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# *Streptococcus equi* Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles—Revised Consensus Statement

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## Streptococcus equi Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles—Revised Consensus Statement

A.G. Boyle , J.F. Timoney, J.R. Newton, M.T. Hines, A.S. Waller, and B.R. Buchanan

This consensus statement update reflects our current published knowledge and opinion about clinical signs, pathogenesis, epidemiology, treatment, complications, and control of strangles. This updated statement emphasizes varying presentations in the context of existing underlying immunity and carrier states of strangles in the transmission of disease. The statement redefines the “gold standard” for detection of possible infection and reviews the new technologies available in polymerase chain reaction diagnosis and serology and their use in outbreak control and prevention. We reiterate the importance of judicious use of antibiotics in horses with strangles. This updated consensus statement reviews current vaccine technology and the importance of linking vaccination with currently advocated disease control and prevention programs to facilitate the eradication of endemic infections while safely maintaining herd immunity. Differentiation between immune responses to primary and repeated exposure of subclinically infected animals and responses induced by vaccination is also addressed.

**Key words:** Equine infectious upper respiratory disease; Guttural pouch; Lymphadenopathy; Nasal discharge.

Disease caused by *Streptococcus equi* in horses, commonly referred to as strangles, was reported by Jordanus Ruffus in 1251. Although the official name of the causative agent is *S. equi* subsp. *equi*, we have decided to use the descriptive term *S. equi* throughout the consensus statement based on its widespread usage in the scientific literature. Strangles is a costly, worldwide, highly infectious upper respiratory disease of the equine. As of 2017, strangles is a reportable disease in the United States and many other countries.

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### Abbreviations:

CK	creatinine kinase
DIVA	differentiating infected from vaccinated animals
iELISA	indirect ELISA
IgG	immunoglobulin G
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
<i>S. equi</i>	<i>Streptococcus equi</i> subsp. <i>equi</i>
TMS	trimethoprim-sulfadiazine

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### Clinical Signs

Infection with *S. equi* is classically characterized by abrupt pyrexia followed by pharyngitis and subsequent abscess formation in the submandibular and retropharyngeal lymph nodes. The disease can occur in horses of any age. In a prospective voluntary surveillance of cases of acute upper respiratory disease with testing of whole blood and nasal swabs via quantitative polymerase chain reaction (qPCR) in the United States, *S. equi* was the most common agent identified in horses of 6–10 years age.<sup>1</sup> However, severity of disease varies greatly depending on the immune status of the animal. 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.

Pyrexia with lethargy become typically the first signs occurring 3–14 days after exposure and before most horses are contagious. The pyrexia is persistent and may exceed

42°C (107.6°F) in some cases.<sup>2</sup> Fever may persist until lymph node abscesses rupture.

A significant pharyngitis frequently accompanies infection with horses reluctant to eat or drink. Many will hold their head in abnormal positions. Nasal discharge is not uncommon with significant pharyngitis. Some horses will develop a soft/mucoid cough, which may be associated with eating. Squeezing the larynx will often cause marked pain, stridor, or gagging followed by coughing. Endoscopy of the upper airway can identify pharyngeal lymphoid hyperplasia and pharyngeal compression from enlarged lymph nodes. Similarly, the nasal and ocular mucosa can become inflamed with purulent ocular discharge from which *S. equi* may be isolated.

Lymphadenopathy is a typical clinical sign. Classically, submandibular and retropharyngeal lymph nodes are involved, although the parotid and cranial cervical lymph nodes are also occasionally involved. Abscesses develop a thick fibrous capsule and typically rupture between 7 days and 4 weeks after infection. The initial evidence of a lymph node abscess is a warm, diffuse swelling. As the abscess matures, serum may ooze from the skin before rupture and drainage of a thick purulent discharge. Depending on the location of the lymph node, the abscess may rupture into the airway or guttural pouch presenting as thick nasal discharge or may erupt externally, through the skin as in the case of the submandibular or parotid lymph nodes. Expulsion of large amounts of discharge from the mouth or nose with coughing, eating, or a lowered head position suggest empyema of the guttural pouch. Parotid and retrobulbar abscesses can cause swelling around the eyelid temporarily obstructing vision. Approximately 50% of horses with guttural pouch empyema exhibit an intermittent unilateral nasal discharge and cough.<sup>3</sup>

Inflammation associated with pharyngitis and lymph node abscess formation/rupture may cause obstruction of the upper respiratory tract (hence the name strangles) necessitating a temporary tracheostomy. Neuropraxia may occur resulting in temporary laryngeal hemiplegia, dysphagia, or both. Damage to the recurrent laryngeal nerve and the subsequent paralysis of the arytenoid cartilage may contribute to the difficulty in breathing associated with upper airway inflammation/swelling. Dysphagia may be noted, occasionally with feed material or water refluxing from the nares.

Not all infections with *S. equi* are confined to the upper respiratory tract with abscesses reported in multiple sites including the brain, abdomen, and mammary gland, with these cases commonly referred to as “bastard strangles.” Lymphangitis of a limb has been observed (B.R. Buchanan, unpublished observations). Additionally, cases of *S. equi* pneumonia have been known to occur.

### Pathogenesis

Upon entering the mouth or nose, *S. equi* attaches to cells within the crypts of the lingual and palatine tonsils and to the follicular-associated epithelium of the pharyngeal and tubal tonsils.<sup>4</sup> There is no evidence of colonization before penetration. Ligands responsible for binding may include exposed surface proteins such as SzPSe. A few hours after infection, the organism is difficult to detect on the mucosal surface, but is visible within epithelial cells and subepithelial tonsillar

follicles. Thus, nasal or nasopharyngeal samples may be culture negative in the early stages of infection. Translocation occurs in a few hours to the mandibular and retropharyngeal lymph nodes that drain the pharyngeal and tonsillar region.

Complement-derived chemotactic factors generated after interaction of complement with bacterial peptidoglycan attract large numbers of polymorphonuclear neutrophils although gross evidence of abscessation is not visible for 3–5 days after *S. equi* enter the lymph node.<sup>5</sup> Failure of neutrophils to phagocytose and kill the streptococci appears to be due to a combination of the hyaluronic acid capsule, anti-phagocytic SeM protein, H factor binding Se18.9, Mac protein, and other undetermined anti-phagocytic factors released by the organism.<sup>6</sup> Final disposal of bacteria is dependent on lysis of the abscess capsule and evacuation of its contents.

Nasal shedding of *S. equi* usually begins 2–3 days after onset of fever and persists for 2–3 weeks in most animals. Some animals that remain without clinical signs and have preexisting immunity never exhibit detectable shedding. In others, shedding may persist much longer should infection persist in the guttural pouch or the sinus.<sup>7–9</sup> Systemic and mucosal immune responses are evident 2–3 weeks after infection and coincide with mucosal clearance.<sup>10</sup>

The infectious dose of organisms propagated in media is probably much higher than that required during natural transmission because virulence factors essential for initial attachment and penetration are more likely to be expressed on in vivo propagated bacteria. (J.F. Timoney, unpublished data). Inocula of less than 10<sup>6</sup> colony forming units are not consistently effective in causing disease because lower numbers of bacteria are likely to be efficiently removed by mucociliary clearance. The larger the intranasal inoculum of cultured *S. equi*, the shorter the incubation period, and the more severe the disease.

If not treated with antibiotics, approximately 75% of horses develop long-term convalescent immunity to strangles as a result of individual immune response as well as natural exposure to disease over time contributing to reboosting and herd immunity.<sup>11–13</sup> Horses in the immediate convalescent phase are resistant to experimental challenge with numbers of *S. equi* greatly exceeding those required to produce the original infection.<sup>10</sup> Approximately 20–25% of convalescent horses become susceptible to a second attack of the disease within several months, which probably represents a failure to produce or maintain an adequate concentration of the relevant mucosal and systemic antibodies.<sup>10</sup> Ongoing exposure to *S. equi* due to the presence of guttural pouch carriers, possibly contributes to the maintenance of increased levels of immunity and extended strangles-free status within isolated herds of previously infected horses.

Older horses with residual immunity, foals with waning maternal antibody protection, and vaccinated animals have limited susceptibility and can develop a mild form of strangles often termed “catarrhal or atypical strangles.” It is important to realize that these animals shed virulent *S. equi* that will produce severe disease in more susceptible, often young, horses.<sup>14</sup>

Milk from mares that have recovered from strangles contains immunoglobulin Gb (IgGb) and IgA with specificities similar to those found in nasopharyngeal mucus of convalescent horses.<sup>15</sup> Suckling foals therefore benefit from the

protective effects of these antibodies until weaned. Colostral antibodies ingested during the first 24 hours of life recirculate to the nasopharyngeal mucosa, thus providing an additional source of protection to the foal during its first weeks.

### Aspects of Pathogenesis Important in Control and Prevention

- Shedding does not usually begin until a day or 2 after the onset of pyrexia making it possible to isolate new cases before they can transmit infection.
- Nasal shedding persists for 2–3 weeks in most animals. Horses may be infectious for at least 6 weeks after their purulent discharges have dried up. Persistent guttural pouch infection may result in intermittent shedding for years.
- Field and experimental data support the conclusion that disease severity is correlated with the dose and frequency of infectious challenge.

## Epidemiology

### Transmission

Active and recovering strangles cases are an important and easily recognizable source of new *S. equi* infections for susceptible horses through their purulent discharges from lymph nodes, nose, and eyes. Transmission of *S. equi* infection occurs when there is either direct or indirect transfer of these purulent discharges between affected and susceptible horses. Direct transmission refers to horse to horse contacts, which occurs through normal equine social behavior involving head-to-head and nose-to-nose contact. Indirect transmission occurs with the sharing of contaminated housing, water sources, feed or feeding utensils, twitches, tack, and other less obvious fomites such as the clothing and equipment of handlers and veterinarians and, anecdotally, even via other animal species.<sup>16</sup>

It is now recognized that transmission originating from outwardly healthy animals may be of greater importance than that from purulent discharges from sick horses in initiating new outbreaks or recurrences in previously affected herds because the source of infection is not obvious. Some horses that are incubating the disease are outwardly healthy and potentially infectious, but do, themselves, go on to develop signs of strangles. It is assumed that nasal secretions are the source of infection in these animals. Also of importance are outwardly healthy convalescent cases that continue to harbor the organism after full clinical recovery.<sup>8,17</sup> It is therefore appropriate to consider that all recovered horses may be potentially infectious for at least 6 weeks after their purulent discharges have dried up. In a proportion of outwardly healthy horses, carriage and at least periodic shedding of *S. equi* occurs for prolonged periods after apparent full and uncomplicated recovery. These horses are commonly referred to as long-term, subclinical *S. equi* carriers and there is strong evidence that they can be a source of new or recurrent disease in well-managed groups of horses.<sup>8,17</sup> Effective strangles control measures require detection, segregation, and treatment of carrier animals.<sup>18–20</sup>

Sequencing genomes of over 200 isolates of *S. equi* has provided a global snapshot of its genetic diversity.<sup>21,22</sup> Persistence in the guttural pouch has been shown to drive both the diversification and decay of its genome (S1).<sup>21,23</sup>

### The Complex Epidemiology of Some Strangles Outbreaks

In most cases, outbreak isolates are highly clonal, consistent with an introduction and onward transmission from a single source.<sup>21,23</sup> However, in some cases, both active and persistent carriage strangles strain were identified in chondroids removed from horses housed in the same stable during a strangles outbreak. Evidence for the persistence of *S. equi* leading to new clinical cases was observed after the genomic analysis of isolates recovered from various large outbreaks.<sup>21</sup>

### Environmental Persistence of *S. equi*

*S. equi* remains viable in water for 4–6 weeks but not in feces or soil. Despite older literature claiming extended survival in the laboratory setting,<sup>24</sup> recent studies using real-world scenarios showed rapid death (1–3 days) of the bacteria on fencing and soil.<sup>25</sup> *S. equi* is sensitive to bacteriocins from environmental bacteria and does not readily survive in the presence of other soil-borne flora.

## Diagnosis

### Bloodwork

Although bloodwork can be variable, a leukocytosis characterized by a neutrophilia found on a complete blood count as well as a hyperfibrinogenemia can be suggestive of infection with *S. equi* when examining an index case and should encourage additional *S. equi*-specific testing.<sup>26,27</sup>

### Sampling

Sensitivity and specificity of testing depends on the stage of infection, the anatomical location from which the sample is taken, the sampling technique, and the testing used.<sup>28–32</sup> See Table 1 for sampling comparison. A needle aspirate from an enlarged or abscessed lymph node is the optimal sample for confirmation of *S. equi* infection; although moistened nasopharyngeal swabs, as well as nasopharyngeal and guttural pouch washes can also be used. As outlined previously under pathogenesis, *S. equi* rapidly invades the lymph nodes of infected horses and is often not isolated from nasal swabs or washes taken during the early stages of disease. Therefore, a negative nasal culture or PCR test does not signify absence of *S. equi* infection—particularly if clinical signs suggest otherwise.

Nasopharyngeal washes had increased sensitivity compared to nasal swabs<sup>29</sup> most likely because a greater surface area within the internal nares is sampled, although these can be problematical as animals can cough and sneeze during sampling leading to loss of potentially contaminated sample into the environment and onto the sampler. Use of rostral nasal swabs may miss the intermittent shedding of *S. equi*



**Table 1.** Comparison of *S. equi* samples.

<i>S. equi</i> Sample	Pros	Cons
Aspirate of mature abscessed lymph node	High yield of bacterial organisms	Requires this stage of disease
Moistened rostral nasal swab <sup>a</sup>	Ease of sampling	Animal needs to have active mucopurulent discharge
Moistened nasopharyngeal swab <sup>a</sup>	Ease of sampling	False negatives possible in early febrile state (not shedding yet) False negatives possible due to intermittent shedding from guttural pouch
Nasopharyngeal wash	Ease of sampling Sampling more surface area Was found to be more sensitive than nasopharyngeal swab <sup>29</sup>	False negatives possible in early febrile state (not shedding yet) False negatives possible due to intermittent shedding from guttural pouch
Guttural Pouch lavage <sup>b</sup>	Best for detection of carrier animals	Special equipment needed Experience entering the guttural pouch More time consuming False negative if lymph nodes have not yet ruptured into the pouch

<sup>a</sup>Synthetic microfiber flocked swabs have not shown increased detection rates over traditional rayon or cotton swabs.

<sup>b</sup>The committee recommends guttural pouch lavage qPCR for the detection of carriers with concurrent visual inspection of the guttural pouch via endoscopy. In order to limit the contamination of the environment and the veterinarians, we recommend collection directly from the guttural pouch, rather than free catch from the nasal passage.

from the guttural pouch into the nasopharynx and are recommended only if there is obvious mucopurulent nasal discharge present that can be readily sampled. The use of flocked swabs (with short fibers arranged perpendicular to the swab shaft) has not improved the recovery of *S. equi* via sampling<sup>29,32</sup> or laboratory processing<sup>28,31</sup> compared to the more commonly used fiber wrapped swabs. Guttural pouch sampling after recovery has been shown to increase the sensitivity of detection of persistent *S. equi* infection compared to repeated nasopharyngeal sampling, but has previously been indicated for confirming subclinical carriage of *S. equi*.<sup>31,32</sup>

Nasopharyngeal sampling involves slowly instilling about 50 mL of warm normal saline either via a 15 cm length of soft latex tubing (5–6 mm diameter) or uterine pipette inserted to the level of the nasal canthus and collecting the washings into a disposable cup or rectal sleeve.<sup>33,34</sup> These washings are centrifuged, and the pellet was tested. Guttural pouch sampling can be performed by introducing 50 mL of warm saline visually via polyethylene tubing using the instrument channel of an endoscope. Sampling blindly via a stiff, bent catheter and recovering the washings can also be performed, although these washings are not necessarily specific to the GPs as the sampling fluid negotiates the pharynx and nasal passages before collection.

### Testing

#### Culture

Specimens should be plated on Columbia CNA (colistin, nalidixic acid) agar with 5% sheep or horse blood added. The presence of other beta hemolytic streptococci, especially *S. zooepidemicus*, may complicate interpretation of cultures. Zoocins produced by *S. zooepidemicus* will kill *S.*

*equi* and so strangles abscesses that rupture quickly can become colonized and dominated by *S. zooepidemicus*. Colonies of commensal *S. zooepidemicus* are also typically nonmucoid whereas fresh isolates from invasive infections are sometimes mucoid. Unlike *S. equi*, *S. zooepidemicus* ferments sorbitol and lactose. Culture provides a slow (results take a minimum of 1–2 days to obtain) but low cost and widely available method to detect *S. equi*, especially when nasal discharges, fever, depression are first noticed in 1 or more animals when they are likely to be actively shedding large numbers of bacteria. Culture may, however, be unsuccessful during the incubation, early clinical phases, and when the bacterial count is low during convalescence. Recovery can be as low as 40%.<sup>28,29,31</sup> *S. equi* is normally not present on the mucosa until 24–48 hours after the onset of fever, and so horses monitored by daily measuring of rectal temperatures during an outbreak may be recognized early and isolated to limit transmission of *S. equi*. Studies highlighting the reduced sensitivity of culture have resulted in most, but not all authors (J.F. Timoney), concluding it is no longer valid as the gold standard for the detection of *S. equi*.<sup>2,28,29,31</sup>

#### Polymerase Chain Reaction

The PCR was originally designed to detect a partial DNA sequence of *SeM*, the gene for the antiphagocytic M protein of *S. equi*.<sup>35</sup> A real-time PCR, otherwise known as quantitative PCR and shortened to qPCR, has since evolved to detect *seeI*, a superantigen-encoding gene.<sup>36</sup> Currently, the *seeI* qPCR is commercially available in the United States. A variety of other sequences and qPCR formats have since been documented in an effort to assure greater specificity and sensitivity including, but not limited to *eqbE* and a triplex qPCR (*eqbE*, SEQ2190, and an internal quality

control).<sup>30,36,37</sup> Since the qPCR test can be completed within 1–2 hours, results may be available on the same day that samples arrive at the laboratory.

Quantitative PCR or other PCR formats used in North America are approximately 3 times more sensitive than culture.<sup>18,35</sup> PCR does not distinguish between dead and live organisms, and so technically false-positive reactions potentially undermine its absolute diagnostic value with respect to detection of actual infection. However, positive experiences of clinical application of qPCR in the diagnosis and control of field outbreaks of strangles in several countries and management settings are testament to the usefulness of qPCR over culture (A.G. Boyle/J.R. Newton/A.S. Waller, unpublished observations). There has been clinical evidence of transmission of strangles from horses with qPCR positive, culture negative guttural pouch lavage samples to naïve animals.<sup>2</sup> Even though, in theory, clinical samples which contain polymerase inhibitors or abundant *S. equi* may give negative PCR results when culture of the same sample confirms the presence of *S. equi*, review of clinicopathologic data in the United Kingdom (J.R. Newton/ A.S. Waller, unpublished data) demonstrates that this is an increasingly rare phenomenon based on use of the triplex qPCR assay.<sup>30</sup>

For practicality, we recommend use of PCR testing of an endoscopically guided guttural pouch lavage for detection of *S. equi* in subclinically infected carrier animals. Visual detection of inflammation of the guttural pouch respiratory epithelium as well as the presence of empyema, chondroids, or enlarged retropharyngeal lymph nodes on the floor of the guttural pouch may suggest strangles even when the lavage is negative for *S. equi*.

### Serology

Different proprietary ELISAs are available for commercial use targeting total IgG antibodies against different *S. equi* surface proteins: SeM and the combined Antigen A (N-terminal fragment of SEQ\_2190 [Se75.3]) and Antigen C (N-terminal fragment of SeM). The purpose of these tests depends on which test is used and in which setting.

#### SeM Antibody Titer

Antibody titers to SeM minus its carboxy terminus (currently commercially available in the United States<sup>a,b</sup> and Europe<sup>c</sup>) peak about 5 weeks after exposure and remain high for at least 6 months.<sup>13,15</sup> Given the possibility that antibodies directed against SzM of *S. zooepidemicus* could cross-react with SeM, incubation of sera with heat-killed *S. zooepidemicus* to remove cross-reactive antibodies was performed to enhance test specificity.<sup>38</sup> However, this process has not been adopted in commercial assays. Considerable variation in the responses of individual horses should be kept in mind when interpreting results of measurement of anti-SeM antibody levels. Horses at risk for development of purpura are hyper-responders and make very strong antibody responses ( $\geq 1:3,200$ ).<sup>39,40</sup> The SeM antibody titer can be used for the following purposes:

- Detection of recent infection evidenced by a 4-fold or greater increase in titer of antibody in paired sera taken 10 days apart.

- To support a diagnosis of existing *S. equi*-associated purpura hemorrhagica (titer  $\geq 12,800$ )
- To support diagnosis of metastatic abscessation (bastard strangles) (titer  $\geq 12,800$ )
- To identify animals with existing high levels of antibody that might predispose to purpura hemorrhagica, especially useful if within 1 year of disease or exposure.<sup>37</sup> Do not vaccinate if value is  $\geq 1:3,200$  (J.F. Timoney, unpublished observations).<sup>39</sup>

Serologic values are not a measure of protection. The SeM-specific titer cannot be used to determine carrier status and a single value is not a measure of active infection. Titers wane over time<sup>14,40</sup> and horses that received antibiotic treatment during an outbreak seem to mount a reduced immune response and remain susceptible to reinfection.<sup>41</sup>

#### Combined Antigen A (SEQ\_2190 N-Terminal Fragment) and Antigen C (SeM N-Terminal Fragment)

An indirect ELISA (iELISA) assay using the N-terminal portion of SeM, which is unique to *S. equi* (antigen C), has been developed to overcome the problem of cross-reactivity with *S. zooepidemicus*.<sup>42</sup> The assay is performed alongside a second iELISA to quantify the levels of antibodies against the *S. equi*-specific portion of SEQ\_2190 (antigen A, also referred to as Se75.3) and a positive result is issued if either or both of the iELISAs exceed the positive cut-off. This resulted in similar sensitivity, but greater specificity when compared to the whole SeM antibody titer.<sup>42</sup> It is currently available in Europe, Australia, and Dubai.<sup>d</sup> The combined Antigen A and C iELISA can be used for the following purposes provided currently available vaccines have not been used in the population of horses:

- To identify recent infection as early as 2 weeks.
- To identify exposed animals without signs that may still be harboring *S. equi*, (carriers) and which may pose an otherwise unsuspected infectious risk to other horses.

A blood sample taken from new arrivals can be used to identify recently exposed or persistently infected horses. If negative, a second blood sample taken 2 weeks later can identify horses that have seroconverted and may have been incubating the infection. If the second sample is also negative and the horse remains free from clinical disease, then it is considered safe to enter the herd. Horses testing positive via the blood test should be investigated further. Ideally, the guttural pouches should be examined by endoscopy to identify signs of persistent infection and a saline wash should be taken for analysis by qPCR. If qPCR tests on these samples are negative then it is considered safe for the horse to enter the herd. If any of the samples test qPCR positive or chondroids, empyema, or both are visible on endoscopy, then debridement followed by topical treatment should be performed. Combined Antigen A and C iELISA and qPCR can be used to identify subclinically infected persistent carriers at the end of an outbreak with it recommended that testing commences no sooner than 3 weeks after resolution of signs in the last clinical case, in order to allow animals that will

clear short-term GP infections to do so without further intervention.

## Vaccination

### *Extract Vaccines*

Currently a single purified M-protein antigen extract vaccine is available in the United States,<sup>c</sup> StrepvaxII, which elicits serum antibody responses 7–10 days later.<sup>15</sup> Naïve horses require a schedule of 3 doses at an interval of 3 weeks. Booster doses are given once annually. An additional booster at 6 months of age is recommended for foals when the initial series is started at less than 3 months of age. Pregnant mares may be boosted a month before expected date of foaling. Although potent with respect to SeM, efficacy of extract vaccines in field studies has been disappointing. A reduction in clinical attack rate of only 50% was reported in vaccinates a few weeks after the final booster.<sup>43</sup> Adverse reactions include soreness or abscesses at injection sites and occasional cases of purpura hemorrhagica.

### *Attenuated Live Vaccines*

An attenuated live, intranasal vaccine,<sup>f</sup> Pinnacle IN, should be administered only to healthy nonfebrile animals free of nasal discharge. The vaccine is given in a schedule of 2 doses at 2–3 week intervals. Annual booster doses are recommended. The intranasal mode of application should be such that an adequate amount of vaccine reaches the pharyngeal and lingual tonsils. Safety issues include residual virulence with formation of slowly developing mandibular abscesses in a proportion of vaccinates,<sup>44–46</sup> nasal discharge, and occasional cases of immune-mediated vasculitis (purpura). Since the vaccine contains live *S. equi*, accidental contamination of remote injection sites or metastatic (hematogenous/lymphatic) transfer from the pharynx will result in abscess formation at these locations. For that reason, ideally no other vaccinations are given concurrently and invasive procedures such as joint injections, dental prophylaxis, and castrations should not be performed during the same visit. No data are available on the effect of concurrently administering a different intranasal vaccine. Vaccination with the modified live intranasal vaccine is not recommended in foals less than 1 year of age due to risk of significant clinical disease (fevers and lymph node enlargement) and increased shedding of the vaccine strain.<sup>44</sup> A genetic deletion has provided a reliable means of identifying the vaccine from wild-type strains using colony characteristics and PCR.<sup>46,47</sup> The live vaccine should not be used during an outbreak except in horses with no known contact with infected or exposed animals. There are no published data to show that vaccination in the face of exposure is detrimental, but there is a risk of transmitting the virulent, wild *S. equi* to other horses as they are vaccinated. Horses that have received intranasal vaccine may test positive on PCR for up to 6 weeks.

Horses known to have had strangles within the previous year or that have signs of strangles should not be vaccinated. *S. equi*-specific serum antibody levels of horses within a year of an outbreak should be assayed before decision to vaccinate.<sup>40</sup> Animals with titers of 1:3,200 or greater in the SeM ELISA should not be vaccinated due to increased risk

of purpura hemorrhagica (J.F. Timoney, unpublished observations).

A live attenuated *aroA* deletion mutant of *S. equi* vaccine,<sup>g</sup> Equilis StrepE, is intermittently available in Europe for administration submucosally on the inside of the upper lip. Immunity to experimental challenge persists for about 3 months. The vaccine lacks DIVA (Differentiating Infected from Vaccinated Animals) capability, which has been demonstrated in serological monitoring of 1 outbreak after use of this vaccine (J.R. Newton, unpublished data). Painful reactions at injection sites may occur.<sup>48</sup> Bacterial replication of the vaccine strain has resulted in rare cases of clinical disease in vaccinated horses.<sup>49</sup> Accidental veterinarian self-injection has occurred.<sup>50</sup>

### *Efficacy of Multicomponent Vaccines*

A multicomponent vaccine, Strangvac,<sup>h,51</sup> in development in Sweden has DIVA capability. Challenge studies with a prototype of this vaccine<sup>52</sup> were completed in September 2016 demonstrating a significant reduction in the severity of disease 2 weeks and 2 months after the initial series of 2 intramuscular injections and 2 weeks post-booster vaccination given 3 months after the initial series of 2 intramuscular injections.<sup>52</sup>

### *Genomic S. equi Evaluations of Potential Relevance to Vaccine Strain Selection*

In genomic studies of *S. equi* strains, 3 major groups of *S. equi* were described. Isolates from commercially available live attenuated vaccines and the Se1866 isolate (the basis of the multicomponent vaccine<sup>h</sup>), were included in the genomic studies to determine the relationships of these vaccines to the currently circulating population of *S. equi*. Interestingly, the increasingly dominant strains in the United Kingdom grouped together, but were genetically distant from the available live attenuated vaccine strains.<sup>53</sup> These data suggest that studies are required to determine how well existing vaccines such as Pinnacle, Strepvax, and Equilis StrepE confer cross-protection against currently circulating strains.

### *Maternal Antibodies after Vaccination*

Transfer of passive immunity to the foal mainly involves antibodies of the IgG<sub>b</sub> isotype, which are distributed to the serum and nasal secretions. Prepartum extract vaccination of the mare significantly increases colostral levels of these antibodies. Foals from vaccinated mares have significantly higher titers of SeM-specific IgG<sub>b</sub> but not IgA in mucosal washes during the first 2 months of life, although colostral levels of SeM-specific IgA are significantly increased by vaccination. Resistance of the foal to strangles during the first months of life appears to be mediated by IgG<sub>b</sub> in mucosal secretions and milk and not by IgA. There are no data available about colostral antibody levels after administration of the intranasal or multicomponent vaccine administered to broodmares.

There is no current consensus on the use of vaccines to prevent *S. equi*. This is limited by geographical restrictions,



varied experience, current lack of accepted proven efficacy, and DIVA capabilities with the latest diagnostic assays.

## Prevention

### *Quarantine and Screening*

Limiting exposure still remains the best method to prevent *S. equi* infections. Biosecurity measures should include: quarantine and screening of all new arrivals, appropriate disinfection and cleaning of potentially contagious equipment, and education of caretakers on proper hygiene.

Appropriate quarantine can be challenging on farms where there is frequent movement of horses during breeding, racing, or show seasons. New arrivals should be isolated for at least 3 weeks. Additional screening for subclinical carriers by guttural pouch endoscopy, culture, and PCR testing should be part of any screening program. If the animal is known to be unvaccinated and lives in a country where there is access to the combined SEQ\_2190 and SeM serology test, screening can be implemented as previously described under “Serology” section.<sup>42</sup>

### *Control of Outbreaks*

At the beginning of a suspected outbreak, a detailed history should be collected from the horse owners, stable managers, and caretakers. Questions pertaining to travel history, management practices, and vaccine history are important. The facility should be evaluated with the owner or manager to discuss and develop a plan that is both logical and practical. The objectives should include identification and segregation of infected horses to prevent further spread of infection, including identifying any subclinical *S. equi* carriers and compliance with local laws regarding reporting and movement restrictions. The general aims and measures for such a strategy are outlined in Table 2.

### *Detection of Carriers with S. equi Infection of the Guttural Pouches*

Overall an average of 10% of horses in strangles outbreaks experience apparent failure of the guttural pouch drainage mechanism resulting in persistent GP empyema.<sup>17,18</sup> A recent retrospective of 108 strangles cases found that 25 of 62 (40%) cases  $\geq$  40 days after initial diagnosis were positive by culture or PCR assay of nasopharyngeal or guttural pouch lavage samples. The median duration of positivity was 60 days (interquartile range 40–75 days).<sup>27</sup> Guttural pouch pathology associated with *S. equi* may persist subclinically for many months or even years.<sup>8,9,17</sup> In these long-standing cases, pus in the pouches inspissates and then eventually forms into discrete, ovoid, smooth concretions known as chondroids that harbor viable *S. equi* on their surface and within their core.<sup>8</sup> Detection of guttural pouch empyema with or without chondroids after strangles is best achieved by direct visual assessment of both pouches using endoscopy. Combined culture and PCR testing of lavage samples collected via a sterile disposable catheter passed through the biopsy channel of the endoscope are recommended to accompany visual examination since infection

may be present in the absence of visible pathology. Diagnosis of guttural pouch empyema with or without chondroids may also be made by radiography of the guttural pouch area, although changes may not be visible in all cases. Identification, treatment, and elimination of guttural pouch *S. equi* carriers has been proven to be effective in eradicating infection in a herd.<sup>18,20</sup> *S. equi* has been cultured from lavages collected by direct percutaneous sampling of the pouch although this is not recommended because of the high risk of injury to important anatomical structures in the region.

### **Treatment of Carriers with *S. equi* Infection of the Guttural Pouches**

The method of treatment of guttural pouch empyema depends on the consistency and volume of the material within the pouches. Repeated lavages of pus-filled pouches via rigid or indwelling catheters using isotonic saline or polyionic fluid accompanied by subsequent lowering of the head to allow drainage or use of a suction pump attached to the endoscope, aid the removal of pus. Sedation aids in implementation of endoscopy and facilitates drainage of flush material from the guttural pouches by lowering the horse's head.

Both topical and prolonged systemic benzylpenicillin administration (10 days) appear to improve treatment success rate. Verheyen et al<sup>20</sup> reported on the method of delivering a gelatin/penicillin mix. A solution of 50 mL gelatin/penicillin is made as follows:

- Weigh out 2 g of gelatin (Sigma G-6650 or household grade) and add 40 mL sterile water.
- Heat or microwave to dissolve the gelatin.
- Cool gelatin to 45–50°C.
- Meanwhile add 10 mL sterile water to 10,000,000 units (10 Mega) sodium benzylpenicillin G.
- Mix penicillin solution with the cooled gelatin to make a total volume of 50 mL.
- Dispense into syringes and leave overnight at 4°C to set.

The gelatin–penicillin mix is retained in the pouches longer than a straight aqueous solution and is a useful way of delivering a large dose of penicillin where it is needed. Installation is easiest through a catheter inserted up the nose and endoscopically guided into the pouch opening or through polyethylene tubing via the endoscope. The catheter works best with the last 2.5 cm bent at an angle to aid entry under pouch flap. Recommendations include elevating the horse's head after infusion. Repeat infusions may be required for more refractory cases.

Topical installation of 20% (w/v) acetylcysteine solution has also been used to aid the treatment of empyema. Acetylcysteine has a denaturing and solubilizing activity by disrupting disulfide bonds in mucoprotein molecules, thus reducing mucus viscosity and so theoretically facilitating natural drainage. Erythema of the mucus membranes lining the guttural pouch has been observed after installation of 20% (w/v) acetylcysteine solution. More long-standing cases in which there is inspissation of the purulent material

**Table 2.** Aims and associated measures used to aid in control and transmission of *S. equi*.

Aim	Measure
1. To prevent the spread of <i>S. equi</i> infection to horses <sup>a</sup>	<ul style="list-style-type: none"> <li>• Quarantine new arrivals for 3 weeks. Additional screening for subclinical carriers by guttural pouch endoscopy, culture, and PCR testing should be part of any screening program. If animal is known to be unvaccinated and is located in a country that has access to the combined SeM and SEQ_2190 serology, then seropositives should be identified and investigated further via endoscopy (See “Serology” section).</li> <li>• Stop all movement of horses on and off the affected premises immediately. Quarantine should last for a minimum of 3 weeks <i>past the resolution of the last clinical case and all cases declared <i>S. equi</i> negative</i>. Animals may be infectious for 6 weeks after discharges clear. Persistent guttural pouch infection may result in intermittent shedding for years.</li> </ul>
2. To prevent indirect cross infection by <i>S. equi</i> from horses in the “dirty” area to those in the “clean” area of the premises	<ul style="list-style-type: none"> <li>• If <i>S. equi</i> infection is suspected, the horse should be isolated immediately to minimize the risk of transmission to in contact animals.</li> </ul>
3. Cohorting	<ul style="list-style-type: none"> <li>• <i>Create 3 color-coded groups</i>, even if limited space dictates that horses must remain in the same paddock only separated by 2 layers of electric fence to avoid nose to nose contact. The red group should include horses that have shown 1 or more clinical signs consistent with strangles. Horses in the amber group are those that have had direct or indirect contact with an infected horse in the red group and may be incubating the infection. The remaining horses, in the green group, are those which have had no known direct or indirect contact with affected animals.</li> <li>• The rectal temperature of all horses in the green and amber groups should be measured twice daily and any febrile horse should be moved to the red group.</li> <li>• Clearly color-coded buckets and other equipment should be used to ensure that indirect mixing between groups does not occur. Eliminate all sharing of water and disinfect water and feed buckets daily.</li> <li>• Wherever possible, dedicated staff should be used for each color-coded group. If separate staff are not an option, staff should always move from the lowest risk to highest risk groups, that is, from green to amber to red groups in that order and not back again.</li> </ul>
4. To establish whether convalescing horses are infectious <i>after</i> clinical recovery.	<ul style="list-style-type: none"> <li>• Testing for potential carrier status should begin no sooner than 3 weeks after resolution of clinical signs or potential exposure with no clinical signs.</li> <li>• Testing horses that have been treated with antibiotics should not commence before 3 weeks after the cessation of antibiotic treatment.</li> <li>• If nasal discharge persists longer than 2 weeks, guttural pouch examination is indicated to identify horses that may have empyema and require additional treatment.</li> <li>• 1 endoscopically guided guttural pouch lavage qPCR on cases and their contacts to screen for carriers provides increased efficiency and sensitivity over 3 nasopharyngeal washes over 3 weeks. All equipment must be disinfected between horses when sampling multiples on a farm.</li> <li>• Continual positive tests despite endoscopically normal guttural pouches should be considered infections and thought given to treatment with systemic antibiotics and sinus radiography. Sites such as the sinuses should be considered in horses that continue to harbor <i>S. equi</i> in the absence of pathology. Purulent discharge at the nasomaxillary opening should be sampled if noted on endoscopy. Sinuscopy and qPCR testing of sinus lavage is possible, but obviously invasive.</li> <li>• If the outbreak is located in a country that has access to the combined SeM and SEQ_2190 serology, then seropositives identified in the in-contact population can be further investigated via endoscopy (See “Serology” section).</li> <li>• Animals are considered safe to move out of isolation on the basis of absence of obvious guttural pouch pathology in conjunction with negative guttural pouch lavage qPCR results.</li> </ul>

<sup>a</sup>There is no current consensus on the use of vaccines to prevent *S. equi*. This is limited by geographical restrictions, varied experience, current lack of accepted proven efficacy, and DIVA (Differentiating Infected from Vaccinated Animals) capabilities with the latest diagnostic assays (See Vaccination).

that does not readily drain into the pharynx are more difficult to treat topically as they can be refractory to large volume irrigation. Use of a memory-helical polyp retrieval basket through the biopsy channel of the endoscope does allow non-surgical removal of chondroids, even when present in very large numbers and in conjunction with empyema. When combined with topical and systemic antimicrobial treatment, this is usually sufficient for cure of severe guttural pouch lesions. Surgical hyovertrebrotomy

and ventral drainage through Viborg’s triangle carries inherent risks of general anesthesia and surgical dissection around major blood vessels and nerves and *S. equi* contamination of the hospital environment. This procedure has been described standing, enabling it to be performed in the isolation setting.<sup>54</sup> Scarring of the pharyngeal openings of the guttural pouch may preclude both natural drainage of purulent material and endoscopic access to the guttural pouches. Such cases may require conventional surgical or

endoscopically guided laser treatments to break down scar tissue and allow access to the pouches.

### Biosecurity

Particular care should be taken with biosecurity measures during strangles outbreaks to prevent indirect transfer of *S. equi* from infectious horses (including potential subclinical carriers) to susceptible animals (see Table 2). Dedicated personnel and equipment must be available. Manure and waste feed from infectious animals should be composted in an isolated location.

It is important to adequately disinfect all potentially contaminated facilities and equipment. Surfaces should be cleaned with a foaming soap agent to remove organic material, rinsed and then thoroughly soaked in an appropriate liquid disinfectant used according to the manufacturer's guidelines and allowed to dry. Use of high pressure systems create risk of aerosolization of bacteria.<sup>55</sup> Wooden surfaces need adequate drying time before treatment with paint or creosote. Replacement with new or alternative material may be most appropriate. Although there is no evidence for prolonged survival of *S. equi* on pastures, those used to hold infectious animals should be rested for several weeks after animals are removed to allow denaturation of *S. equi* through the effects of drying and direct sunlight, which are best achieved during hot dry periods of prevailing weather. Exposure to direct sunlight has been shown to be beneficial, as cultured *S. equi* was shown to survive less than 24 hours on wood, rubber, and metal surfaces when in direct sunlight.<sup>25</sup> Horse vans should be cleaned and disinfected after each use. Stalls should be held open after cleaning/disinfection to allow for adequate contact time with disinfectant and ideally through thorough drying of the surfaces.

*Streptococcus spp.*, including *S. equi*, are relatively susceptible to disinfection. Some products commonly used include hypochlorites (primarily household bleach), quaternary ammonium compounds,<sup>i</sup> phenolic compounds,<sup>j</sup> potassium peroxymonosulfate,<sup>k</sup> and accelerated hydrogen peroxides.<sup>l</sup> Chlorine compounds and quaternary ammoniums are not active in the presence of organic material, therefore it is particularly important to thoroughly clean the surfaces first. Diluted bleach and foot baths contaminated by organic debris quickly become inactivated.<sup>56</sup>

### Zoonotic Risks

Cases of *S. equi* infection in debilitated humans have been reported, but are rare since *S. equi* is highly host adapted.<sup>57–60</sup> Animal handlers, care takers, veterinary practitioners, pathologists, and equine post mortem attendants should take particular care to avoid unnecessary contamination from infectious horses, especially avoiding respiratory and oral contamination by purulent material.

### Treatment

Appropriate treatment of horses with strangles usually depends on the stage and severity of the disease. The majority of strangles cases require no treatment other than proper rest and a dry, warm stall and provision of soft, moist, and

palatable food of good quality while letting the disease run its course. Food and water should be easily accessible to the horse. In conditions of high summer temperature acute febrile cases in stalls should have fan assisted ventilation.

Veterinary opinion as to whether or not to use antibiotic treatment remains markedly divided.<sup>61</sup> Clinical and experimental evidence evaluating the effects of antibiotic use in strangles is limited, and there are no reported prospective studies comparing horses treated with antimicrobials to untreated horses. In many cases, antibiotics are unnecessary, and several potential concerns regarding their use have been put forward. Some of these concerns include a delay in maturation of abscesses or a recurrence of abscesses when antibiotics are discontinued. Conclusive data regarding the role of antibiotics on increasing the risk of occurrence of metastatic abscesses ("bastard strangles") is lacking. In addition, antibiotic treatment could potentially inhibit the synthesis of protective antigens and affect the bacterial cell wall, which in turn could diminish the development of protective immunity.<sup>41</sup> A suboptimal immune response could leave horses susceptible to reinfection.

Antibiotics may be indicated in some cases, although these are always at the discretion of the attending veterinarian including

- acutely infected animals with very high fever and malaise before abscess formation,
- horses with profound lymphadenopathy and respiratory distress,
- horses with metastatic abscessation,
- cases of purpura hemorrhagica treated with corticosteroids,
- guttural pouch infections treated locally and systemically to eliminate the carrier state.
- Antibiotics should *NOT* be used as a preventative in animals that may have been exposed. Overuse of antibiotics, promotes resistance, provides a false sense of security, and convalescent immune responses may not be induced.

### Horses with Early Clinical Signs

During an outbreak, immediate antibiotic therapy of new cases in the early acute phase of infection with fever and lethargy may be curative and may prevent focal abscessation. Usually, the time and dose of natural infection is not known, thus making it difficult to determine the duration of treatment needed for these animals. Premature cessation of antibiotics can therefore result in prolonged disease rather than shortening. Good infection control and biosecurity is essential to prevent re-exposure after discontinuation of treatment.

### Horses with Lymph Node Abscessation

Once external lymphadenopathy is detected, antibiotic therapy is generally ineffective. These cases primarily require good nursing care. Specific therapy should be directed toward enhancing maturation and drainage of the abscesses. Topical treatments such as ichthamol or a hot pack

may be applied to promote maturation of the lymph node abscess. Surgical drainage of lymph node abscesses may be indicated if the abscesses do not rupture spontaneously; however, it is critical to wait until the abscess has matured and thinned out ventrally. Earlier surgical intervention may only result in minimal exudate drainage and continued lymph node swelling, because the abscess has enough internal structure (honeycomb loculations) to block drainage through a single surgical incision. Daily flushing of the open abscess with a 3–5% povidone iodine solution should be continued until the discharge ceases.

The use of non-steroidal anti-inflammatory medications such as phenylbutazone or flunixin meglumine may improve the horse's demeanor by reducing fever, pain, and inflammatory swelling at the site of the abscesses. This may in turn encourage eating and drinking. Consideration must be given to the complications seen after the use of non-steroidal anti-inflammatory medications in dehydrated and anorectic horses. Rarely, affected horses may require intensive supportive therapy, including intravenous fluids, feeding by nasogastric tube, and tracheostomy.

Antibiotic therapy is indicated to decrease abscess size and prevent complete airway obstruction in cases with signs of respiratory distress including stridor. An animal requiring a tracheostomy should be given systemic antimicrobial drugs to prevent secondary bacterial infections of the lower respiratory tract. Ideally, the duration of any treatment should be guided with biweekly or weekly serial measurements of inflammatory proteins, such as fibrinogen, to ensure long enough treatment has been implemented.

### **Drugs of Choice for Treatment**

Penicillin [22,000–44,000 iu/kg bwt IM q12h or IV q6h] is generally considered the drug of choice for the treatment of non-pneumococcal streptococcal disease, with the selection of alternative drugs depending on susceptibility, ease of administration or the site of infection. *S. equi* is consistently susceptible to penicillin. Laboratories (J.F. Timoney and J.R. Newton, personal communications) handling hundreds of *S. equi* strains have noted no emerging antibiotic resistance to penicillin by *S. equi* or *S. zooepidemicus*. In general, the incidence of resistance to most other drugs is low in *S. equi* with the exception of aminoglycoside resistance, including gentamicin, which is consistently observed.<sup>62</sup> Recently, an increase in the percentage of all streptococcal species resistant to enrofloxacin has been reported.<sup>63</sup>

The use of alternatives to penicillin therapy is often driven by concerns about ease of administration, especially in those horses that may require long-term treatment. Susceptibility testing may help in antibiotic selection. Common alternatives to penicillin include cephalosporins and macrolides when age appropriate. Extra-label usage of ceftiofur has been advocated for the treatment of *S. equi* when antibiotics are indicated. Isolates of both *S. zooepidemicus* and *S. equi* have been shown to be susceptible to ceftiofur in vitro and the sustained release ceftiofur suspension has been shown to be effective in the treatment of lower respiratory tract infection associated with *S. equi* subspecies *zooepidemicus*.<sup>64–66</sup> Other than one anecdotal report of long acting ceftiofur

[6.6 mg/kg IM q96h] use during a large strangles outbreak improving treatment compliance and resulting in final resolution, there are no data on its efficacy in vivo.<sup>67</sup> In order to honor antibiotic use stewardship, cephalosporins should be reserved for those animals in which compliance is difficult.

Anecdotal reports of the efficacy of trimethoprim-sulfadiazine (TMS) [30 mg/kg PO q12h] in the treatment of strangles are variable. Based on in vitro antimicrobial susceptibility testing where testing methods follow the Clinical and Laboratory Standards Institute's guidelines, the majority of *S. equi* isolates are susceptible to TMS.<sup>68,69</sup> However, this may not translate into in vivo efficacy. While there is evidence that TMS did not eliminate *S. zooepidemicus* infection in tissue chambers implanted SC in ponies, the study did not determine its effectiveness against *S. equi*.<sup>70</sup> TMS has been seen to fail repeatedly in the treatment of strangles (A.G. Boyle, unpublished observations).

### **Complications Associated with *S. equi* Infection**

The overall complication rate increases with duration and intensity of exposure and may be as high as 20%.<sup>71,72</sup> Isolation of infectious horses is therefore critical in reducing the complication and case fatality rates. In a study in which data were collected from a farm with 235 horses; 74 horses had strangles, 15 of which (20.3%) had complications.<sup>71</sup>

Overall case fatality rates can be as high as 8.1%<sup>69</sup> to 9.7%<sup>67</sup> on large farm outbreaks. A more recent field study comparing 108 strangles cases from smaller farms (<50 horses) to 215 cases with fevers of other origin over a 7-year period found a much lower strangles case fatality rate of 0.9% compared to the controls (3.2%).<sup>27</sup>

A variety of complications can occur as a result of strangles. These can be generally grouped as:

- Those associated with the spread of infection from the head and neck region to other locations excluding the guttural pouches (an inevitable percentage of animals in which retropharyngeal lymph node abscessation will burst into the guttural pouch as the weakest line of resistance).
- Immune-mediated processes, including purpura hemorrhagica and myopathies

Another rare complication of strangles is extension of infection to the sinuses.<sup>7,8,20</sup> Horses with infection in the sinuses may become carriers. Other reported sequelae associated with *S. equi* infection include anemia,<sup>26</sup>agalactia, myocarditis, endocarditis, panophthalmitis, periorbital abscesses, ulcerative keratitis, paravertebral abscesses, meningitis, funiculitis, septic arthritis, and tenosynovitis.<sup>26,73–77</sup>

### **Complications Associated with Metastatic Spread of Infection**

*S. equi* may potentially infect any anatomic site. The term bastard strangles is often used to describe metastatic abscessation. Spread of the organism may occur through several routes, including hematogenous spread, lymphatic migration, or via close association with a septic focus, for



example, when connecting structures, such as cranial nerves, allow transport of the organism or when there is direct aspiration of purulent material into the lower respiratory tract.

Common sites of infection include the lung, mesentery, liver, spleen, kidneys, and brain. Respiratory distress may occur due to tracheal compression resulting from enlargement of the cranial mediastinal lymph nodes. Suppurative bronchopneumonia is one important sequela of strangles. Of 15 horses with complications associated with strangles, 5 had pneumonia or pleuropneumonia, 3 of which resulted in death.<sup>71</sup> In an earlier study, 22/35 cases with complications (62%) died of pneumonia secondary to strangles.<sup>72</sup>

The diagnosis and treatment of *S. equi* infections that have spread are potentially more difficult than in cases of uncomplicated strangles. The specific means of diagnosis vary depending on the site of infection and whether there are concurrent signs of classic strangles. For infections such as bronchopneumonia, guttural pouch empyema or sinusitis, appropriate samples can be collected for laboratory testing as considered appropriate by the attending veterinarian. However, for some internal abscesses, a specific diagnosis may be difficult. A history of exposure to *S. equi*, intermittent low-grade fevers responsive to penicillin, very increased SeM specific antibody titers, and laboratory results consistent with chronic infection, such as anemia, hyperfibrinogenemia, neutrophilia, and hyperglobulinemia, are supportive of the diagnosis of metastatic abscessation. Mesenteric abscesses may be accompanied by an immune ascites with increased SeM-specific antibody in ascitic (peritoneal) fluid (J.F. Timoney, unpublished observations). Treatment of *S. equi* infection that has spread frequently involves long-term antimicrobial therapy, and appropriate local treatment or drainage of abscesses if possible.<sup>78,79</sup>

The prevalence of metastatic abscessation is generally low.<sup>27</sup> However, in a study in which outbreaks of strangles on 2 farms were investigated, 7/25 (28%) developed metastatic abscessation.<sup>80</sup> Of these, euthanasia was performed in 5 horses, 4 of which had neurologic signs and confirmed cerebral abscesses. The reason for the high incidence of complications, and particularly neurologic disease, on these farms is unclear, but possible theories include a high infectious dose, the virulence of the strains involved, and differences in host susceptibility. None of the horses were on antibiotics before complications were identified. Again, while antimicrobial therapy has been hypothesized to be a risk factor for metastatic abscessation, data supporting this theory are lacking,<sup>61</sup> and it is clear that metastatic abscessation can occur without prior antimicrobial therapy.

### ***Immune-Mediated Complications***

#### ***Purpura Hemorrhagica***

Purpura hemorrhagica is an aseptic necrotizing vasculitis characterized primarily by edema and petechial or ecchymotic hemorrhage. While the exact pathogenesis of purpura hemorrhagica is not fully understood, it appears to be a vasculitis caused by the deposition of immune complexes in blood vessel walls. Although commonly associated with *S. equi* infection, purpura may also occur in response to a number of non *S. equi* antigens. Of 53 horses with purpura, 17

were exposed to or infected with *S. equi* and 5 were vaccinated with *S. equi* M protein, while the remaining 31 cases were either associated with other organisms or had no known causes.<sup>81</sup>

The risk of developing purpura hemorrhagica after exposure to *S. equi* through infection or vaccination is not known. Four of 74 horses with strangles developed purpura and all 4 were male yearlings that had been vaccinated with an M protein vaccine and all developed signs of purpura hemorrhagica within 2–6 days after the onset of strangles.<sup>71</sup> A preexisting high serum antibody titer to *S. equi* antigens or exaggerated immunological response to *S. equi* may predispose horses to the development of purpura hemorrhagica.

### ***Clinical Signs and Diagnosis of Purpura Hemorrhagica***

The severity of clinical signs seen with purpura varies from a mild, transient reaction to a severe, fatal disease.<sup>81</sup> The typical clinical signs seen as a result of the vasculitis include subcutaneous edema, most frequently involving the head; limbs, trunk, or both; and petechiation and ecchymoses of the mucous membranes. Severe edema may result in oozing from the skin surfaces and sloughing of the skin. In some cases, the vasculitis may affect other sites such as the gastrointestinal tract, lungs, and muscle, resulting in signs such as colic, respiratory difficulties and muscle soreness. Multiple small-intestine intussusceptions were reported as a complication of purpura hemorrhagica in a horse.<sup>82</sup> Intussusception is a well-known complication in children with Henoch-Schoenlein purpura, a human autoimmune disease that resembles purpura hemorrhagica in horses.

Leukocytoclastic vasculitis on histologic exam of skin biopsy is consistent with a diagnosis of purpura hemorrhagica. In those cases associated with *S. equi*, isolation of the organism and demonstration of increased IgA and IgG titers to SeM are also supportive.

### ***Treatment and Prognosis of Purpura Hemorrhagica***

Corticosteroids are the primary treatment for purpura. Generally, dexamethasone at 0.1–0.2 mg/kg is used followed by a tapering dose regime over 2–4 weeks, reducing by 15% every 2 days.<sup>81</sup> In those cases where purpura is associated with active bacterial infection or the horse is considered at high risk of developing infection, appropriate antibiotic therapy is also indicated. Non-steroidal anti-inflammatory drugs may be of some benefit in some cases of purpura. Supportive care, including intravenous fluids, hydrotherapy, and bandaging may also be indicated. The majority of the 53 horses with purpura were treated for more than 7 days.<sup>81</sup>

Purpura hemorrhagica can be a serious often fatal complication of strangles. One of the 4 cases with purpura were euthanized due to the severity of the skin necrosis.<sup>71</sup> Similarly, 3 of 22 horses with purpura secondary to exposure to *S. equi* did not survive.<sup>81</sup>

### Myositis

Three types of myopathies have been documented in horses after exposure to *S. equi*: muscle infarctions,<sup>83</sup> rhabdomyolysis with acute myonecrosis and presence of *S. equi*,<sup>84</sup> and rhabdomyolysis with progressive atrophy.<sup>85</sup>

#### Muscle Infarctions

This syndrome is most likely a severe manifestation of purpura hemorrhagica. Many horses with purpura exhibit mild elevations in serum creatine kinase (CK) activity due to immune-mediated vasculitis within the muscle and mild muscle necrosis, but these horses will have marked increases. Titers of SeM-specific antibody may exceed 1:6,400. Some horses develop a severe vasculopathy characterized by infarction of skeletal muscle, skin, gastrointestinal tract, and lungs.<sup>83,86</sup> Horses present with muscle stiffness, lameness, and elevations in muscle enzymes in conjunction with other signs, such as abdominal pain and subcutaneous swelling. On histopathology, there is acute coagulative necrosis of muscle with infarctions. Also, pulmonary hemorrhage and gastrointestinal infarctions may be present. Even with aggressive corticosteroid therapy and antibiotics, the prognosis is guarded.

#### Rhabdomyolysis with Acute Myonecrosis

Acute severe rhabdomyolysis has been described in horses with clinically evident strangles.<sup>84</sup> Affected horses develop a stiff gait and become recumbent. Swelling and pitting edema may be present along the epaxial and gluteal muscles. Clinicopathologic findings include mature neutrophilia, hyperfibrinogenemia, and marked elevations in CK and aspartate aminotransferase. Large multifocal, pale, friable areas are present in affected muscle at necropsy. *S. equi* are visible in sections of affected muscle. While the mechanism is not known, it has been hypothesized that the rhabdomyolysis is due to either an inflammatory cascade similar to streptococcal toxic shock syndrome or potentially direct toxic effects of *S. equi* in muscle tissue.

#### Rhabdomyolysis with Progressive Atrophy

Significant rhabdomyolysis with muscle atrophy has been identified in 4 Quarter Horses after exposure to *S. equi*.<sup>86–88</sup> Some of these horses had underlying polysaccharide storage myopathy and developed rhabdomyolysis while ill. Others developed myositis without an underlying problem and exhibited malaise and a rapidly progressive atrophy of the epaxial and gluteal muscles. Muscle enzymes were increased and muscle biopsies revealed chronic active rhabdomyolysis with regeneration, prominent macrophage infiltration, atrophy of fast-twitch fibers, and lymphocytic vasculitis.<sup>86</sup> Fibrosis developed around blood vessels. The presence of concurrent signs of strangles was variable.

Horses affected with myositis should be treated with corticosteroids. In cases with atrophy, muscle mass may return to normal. If there are signs consistent with concurrent infection, antibiotics are also indicated.

### Myocarditis

Streptococcal antigens have been suggested as a trigger for development of myocarditis<sup>72,83</sup> which may account for

electrocardiographic abnormalities reported in convalescent horses in Sweden.<sup>89</sup>

### Concluding Remarks Including Future Directions

Our knowledge of *S. equi* including the mechanisms it employs to cause disease is growing at an unprecedented rate. The importance of identifying and treating persistently infected horses is clear at both national and international levels. However, there remains a relatively low level of adoption of even basic biosecurity and testing measures to prevent incursions of strangles or other infectious diseases. The ability to differentiate strains of *S. equi* in source tracing provides a basis for possible litigation and will serve as an impetus to galvanize horse owners and veterinarians into taking pre-emptive preventative action.

Vaccines that generate sterile immunity and serum antibody responses distinguishable from those induced by infection (DIVA) are urgently required. Unfortunately, little is known about the protective immunogens involved and how they must be presented in the horse to induce high level protective immunity. More research in this area is necessary in order to improve the effectiveness of emerging vaccines. Studies are also needed to explain shedding of virulent *S. equi* from persistently infected guttural pouches, a location in which the organism is experiencing genetic decay, massive reduction in numbers, and loss of virulence and fitness.

With proper biosecurity implementation, new capabilities in serology, vigilant detection of subclinically infected carrier animals, and emerging vaccines with DIVA capabilities, prevention against and efficient elimination of strangles from a property is becoming ever more feasible.

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### Footnotes

<sup>a</sup> IDEXX Laboratories, Inc, Westbrook, ME

<sup>b</sup> Equine Diagnostic Systems LLC, Lexington, KY

<sup>c</sup> IDScreen Streptococcus equi Indirect, ID.vet Innovative Diagnostics, France

<sup>d</sup> Animal Health Trust, Newmarket, UK

<sup>e</sup> StrepvaxII, Boehringer Ingelheim, St. Joseph, MO

<sup>f</sup> Pinnacle I.N., Zoetis, Parsippany, NJ

<sup>g</sup> Equilis StrepE, Intervet International, the Netherlands

<sup>h</sup> Strangvac, Intervacc, Sweden

<sup>i</sup> Roccal-D, Zoetis, Parsippany, NJ

<sup>j</sup> Bio-tek Industries, Inc, Atlanta, GA

<sup>k</sup> Vetoquinol, Fort Worth, TX

<sup>l</sup> Intervention, Virox Animal Health, Oakville, ON

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Grayson Jockey-Club Foundation, Boehringer Ingelheim, and the American Quarter Horse Association Foundation. One of the authors (Timoney) shares a patent on the SeM sequence and its use (US Patent # 6,458,358 Oct. 1, 2002). One of the authors (Waller) shares patents on a live attenuated strangles vaccine strain (US8187610), a diagnostic test for *S. equi* (US20110201007) and internal control strain (US20150051082); all rights reside with the Animal Health Trust. One of the authors (Waller) conducted the experiments to determine the safety and efficacy of a new multi-component vaccine, all rights reside with the sponsor.

**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

**Institutional Animal Care and Use Committee (IACUC) or Other Approval Declaration:** Authors declare no IACUC or other approval was needed.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Data S1.** Population structure of *S. equi*.