The persistence of equine strangles

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Abstract

Streptococcus equi subspecies equi (*S. equi*) is the causative organism of the upper respiratory disease of equids, strangles, characterised by pyrexia, lymphadenopathy, and mucopurulent nasal discharge. Strangles was first reported over 750 years ago and continues to be of significance in equine populations across the globe. This review discusses how *S. equi* has adapted, the clinical manifestation of strangles, and how clinicians and caregivers can tackle the disease in the future. *S. equi* evolved from the commensal, and occasionally opportunistic pathogen, *Streptococcus equi subspecies zooepidemicus* refining its capabilities as it became host restricted. The success of *S. equi* can be attributed to its ability to cause both acute and persistent infection, the latter occurring in about 10% of those infected. In this carrier state, *S. equi* persists in the guttural pouch without causing clinical signs, intermittently shedding into the environment, and encountering naïve animals. Insight into the *S. equi* genome and lifestyle has led to advances in diagnostic assays and the development of a safe and efficacious recombinant-fusion vaccine, giving clinicians and caregivers the tools to better combat this infection. Alongside rigorous biosecurity protocols and pragmatic control measures such as screening new arrivals for exposure and carrier status, these new technologies demonstrate that strangles can be an increasingly preventable infection.

Introduction

Strangles was first described in 1256 (Ruffo, 1256), although the disease and its causative organism *Streptococcus equi subspecies equi* (*S. equi*), first identified by Schütz (1888), have likely been around for much longer. In the 17$^{\text{th}}$ century, strangles was considered an inevitability; indeed, it was suggested that the disease was transmitted *in utero* due to the high numbers of horses that contracted the infection across varied backgrounds, genetic profiles, and management systems (Solleysel, 1664, Paillot et al., 2017). By the 20$^{\text{th}}$ century, risk factors had been determined (Todd, 1910), including age and body condition; however, these are still in dispute (Ling et al., 2011). Management-related factors are commonly the only factors consistently associated with outbreaks (Libardoni et al., 2016, Laing et al., 2021).

As was long suspected (Todd, 1910) and later confirmed (George et al., 1983, Newton et al., 1997a, Timoney et al., 1998), *S. equi*persists in the guttural pouch, without causing clinical disease in a proportion of animals. *S. equi* survives in this low nutrient state, intermittently shedding into the environment, allowing the organism to spread to naïve individuals; indeed, its success as a pathogen can be attributed to the ability to cause both acute and persistent disease. Chronically infected equids rarely show clinical signs, presenting a major obstacle to the prevention and control of outbreaks (Verheyen et al., 2000). The challenges associated with detecting carriers are a key reason for the perpetual spread of *S. equi* (Pringle et al., 2020b).

Streptococcus equi

Pathogenesis

S. equi has been shown to persist in the environment for up to 34 and 13 days in wet and dry sites, respectively (Durham et al., 2018). Equids become infected via the oronasal route, likely through ingestion of contaminated material (Boyle et al., 2018). Upon entry, *S. equi* attaches to the crypt cells of the lingual and palatine tonsillar tissue, before translocating to regional lymph nodes (Timoney and Kumar, 2008).

Virulence factors act to mitigate the effects of the host immune response: the hyaluronic acid capsule aids immune evasion (Woolcock, 1974), IgG endopeptidases are secreted to cleave antibodies (Lannergard and Guss, 2006), and antiphagocytic binding proteins such as Se18.9 are secreted (Tiwari et al., 2007) Additionally, SeM surface proteins block immune activity by binding to fibrinogen and immunoglobulin (Timoney et al., 1997, Meehan et al., 2009). High morbidity is achieved through this antiphagocytic activity, resulting in intra and extracellular multiplication in tonsillar and lymphoid tissue, including regional lymph nodes (Timoney and Kumar, 2008). Additionally, *S. equi* can produce a microscopic biofilm with potential adhesive functions (Steward et al., 2017) that may play a role in persistence.

Abscessation of the lymph nodes is not visible until 3-5 days after their infiltration, as large numbers of neutrophils are attracted to the site through the interaction of complement-derived factors and pathogen-associated molecular patterns such as peptidoglycan (Muhktar and Timoney, 1988). The ability of *S. equi* to import iron has been linked to its growth within these abscesses, with the secreted molecule equibactin facilitating this acquisition (Heather et al., 2008, Harris et al., 2015). Abscesses commonly rupture into the airways, guttural pouches or through the skin 7-28 days after initial infection (Waller, 2014): abscesses of the retropharyngeal lymph nodes typically rupture into the guttural pouches, draining into the nasopharynx and subsequent nasal passages resulting in copious mucopurulent discharge.

Severity is dose-dependent with around 10,000 colony-forming units required to cause disease in a mature and immunocompetent equid (Boyle et al., 2018). Increasing the number of colony-forming units will result in more severe disease and a shorter incubation period, which can vary from 1-28 days (Boyle et al., 2018, Judy et al., 1999).

Shedding of *S. equi* typically commences 1-2 days after the onset of pyrexia and persists for 2-3 weeks; equids remain infectious for over six weeks after purulent nasal discharge has dried up (Boyle et al., 2018).

Streptococcus equi evolution

S. equi is a host-restricted pathogen of equids, thought to have evolved from the opportunistic pathogen *Streptococcus equi subspecies zooepidemicus* (*S. zooepidemicus*) (Waller et al., 2011, Holden et al., 2009, Harris et al., 2015). *S. equi* and*S. zooepidemicus* are closely related, sharing over 97% of their DNA (Holden et al., 2009).

S. equi and *S. zooepidemicus* share a common phage pool that has enhanced their cross-species evolution: their divergent evolution is a result of functional loss, pathogenic adaptation, and genetic exchange (Holden et al., 2009). The deletion of the clustered regularly interspaced short palindromic repeats (CRISPR) locus in*S. equi* is thought to have favoured the acquisition of genetic elements, at the expense of genome stability (Waller and Robinson, 2013). *S. equi* and *S. zooepidemicus* have many structural and functional differences (Bannister et al., 1985, Holden et al., 2009, Lindmark et al., 2001), as *S. equi* has refined its requirements and capabilities similar to other host-restricted pathogens (Parkhill et al., 2003). A notable difference between the two genomes is the presence of the equibactin locus in *S. equi* , involved in iron acquisition (Heather et al., 2008). This species difference is indicative of how novel functions were introduced, at the expense of ancestral capabilities (Holden et al., 2009); indeed, its acquisition may have been the speciation event that distinguishes *S. equi* from*S. zooepidemicus* (Holden et al., 2009, Heather et al., 2008, Harris et al., 2015).

The *S. equi* genome is larger than that of *S. zooepidemicus* , in-part because of its plasticity and the procurement of many mobile genomic elements (Holden et al., 2009). This has been crucial in the development of *S. equi* as a pathogen; although, the loss of genes not required to cause has resulted in host-restriction, only being able to cause disease in equids (Waller, 2016).

Streptococcus equi genome changes during persistent infection

S. equi has been characterised as possessing a dynamic genome with the ability to diversify and decay; mutations relating to metabolic streamlining and the loss of virulence have been noted in chronically infective isolates (Harris et al., 2015). The endemicity of *S. equi* can, in part, be attributed to its ability to persist in the guttural pouch following an infection, surviving in a low-nutrient state yet intermittently shedding bacteria and thus exposing naïve animals.

Genomic decay during persistent infection may reduce transmissibility and result in a lessened ability to cause severe acute disease, such as with the deletion of the equibactin locus, which is linked to the development of lymph node abscesses; although, the organism undoubtedly remains infectious (Harris et al., 2015). Furthermore, individuals with residual immunity such as equids that are older or vaccinated, and foals with maternal antibodies can present with 'atypical' strangles where typical clinical signs are not presented (Prescott et al., 1982, Tscheschlok et al., 2018); this presentation may be caused by a reduction in virulence (Waller, 2016).

A complex interplay between the host and causative agent is suggested (Harris et al., 2015, Morris et al., 2021) in which genomic plasticity could play a central role; this is an opportunity for further research with an emphasis on understanding host, as well as pathogenic, factors such as immunity.

Global endemicity

Equids are widely used and transported between geographic regions and strangles continues to spread as rapidly as ever (Mitchell et al., 2021, Leadon et al., 2008). Strangles is endemic worldwide, with only Iceland remaining free from the disease, due to a self-imposed import ban of equids and geographical isolation (Björnsdóttir et al., 2017). Population analysis of 670 isolates from 19 countries (Mitchell et al., 2021) revealed the extent of the international transmission that results in the endemicity of strangles across the world. The international transmission of *S. equi* , as demonstrated by Mitchell et al. (2021), is in accordance with the first criterion of the World Organisation of Animal Health listing of terrestrial animal diseases. The other three criteria are demonstrated elsewhere (Björnsdóttir et al., 2017, Boyle et al., 2018); therefore, it was recommended strangles be added to this listing (Mitchell et al., 2021).

Clinical manifestation

Acute *Streptococcus equi* infection

Strangles is characterised by sudden pyrexia, mucopurulent intermittent nasal discharge and the abscessation of the submandibular and retropharyngeal lymph nodes (Timoney et al., 1998). Less common clinical signs include respiratory signs, pharyngeal swelling, lethargy, inappetence, dysphagia, depression, and the presence of chondroids (Rendle et al., 2021). Although strangles has a low mortality rate, severe swelling of abscesses in the lymph nodes can lead to significant inflammation, asphyxia and, ultimately, death (Gharieb et al., 2019).

Pyrexia can exceed 42°C (Boyle et al., 2018), and is typically accompanied by lethargy, occurring 3-14 days after initial exposure. Fever ordinarily precedes bacterial shedding by 1-2 days; thus, identification of its onset can be of paramount importance to isolate individuals and control outbreaks (Waller, 2014).

Pharyngitis can be significant, often with concurrent nasal discharge, inappetence, dysphagia, a mucoid cough, and laryngeal-associated pain (Boyle et al., 2018). Affected equids may stand with their heads in an abnormal, extended position (Waller, 2014).

As abscesses form and subsequently rupture, empyema of the guttural pouch or upper respiratory tract can occur. Intermittent expulsion of this thick highly infectious pus is important for the resolution of the infection and removal of bacteria (Boyle et al., 2018); it results in mucopurulent nasal discharge and a cough, present in around half of horses with guttural pouch empyema (Judy et al., 1999). Abscessation and pharyngitis can obstruct the upper respiratory tract, resulting in dyspnoea and dysphagia, alongside potential temporary laryngeal hemiplegia (Boyle et al., 2018).

Systemic and mucosal immune responses are evident 2-3 weeks post-infection, and this immunity wanes over time (Boyle et al., 2018). Hamlen et al. (1994) showed that 75% of foals exposed to *S. equi*6 months after recovering from strangles were protected from severe infection, corroborated by historical and contemporary literature (Todd, 1910, Boyle et al., 2018), although no animals were completely protected from mild clinical signs. The use of antimicrobial therapy has been demonstrated to interfere with the persistence of humoral immunity (Pringle et al., 2020a).

Neonates can derive protection from colostral antibodies from exposed dams, and subsequently, IgA and IgG in milk confer some protection by coating the upper respiratory and oral mucosa until the time of weaning (Galan et al., 1986). Individuals with residual immunity may develop a milder form of the disease with short-lived clinical signs, termed 'atypical' strangles, although these animals can still shed *S. equi* to susceptible animals (Sheoran et al., 1997, Prescott et al., 1982).

Complications of*Streptococcus equi* infection

S. equi has the potential to spread haematogenously, via lymphatics, septic focus, or by direct aspiration of purulent material (Boyle, 2017). Common sites include the lung, mesentery, liver, spleen, kidney, and brain (Boyle et al., 2018, Sweeney et al., 1987); additional clinical signs are dependent on the location of abscesses. This presentation is known as metastatic or 'bastard' strangles and has been documented since the 17$^{\text{th}}$ century (Solleysel, 1664). Prevalence of these complications ranges from 2-28% across outbreaks (Spoormakers et al., 2003, Sweeney et al., 1987, Duffee et al., 2015); metastatic abscessation has consistently been shown to increase mortality (Ford and Lokai, 1980).

S. equi infection is the most common cause of purpura haemorrhagica, but vaccination with M-protein-containing vaccines, other bacteria, viruses, and neoplasia can similarly result in purpura complexes and vasculitis (Mallicote, 2015). Purpura haemorrhagica is caused by a type III hypersensitivity reaction, resulting in necrotising vasculitis secondary to immune-complex deposition (Whitlock et al., 2019). Its presentation can vary from innocuous to a potentially fatal complication (Boyle et al., 2018).

Myopathies can be seen with *S. equi* infection, with three predominant presentations (Boyle et al., 2018): muscle infarctions (Kaese et al., 2005) and rhabdomyolysis with either acute myonecrosis or progressive atrophy (Sponseller et al., 2005, Valberg et al., 1996).

Other complications associated with strangles include anaemia, agalactia, meningitis, septic arthritis, and endocarditis (Boyle et al., 2018).

Persistent*Streptococcus equi* infection

Once ruptured, abscesses of the retropharyngeal lymph nodes typically drain into the guttural pouches, resulting in guttural pouch empyema. If the purulent material is not cleared and loses fluid, this can form chondroids; both empyema and chondroids can act as chronic reservoirs of*S. equi* (Newton et al., 1997a, Judy et al., 1999). This infectious material, inspissated or otherwise, has also been reported in the sinuses, albeit rarely (Newton et al., 1997b).

Carriage of *S. equi* occurs in equids that are chronically infected; most strangles cases are cleared within 6 weeks, but some animals can enter a carrier state (Newton et al., 1997a, Newton et al., 1997b). An average of 10% of infected individuals in an outbreak develop into carriers (Boyle et al., 2018, Sweeney et al., 2005); although, this figure may be an underestimate, with detection rates being limited by current diagnostic sensitivity (Pringle et al., 2019). Carriers intermittently shed bacteria into the environment, leading to recurrence and perpetuation of strangles within their herd as well as transmission to naïve individuals (Mallicote, 2015).

Diagnosis of strangles

Diagnosis of acute disease

The diagnosis of strangles relies on a thorough understanding of an animal's history, with particular respect

to onset, management structures, and possible exposure, including the history of travel, or new arrivals to the farm (Boyle et al., 2018). Clinical signs can be variable and non-specific; indeed, not all animals develop clinical signs (Duran and Goehring, 2021, Boyle et al., 2018). Nevertheless, they form a vital part of any clinical diagnosis, especially during an outbreak where the testing of all affected individuals may not be necessary (Rendle et al., 2021). Many diagnostic modalities can aid in the diagnosis of strangles and its complications, including radiography, ultrasonography, and endoscopy, as well as clinical pathology (Boyle et al., 2018, Duffee et al., 2015, Van de Kolk and Kroeze, 2013).

Pathogen identification historically relied on the culture of *S. equi* due to its low cost and wide availability (Waller, 2014). However, sensitivity can be as low as 30-40% (Lindahl et al., 2013, Boyle et al., 2012, Pusterla et al., 2021), and other beta-haemolytic Streptococci such as *S. zooepidemicus* can complicate interpretation (Boyle et al., 2018). Low levels of bacterial shedding, the presence of host-produced growth inhibitors, sample site and poor sampling technique can also lead to false negative results (Pusterla et al., 2021).

Advances in polymerase chain reaction (PCR) (Webb et al., 2013, Noll et al., 2020, Willis et al., 2021) and loop-mediated isothermal amplification (LAMP) assays (Boyle et al., 2018), with their shorter turnaround times, have improved the sensitivity and specificity of the detection of *S. equi* and these assays are now regarded as the gold standard (Boyle et al., 2018). PCR and LAMP assays detect DNA of live and dead bacteria indiscriminately; although efforts to determine the physiological state and viability of *S. equi* using molecular approaches show promise (Pusterla et al., 2018). Despite the potential for false positive results, all positive PCR cases should be taken seriously, even if they are culture negative (Rendle et al., 2021, Boyle et al., 2018, Waller, 2014, Pusterla et al., 2018). Identification of animals with clinical signs consistent with strangles, regardless of the PCR result, should result in strict movement restrictions and biosecurity protocols (Willis et al., 2021, Rendle et al., 2021).

Advances in diagnostics and surveillance are interlinked: techniques such as quantitative PCR (Webb et al., 2013), nested PCR (Noll et al., 2020), and LAMP assays (Boyle et al., 2021) are rapid and possess high sensitivities and specificities These technologies allow for the creation of clinically valuable surveillance schemes (McGlennon, 2019), with both laboratory and veterinary contributors. Point-of-care assays have limitations in detection threshold, but have the potential to reduce diagnostic turnaround times and provide a simpler option to caregivers (Slovis et al., 2020). This would allow for the screening of high-risk animals, reducing diagnostic guesswork, and ensuring well-timed enaction of biosecurity measures.

The successful identification of *S. equi* , whether through bacterial culture or molecular methods, is dependent on the stage of infection (Rendle et al., 2021) and the sampling site and technique used (Boyle et al., 2017). A single negative test result does not equate to the absence of infection (Boyle et al., 2018). *S. equi* is only present transiently on the nasal mucosa and is often undetectable until the lymphoid abscesses rupture, which typically occurs 1-4 weeks after infection (Rendle et al., 2021). Consequently, nasal swabs and washes will often yield negative results in the initial stages of infection (Boyle et al., 2018). Using quantitative PCR, it was found that nasopharyngeal lavage was the optimal sampling technique with the highest sensitivity, followed by nasopharyngeal swabbing and then nasal swabbing (Lindahl et al., 2013).

Diagnosis of persistent infection

Carriers of *S. equi* do not differ clinically and cannot be diagnosed on the basis of inflammatory markers, including white blood cell counts and serum amyloid A (Pringle et al., 2020b, Christoffersen et al., 2010, Davidson et al., 2008); therefore, carrier status has little impact on systemic inflammation. There is conflicting evidence on the utility of endoscopy scoring since many carriers have grossly normal guttural pouches (Pringle et al., 2020b, Riihimäki et al., 2016); however, Boyle et al. (2017) found distinct differences are visible in many carriers.

Endoscopically guided guttural pouch lavage followed by quantitative PCR is recommended for the detection of persistent infections (Boyle et al., 2018). This technique provides visualisation of the guttural pouch, allowing identification of chondroids, inflammation, or empyema; although, contamination of equipment can result in false positive results (Svonni et al., 2020). LAMP assays have been demonstrated to be comparable

to PCR for this purpose (Boyle et al., 2017). Guttural pouch lavage has been validated as superior to a single nasopharyngeal swab or lavage (Boyle et al., 2017). However, nasopharyngeal lavage on three separate occasions has been demonstrated to predict freedom from persistent infection (Pringle et al., 2022, Sweeney et al., 2005), with repeated testing mitigating the possibility of false negatives. Serological testing is unable to identify carrier animals (Durham and Kemp-Symonds, 2021) and does not replace these other more invasive, expensive and time-consuming methods of detection. Guttural pouch lavage combined with quantitative PCR is considered the best, albeit imperfect, method for carrier detection (Svonni et al., 2020, Boyle et al., 2018, Rendle et al., 2021).

Serological testing

Indirect ELISAs detect antibodies generated by the host: they are used for screening animals (Craig, 2021), identifying exposure following an outbreak (Robinson et al., 2013, Rendle et al., 2021), and diagnosing the complications of strangles (Boyle et al., 2009). Carrier status cannot be determined using commercially available ELISAs (Durham and Kemp-Symonds, 2021, Van Maanen et al., 2021). Commercially available ELISAs detect antibodies produced against the SeM surface protein, or both antigen A (SEQ2190, a non-SeM target) and antigen C (a fragment of SeM) of *S. equi* , the so-called dual-target ELISA (Robinson et al., 2013, Boyle et al., 2018).

SeM-based ELISAs can be used to aid in the diagnosis of purpura haemorrhagica or metastatic abscessation ([?]12,800), as well as identify animals predisposed to developing purpura haemorrhagica (>1:3,200) (Boyle et al., 2018, Boyle et al., 2009). They can also be used to indicate recent infection ([?]4-fold increase in paired samples ten days apart) (Boyle et al., 2009, Boyle et al., 2018); although, a single reading is not a measure of protection or active infection.

Cross-reactivity with a SeM homologue in *S. zooepidemicus* (Kelly et al., 2006) combined with the failure of the SeM-based ELISA to detect*S. equi* strains not containing SeM (Harris et al., 2015) led to the development of the dual-target ELISA (Duran and Goehring, 2021, Robinson et al., 2013). Following an outbreak, it is advised to use the dual-target ELISA to identify horses exposed to *S. equi* (Boyle et al., 2018, Duran and Goehring, 2021). The dual-target ELISA is reported to have similar sensitivity but greater specificity than the single target SeM-based ELISA (Robinson et al., 2013). It can be used to identify recent exposure, from as little as two weeks post-infection, and has been used to determine exposure in populations across the globe (Ling et al., 2011, Štritof et al., 2021, Ivens et al., 2011).

Clinical management

Treatment of acute *Streptococcus equi* infection

Most equids with acute strangles exhibit non-specific signs of generalised respiratory infection with presentation depending on challenge dose and host immunity, often responding well with only supportive and nursing care (Rendle et al., 2021, Whitelegg and Saunders, 2021). Acute disease can quickly deteriorate into severe cases, emphasising the need for regular monitoring (Rendle et al., 2021).

Nursing for an animal with strangles is vital and wide-ranging: good nursing provision will include an environment that encourages rest, appropriate nutrition, regular monitoring (TPR), abscess management, and a quarantine protocol (Whitelegg and Saunders, 2021). A soft, calorific, and palatable diet alongside water, to facilitate deglutition, both provided from a height, can help equids with profound lymphadenopathy; assisted nutrition may be indicated (Rendle et al., 2021). The experience of individual equids must be considered during a strangles outbreak, as small changes in diet and environment can aid in assuaging the effects of infection with *S. equi* .

Individuals with visible lymphadenopathies require good supportive and nursing care, with a focus on facilitating the maturation and subsequent drainage of abscesses (Boyle et al., 2018); the use of a 'hot pack' can enhance this process. Surgical drainage may be required if the abscesses are not spontaneously rupturing, although care must be taken to ensure the abscess is mature to enable maximal drainage (Boyle et al., 2018).

Once open, abscesses should be lavaged with saline or antiseptic solutions, followed by daily flushing so long as discharge persists (Rendle et al., 2021).

NSAIDs can be employed to provide analgesia and reduce pyrexia; it has been suggested that their use can slow the development of abscesses, but this claim lacks evidence (Rendle et al., 2021). Paracetamol has also been recommended since it does not inhibit inflammation but possesses anti-pyretic and analgesic actions, resulting in improved appetite and welfare (Rendle et al., 2021). Phenylbutazone or flunixin meglumine could also be considered (Boyle et al., 2018).

Antimicrobial therapy in strangles is controversial (Ramey, 2007): their use is encouraged between initial exposure and abscessation (Boyle et al., 2018). This window is not always adhered to since abscesses can develop within days (Timoney and Kumar, 2008). Although antimicrobial therapy can decrease the size of abscesses and should be considered in equids with stridor, dyspnoea or dysphagia on welfare grounds, their effects are limited following the detection of lymphadenopathy (Boyle et al., 2018).

Penicillin is the drug of choice for *S. equi* infection; however, population analysis (Morris et al., 2020) revealed that pbp2x mutations are emerging. This mutation is in the penicillin-binding site and is associated with penicillin resistance in *Streptococcus pneumoniae*(Maurer et al., 2012, Nichol et al., 2002). Penicillin resistance is variably seen in *S. equi* isolates (Fonseca et al., 2020, Clark et al., 2008, Johns and Adams, 2015) and constitutes a growing concern that clinicians will be less able to treat severely afflicted horses in the future. Antimicrobial therapy has a role in combatting *S. equi*infections, but it must be employed judiciously on an individual tailored basis, with careful consideration to minimise the development of antimicrobial resistance (Jaramillo-Morales et al., 2022, Boyle et al., 2018).

Treatment of persistent*Streptococcus equi* infection

Persistent infections of the guttural pouch are typically treated with topical and prolonged systemic antimicrobial therapy (Boyle et al., 2018). Administration of penicillin systemically and an endoscopically-guided gelatin-penicillin mix topically, has been regarded as broadly successful (Verheyen et al., 2000).

The removal of purulent material and chondroids from the guttural pouches is required for the elimination of the carrier state (Boyle et al., 2018). Endoscopic intervention is preferable to surgical intervention due to inherent risks of general anaesthesia, surgical dissection around vital structures, and *S. equi* environmental contamination (Boyle et al., 2018). Topical application of 20% acetylcysteine (w/v) solution can facilitate drainage of non-inspissated mucopurulent material through the nasal passages by disrupting disulphide bonds, thereby reducing mucus viscosity (Boyle et al., 2018).

Specific treatment methods depend on the individual presentation and the type of material within the guttural pouches. Many carriers do not present with empyema or chondroids, and it has been reported that the carrier state can self-resolve without treatment (Pringle et al., 2019).

Outbreak prevention and management

Strangles was once considered an inevitability (Solleysel, 1664), but has since been demonstrated to be a very preventable infection (Rendle et al., 2021). Outbreaks can be prevented by limiting exposure to the infectious agent, through enacting rigorous biosecurity protocols, using appropriate quarantining and screening facilities, and understanding of the pathogenesis of *S. equi* (Boyle et al., 2018). Outbreaks of strangles are controlled through the cessation of movement to and from the farm, isolating animals that are infected and where infection is suspected. A tiered 'traffic light' system with segregation based on exposure and no mixing between groups should be adhered to (Boyle et al., 2018). Following the outbreak, all animals should be tested for exposure and persistent infection.

Long-term control strategies should consider the vaccination of unexposed animals, the identification and treatment of carrier animals, and caregiver education on clinical signs associated with acute disease (Duran and Goehring, 2021).

Vaccination

The ideal strangles vaccine should provide a high degree of protection against *S. equi* , a long duration of immunity, the ability to be administered intramuscularly safely, and permit the differentiation of infected from vaccinated animals (DIVA) (Waller and Jolley, 2007). DIVA capability is important since the current commercially available enzyme-linked immunosorbent assays (ELISAs) do not differentiate between recently exposed horses and those animals vaccinated with live-attenuated vaccines, with implications for screening animals, movement restrictions and disease control (Duran and Goehring, 2021).

The first strangles vaccines were developed in the 1940s, using heat-killed bacteria, conferring a limited degree of protection but often resulting in adverse effects, including injection site reactions and pyrexia (Bazeley, 1940a, Bazeley, 1940b, Bazeley, 1942a, Bazeley, 1942b, Bazeley, 1943). Cell-free variations of this vaccine still exist (Waller, 2014), although the incidence of adverse reactions and the lack of DIVA capability have limited their use. A recent attempt to combine the *S. equi* bacterin and recombinant SeM protein in a vaccine yielded promising results in mice, with all demonstrating a humoral response (Rosa et al., 2021); evaluation of its safety and efficacy in horses is ongoing.

M-protein-containing extract vaccines have demonstrated some efficacy in reducing the frequency and severity of disease; although adverse reactions are common and they possess no DIVA capability (Hoffman et al., 1991). In a double-blind randomised clinical trial in foals, 29% (17/59) of vaccinates developed cervical lymphadenopathy, compared to 71% (39/55) of sham-vaccinated controls (Hoffman et al., 1991). Commercially available options, although none are available in the UK, include Strepvax II (Boehringer Ingelheim), Equivac S (Zoetis New Zealand), and Strepguard (MSD Animal Health) (Duran and Goehring, 2021).

Live-attenuated vaccines have been at the forefront of strangles prevention since the early 21st century; a 109 dose of an avirulent strain of *S. equi* , was shown to prevent lymphadenopathy in 100% (5/5) and 50% (2/4) of ponies, respectively, across two experiments conducted by Jacobs et al. (2000). The Equilis StrepE (MSD Animal Health) is administered submucosally, and the Pinnacle IN (Zoetis) is administered intranasally; they are commercially available in Europe and North America, respectively, as well as other countries intermittently (Duran and Goehring, 2021). Adverse reactions were reported upon intramuscular administration, and these live-attenuated vaccines possess no DIVA capability (Kemp-Symonds et al., 2007, Borst et al., 2011, Livengood et al., 2016, Lanka et al., 2010). Furthermore, the Equilis StrepE (MSD Animal Health) vaccine has been linked to *S. equi* replication, resulting in lymph node abscesses (Kemp-Symonds et al., 2007, Kelly et al., 2006, Mitchell et al., 2021, Harris et al., 2015).

Strangvac (Intervacc AB) is a recombinant fusion protein vaccine that is administered intramuscularly and has been shown to provide immunity in up to 94% (15/16) of ponies when challenged two weeks following third vaccination (Robinson et al., 2020). Strangvac has DIVA capability as the vaccine does not contain live *S. equi* , S. *equi* DNA nor the SeM and SEQ2190 antigens that are targeted by culture, PCR, or ELISA diagnostic tests (Robinson et al., 2018). Future studies will be needed to evaluate the utility of Strangvac (Intervacc AB) in clinical practice.

Vaccination as a tool for outbreak prevention has been limited by efficacy, safety, practicality, clashes with other vaccination schedules, DIVA capability, geographical restrictions, differences in circulating *S. equi* strains and owner compliance (Boyle et al., 2018, Mitchell et al., 2021). Advancements such as the Strangvac vaccine represent a promising development, potentially allowing vaccination to become a more efficacious control measure. However, continued work is required from veterinary professionals to build trust with owners and caregivers over the use of any strangles vaccines due to past difficulties (White et al., 2021).

Conclusion

Understanding *S. equi* is crucial to combatting strangles, and much work has been carried out to characterise its evolution (Holden et al., 2009), genome (Harris et al., 2015), epidemiology (Mitchell et al., 2021), survivability (Durham et al., 2018), resistance profile (Fonseca et al., 2020) and pathogenicity (Timoney and Kumar, 2008, Timoney, 2004). This increased understanding has enabled the development of more targeted diagnostic assays (Noll et al., 2020, Webb et al., 2013, Willis et al., 2021, Boyle et al., 2021), better outbreak prevention and management protocols (Rendle et al., 2021) and a safe and efficacious vaccine with DIVA

capability (Robinson et al., 2020). These advances better equip clinicians and caregivers to treat and prevent strangles.

Further research is required to investigate the role of *S. zooepidemicus* as a primary respiratory pathogen in equids (Waller and Wilson, 2021, Waller, 2017), and to better understand the growing concern of antibiotic resistance in both *S. equi* and *S. zooepidemicus* (Fonseca et al., 2020, Johns and Adams, 2015). The success of *S. equi* as a pathogen can be attributed to the carrier state allowing infection to spread to naïve animals. Understanding the host and pathogenic factors that predispose equids to persistent infection and validating a gold-standard method of diagnosis will help prevent future outbreaks and safeguard animal welfare.

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