Unsaddling *Streptococcus equi* infection of horses

Infection with *Streptococcus equi,* which forms abscesses in the lymph nodes of the head and neck in horses, is endemic in almost all countries around the world. The identification and isolation of horses with fever, an early sign of disease, is critical to minimising the number of horses affected and the severity of an outbreak, while the identification and treatment of persistently infected 'carrier' horses can reduce the risk of recurrent outbreaks and transmission between equine populations. Rapid diagnostic testing plays a key role in the identification of infected horses, which can then be isolated before the development of acute disease or treated to clear persistent infection. Vaccination can also be used to reduce the number of horses that become infected and the severity of their ensuing disease. This review describes the tools available to veterinarians and the journey towards the development and launch of a multi-component fusion protein vaccine that does not trigger positive diagnoses with any of the available diagnostic tests for strangles. The use of vaccination, alongside conventional methods of biosecurity and diagnostic testing, has the potential to unsaddle *S. equi*, reducing the number of strangles outbreaks and enhancing the health of horses.

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Strangles, caused by *Streptococcus* subspecies *equi* (*S. equi*), was first described in 1251 by Jordanus Rufus (Ruffo, 1256). However, the disease remains endemic in horse populations throughout the world, with the notable exception of Iceland, where a ban on the import of horses has been in place for over 1000 years (Björnsdóttir et al, 2017; Mitchell et al, 2021). This article describes the mechanisms that underpin the transmission of *S. equi* and the tools available for preventing and resolving this important disease.

Pathogenesis of strangles

Horses become infected with *S. equi* via the nose or mouth, most likely through ingestion of contaminated food or water (*Figure 1*) (Boyle et al, 2018). The bacteria attaches to and invades the mucosal surface of the nasopharynx, using an array of 'sticky' proteins on its surface, including the collagen-binding protein, CNE, which is believed to stick out from the surface of *S. equi* on hair-like projections (Lannergård et al, 2003; Holden et al, 2009; Steward et al, 2017). However, *S. equi* does not colonise the nasopharynx and samples taken from recently infected horses and those incubating the disease often return a negative culture or polymerase chain reaction (PCR) result, confounding their diagnosis (Boyle et al, 2018; Rendle et al, 2021). Instead, *S. equi* translocates to the submandibular and retropharyngeal lymph nodes within only a few hours of infection (Timoney and Kumar, 2008).

Once within the lymph nodes, *S. equi* produces an arsenal of virulence factors that misdirect the equine immune response, initiating the development of abscesses. As an example, *S. equi* produces an anti-phagocytic hyaluronic acid capsule, which camouflages it from the equine immune response (Woolcock, 1974; Anzai et al, 1999; Harris et al, 2015). An IgG endopeptidase called IdeE is secreted by *S. equi* into the surrounding tissues where it cleaves antibodies, reducing the immune response's effectiveness (Lannergard and Guss, 2006). Many other factors including the surface-attached SeM and EAG proteins, are also deployed to block the activity of the immune response (Galán and Timoney, 1987; Boschwitz and Timoney, 1994; Jonsson et al, 1995; Lindmark et al, 1999; Meehan et al, 2001, 2009). Therefore, it is not surprising that morbidity can reach 100% in some outbreaks, highlighting the importance of biosecurity measures to minimise horses' exposure to *S. equi* (Waller, 2014; Boyle et al, 2018; Rendle et al, 2021).

Infected horses develop fever over a period of 3–14 days, which can exceed 42°C in some cases. Importantly, signs of fever usually precede the shedding of *S. equi*, and so isolation of affected horses and good biosecurity at this stage can minimise both the number of horses affected and disease severity in an outbreak (Waller, 2014; Boyle et al, 2018; Rendle et al, 2021). Abscesses in the lymph nodes grow over a period of 7–21 days, potentially obstructing the airway, hence the name 'strangles' (*Figure 2*). The rupture of abscesses releases copious volumes of highly infectious pus that drains into the local environment, either via the horse's nose or through the skin. Further complications may arise if *S. equi* disseminates beyond the lymph nodes of the head and neck,

Figure 1. Streptococcus equi *can survive for up to 6 weeks in drinking water, facilitating horse-to-horse transmission.*

Figure 2. A foal showing the classic signs of strangles as a result of enlargement of abscesses in the lymph nodes, which are obstructing the airway.

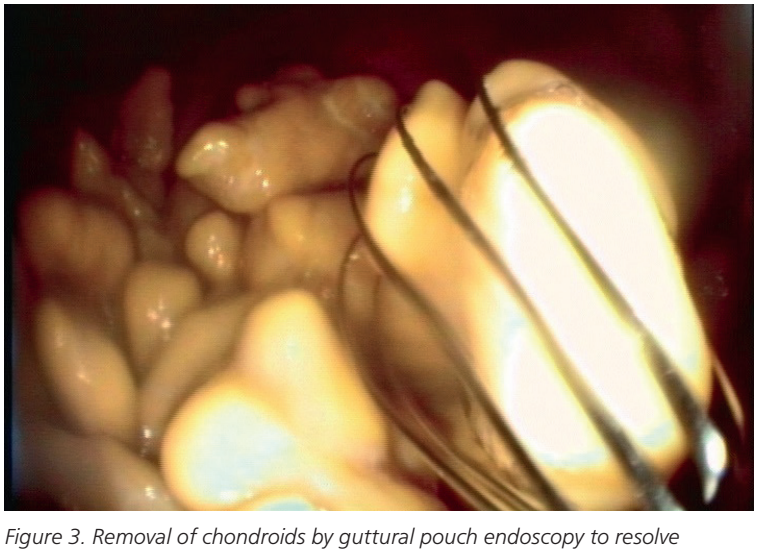

Figure 3. Removal of chondroids by guttural pouch endoscopy to resolve persistent infection with Streptococcus equi.

a condition known as 'bastard strangles', or if the infection causes immune-mediated conditions such as purpura haemorrhagica (Boyle et al, 2018).

Diagnosis of *Streptococcus equi* infection

The diagnosis of horses with acute disease can be confirmed by testing aspirates of lymph node abscesses or nasopharyngeal swab samples. The culture assay involves inoculating clinical samples onto blood agar and incubating overnight at 37°C. β-haemolytic colonies are picked and grown in nutrient broth overnight to enable differentiation of *S. equi* from other streptococci by sugar fermentation assays (Jones, 1919; Bannister et al, 1985). However, the identification of *S. equi* using culture is time consuming, requiring a minimum of 48 hours from receipt of clinical samples, and the test can be confounded by contamination of the sample with other bacteria.

Building on access to the DNA sequence of *S. equi* (Holden et al, 2009), PCR-based tests identify the presence of *S. equi* DNA and are much more sensitive, specific and rapid, being capable of returning a diagnosis within hours of sample receipt (Båverud et al, 2007; Webb et al, 2013). Recent data from the Surveillance of Equine Strangles project showed that 95.4% of positive cases of *S. equi* infection in the UK between 2015 and 2019 were identified by PCR (McGlennon et al, 2021). These data highlight that veterinarians in the UK are using quicker and more sensitive PCR assays to optimise the management and prevention of strangles. However, it remains vital that all horses with clinical signs of strangles, or those suspected of being infected, are isolated as soon as possible to minimise the transmission of *S. equi*. It is also important to retest horses that return negative diagnostic results if this is in conflict with the clinical diagnosis, especially during the initial phases of disease when *S. equi* may not be detected within the nasopharynx.

Persistence of *Streptococcus equi*

Despite the severity of signs in some horses, most (99%) make a full recovery over a period of a few weeks (Boyle et al, 2018). An immune response against *S. equi* can be detected within 2 weeks of infection (Galan and Timoney, 1985a). However, regardless of the development of these immune responses, *S. equi* continues to persist in the guttural pouches of around 10% of recovered 'carrier' horses in dried balls of pus called chondroids, or as a biofilm (Newton et al, 1997, 2000; Verheyen et al, 2000; Steward et al, 2017). The identification and treatment of carriers by removing residual purulent material and administering penicillin is effective at preventing further cases of strangles (*Figure 3*) (Verheyen et al, 2000).

In order to minimise the risks posed by carriers, ideally all new horses arriving at a yard would be placed into quarantine for a period of 3 weeks pending examination by guttural pouch endoscopy and testing by PCR (Waller, 2014; Newton et al, 2000). Similarly, all horses at an affected yard should be examined by guttural pouch endoscopy approximately 4 weeks following the resolution of all clinical cases to identify those that had developed persistent infection. However, the cost and time required to conduct such a high number of procedures can be substantial.

An alternative approach involves examining the guttural pouches of horses that have recovered from acute disease and horses that remained healthy, but were found to have antibodies directed towards two cell surface proteins SEQ2190 and SeM using a dual antigen indirect enzyme-linked immunosorbent assay (iELISA), which indicates recent exposure to *S. equi* (Robinson et al, 2013). While the dual antigen iELISA can identify all horses that have been exposed to *S. equi* during the acute phase of some outbreaks (Pringle et al, 2020), concerns have been raised about its ability to identify long-term, persistently infected horses where the antibody response post-acute disease may have waned to below detectable levels (Pringle et al, 2020; Durham and Kemp-Symonds, 2021). The dual antigen iELISA also fails to discriminate between horses that have recently been exposed to *S. equi* and those that remain persistently infected (Pringle et al, 2020). However, despite these deficiencies, the screening of horses using this tool has been widely adopted in the UK, directing further sampling that accounted for the identification of 8.5% of all PCR- and/or culture-positive horses in the period 2015–2019 (McGlennon et al, 2021). The removal of persistently infected carriers from the UK horse population is likely to yield significant long-term benefits to equine health and may already be reflected by an apparent decline in the number of cases of strangles identified over recent years (Parkinson et al, 2011; McGlennon et al, 2021).

Unsaddling *Streptococcus equi* infection

The application of quarantine and diagnostic testing procedures can greatly reduce the risk of *S. equi* entering a yard (Waller, 2014; Boyle et al, 2018). However, the implementation of these measures following the return of horses from equestrian events may not always be possible. Restricting horses' contact with others at events and avoiding horses sharing feed and water is a cost-effective measure that can minimise exposure to *S. equi*. Alongside these measures, vaccinating horses to increase their resistance to *S. equi* may slow down the disease and help the equine immune response to kill *S. equi* before abscesses form.

Approximately 75% of horses that recover from strangles develop a protective immune response that may persist for up to 5 years (Hamlen et al, 1994), providing evidence supporting the use of vaccines to prevent infection. Early vaccines employed cultures of *S. equi*, that were gently heat-killed at 55°C for 12 minutes to maintain potentially protective antigens (Bazeley and Battle, 1940; Bazeley, 1940, 1942a, 1942b, 1943). In trials, the vaccine was administered via subcutaneous injection to horses in the Australian army. Of approximately 2500 vaccinated horses, 29 developed strangles compared with 101 of approximately 1900 unvaccinated horses ($P<0.0001$) (Bazeley, 1942a). However, severe adverse reactions occurred at the site of injection and vaccines of this type have failed to enter commercial production.

Extract vaccines

Cell surface extract-based vaccines apply proprietary acid or enzymatic treatments to extract immunogenic protein components from the surface of *S. equi*. These extracts contain the SeM protein and are approved for intramuscular administration in horses in the USA, Australia and some other regions around the world. the same schedule. Examination of foals 2 weeks after the third vaccination identified cervical lymphadenopathy in 17 of 59 (29%) of vaccinates and 39 of 55 (71%) controls ($p<0.0001$), providing evidence for a 59% reduction in the clinical attack rate (Hoffman et al, 1991) Samples from horses vaccinated with extract vaccines may test positive by iELISA (El-Hage et al, 2019), but not by culture or PCR tests.

Adverse reactions to vaccination with extract vaccines included soreness or abscesses at injection sites in 44% of vaccinated foals and 2% of control foals (Hoffman et al, 1991). A total of 17 out of 53 cases of purpura haemorrhagica were associated with vaccination with SeM-containing vaccines (Pusterla et al, 2003). Immune complexes that were associated with cases of purpura haemorrhagica were found to contain IgA and *S. equi*-specific antigens that were similar to those found in acid extracts of *S. equi* (Galan and Timoney, 1985b). A recent study showed that six of eight (75%) horses with complications following natural infection with *S. equi* and 10/48 (21%) healthy horses had SeM antibody titres ≥1:12 800 8 weeks post-infection ($P=0.009$, two-tailed Fisher's exact test) (Delph et al, 2019).

Live attenuated vaccines

Live attenuated vaccines against strangles are widely used for the prevention of strangles in several countries around the world. These vaccines are based on strains that have been disabled, either by treatment with DNA-damaging agents (Lanka et al, 2010; Harris et al, 2015; Livengood et al, 2016), or the removal of a gene, *aroA*, required for the production of aromatic amino acids (Jacobs et al, 2000; Kelly et al, 2006), and are administered intranasally (Borst et al, 2011) or submucosally into the upper lip (Jacobs et al, 2000), respectively. In one study, three of 22 horses vaccinated with a high dose, and two of 22 horses vaccinated with a low dose, of the intranasal live attenuated vaccine, developed strangles compared with 9/15 controls ($P=0.005$ and $P=0.002$, respectively) (Waller, 2014). Submucosal vaccination protecteda all five, (100%) and two of four (50%) horses at 2 weeks after second vaccination ($P=0.05$ and $P=0.43$, respectively) (Jacobs et al, 2000). However, samples from horses vaccinated with live attenuated vaccines may test positive in culture, PCR and iELISA tests for strangles (Boyle et al, 2018, 2022).

Adverse events caused by live attenuated vaccines include the formation of rare mandibular abscesses (Jacobs et al, 2000; Kemp-Symonds et al, 2007; Lanka et al, 2010; Borst et al, 2011; Cursons et al, 2015; Livengood et al, 2016). Accidental contamination of remote injection sites in the horse can result in abscess formation (Kemp-Symonds et al, 2007), and a veterinary surgeon who suffered an accidental needlestick injury developed an inflamed thumb and lymphangitis progressing proximally along her left arm that resolved following the administration of intravenous antibiotics (Thompson and McNicholl, 2010).

Live attenuated vaccines contain the SeM protein and it is not recommended to vaccinate horses that have had strangles within the previous year or that have signs of strangles (Boyle et al, 2018). It is also recommended that horses with SeM antibody titres of 1:3200 or greater should not be vaccinated because of the increased risk of purpura hemorrhagica (Boyle et al, 2017, 2018, 2022).

To reduce the risk of purpura haemorrhagica and facilitate vaccination via the intramuscular route, a six-gene deletion strain of *S. equi* 4047 was generated. The strain lacked the genes *sagA*, *hasA*, SeM, *aroB*, *pyrC* and *recA*, which encode proteins involved in production of the β-haemolytic toxin, hyaluronic acid capsule, SeM, aromatic amino acids, pyrimidine DNA bases and DNA recombination repair, respectively. Intramuscular vaccination with a dose of 1 x 10^8 colony forming units (cfu) protected six of seven ponies from *S. equi* infection 52 days after second vaccination, while all nine control ponies developed strangles (P=0.0009) (Robinson et al, 2015). However, four of nine vaccinated ponies developed abscesses at the intramuscular injection site post-first vaccination, and two of these ponies were withdrawn from the study, demonstrating that this six-gene deletion strain was not suitable for further development for intramuscular administration (Robinson et al, 2015).

Protein subunit vaccines

Vaccination with recombinant SeM protein conferred significant levels of protection against infection with *S. equi* in mice (Meehan et al, 1998), but not horses (Sheoran et al, 2002). Another vaccine based on the IL8-cleaving protein SeCEP was shown to protect mice (Turner et al, 2009), but no data are available regarding its application to protect horses.

To broaden the immune response generated in horses, two combinations of recombinant proteins derived from *S. equi* (SzPSe, CNE, Se51.9, Se44.2 (IdeE2) and Se46.8 or SeM, Se44.2, Se75.3, Se42.0, Se110.0 and Se18.9) were tested as vaccines against strangles, but neither of these combinations protected horses from infection (Timoney et al, 2007).

However, a multi-component subunit vaccine comprising eight protein antigens from *S. equi* conferred significant levels of protection to horses from intranasal challenge with *S. equi* that induced strangles in all control animals (*Figure 4*) (Robinson et al, 2020). The pathway to identify the eight antigens and optimise their production and the protection conferred by fusing individual antigens together is described in a series of publications that began with the identification of the protein G-like cell surface protein ZAG in *Streptococcus zooepidemicus* 27 years ago (Jonsson et al, 1995). ZAG was shown to bind alpha 2-macroglobulin, serum albumin, and IgG, protecting *S. zooepidemicus* from recognition by the equine immune response (Jonsson et al, 1995) Further analysis revealed the presence of a homologous protein, EAG, in all isolates of *S. equi* (Lindmark et al, 1999). EAG was immunogenic in horses and conferred protection against *S. equi* infection in mice (Flock et al, 2004).

The collagen-binding *S. equi* cell surface protein, CNE, was discovered in 2003 and proposed to assist *S. equi* to attach to the equine mucosa during the initial stages of infection (Lannergård et al, 2003). In contrast, the function of another cell surface-attached collagen-like protein, SclC, remains unknown, despite its discovery in 2004 (Karlström et al, 2004, 2006). Vaccinating mice with EAG, CNE and SclC protected against *S. equi* infection, with a combination of EAG and CNE appearing to have an enhanced effect (Flock et al, 2006). Moreover, ponies vaccinated with the combination of EAG, CNE and SclC, formulated with a saponin-derived adjuvant had significantly reduced shedding of *S. equi* (P=0.02) and nasal discharge (P=0.0004), following intranasal challenge with 1 x 10^8 cfu of *S. equi* strain 4047 (Waller et al, 2007). The addition of two other surface proteins, Eq5 (SEQ0256) and Eq8 (SEQ0402), to the EAG, CNE and SclC vaccine fully protected one pony and significantly reduced the burden of infection identified post-mortem (P=0.01) at 2 weeks after a fourth vaccination (Guss et al, 2009).

The secreted IgG endopeptidase, IdeE, was discovered in 2006 (Lannergård and Guss, 2006) followed by a second IgG endopeptidase, IdeE2, shortly thereafter (Hulting et al, 2009). These enzymes cleave immunoglobulin at the hinge region, reducing the ability of antibodies to target *S. equi* (Timoney et al, 2008; Hulting et al, 2009), and their administration conferred protection to mice (Hulting et al, 2009). Their addition, to make a seven-component vaccine comprising IdeE, IdeE2, EAG, CNE, SclC, Eq5 and Eq8, protected six of seven (86%) ponies from strangles, significantly reducing signs of fever (P=0.0001) and signs of infection identified post-mortem (P=0.005) following challenge with *S. equi* at 2 weeks after the third vaccination (Guss et al, 2009). However, while this vaccine conferred significant levels of protection, the production and formulation of seven individual protein components would have significant cost implications.

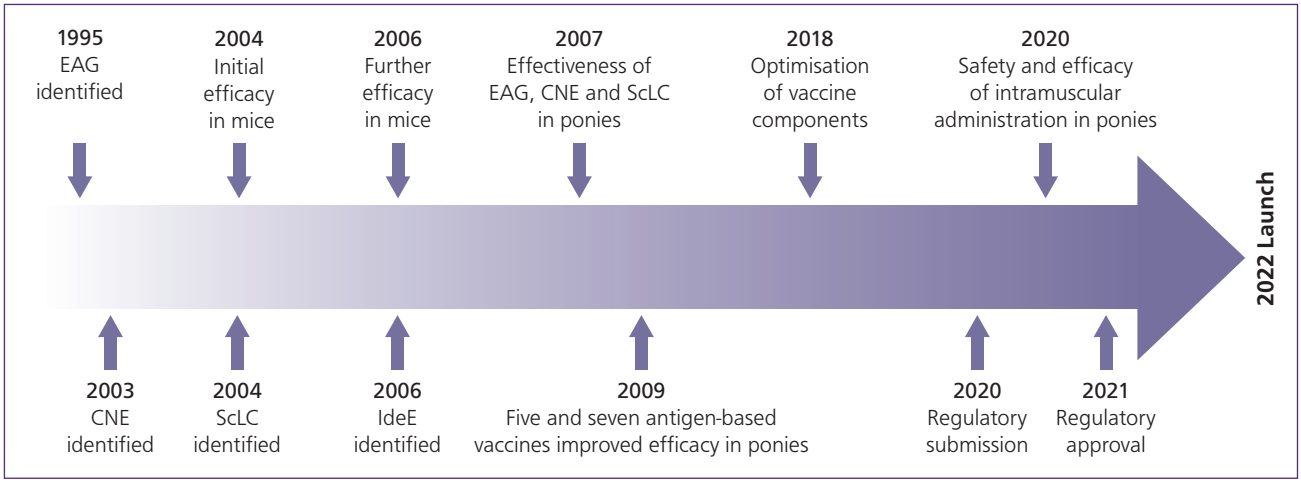

Figure 4. Timeline for the development of an eight-component fusion protein vaccine.

To improve the ease of production, the efficacy of five different combinations of five different fusion proteins, IdeE and IdeE2, encompassing a total of 11 different *S. equi* proteins were measured in three experiments in ponies. A combination of two fusion proteins (CCE containing CNE, EAG, SclC, SclF and SclI; and Eq85 containing Eq8 and Eq5) with IdeE was found to optimise the ease of production and the efficacy conferred against *S. equi* challenge (Robinson et al, 2018). However, this vaccine was administered via intranasal spray into both nostrils in combination with two subcutaneous injections near to the retropharyngeal lymph nodes, which is not practical for use in the field.

Therefore, the safety, immunogenicity and efficacy of this fusion protein vaccine following intramuscular administration was quantified in a series of four experiments in ponies. The vaccine was found to induce adverse effects of fever and pain, swelling and heat at the injection site, which lasted for up to 5 days and resolved without veterinary intervention (Robinson et al, 2020). Antibody responses to the vaccine components in the blood of vaccinated ponies increased after first and second vaccinations, which were given 4 weeks apart. Antibody responses then declined, but remained above the levels pre-first vaccination up to 1 year after the second vaccine dose (Robinson et al, 2020). A booster vaccination administered at 3, 6 or 12 months after the second dose restored peak antibody levels. Antibody responses to vaccine components were also detected in samples taken from the nasopharynx of vaccinated ponies, highlighting that intranasal vaccination was not required to induce a mucosal antibody response (Robinson et al, 2020). Furthermore, sera from vaccinated ponies neutralised the collagen-binding activity of *S. equi* cells in vitro and the ability of IdeE to cleave immunoglobulin (Righetti et al, 2021). Significant levels of IdeE-neutralisation were maintained up to 6 months after second vaccination, and increased after the administration of a third vaccination whether this was 3, 6 or 12 months after the second dose, providing the first insights into one possible mechanism by which this vaccine may protect horses (Righetti et al, 2021).

The challenge of ponies at 2 weeks after the second vaccination induced signs of fever in all 16 control ponies and 11 of 16 (69%) vaccinates (P=0.04). Vaccinated ponies developed a median of two lymph node abscesses compared with three in the control group (P=0.01), and the onset of fever and shedding of *S. equi* was significantly delayed in vaccinated ponies (P=0.000003 and P=0.05, respectively). Challenge of ponies at 2 months after second vaccination induced signs of fever in all four control ponies and five of 12 (42%) vaccinates (P=0.09), although these data were significant following the addition of historical control data from similar groups of control ponies (P=0.005) (Robinson et al, 2020). Vaccinated ponies developed a median of 0.5 lymph node abscesses compared with two in the control group (P=0.02), the onset of fever was significantly delayed in vaccinated ponies (P=0.008) and the amount of *S. equi* that was shed from vaccinated ponies was lower (P=0.02). The level of protection against challenge with *S. equi* that was conferred at 2 weeks after a third dose of vaccine increased further with one of 16 (6%) vaccinated ponies developing fever compared with all 15 control ponies (P<0.0001), and only one (6%) of the vaccinated ponies developed a lymph node abscess compared with 14 of 15 (93%) controls (P<0.0001). None of the vaccinated ponies, but five control ponies shed *S. equi* in the period post-challenge (P=0.02) (Robinson et al, 2020).

Analysis of the eight antigen sequences that were extracted from 759 genomes of isolates recovered from 19 countries found very high levels of conservation. The predicted amino acid sequences of SclC, SclI and IdeE were identical across all genomes and at least 1579 of 1580 predicted amino acids in the eight antigens were identical in 743 (97.9%) of the genomes, suggesting that immune responses generated towards these antigens will target all types of *S. equi* that have been identified to date (Frosth et al, 2022).

The multicomponent fusion protein vaccine contains no live bacteria, no *S. equi* DNA and the antigens within the vaccine do not include SEQ2190 or SeM. During the vaccination/challenge experiments, none of the 44 vaccinated ponies tested positive by quantitative PCR or iELISA prior to experimental challenge (Robinson et al, 2020). Therefore, it is possible for this vaccine to be used alongside conventional strategies of biosecurity and diagnostic testing, providing new opportunities for the prevention of strangles.

KEY POINTS

- Isolating horses with early signs of strangles, such as fever, can reduce the number of horses affected and the severity of disease in an outbreak.
- Identifying and treating carriers carriers can prevent recurrent disease and transmission of *Streptococcus equi* to other populations of horses.
- Polymerase chain reaction testing facilitates rapid diagnosis of infection.
- Serological screening of horses can identify those exposed to *S. equi* during an outbreak, which may have become persistently infected.
- Vaccination can reduce the number of affected horses and the severity of ensuing disease.
- The application of vaccination alongside biosecurity and diagnostic testing strategies has the potential to finally unsaddle *S. equi.*

Conclusions

Strangles remains an endemic disease of horses with only the geographically isolated population of horses in Iceland remaining free of infection. The completion of the *S. equi* genome sequence facilitated the development of more sensitive and specific diagnostic assays and their use alongside biosecurity measures can reduce the risk of new outbreaks. However, further research to develop more sensitive assays for the detection of long-term persistently infected horses is warranted. Vaccination of horses provides another tool that can reduce the number of horses that become infected and the severity of their disease. The application of knowledge gained from genome sequencing of *S. equi*, so-called reverse vaccination, has led to the development of a multi-component fusion protein vaccine that can be administered via intramuscular injection. The vaccine did not trigger diagnostic tests for *S. equi* and protected up to 94% of ponies from the development of fever and lymph node abscesses at 2 weeks after a third dose. Further studies are required to determine if the reduced number of lymph node abscesses in vaccinated animals leads to a reduction in the

number of horses that experience complications or become persistently infected. The application of vaccines alongside effective biosecurity and diagnostic testing has the potential to eliminate strangles from properties, opening up the possibility of other nations to rid themselves of this equine scourge. EQ

Conflicts of interest

The author is Chief Scientific Officer at Intervacc AB, Stockholm.

References

Anzai T, Timoney JF, Kuwamoto Y, Fujita Y, Wada R, Inoue T. In vivo pathogenicity and resistance to phagocytosis of *Streptococcus equi* strains with different levels of capsule expression. Vet Microbiol. 1999;67(4):277–286. https://doi.org/10.1016/S0378-1135(99)00051-6

Bannister MF, Benson CE, Sweeney CR. Rapid species identification of group C streptococci isolated from horses. J Clin Microbiol. 1985;21(4):524–526. https://doi.org/10.1128/jcm.21.4.524-526.1985

Båverud V, Johansson SK, Aspan A. Real-time PCR for detection and differentiation of *Streptococcus equi* subsp. *equi* and *Streptococcus equi* subsp. *zooepidemicus*. Vet Microbiol. 2007;124(3-4):219–229. https://doi.org/10.1016/j.vetmic.2007.04.020

Bazeley PL. Studies with equine streptococci 2. Aust Vet J. 1940;16(6):243–259. https://doi.org/10.1111/j.1751-0813.1940.tb06315.x

Bazeley PL. Studies with equine streptococci 3. Aust Vet J. 1942a;18(4):141–155. https://doi.org/10.1111/j.1751-0813.1942.tb06344.x

Bazeley PL. Studies with equine streptococci 4. Aust Vet J. 1942b;18(5):189–194. https://doi.org/10.1111/j.1751-0813.1942.tb06359.x

Bazeley PL. Studies with equine streptococci 5. Aust Vet J. 1943;19(3):62–85. https://doi.org/10.1111/j.1751-0813.1943.tb04318.x

Bazeley PL, Battle J. Studies with equine streptococci 1. Aust Vet J. 1940;16(4):140–146. https://doi.org/10.1111/j.1751-0813.1940.tb01317.x

Björnsdóttir S, Harris SR, Svansson V et al. Genomic dissection of an Icelandic epidemic of respiratory disease in horses and associated zoonotic cases. MBio. 2017;8(4):8. https://doi.org/10.1128/mBio.00826-17

Borst LB, Patterson SK, Lanka S, Barger AM, Fredrickson RL, Maddox CW. Evaluation of a commercially available modified-live *Streptococcus equi* subsp *equi* vaccine in ponies. Am J Vet Res. 2011;72(8):1130–1138. https://doi.org/10.2460/ajvr.72.8.1130

Boschwitz JS, Timoney JF. Inhibition of C3 deposition on *Streptococcus equi* subsp. *equi* by M protein: a mechanism for survival in equine blood. Infect Immun. 1994;62(8):3515–3520. https://doi.org/10.1128/iai.62.8.3515-3520.1994

Boyle AG, Smith MA, Boston RC, Stefanovski D. A case-control study developing a model for predicting risk factors for high SeM-specific antibody titers after natural outbreaks of *Streptococcus equi* subsp equi infection in horses. J Am Vet Med Assoc. 2017;250(12):1432–1439. https://doi.org/10.2460/javma.250.12.1432

Boyle AG, Timoney JF, Newton JR, Hines MT, Waller AS, Buchanan BR. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles-revised consensus statement. J Vet Intern Med. 2018;32(2):633–647. https://doi.org/10.1111/jvim.15043

Boyle AG, Mitchell C, Stefanovski D, Waller AS. Horses vaccinated with live attenuated intranasal strangles vaccine seroconvert to SEQ2190 and SeM. Equine Vet J. 2022;54(2):299-305. https://doi.org/10.1111/evj.13443

Cursons R, Patty O, Steward KF, Waller AS. Strangles in horses can be caused by vaccination with Pinnacle I. N. Vaccine. 2015;33(30):3440–3443. https://doi.org/10.1016/j.vaccine.2015.05.009

Delph KM, Beard LA, Trimble AC, Sutter ME, Timoney JF, Morrow JK. Strangles, convalescent *Streptococcus equi* subspecies *equi* M antibody titers, and presence of complications. J Vet Intern Med. 2019;33(1):275–279. https://doi.org/10.1111/jvim.15388

Durham AE, Kemp-Symonds J. Failure of serological testing for antigens A and C of *Streptococcus equi* subspecies equi to identify guttural pouch carriers. Equine Vet J. 2021;53(1):38–43. https://doi.org/10.1111/evj.13276

El-Hage CM, Bannai H, Wiethoelter AK et al. Serological responses of Australian horses using a commercial duplex indirect ELISA following vaccination against strangles. Aust Vet J. 2019;97(7):220–224. https://doi.org/10.1111/avj.12825

Flock M, Jacobsson K, Frykberg L et al. Recombinant *Streptococcus equi* proteins protect mice in challenge experiments and induce immune response in horses. Infect Immun. 2004;72(6):3228–3236. https://doi.org/10.1128/IAI.72.6.3228-3236.2004

Flock M, Karlström A, Lannergård J, Guss B, Flock J. Protective effect of vaccination with recombinant proteins from *Streptococcus equi* subspecies *equi* in a strangles model in the mouse. Vaccine. 2006;24(19):4144–4151. https://doi.org/10.1016/j.vaccine.2006.02.016

Frosth S, Morris ERA, Wilson H et al. Conservation of vaccine antigen sequences encoded by sequenced strains of *Streptococcus equi* subsp. *equi*. Equine Vet J. 2022;evj.13552. https://doi.org/10.1111/evj.13552

Galan JE, Timoney JF. Mucosal nasopharyngeal immune responses of horses to protein antigens of *Streptococcus equi*. Infect Immun. 1985a;47(3):623–628. https://doi.org/10.1128/iai.47.3.623-628.1985

Galan JE, Timoney JF. Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of *Streptococcus equi*. J Immunol. 1985b;135(5):3134–3137

Galán JE, Timoney JF. Molecular analysis of the M protein of *Streptococcus equi* and cloning and expression of the M protein gene in *Escherichia coli*. Infect Immun. 1987;55(12):3181–3187. https://doi.org/10.1128/iai.55.12.3181-3187.1987

Guss B, Flock M, Frykberg L et al. Getting to grips with strangles: an effective multi-component recombinant vaccine for the protection of horses from *Streptococcus equi* infection. PLoS Pathog. 2009;5(9):e1000584. https://doi.org/10.1371/journal.ppat.1000584

Hamlen HJ, Timoney JF, Bell RJ. Epidemiologic and immunologic characteristics of *Streptococcus equi* infection in foals. J Am Vet Med Assoc. 1994;204(5):768–775

Harris SR, Robinson C, Steward KF et al. Genome specialization and decay of the strangles pathogen, *Streptococcus equi*, is driven by persistent infection. Genome Res. 2015;25(9):1360–1371. https://doi.org/10.1101/gr.189803.115

Hoffman AM, Staempfli HR, Prescott JF, Viel L. Field evaluation of a commercial M-protein vaccine against *Streptococcus equi* infection in foals. Am J Vet Res. 1991;52(4):589–592

Holden MTG, Heather Z, Paillot R et al. Genomic evidence for the evolution of *Streptococcus equi*: host restriction, increased virulence, and genetic exchange with human pathogens. PLoS Pathog. 2009;5(3):e1000346. https://doi.org/10.1371/journal.ppat.1000346

Hulting G, Flock M, Frykberg L, Lannergård J, Flock JI, Guss B. Two novel IgG endopeptidases of *Streptococcus equi*. FEMS Microbiol Lett. 2009;298(1):44–50. https://doi.org/10.1111/j.1574-6968.2009.01698.x

Jacobs AAC, Goovaerts D, Nuijten PJM, Theelen RPH, Hartford OM, Foster TJ. Investigations towards an efficacious and safe strangles vaccine: submucosal vaccination with a live attenuated *Streptococcus equi*. Vet Rec. 2000;147(20):563–567. https://doi.org/10.1136/vr.147.20.563

Jones FS. The Streptococci of Equines. J Exp Med. 1919;30(2):159–178. https://doi.org/10.1084/jem.30.2.159

Jonsson H, Lindmark H, Guss B. A protein G-related cell surface protein in *Streptococcus zooepidemicus*. Infect Immun. 1995;63(8):2968–2975. https://doi.org/10.1128/iai.63.8.2968-2975.1995

Karlström A, Jacobsson K, Flock M, Flock JI, Guss B. Identification of a novel collagen-like protein, SclC, in *Streptococcus equi* using signal sequence phage display. Vet Microbiol. 2004;104(3–4):179–188. https://doi.org/10.1016/j.vetmic.2004.09.014

Karlström A, Jacobsson K, Guss B. SclC is a member of a novel family of collagen-like proteins in *Streptococcus equi* subspecies equi that are recognised by antibodies against SclC. Vet Microbiol. 2006;114(1–2):72–81. https://doi.org/10.1016/j.vetmic.2005.10.036

Kelly C, Bugg M, Robinson C et al. Sequence variation of the SeM gene of *Streptococcus equi* allows discrimination of the source of strangles outbreaks. J Clin Microbiol. 2006;44(2):480–486. https://doi.org/10.1128/JCM.44.2.480-486.2006

Kemp-Symonds J, Kemble T, Waller A. Modified live *Streptococcus equi* ('strangles') vaccination followed by clinically adverse reactions associated with bacterial replication. Equine Vet J. 2007;39(3):284–286. https://doi.org/10.2746/042516407X195961

Lanka S, Borst LB, Patterson SK, Maddox CW. A multiphasic typing approach to subtype *Streptococcus equi* subspecies *equi*. J Vet Diagn Invest. 2010;22(6):928–936. https://doi.org/10.1177/104063871002200612

Lannergård J, Frykberg L, Guss B. CNE, a collagen-binding protein of *Streptococcus equi*. FEMS Microbiol Lett. 2003;222(1):69–74. https://doi.org/10.1016/S0378-1097(03)00222-2

Lannergård J, Guss B. IdeE, an IgG-endopeptidase of *Streptococcus equi* ssp. equi. FEMS Microbiol Lett. 2006;262(2):230–235. https://doi.org/10.1111/j.1574-6968.2006.00404.x

Lindmark H, Jonsson P, Engvall E, Guss B. Pulsed-field gel electrophoresis and distribution of the geneszagandfnzin isolates of *Streptococcus equi*. Res Vet Sci. 1999;66(2):93–99. https://doi.org/10.1053/rvsc.1998.0250

Livengood JL, Lanka S, Maddox C, Tewari D. Detection and differentiation of wild-type and a vaccine strain of *Streptococcus equi* ssp. *equi* using pyrosequencing. Vaccine. 2016;34(34):3935–3937. https://doi.org/10.1016/j.vaccine.2016.06.035

McGlennon A, Waller A, Verheyen K et al. Surveillance of strangles in UK horses between 2015 and 2019 based on laboratory detection of *Streptococcus equi*. Vet Rec. 2021;189(12):e948. https://doi.org/10.1002/vetr.948

Meehan M, Nowlan P, Owen P. Affinity purification and characterization of a fibrinogen-binding protein complex which protects mice against lethal challenge with

Streptococcus equi subsp. *equi.* Microbiology. 1998;144(4):993–1003. https://doi.org/10.1099/00221287-144-4-993

Meehan M, Owen P, Lynagh Y, Woods C. The fibrinogen-binding protein (FgBP) of *Streptococcus equi* subsp. *equi* additionally binds IgG and contributes to virulence in a mouse model. Microbiology. 2001;147(12):3311–3322. https://doi.org/10.1099/00221287-147-12-3311

Meehan M, Lewis MJ, Byrne C, O'Hare D, Woof JM, Owen P. Localization of the equine IgG-binding domain in the fibrinogen-binding protein (FgBP) of *Streptococcus equi* subsp. *equi.* Microbiology. 2009;155(8):2583–2592. https://doi.org/10.1099/mic.0.028845-0

Mitchell C, Steward KF, Charbonneau ARL et al. Globetrotting strangles: the unbridled national and international transmission of *Streptococcus equi* between horses. Microb Genom. 2021;7(3):7. https://doi.org/10.1099/mgen.0.000528

Newton JR, Wood JLN, Dunn KA, DeBrauwere MN, Chanter N. Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with *Streptococcus equi.* Vet Rec. 1997;140(4):84–90. https://doi.org/10.1136/vr.140.4.84

Newton JR, Verheyen K, Talbot NC et al. Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of *Streptococcus equi*. Equine Vet J. 2000;32(6):515–526. https://doi.org/10.2746/042516400777584721

Parkinson NJ, Robin C, Newton JR, Slater J, Waller AS. Molecular epidemiology of strangles outbreaks in the UK during 2010. Vet Rec. 2011;168(25):666. https://doi.org/10.1136/vr.d1485

Pringle J, Venner M, Tscheschlok L, Waller AS, Riihimäki M. Markers of long term silent carriers of *Streptococcus equi* ssp. *equi* in horses. J Vet Intern Med. 2020;34(6):2751–2757. https://doi.org/10.1111/jvim.15939

Pusterla N, Watson JL, Affolter VK, Magdesian KG, Wilson WD, Carlson GP. Purpura haemorrhagica in 53 horses. Vet Rec. 2003;153(4):118–121. https://doi.org/10.1136/vr.153.4.118

Rendle D, de Brauwere MN, Hallowell G et al. *Streptococcus equi* infections: current best practice in the diagnosis and managment of 'strangles. UK-Vet Equine. 2021;5 Suppl 2:s1–s16. https://doi.org/10.12968/ukve.2021.5.2.S.3

Righetti F, Flock M, Hentrich K et al. Functional activities of antibody responses following vaccination of ponies with a multicomponent subunit vaccine against strangles. Presented at the proceedings of the International Equine Infectious Diseases Conference, 27th September – 1st October 2021

Robinson C, Steward KF, Potts N et al. Combining two serological assays optimises sensitivity and specificity for the identification of *Streptococcus equi* subsp. *equi* exposure. Vet J. 2013;197(2):188–191. https://doi.org/10.1016/j.tvjl.2013.01.033

Robinson C, Heather Z, Slater J et al Vaccination with a live multi-gene deletion strain protects horses against virulent challenge with Streptococcus equi. Vaccine. 2015;33(9):1160–1167. https://doi.org/10.1016/j.vaccine.2015.01.019

Robinson C, Frykberg L, Flock M, Guss B, Waller AS, Flock JI. Strangvac: A recombinant fusion protein vaccine that protects against strangles, caused by *Streptococcus equi.* Vaccine. 2018;36(11):1484–1490. https://doi.org/10.1016/j.vaccine.2018.01.030

Robinson C, Waller AS, Frykberg L et al. Intramuscular vaccination with Strangvac is safe and induces protection against equine strangles caused by *Streptococcus equi.* Vaccine. 2020;38(31):4861–4868. https://doi.org/10.1016/j.vaccine.2020.05.046

Ruffo G. De Medicina Equorum. 1251. http://wellcomelibrary.org/player/b19689755 (accessed 23 February 2022)

Sheoran AS, Artiushin S, Timoney JF. Nasal mucosal immunogenicity for the horse of a SeM peptide of *Streptococcus equi* genetically coupled to cholera toxin. Vaccine. 2002;20(11–12):1653–1659. https://doi.org/10.1016/S0264-410X(01)00488-1

Steward KF, Robinson C, Maskell DJ, Nenci C, Waller AS. Investigation of the Fim1 putative pilus locus of *Streptococcus equi* subspecies equi. Microbiology. 2017;163(8):1217–1228. https://doi.org/10.1099/mic.0.000506

Thompson RN, McNicholl BP. Needlestick and infection with horse vaccine. BMJ Case Rep. 2010;2010:bcr1120092444. https://doi.org/10.1136/bcr.11.2009.2444

Timoney JF, Kumar P. Early pathogenesis of equine *Streptococcus equi* infection (strangles). Equine Vet J. 2008;40(7):637–642. https://doi.org/:10.2746/042516408X322120

Timoney JF, Qin A, Muthupalani S, Artiushin S. Vaccine potential of novel surface exposed and secreted proteins of *Streptococcus equi.* Vaccine. 2007;25(30):5583–5590. https://doi.org/10.1016/j.vaccine.2007.02.040

Timoney JF, Yang J, Liu J, Merant C. IdeE reduces the bactericidal activity of equine neutrophils for *Streptococcus equi.* Vet Immunol Immunopathol. 2008;122(1–2):76–82. https://doi.org/10.1016/j.vetimm.2007.10.017

Turner CE, Kurupati P, Wiles S, Edwards RJ, Sriskandan S. Impact of immunization against SpyCEP during invasive disease with two streptococcal species: *Streptococcus pyogenes* and *Streptococcus equi.* Vaccine. 2009;27(36):4923–4929. https://doi.org/10.1016/j.vaccine.2009.06.042

Verheyen K, Newton JR, Talbot NC, Brauwere MN, Chanter N. Elimination of guttural pouch infection and inflammation in asymptomatic carriers of *Streptococcus equi.* Equine Vet J. 2000;32(6):527–532. https://doi.org/10.2746/042516400777584703

Waller A, Flock M, Smith K et al. Vaccination of horses against strangles using recombinant antigens from *Streptococcus equi.* Vaccine. 2007;25(18):3629–3635. https://doi.org/10.1016/j.vaccine.2007.01.060

Waller AS. New perspectives for the diagnosis, control, treatment, and prevention of strangles in horses. Vet Clin North Am Equine Pract. 2014;30(3):591–607. https://doi.org/10.1016/j.cveq.2014.08.007

Webb K, Barker C, Harrison T et al. Detection of *Streptococcus equi* subspecies *equi* using a triplex qPCR assay. Vet J. 2013;195(3):300–304. https://doi.org/10.1016/j.tvjl.2012.07.007

Woolcock JB. The capsule of Streptococcus equi. J Gen Microbiol. 1974;85(2):372–375. https://doi.org/10.1099/00221287-85-2-372