REVIEW ARTICLE

Equine strangles: An update on disease control and prevention

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ABSTRACT. *Streptococcus equi spp. equi* (SEE) causes a disease in horses commonly referred to as strangles. Carrier or reservoir equids are important for the maintenance of the bacteria between epizootics and the initiation of outbreaks on premises, they also make the control and prevention of the disease more difficult. Disease outbreaks are common in many countries, affecting negatively equine health and causing major economic losses to the equine industry. This review describes general aspects of the disease caused by SEE in horses (clinical signs, pathogenesis, epidemiology, treatment, complications) and then focuses on prevention, control and eradication mechanisms.

Key words: *Streptococcus equi spp. equi*, lymphadenopathy, guttural pouch, empyema, strangles.

GENERAL BACKGROUND

Over the years our knowledge and understanding of equine infectious diseases have been increasing, but despite these advances, the control of many important equine infectious diseases remains challenging. Furthermore, a number of significant infectious diseases are (re-)emerging in many countries and regions, causing detrimental effects on animals' welfare and impacting negatively the equine industry.

The nature of the equine industry, where horses are regularly moved to and from competitions or between breeding farms, makes it difficult to control and prevent highly contagious infectious diseases. Incidents of disease introduction associated with national and international movement of horses are regularly reported[1] (Dominguez *et al* 2016, Moreira *et al* 2019, Pusterla *et al* 2011, Christmann and Pink 2017). Nevertheless, the spread of infectious and contagious diseases carried by animals with subclinical infection remains a challenge for national and international trade, in part because government prevention and control programs are mainly focused on exotic diseases, whereas the control and prevention of endemic diseases (e.g. equine influenza, equid herpesvirus-infection, contagious equine metritis, strangles) still needs more attention.

Streptococcus equi spp. equi (SEE) causes the disease commonly referred to as strangles (also paperas, gurma, gourme, Druse, etc.), which is a highly infectious and contagious bacterial infection that affects horses, donkeys and mules of any age. The disease has a worldwide distribution but outbreaks may occur when large numbers of horses are gathered together or re-introduction *after* population immunity has decreased (Dominguez *et al* 2016, Pusterla *et al* 2011, Boyle *et al* 2018). The bacterium is highly contagious and produces high morbidity and low mortality in susceptible populations previously free of disease. Transmission occurs via direct contact with infectious exudates and via fomite transmission. After infection, some animals keep harbouring the bacterium in their upper airways (more commonly in their guttural pouches). Carrier animals are important for maintenance of the bacteria between epizootics and initiation of outbreaks on premises, they also make the control and prevention of the disease more difficult (Boyle *et al* 2018, Ivens *et al* 2011, Boyle *et al* 2009). The disease causes major economic losses to the equine industry worldwide due to its prolonged course, extended recovery period and associated serious complications.

The review describes general aspects of the disease caused by SEE in horses (clinical signs, pathogenesis, epidemiology, treatment, complications) and then focuses on prevention, control and eradication mechanisms.

CLINICAL SIGNS

Infection with SEE can occur in horses of all ages, more severe clinical signs are seen commonly in younger horses (Sweeney *et al* 2005, Pusterla *et al* 2011) whereas older horses are often less severely affected and recover more rapidly, probably due to their immune status (Pusterla *et al* 2011, Boyle *et al* 2018).

Clinical signs vary among animals but are generally characterised by abrupt onset of pyrexia followed by pharyngitis and abscess formation in the mandibular and retropharyngeal lymph nodes (Boyle *et al* 2018, Sweeney *et al* 2005). The first signs, 3 to 14 days after exposure, are lethargy and fever (>40°C) (Waller 2014). Pharyngitis and lymphadenopathy develop in most horses, causing reluctance to eat, drink, abnormal position of the head (neck extension) and even upper airway obstruction (strangulation

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[1]   Animal Health Trust. 2019. Equine influenza outbreaks reported in 2019. Available at: https://www.aht.org.uk/wp-content/uploads/2019/04/Equiflunet-outbreaks-to-15-April-2019.pdf

- strangles) necessitating a tracheostomy in severe cases (Mallicote 2015). Nasal discharge is common, only some horses develop cough (Mallicote 2015, Sweeney *et al* 2005, Boyle *et al* 2018). Inflammation of the larynx and pharynx makes this area sensitive to the touch, and palpation usually causes pain, stridor, gaging and cough. Although lymphadenopathy commonly develops in mandibular and retropharyngeal lymph nodes, parotid and cranial cervical lymph nodes can also be affected. Abscesses have a firm capsule and generally rupture 1 to 4 weeks after infection into the airway/guttural pouch (causing thick nasal discharge) or break through the skin (mandibular or parotid lymph nodes) (Judy and Chaffin 1999).

Complications like neuropraxia (laryngeal hemiplegia, dysphagia, or both) and damage to the recurrent laryngeal nerve with subsequent paralysis of the arytenoid cartilage may occur, contributing to breathing difficulty (Judy and Chaffin 1999, Mallicote 2015). Infection with SEE can spread occasionally to other locations, abscesses can form in multiple locations (abdomen, mammary gland, brain, etc.), a condition that is commonly called "bastard strangles". Also, SEE pneumonias and immune-mediated vasculitis leading to limb or head swelling have been described (Sweeney *et al* 2005).

PATHOPHYSIOLOGY AND IMMUNITY

SEE, a β-hemolytic Lancefield group C Streptococcus, is generally associated with disease in equids and is not considered a normal commensal of the respiratory tract. Its hyaluronic capsule and surface protein SeM enables SEE to evade phagocytosis, while many other cell surface antigens contribute to its virulence. Intensive research has been directed toward identifying cell surface antigens for developing better diagnostics and vaccines.

The bacteria enters the nose or mouth, then it attaches to and invades the cells within the tonsillar crypts of the lingual and palatine tonsils and the follicular epithelium of the pharyngeal and tubal tonsils, reaching the lymph nodes of the head and neck within 3 hours after infection (Timoney and Kumar 2008). There is no colonisation before penetration, thus a few hours after infection SEE is difficult to detect on the mucosal surface via culture but potentially visible within epithelial cells and subepithelial tonsillar follicles (Timoney and Kumar 2008). Hence, nasal or nasopharyngeal samples may be culture negative in the early stages of infection (Timoney and Kumar 2008, Boyle *et al* 2018). A few hours later, SEE is then translocated to the mandibular and retropharyngeal lymph nodes that drain the pharyngeal and tonsillar region. The interaction of complement with SEE causes migration of large numbers of polymorphonuclear neutrophils, causing abscessation, visible 3 to 5 days after SEE has entered the lymph nodes (Boyle *et al* 2018, Sweeney *et al* 2005).

SEE releases a hyaluronic acid capsule, antiphagocytic SeM protein, H factor binding Se18.9, Mac protein, and other undetermined antiphagocytic factors that modulate the proliferation and activity of neutrophils and macrophages (Timoney and Kumar 2008, Waller 2014), causing failure to phagocytose and kill the bacteria (Waller 2014, Boyle *et al* 2018). Relatively few organisms are present at the time of initial colonisation, but substantial bacterial propagation by the time of onset of fever has been detected (Mallicote 2015).

With lysis of the abscess capsule and evacuation of the contents, bacteria are eliminated. Nasal shedding of SEE begins 2-3 days after onset of fever and persists for 2-3 weeks in most animals. Shedding may persist much longer should infection persist in the guttural pouch or a sinus cavity (Chanter *et al* 1998, Newton *et al* 1997). Some animals with preexisting immunity and without clinical signs never exhibit detectable shedding (Boyle *et al* 2018).

Rupture of the abscesses allows for easy contamination of the environment and infection of other horses (Boyle *et al* 2018, Mallicote 2015).

Most horses recover from strangles over a period of weeks (~98%). Two to 3 weeks after infection mucosal and systemic immune responses are detected, coinciding with mucosal clearance of SEE (Galan and Timoney 1985, Boyle *et al* 2009). Long-term immunity to strangles is built in most horses after infection (approx. 75%), if not treated with antibiotics (Boyle *et al* 2018, *et al* 2002, Tiwari *et al* 2007, Galan and Timoney 1985). Just after the convalescent phase horses are resistant to re-infection (Boyle *et al* 2018, Galan and Timoney 1985).

Despite the development of antibody responses, some horses fail to clear all abscess material from their guttural pouches or sinuses (approx. 10%) and the residual purulent material forms chondroids that can remain in the horse for several years and even a lifetime (Newton *et al* 1997, Verheyen *et al* 2000). SEE persists in chondroids (visible) or in mucosal surface biofilms (invisible - inapparent) and is intermittently shed from carriers into the environment. Ongoing exposure to SEE due to the presence of carriers likely contributes to the maintenance of increased levels of immunity and extended strangles-free status within isolated herds of previously infected horses. Older horses, with waning immunity, and vaccinated animals have limited susceptibility to SEE and can develop a mild form of strangles ("atypical or catarrhal strangles"). Nevertheless, these animals still shed virulent SEE, able to cause severe disease in more susceptible horses (Sheoran *et al* 1997).

Recovered mares shed immunoglobulin Gb (IgGb) and IgA in their milk and colostrum, with specificities similar to those found in nasopharyngeal mucus of convalescent horses (Galan and Timoney 1987). Milk and colostral antibodies protect suckling foals during the first weeks and months of their lives (Boyle *et al* 2009).

SEE does not survives for long in the environment (surfaces exposed to direct sunlight), but it can persist for up to 1 month in sufficiently moist areas (Weese *et al* 2009). Transmission of SEE to naïve horses from acutely

or persistently infected ones is via direct contact (nose or mouth), or through contaminated drinking water, tack, and other fomites.

The persistent SEE infection status is critical to interepizootic transmission, the recurrence and the high incidence of disease, making it very challenging to control and prevent the disease around the world.

COMPLICATIONS

Most strangles cases progress as described above and resolve after rupture of the abscessed lymph nodes (Boyle *et al* 2018, Mallicote 2015, Waller 2014). However, complication rates vary with the duration and intensity of exposure to SEE, increasing up to 20% in some cases (Sweeney *et al* 1987, Piche 1984, Ivens *et al* 2011). Case fatality rates, although generally low, can be as high as 10% during farm outbreaks (Boyle *et al* 2018, Christmann and Pink 2017). Isolation of infectious horses is therefore critical in reducing the complication and case fatality rates (Boyle *et al* 2018).

After SEE infection various sequelae can occur: establishment of chronic carriers, spread of infection from the head and neck region to other locations (metastatic abscessation), and immune-mediated complications.

METASTATIC ABSCESSATION

Commonly referred to as "bastard strangles", it occurs when the infection spreads to lymph nodes or tissues distant from the lymph nodes of the head and neck. Diagnosis is reached with a history of exposure to SEE and laboratory results consistent with chronic infection, anaemia, fever that responds to penicillin, hyperfibrinogenemia, and hyperglobulinemia. Treatment requires long-term antimicrobial therapy, and appropriate local treatment or drainage of abscesses if possible. Nevertheless, bastard strangles often results in the death of patients, especially when lungs, lymphoid tissue including the spleen in the abdomen, liver, kidneys, or brain are involved (Waller 2014).

IMMUNE-MEDIATED COMPLICATIONS

Purpura haemorrhagica (aseptic necrotizing vasculitis). It is the most frequent type of immunologic complication (immune-mediated type III hypersensitivity reaction) in response to several different SEE antigens, including anti-SeM antibody responses that result in deposition of immune complexes in blood vessels causing severe vasculitis, substantial ventral oedema, and necrosis (Sweeney *et al* 1987, Mallicote 2015, Pusterla *et al* 2003). It occurs most frequently 3 to 4 weeks after strangles or the administration of strangles vaccine (Mallicote 2015). Animals hypersensitised to SEE antigens (titers greater than 1:1,600) are at increased risk of developing purpura haemorrhagica (Sweeney *et al* 2005). Treatment includes

corticosteroids (dexamethasone) and supportive care (intravenous fluids, hydrotherapy, bandaging, etc.). Most horses can recover if given good veterinary care, but mortalities have been reported (8-25%) (Waller 2014, Pusterla *et al* 2003, Sweeney *et al* 1987, Heath *et al* 1991).

Myositis. Muscle infarctions, rhabdomyolysis with acute myonecrosis, and rhabdomyolysis with progressive atrophy after SEE infection are a relatively rare, localised immunologic complication that presents as various syndromes (Mallicote 2015). Although infarctions are a severe manifestation of purpura hemorrhagica, the mechanisms of rhabdomyolysis are not known, but inflammatory cascades as with streptococcal toxic shock syndrome or direct toxic effects of SEE in muscle tissue have been hypothesised (Boyle *et al* 2018). Widespread lymphocytic inflammatory infiltrates with marked change most evident in atrophied muscle samples have been reported in the histopathologic evaluation of muscle tissue (Mallicote 2015). Horses with myositis should be treated with corticosteroids, if there are signs consistent with concurrent infection, antibiotics are also indicated (Boyle *et al* 2018).

Myocarditis. Antigens of SEE were also reported to trigger inflammation of the myocardium, causing electrocardiographic abnormalities in convalescent horses (Boyle *et al* 2018, Mallicote 2015).

DIAGNOSIS

The diagnosis of SEE infection traditionally relied on bacterial culture out of material recovered from swabs, washes from the upper respiratory tract or abscess content. Advances in molecular technology (quantitative PCR or other PCR formats) have shown deficiencies in the culture test, proving that this method is no longer the gold-standard method for the detection of SEE, diagnosis of strangles or to detect carrier status. Serology is used to identify exposure, higher titers have been found in carrier animals and are also associated with a higher risk of developing immunomodulated complications. While still cited by some as the gold standard of diagnosis, culture results must be carefully interpreted, especially when used to screen samples collected from the upper respiratory tract.

CULTURE

Culture details are reported elsewhere (Mallicote 2015, Boyle *et al* 2018, Waller 2014). Briefly, samples are cultured in blood agar containing colistin and nalidixic acid. After overnight incubation, beta-haemolytic colonies of SEE are picked and used to inoculate Todd-Hewitt nutrient broth. Turbid cultures are also used to inoculate purple broth cultures containing trehalose, lactose, or sorbitol (SEE fails to ferment these sugars, whereas *S. zooepidemicus* ferments lactose and sorbitol, and *S. dysgalactiae* subsp. *S equisimilis* ferments trehalose) (Bannister *et al* 1985).

The isolation and identification of SEE is time consuming (at least 48h) and confounded by the presence of other beta-hemolytic bacteria (*S zooepidemicus*, *S equisimilis*) that tend to overgrow SEE in culture. In samples collected from the upper respiratory tract SEE has to outcompete other normal respiratory flora in order to be readily identified in culture. Thus, culture results must be carefully interpreted, especially when used to screen samples collected from the upper respiratory tract (Boyle *et al* 2018, Mallicote, 2015). The reporting delay could also have consequences for the spread of SEE if suspected animals are not isolated when samples are collected.

PCR ASSAYS

Different PCR-based tests have been developed. The first test that targeted the 50 region of the SeM gene was reported to be 3 times more sensitive than culture (Båverud *et al* 2007, Webb *et al* 2013). However, the region is highly variable, and some SEE strains even lack the target. Therefore, a variety of other sequences and qPCR formats were developed to assure even greater specificity and sensitivity (Webb *et al* 2013, Båverud *et al* 2007), also providing fast results (qPCR test can be completed within 1-2 hours, results may be available on the same day that samples arrive at the laboratory) (Boyle *et al* 2018, Webb *et al* 2013, Mallicote 2015, Waller 2014).

Recently, a triplex qPCR assay was developed (Webb 2013) that targets two SEE specific genes (eqbE, SEQ2190) and an internal control strain of *S. zooepidemicus* (within-in-assay control, to reduce false-negatives). This assay has an overall sensitivity and specificity of 93.9 and 96.9%, respectively, and is able to detect 10-fold fewer quantities of SEE than culture, regardless of the presence of contaminating bacteria (Webb *et al* 2013). Therefore, the triplex qPCR sets a new benchmark for quality control and sensitivity and is now regarded as the new gold-standard test for the detection of SEE (Waller 2014). Culture assays failed to identify 39.7% of qPCR-positive samples (Webb *et al* 2013). Historically, this poor sensitivity of culture assays and its failure to correctly identify qPCR-positive samples was excused by the claim that PCR does not distinguish between dead and live organisms, and so technically false-positive reactions affect the diagnostic value of PCRs with respect to detection of actual infection (Sweeney *et al* 2005). Although this is technically correct, as DNA does not persist on mucosal surfaces, experts emphasise that any culture-positive or qPCR-positive result should be taken seriously (Boyle *et al* 2018). Positive results of the clinical application of qPCR in the diagnosis and control of field outbreaks of strangles in several countries and management settings are considered a testament to the usefulness of qPCR over culture (Boyle *et al* 2018). Experts have also recommended the use of PCR testing of an endoscopically guided guttural pouch lavage for detection of SEE in subclinical infected carrier animals.

Also, the 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 SEE.

SEROLOGY

Indirect enzyme-linked immunosorbent assays (iELISA) are available to detect anti-SEE antibodies (anti-SeM, anti-antigen A (SEQ2190 N-Terminal Fragment) and -antigen B or C (SeM N-Terminal Fragment).

SeM-based-iELISAs were developed to detect antibody titers to SeM that peak about 5 weeks after exposure and remain high for at least 6 months (Timoney *et al* 2007, Galan and Timoney 1985, Galan and Timoney 1987).

The SeM antibody titer can be used to: 1) detect recent infection (4-fold or greater increase in titers, 10-14 days apart); 2) support the diagnosis of SEE-associated purpura hemorrhagica or metastatic abscessation (titer ≥ 1:12,800); and 3) identify high risk of developing purpura hemorrhagica (animals with titers >1:3,200 should not be vaccinated) (Boyle *et al* 2018, Boyle *et al* 2009).

Nevertheless, false positive results were associated with cross-reactivities between anti-SeM and anti-SzM40 antibodies, produced in response to the homologue protein to SeM expressed by *Streptococcus zooepidemicus* (Waller 2014). This was overcome with preincubation of the serum with heat-killed *S. zooepidemicus* which allowed to remove cross-reactive antibodies from the samples (Davidson 2008), but the step was not incorporated in full-length SEM assays (Waller 2014). Therefore, a SeM-specific titer should not be used to determine carrier status and a single value cannot be used as a measure of active infection or protection to reinfection (Boyle *et al* 2018). SeM titers wane over time (Boyle *et al* 2017, Sheoran *et al* 1997) and horses treated with antibiotics during an outbreak seem to mount a reduced immune response, remaining susceptible to reinfection (Piche 1984).

Dual antigen or combined antigen iELISAs were then developed to overcome the cross-reactivities with *S. zooepidemicus*. This combined iELISA test detect antibodies against antigen A (SEQ2190 N-Terminal Fragment; also known as Se75.3), antigen B or C (SeM N-Terminal Fragment) of SEE, if one or both (A and B or C) antibody results exceed the cutoff, the sample is considered positive (Robinson *et al* 2013, Knowles *et al* 2010). These tests were successfully used to determine the prevalence of exposure to SEE in horse populations in Lesotho (Ling *et al* 2011), UK (Knowles *et al* 2010, Waller 2014, Ivens *et al* 2011) and Sweden (Riihimaki *et al* 2017). The antigen A and C dual antigen iELISA test was compared to a commercial iELISA (IDvet) based on the full SeM protein, showing a similar sensitivity (89.9 vs 93.3%), but higher specificity (77.0 vs 99.3%), highlighting the application of this dual antigen anti-A and -C antigen test to identify

potential carriers before they can transmit the infection (Robinson *et al* 2013). Recently, this combined antigen A and C iELISA test was recommended to identify recent infection as early as 2 weeks after infection and exposed carrier animals without signs, provided currently available vaccines have not been used (Boyle *et al* 2018).

TREATMENT OF HORSES WITH STRANGLES

In animals with lymph node abscessation hot packing and topical softening agents can help in the development and maturation of the masses, speeding up the resolution (Sweeney *et al* 2005, Mallicote *et al* 2015). Surgical intervention of sufficiently mature and soft masses can be required when abscesses do not rupture spontaneously (Sweeney *et al* 2005, Boyle *et al* 2018). Once drainage occurs, daily lavage of the abscess with dilute povidone iodine solution should be instituted (Sweeney *et al* 2005).

The use of antibiotics in the treatment of SEE infected horses is still controversial. The bacterium is sensitive to many antibiotics, but reaching SEE in organised abscesses remains challenging. Penicillin is considered the drug of choice and antibiotic resistance has not yet been reported in SEE (Boyle *et al* 2018) but has begun to emerge in some strains of *S. equi spp. zooepidemicus* (Chalker *et al* 2012).

The treatment of isolated clinically healthy in-contact animals for 3 to 5 days can prevent the development of clinical signs, but it also prevents treated animals to develop SEE immunity (Sweeney 2005) and it delays abscess maturation, extending recovery times (Waller 2014, Boyle *et al* 2018). With severe cases, antibiotic treatment is often needed to reach clinical improvement, reducing fever, lethargy and upper airway obstruction (Mallicote 2015).

PREVENTION, CONTROL AND ERADICATION MECHANISMS

In many countries strangles is an endemic disease that generally comes in waves or outbreaks when naïve population is exposed to carrier animals. Historically, SEE was considered by veterinarians and the equine industry as a common pathogen that caused a limiting disease in young horses. Nevertheless, nowadays probably due to the increase in horse movements, waning immunity and other factors, strangles outbreaks are more common and frequent, reemerging even in countries where strangles have long been a rarity. The disease continues to cause major economic losses to the equine industry worldwide, by the direct effects of the disease (isolation, treatments, prolonged disease course, extended recovery period, serious complications) and indirectly by limiting horse movements and causing the cancellation of equestrian events.

Therefore, the authors believe national and international efforts should be directed to prevent and control the disease, starting by making the disease reportable and focusing on identifying and treating carrier animals

and screening regularly possible exposure of moving equids. Shedding of the SEE from carrier animals enables the onward transmission of SEE and further outbreaks of disease. Identification and treatment of persistently infected carriers is critical to break the cycle of infection and eradicate SEE.

CONTROL DURING AND AFTER OUTBREAKS

Early identification of the infectious agent is essential to minimise the impact of the disease. Measuring rectal temperatures once or ideally twice daily aids in early identification of suspect outbreak cases.

Initial clinical signs of strangles (pyrexia, nasal discharge, and enlarged mandibular lymph nodes) vary among horses and are obviously not restricted to SEE infection. However, if infection is suspected, affected animals should be isolated and biosafety measures must be applied to minimise the transmission to in-contact animals. Confirmation of clinical cases can be achieved quickly by qPCR analysis of needle aspirates from enlarged or abscessed lymph nodes (Sweeney *et al* 2005). SEE rapidly invades (draining) lymph nodes of convalescent animals, hence in initial stages of disease negative results from qPCR analysis of nasal/retropharyngeal swabs or lavages can occur, and do not necessarily rule out SEE infection, especially if clinical signs suggest otherwise (Waller 2014, Boyle *et al* 2018).

General biosecurity measures are described in detail elsewhere (Waller 2014, Boyle *et al* 2018, Mallicote 2015). Nose to nose contact and contact with nasal discharge and purulent material from erupted abscesses must be avoided.

To minimise the spread of disease, horse movements should be stopped, and farms or equine facilities can be divided into 3 color-coded groups. The *red group* includes horses that have shown 1 or more clinical signs consistent with strangles. *Yellow/Orange/Amber group* horses are those that have had direct or indirect contact with an infected horse in the red group and may be incubating the infection. Horses in the *green group* have had no contact with infected or suspect animals. Body temperatures of horses in the yellow and green group should be measured twice daily to identify new cases and move them to the red group. Equipment used with horses can be colour coded as well to avoid cross contamination. Ideally, different equipment and personnel should be assigned to the different groups, if not possible, staff should always move from the lowest to the highest risk group (from green to red).

Screening by dual antigen ELISA A and C iELISA to identify SEE-persistently-infected-horses can commence at least three weeks after the resolution of the last clinical case. Horses in the yellow and green groups must be included in the screening, otherwise carrier horses, which remained subclinical or were exposed before and did not get infected, will remain undetected and stay as a source of future re-infections. All horses from the red group and those from the yellow and green group that tested positive by

iELISA should be examined further. Ideally, upper airway with guttural pouch endoscopy should be performed, followed by the collection of bilateral guttural pouch washes (Waller 2014). If not possible, nasopharyngeal swabbing/washing samples should be collected (Boyle *et al* 2018, Waller 2014). Samples should be analysed by qPCR to maximise sensitivity. Identified carriers should be treated as described in figure 1.

PREVENTION - ERADICATION

Identification and treatment of carrier animals. Horses that are SEE carriers are persistently infected but seem clinically normal and generally remain unaffected during strangles outbreaks. In most farms, once clinical signs of affected animals resolve, the screening of unaffected animals is considered unnecessary but it should not be the case, as it is increasing the chances of keeping possible carrier animals and a future infection source in the population. Nevertheless, screening by dual antigen A and C iELISA has been successful in identifying persistently infected horses (Boyle *et al* 2009, Riihimaki *et al* 2017, Robinson *et al* 2013).

Serology for identification of infected animals. To prevent the entry of carrier animals into a herd, before entry horses should stay in quarantine and serology of blood samples collected on arrival should be performed (Waller 2014). If negative, the analysis should be repeated 14 days later to identify horses that have been incubating the infection and seroconvert after arrival. If the second sample is still negative, it should be safe to enter the new arrivals into the herd. If positive, on day zero or 14, further diagnostics are required (Waller 2014). Endoscopy of the upper airway and guttural pouches should be examined to identify obvious signs of persistent infection (empyema, chondroids, follicular hyperplasia, etc.). During this procedure, (saline wash) samples from both guttural pouches should be taken for qPCR analysis. If visually the airways are normal and qPCR analysis results are negative, it should be safe to introduce the horse into the herd. If qPCR samples test positive or chondroids are visible on endoscopy, the horse should be treated to eliminate the carrier status before entry into the premises (Waller 2014).

Treatment of carrier animals. Once carrier animals have been identified, elimination of SEE from the guttural pouches should be accomplished by endoscopic guttural pouch lavage. If large numbers of chondroids are present, surgical hyovertebrotomy and ventral drainage through the Viborg triangle can be performed, but the procedure carries inherent risks of general anesthesia and surgical dissection around major blood vessels and nerves, plus the contamination with SEE of the hospital environment (Waller 2014).

For guttural pouch lavages, horses are sedated to facilitate drainage of flush material, if chondroids are present, they can be removed with basket tools passed through the biopsy channel of the endoscope (Waller 2014, Verheyen e *et al* 2000). Lavages with isotonic saline or polyionic fluid using rigid or indwelling catheters or with a suction pump attached to the endoscope should be repeated until negative qPCR results are obtained.

Topical instillation of acetylcysteine solution (20%, weight/volume) into guttural pouches can be performed to assist empyema treatment. Once the lavage is finished,
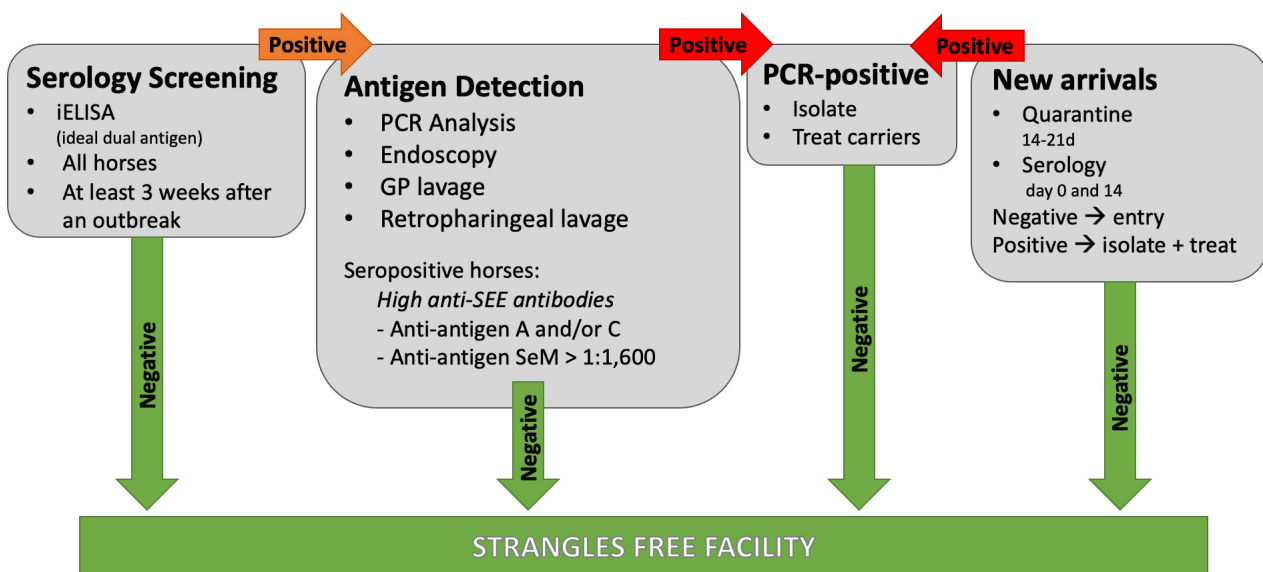

Figure 1. Diagram of procedures to reach a strangles free facility. SEE: *Streptococcus equi* subspecies *equi*; GP: guttural pouch; iELISA: indirect enzyme-linked immunosorbent assays; PCR: Polymerase C Reaction; d: days.

topical benzylpenicillin mixture can be instilled into the guttural pouches to facilitate SEE elimination Waller 2014, Boyle *et al* 2018). The administration of systemic antibiotics may further improve treatment success (Waller 2014, Boyle *et al* 2018).

In two-weeks intervals the guttural pouches should be resampled for qPCR analysis to confirm SEE elimination. Two to 3 qPCR negative results, two weeks apart, are considered sufficient to confirm elimination of carrier status.

Vaccination. Ideally, vaccines should provide adequate levels of protection against circulating SEE strains with a long duration of immunity, especially for horses that travel and are exposed to other horses from different origins. They should also be safe to be administered and capable to differentiate infected animals from vaccinated ones, which enables normal movement of vaccinated horses but also permits the identification of vaccinated horses that were exposed to and are protected from SEE (differentiate infected from vaccinated animals - DIVA) (Waller 2014).

–*Killed and cell extract vaccines:* These are the first types of vaccines that were developed. Nowadays, commercially available ones which are administered by the intramuscular route, include Equivac S (Zoetis New Zealand), Strepguard (MSD Animal Health), and Strepvax II (Boehringer Ingelheim). Little is known about the real efficacy of these vaccines. However, they were reported to reduce the severity and frequency of strangles but adverse reactions were common, the protection conferred was short-lived and DIVA is not possible (Hoffman *et al* 1991).

–*Live-attenuated vaccines:* There is one live-attenuated aroA deletion mutant available in Europe based on an isolate from Holland (Equilis StrepE, Europe; MSD Animal Health) (Kelly *et al* 2006, Jacobs *et al* 2000). Submucosal and intramuscular (IM) injection protects from lymph node abscessation (Jacobs *et al* 2000), but adverse reactions from IM injections made this administration route less practicable (Kelly *et al* 2006, Jacobs *et al* 2000, Kemp-Symonds *et al* 2007). The vaccine contains the same genetic material as virulent strains of SEE, and therefore it has no DIVA properties.

Another live-attenuated vaccine is the Pinnacle IN (Zoetis), available in North America and some other countries. It is for intranasal administration, based on the CF32 strain isolated in the USA (New York 1981) and attenuated with nitrosoguanidine. Lymph node abscess formation and bacterial shedding after vaccination were reported, it caused severe adverse effects when injected intramuscularly and it does not permits DIVA (Waller 2014). Pinnacle IN vaccine-like SEE strains were isolated in strangles cases in New Zealand, suggesting an increased sensitivity to vaccine reactions and possible revert to virulence (Patty and Cursons 2014). Efficacy has not been reported, but Zoetis claims that following

experimental challenge live-attenuated vaccines for strangles can confer significant levels of protection. However, they also lack DIVA properties and adverse reactions occur (Waller 2014).

Sequence analysis of the SeM gene identified differences between strains of SEE (Parkinson *et al* 2011, Anzai *et al* 2005, Kelly *et al* 2006, Patty and Cursons 2014, Ivens *et al* 2011), suggesting that the population of SEE is changing over time as the organism is evolving with dominations of SeM-9 strains in the UK (Parkinson *et al* 2011, Ivens *et al* 2011). The vaccines Pinnacle and Equilis were derived from strains that are distantly related to dominant SeM-9 strains. It is likely that antibody responses cross-react among SEE strains (Galan and Timoney 1988) but the level of protection conferred by the vaccines against circulating SEE remains unknown (Waller 2014).

–*Subunit vaccines:* Recombinant SEE proteins have also been produced to generate vaccines. They are safer as they only contain target proteins and no SEE DNA, making it possible to DIVA.

Nevertheless, vaccination of horses with three different subunit vaccines (recombinant SeM; SEE-specific proteins; SEE adhesin proteins) did not protect against *in vivo* challenge with SEE (Sheoran *et al* 2002, Timoney *et al* 2007), despite the generation of serum antibody response. The inclusion of immunoglobulin-cleaving proteins IdeE and IdeE2 in subunit vaccines were found to be important for effective protection (Guss *et al* 2009). A developed vaccine (known as Strangvac), based on a SeM-9 SEE strain isolated in Sweden (Guss *et al* 2009), included immunoglobulin-cleaving proteins IdeE and IdeE2 but not SeM or SEQ2190 proteins, to reach DIVA properties. The strain used is more closely related to the circulating strains in Europe (UK) and was found to provide protection against *in vivo* challenge (Guss *et al* 2009).

CONCLUSIONS

Strangles outbreaks are common in many countries and they continue to affect equine health negatively and cause major economic losses to the equine industry worldwide.

National and international efforts should be directed to establish effective and on-going disease surveillance together with the identification and treatment of persistently infected carriers.

Developed diagnostic tests (qPCR and serology) have greatly improved over the years and should become available and accessible worldwide to assist the identification of persistently infected equids to prevent future outbreaks.

Although strangles vaccination is used in many equine facilities to reach herd immunity, conferred protection by available vaccines is limited and their antibody response cannot be differentiated by existing serology tests. Further research is needed to generate and test effective vaccines and to use diagnostic testing alongside vaccinations that provide adequate herd immunity.

For now, we believe that the surveillance of outbreaks as well as the testing and treating of SEE carriers can reach disease control and prevention and will lead to the break of the cycle of infection, and, eventually, to disease eradication.

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