeal swabs is that they are easily obtained, often without the need for sedating the horse. For nasopharyngeal samples, a Tieglund swab is advanced through the ventral nasal meatus to the level of the pharynx and rotated gently over the pharyngeal tissue. If both microbial culture and PCR testing will be performed, it may be necessary to use two separate swabs. (Check with the laboratory but most will not perform PCR on samples placed in Ames media).

Nasal washes can be obtained using a 20 cm #10 Fr polypropylene catheter that is passed through the ventral nasal meatus approximately 10 cm. With the horse’s head lowered, 60 ml of sterile saline is infused into the area and the “wash” is collected in a rectal sleeve or collection cup for submission to the laboratory. This procedure is best performed on sedated horses to reduce spread of wash material by head tossing.

Endoscope-guided gullet pouch lavage offers the advantage of identifying horses with concurrent GP pathology such as enlargement of the retropharyngeal lymph nodes or evidence of empyema, chondroids formation.
perform a GP lavage, a 1-m endoscope is passed nasally into each guttural pouch following sedation of the horse. Use caution when sedating horses with upper airway compromise as the alpha-2 agonists increase laxity of the pharyngeal muscles, further narrowing the diameter of a compromised upper airway. Following visual inspection of the pouch for evidence of inflammation, empyema, chondroids or protruding lymph nodes, a sterile polyethylene tubing is advanced through the biopsy port of the endoscope. Then, 30 to 60 ml of sterile saline is gently instilled and aspirated. “Blind” lavage of the pouches may also be achieved using a Chamber’s catheter that is passed nasally to the level of the medial canthus and rotated laterally to open the external flap of the guttural pouch.

PCR analysis of samples for *S. equi* genes is considered to be 2-3 times more sensitive than microbial culture alone in detecting the organism but this technique is not without its limitations. False negatives may occur in samples that have heavy growths of *S. equi* and/or in samples with large amounts of purulent material that contain high concentrations of DNA polymerase inhibitors. False positives may occur when the organism has been killed but the PCR technology still detects residual DNA. At our institution, we routinely use both microbial culture and PCR analysis of GP lavage samples to maximize detection of *S. equi* in suspected cases.

Serological diagnosis of *S. equi* infections. A commercially available ELISA detects serum antibodies (IgG) generated against the SeM protein of *S. equi*. During natural infections, serum IgG titers to SeM peak approximately 5 weeks post-exposure and remain high for at least 6 months or more. Responses to commercial extract vaccines (IM) peak around 2 weeks post vaccination and remain high for 6 months. Intranasal (IN) vaccination with the attenuated *S. equi* strain induces a low systemic humoral response but, as expected, a significant increase in nasal mucosal IgA, IgG titers to the SeM protein. ELISA results are reported as negative; weak positive (1:200-1:400); moderate positive (1:800-1:1,600); high positive (1:3,200-1:6,400) and very high positive (> 1:12,800). The ELISA does not allow one to distinguish between immune responses to vaccine versus natural infection with *S. equi*. Overlapping titer breakpoints in normal and convalescent horses also complicate the interpretation of test results. Furthermore, identification of animals with subclinical disease (early in the course of infection or those with persistent infection) is also not possible. Serological tests are generally not considered to be useful in the diagnosis of uncomplicated cases of strangles. The company technical report suggests that the ELISA results may be useful for the detection of horses recently vaccinated as well as horses with purpura hemorrhagica, streptococcal myopathies and/or metastatic strangles. Titers should always be interpreted in the context of clinical findings.

Treatment of the “typical” is still controversial! During the acute phase of the disease when the horse exhibits a fever and depression, penicillin therapy may prevent abscess formation. However, once antimicrobial therapy is stopped and if protective immunity has not been established, the horse will be susceptible to re-infection.

When external lymphadenopathy is evident and the horse remains bright and alert, one approach is to not administer penicillin but rather to hot pack the abscesses and to allow their maturation and eventual rupture. Anti-inflammatories may be necessary to provide pain relief from the pharyngitis. Soft feed materials should be available to the horse.

In cases with retropharyngeal lymphadenopathy and guttural pouch empyema, ensure that the horse has an adequate airway and is able to eat and drink normally without aspirating. For horses experiencing respiratory stridor or respiratory distress, a tracheostomy should be performed and the tube changed at least once a day. In horses with GP empyema or erupted retropharyngeal lymph nodes, GP lavage (2-3
liters of 0.9% saline or polyionic solutions) followed by instillation of a penicillin-gelatin mixture is recommended. At our institutions, we generally perform lavage and local treatment every other day although it may take weeks for lymphadenopathy to resolve. Lavage may also remove small chondroids, but physical removal by endoscopic-guided grabbing forceps, a basket snare or a memory-helical polyp retrieval basket may be required. In cases in which GP involvement (pharyngeal plexus, retropharyngeal lymphadenopathy) predispose the horse to aspiration pneumonia, broad spectrum parenteral antimicrobials (K+ penicillin 22,000 IU/kg q 6h IV; gentamicin 6.6 mg/kg q 24 h IV; metronidazole 25-25 mg/kg q 6-8 h per rectum) and intravenous fluid support (60 mL/kg/24h) are necessary. To prevent endotoxemia, anti-inflammatory therapy (flunixin meglumine 0.25 mg/kg q 8h IV) and laminitis prophylactic measures (cryotherapy, pentoxifylline) should be added to the treatment regimen.

Vaccination. During an outbreak, some clinicians advocate the vaccination of healthy, non-infectious horses that have not been exposed to or have had contact with infected horses in an effort to reduce the spread of the disease. Recall that effective immune responses following vaccination require at least 10-14 days to be generated and that vaccination may lessen the severity of clinical signs but not prevent shedding of the organism. Depending upon the vaccine used, there is also a potential risk of inducing purpura hemorrhagica.

**ATYPICAL CASES OF S. EQUI**

Atypical presentations occur in approximately 20% of the outbreaks and include disseminated (metastatic) strangles, purpura hemorrhagica (PH) and myopathies.

**Internal abscessation (metastatic strangles).** The factors contributing to spread of the organism to the thoracic cavity, brain, mesentry, liver, spleen and/or kidneys are not known. The route of infection—hematogenous or lymphatic; cell associated or free—is also unknown. There is currently no evidence to suggest that the initiation of antimicrobial therapy early in the infection predisposes the horse to the development of internal abscesses.

In a retrospective study of 10 horses diagnosed with *S. equi* internal abdominal abscesses, the chief complaints on presentation included weight loss, fever and colic. The mean duration of illness was 25 days, with nine of the 10 horses having been stabled at facilities which had experienced an outbreak of *S. equi* infection 1-4 months earlier. All of the horses in the study had had signs compatible with strangles. Hematological and serum biochemical abnormalities in affected horses included anemia, neutrophilic and monocytic leukocytosis, hyperfibrinogenemia, hyperglobulinemia and hypoalbuminemia. Peritoneal fluid analysis revealed a neutrophilic leukocytosis (total nucleated cell counts/μL ranged from 19,00 – 166,000) and increased total protein (4.3-7.9 g/dL) but culture of the abdominal fluid was positive in only 1 of the 10 cases. Differentiating abdominal abscesses from neoplasia can be challenging, requiring a myriad of diagnostic techniques such as serology, rectal palpation, abdominal (thoracic) ultrasonography, CT (miniature horse, pony) and possibly nuclear scintigraphy. In the review by Pusterla (2007), abdominal ultrasonography (transabdominal, transrectal or both) in 7 of the 9 cases detected circular encapsulated hypoechoic masses of either homogenous or mixed echogenicity. Serology, performed on 6 of the horses, demonstrated high titers (> 1:3,200) against SeM; in another horse, culture of a draining mandibular lymph node was positive for *S. equi*. Although surgical exploration was not performed in any of the cases from that study, it may be necessary to confirm the presence of suspected abscesses not imaged by ultrasonography. Surgical intervention may allow drainage and/or marsupialization as part of the treatment. In affected horses, a protracted course of antimicrobials (average duration =

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72 days) initially consisting of a beta-lactam (ceftiofur, ampicillin, procaine penicillin, etc) for 7-10 days followed by administration of an oral antimicrobial (chloramphenicol, trimethoprim/sulfa) is recommended. The prognosis is still guarded to poor with survival rates reported to be 40%.

Purpura hemorrhagica (PH) is a type III immune-mediated hypersensitivity vasculitis that occurs following natural infection or vaccination against *S. equi*. The condition has been likened to Henoch-Schönlein purpura in human beings. The prevalence of PH following natural outbreaks is not known. In a retrospective study of 53 horses diagnosed with PH at a veterinary teaching hospital, 17 had confirmed exposure to *S. equi* while 5 had received vaccines with the SeM protein. Clinically affected horses exhibit increased IgA titers to the SeM but as the horse improves, IgG against SeM becomes the predominant isotype. The immunological basis for the high serum IgA titers is not known but might reflect uncontrolled expansion of B cell populations that produce IgA against antigens of *S. equi*; failure of IgA removal mechanisms; delayed, defective or suppressed production of IgG; or neutralization or excess utilization of IgG. The clinical signs of PH range from a mild transient reaction to a severe and fatal form with signs occurring within 8 weeks of a respiratory infection (vaccination). Horses exhibit pitting edema of the limbs, head and ventral abdomen and petechiation and ecchymoses of the mucous membranes. The vasculitis may produce skin sloughing, colic and myopathies. Death may occur as a result of pneumonia, cardiac arrhythmias, renal failure, or a severe infarctive myopathy. Clinical pathological alterations in affected horses include the presence of anemia, hyperproteinemia, hyperglobulinemia, hyperfibrinogenemia, findings similar to those in a “typical” strangles case. Thrombocytopenia is not usually a feature of the syndrome. In horses with PH, serum cardiac troponin I concentrations should be evaluated and monitored for response to therapy. In cases of infarctive myopathy, serum elevations of aspartate aminotransferase and creatine kinase are typically found. The diagnosis of PH is confirmed by skin biopsy (leukocytoclastic vasculitis; acute coagulative necrosis of muscle with evidence of infarctions) and evidence of a current or past *S. equi* infection (culture, PCR, elevated IgA or IgG SeM titers).

Treatment. Horses with PH require intensive management and benefit by being referred to a tertiary care center. Cases are treated with IV fluids (50-60 ml/kg/day), IV sodium or potassium penicillin (22,000 IU/kg q6 h) and IV dexamethasone (0.1 mg/kg q24-36 h). A feeding tube is placed so that enteral nutrition may be provided as needed. Treatment may be necessary for more than 7 days. Clinical recovery occurs as the source of antigen is removed and as the immune response is suppressed.

Myopathies. In addition to the infarctive myopathy of PH, a second type of muscle disorder characterized by acute, severe rhabdomyolysis without evidence of infarction has been described in Quarter Horses exposed to *S. equi*. Affected horses exhibit a stiff gait and rapidly progressing firm, swollen epaxial and gluteal muscles. Most affected horses become recumbent and unresponsive to analgesics, necessitating euthanasia. Clinical pathology data demonstrate a neutrophilic leukocytosis, hyperfibrinogenemia and marked increases in muscle enzymes (CK, AST). Diagnosis is dependent upon histopathological evaluation of muscle biopsies – severe acute myonecrosis with macrophage infiltration. IgG titers against SeM are usually low but titers against the myosin binding protein have been found to be increased in a small number of cases. *S. equi* can be isolated from some cases. It is uncertain if this disorder represents a streptococcal myositis characterized by production of exotoxins or proteases within the skeletal muscle. A high mortality rate has been reported but may be improved if the disorder is recognized early. Suggested treatment approaches include intravenous fluids; intravenous penicillin, high doses of short-acting corticosteroids (prednisolone), analgesics (flunixin meglumine, lidocaine CRI) and aggressive nursing care (deeply bedded stall, sling the horse when possible).
PROPHYLACTIC STRATEGIES

Ideally one should prevent newly acquired and potentially infected animals from co-mining with resident horses. Whenever possible, all new acquisitions should be isolated for at least 3 weeks and screened for *S. equi* by repeated swabs or lavages at weekly intervals.

REFERENCES