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Does antibiotic treatment in horses with strangles affect the development of S. equi ssp. equi specific antibodies?

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Does antibiotic treatment in horses with strangles affect the development of S. equi ssp. equi specific antibodies?

Påverkar antibiotikabehandling hos hästar med kvarka utvecklingen av specifika antikroppar mot S. equi ssp. equi?

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SUMMARY

Strangles, one of the most common infectious diseases in equids worldwide, is caused by *Streptococcus equi* ssp. *equi*, and is characterized by clinical signs like fever, nasal- and ocular discharge, lymphadenopathy, inappetence and depression. Several complications, like bastard strangle, immunomediated reactions and chronic carriage, are known. Outbreaks with *S. equi* can be extensive and proceed for longer periods. Treatment of horses with strangles is debated. It is argued that early treatment with antibiotics affect the development of antibodies but there is no evidence for this. The aim with this project is to look closer into how treatment with antibiotics affect immunity in horses with strangles.

For the prospective project, 41 naturally strangles infected horses on a Swedish Icelandic horse farm were classified into 3 groups. Group 1 included horses treated with antibiotics (penicillin) within 16 days after the first horse started showing symptoms (fever), group 2 included horses treated between 16 days and 22 days after the first horse started showing symptoms and group 3 included horses not treated with antibiotics during the acute period. Diagnostics were made by qPCR and culture and iELISA serology samples were taken at 7 occasions as a measure of the antibody response. Horses with iELISA od units ≥ 0.5 were classified as seropositive. The proportion seropositive horses was recorded for each group and the results were compared with Fisher's exact test.

All horses were classified as seropositive against antigen A on one or more occasions whereas for antigen C 3 horses were negative through all sampling occasions. Significant differences between group 1 and 2 (higher percentage seropositive horses in group 2 than 1) were seen in August 2015 (five months post first clinical sign) for both antigen A and C (P=0,0152) and in March 2016 (12 months post first clinical sign) for antigen A (P=0,0152). Between group 1 and 3, significant differences (higher percentage seropositive horses in group 3 than 1) were only observed for antigen A in August 2015 (P=0,0305) and in March 2016 (P=0,0063). No significant differences were observed during the first and second sampling occasions (about 4 and 6 weeks after the beginning of the outbreak) when most horses were positive.

These results provide support to the hypothesis that antibiotic treatment affects the development of immunity. However, many factors should be taken into consideration when interpreting the results, including individual variations in immune status between the horses, and that horses were continuously immunostimulated during the sampling period. Immunology is a complex subject and much is unknown regarding immunology and strangles; in particular whether serum antibodies fully reflect the level of immunity to strangles infections. Restrictive use of antibiotics is always important but for more evidence based recommendations regarding the antibiotic use to horses with strangles, more research is needed.

SAMMANFATTNING

Kvarka, en av de vanligaste infektionssjukdomarna hos hästdjur världen över, orsakas av bakterien *Streptococcus equi* ssp. *equi*, och kännetecknas av sjukdomstecken som feber, näs- och ögonflöde, lymfadenopati, inappetens och depression. Flera komplikationer, som kastad kvarka, immunmedierade reaktioner och kroniskt bärarskap, är kända. Utbrott med *S. equi* kan vara omfattande och fortgå under längre perioder. Antibiotikabehandling vid kvarka är omdiskuterat. Det hävdas att tidig behandling med antibiotika påverkar utvecklingen av antikroppar men inga bevis finns i litteraturen. Syftet med detta projekt är att se närmare på hur behandling med antibiotika påverkar immuniteten hos hästar med kvarka.

I den prospektiva studien delades 41 naturligt kvarkainfekterade hästar, från en svensk islandshästgård, in i 3 grupper. Grupp 1 innefattade de hästar som behandlats med antibiotika (penicillin) inom 16 dagar efter det att den första hästen började visa symtom (feber), grupp 2 de hästar som behandlades mellan 16 dagar och 22 dagar efter att den första hästen började visa symtom och grupp 3 de hästar som inte behandlats med antibiotika under den tidiga fasen av utbrottet. Diagnostiska metoder som användes var qPCR-analys och odling av nässköljprover. Serologiprover analyserades med iELISA vid 7 tillfällen och resultatet användes som ett mått på antikroppssvaret. Hästar med iELISA od värden ≥ 0.5 klassades som seropositiva. Andelen seropositiva hästar registrerades för varje grupp och resultatet jämfördes med Fishers exakta test.

Alla hästar var positiva för antigen A vid ett eller flera tillfällen och endast 3 hästar var negativa för antigen C under alla provtagningstillfällena. Signifikanta skillnader mellan grupp 1 och 2 (högre andel positiva hästar i grupp 2 än grupp1) sågs i augusti 2015 (fem månader efter första kliniska tecknet) för både antigen A och C (P=0,0152) och i mars 2016 (12 månader efter första kliniska tecknet) för enbart antigen A (P=0,0152). Mellan grupp 1 och 3 observerades signifikanta skillnader (högre andel positiva hästar i grupp 3 än grupp 1) endast för antigen A i augusti 2015 (P = 0,0305) och i mars 2016 (P = 0,0063). Inga signifikanta skillnader sågs under de två första provtagningstillfällena (ca 4 och 6 veckor efter utbrottets början) då flest hästar var seropositiva.

Resultatet ger visst stöd till hypotesen om att antibiotikabehandling påverkar utvecklingen av immuniteten. Hänsyn bör dock tas till flera faktorer vid tolkning av resultatet. Variationer i immunstatus mellan olika hästar, studiedesignen och specifika händelser under utbrottet kan ha spelat roll. Hästarna var kontinuerligt stimulerade med antigen under provtagnings-perioden. Immunologi är ett komplext ämne och mycket är okänt när det gäller immunologi och kvarka; speciellt beträffande om antikroppar i serum helt reflekterar graden av immunitet vid kvarkainfektion. Restriktiv användning av antibiotika är alltid viktigt, men för mer evidensbaserade rekommendationer om antibiotikabehandling vid kvarka, behövs mer forskning.

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INTRODUCTION

Strangles is one of the most common infectious diseases in equids worldwide. The bacteria causing strangles, *Streptococcus equi* ssp. *equi* (hereafter *S. equi*), is highly contagious and can cause severe symptoms especially in younger horses (Reed, Bayly & Sellon, 2010). In Sweden, strangles were on average diagnosed in 53 stables per year between 2005 and 2014 (SVA, 2017b). Outbreaks can be extensive and proceed for longer periods. It is difficult to limit the spread of strangles and as yet there is no uniformly applicable treatment regime for affected horses or entire stables.

How to best treat horses with strangles is debated. There are some indications that early antibiotic treatment inhibits the immunological response and that treatment of horses with strangles only prolongs the course of the disease (Piché, 1984). Another hypothesis is that antibiotic treatment of horses with abscesses can increase the risk of bastard strangles (Harrington, Sutcliffe & Chanter, 2002; Sweeney *et al.,* 2005; Reed, Bayly & Sellon, 2010; SVS, 2013; Waller, 2014). However, most of these hypotheses are based on limited research or lacking hypothesis based studies. More research, using improved diagnostic methods, is needed to examine what evidence there is for such statements and thereby assist in the establishment of scientifically based treatment guidelines.

The aim of this study is to examine whether there is any association between treatment with antibiotics in horses in an early stage of strangles and the development of seropositivity to *S. equi* after treatment.

LITERATURE REVIEW

Etiology

Strangles is caused by the gram positive beta hemolytic bacteria *Streptococcus equi* ssp. *equi* (hereafter termed *S. equi*). The DNA of *S. equi* is almost identical to the DNA of *Streptococcus equi* ssp. *zooepidemicus* (hereafter termed *S. zooepidemicus*) and is suggested to have evolved from an archetype of the latter. However, the bacteria differ greatly in biology and pathogenicity. *S. zooepidemicus* generally does not cause respiratory disease in equids and is sometimes found in the upper respiratory tract of healthy horses. In certain cases, however *S. zooepidemicus* can cause strangles-like disease in horses (Lindahl, 2013). Immunity against *S. zooepidemicus* does not protect against *S. equi* (Timoney, 2004; Waller & Jolley, 2007; Reed, Bayly & Sellon, 2010; Lindahl *et al.*, 2013; Waller, 2014).

Pathogenesis

The incubation time for *S. equi* is 3 to 14 days (Sweeney *et al.,* 2005). The bacteria enter the horse via the mouth or nose and attaches to naso- and oropharyngeal tonsil cells as well as to the epithelium of pharynx. Within hours it reaches deeper tonsil tissues and lymph nodes (Timoney & Kumar, 2008). Chemotactic complement factors attract a large number of granulocytes. Examples of antiphagocytic virulence factors, that seem to inhibit granulocytes from phagocytosis of the bacteria, are the hyaluronic acid capsule, the SeM-protein and a leukocidal toxin (acts toxic to leukocytes) released by the organism. The SeM-protein is an immunogenic cell wall protein that binds to fibrinogen and IgG. Fibrinogen binding inhibits

deposition of the complement factor C3b on the bacterial surface and phagocytosis by neutrophils. A loss of the SeM expression lead to loss of full virulence of the bacteria. The antiphagocytic virulence factors lead to an accumulation of bacteria and inflammatory cells. Abscesses are formed in the lymph nodes and drainage of these appears to be necessary for natural disposal of the organism (Harrington, Sutcliffe & Chanter, 2002; Timoney, 2004; Waller & Jolley, 2007).

Infection sometimes spreads from upper airways and associated lymph nodes. This condition has several names like malignant strangles, bastard strangles and metastatic abscessation (Todd, 1910; Boyle, 2017). Spreading may occur hematogically or via lymph vessels. Bacteria form metastasis abscesses in organs of thorax and the abdomen (Timoney 2004; Boyle, 2016) and there are also cases when abscesses have been found in the brain (Ford & Lokai, 1980; Henderson, 2011).

Pneumonia can occur secondary to rupturing abscesses in horses with bastard strangles but can also develop due to aspiration of *S. equi*-containing discharge (Boyle, 2016).

Another complication to strangles, associated with an aseptic necrotizing vasculitis, is called purpura haemorrhagica. It is hypothesized to be cause be caused by a type 3 hypersensitivity reaction following infection or vaccination. Galan and Timoney (1985b) found that there were immune complexes in sera of four horses with signs of purpura haemorrhagica and showed that these horses had significantly higher levels of IgA-antibodies than horses without signs of purpura haemorrhagica (Galan & Timoney, 1985b; Boyle, 2016). Purpura haemorrhagiga appears to be more common in anemic horses (Duffee *et al.,* 2015).

Myopathies (muscle infarctions and rhabdomyolysis) are other not commonly diagnosed complications to strangles. Muscle infarctions are assumed to be secondary to immune mediated vasculitis in horses with purpura haemorrhagica while rhabdomyolysis is thought to be caused by an immunological cross-reaction between the SeM-protein and myosin (Boyle, 2017).

Rupturing of retropharyngeal lymph nodes into the guttural pouches can cause empyema and in some cases persisting/chronic infection. Remaining pus in the guttural pouches may dry and hardens into so called chondroids (Mallicote, 2015; Boyle, 2016). Why only certain horses become chronical carriers is not known but previous exposure, disease severity, immunological factors and virulence of the bacteria may play a role (Newton *et al.,* 1997). There is some evidence that mutations leading to loss of virulence is more common in *S. equi* isolates from persistent carriers (Chanter *et al.,* 2000; Harris *et al.,* 2015).

Clinical signs

Early clinical signs in of strangles are fever and nasal- and ocular discharge. As the disease progresses some horses develop lymphadenopathy/lymph node abscesses, cough, depression and inappetence. Most commonly abscesses are found in the mandibular and retropharyngeal lymph nodes. Narrowing of the pharynx due to enlargement of the retropharyngeal lymph nodes can lead to clinical signs like dyspnea, dysphagia and neck extension. External swelling is not

always present. The enlarged lymph nodes eventually rupture spontaneously (Evers, 1968; Duffee *et al.*, 2015; Boyle, 2017).

In cases of bastard strangles, clinical signs like colic and dyspnea can be observed. This is depending on which organ that is involved. Abscesses in the abdomen often lead to peritonitis. Respiratory signs can also be observed in cases of pneumonia caused by aspiration (Boyle, 2017).

Horses with purpura haemorrhagica develop clinical signs like edema of the head, trunk and distal limbs and petechiations and ecchymoses of mucous membranes. In some cases, the antigen-antibody complexes affect other sites and cause symptoms from the gastrointestinal tract, muscles, lungs and kidneys (Boyle, 2017).

Epidemiology

Strangles is a highly contagious disease with a morbidity up to 100% and a mortality up to 10% in naïve populations (Sweeney *et al.*, 2005). However, different definitions of rate measure and diagnostic methods with different sensitivity are used in different reports, making it difficult to compare different outbreaks (Newton *et al.*, 2000). As a worst case scenario, Ford and Lokai (1980) present an outbreak with a morbidity of 100% and a mortality as high as 10%. However, management factors likely influenced this mortality level. During this outbreak, weanlings arrived at a farm that earlier had problems with strangles. Many of the new yearlings were sick at arrival and were mixed with healthy weanlings. Nonetheless, even in a more well managed outbreak Piché (1984) reports of a case specific mortality of 3,6% (Piché, 1984). Other reported morbidity rates are 84% (Dalgleish *et al.*, 1993) and 53% (Tscheschlok *et al.*, 2017), both based on clinical scoring.

Younger horses between one and five years of age are generally more sensitive and do often show more severe clinical signs (Piché, 1984; Sweeney *et al.*, 2005; Reed, Bayly & Sellon, 2010; Neamat-Allah & El Damaty, 2016). Foals up to three months of age, with immune mares, appear to be resistant to early strangles but can develop disease after weaning. Maternal antibodies initially reach the foals circulation by passive transfer after colostral ingestion. Later, during the first months of the foal's life, passively ingested immunoglobulins seem to reach the nasal mucosa during milk ingestion (Galan & Timoney, 1985a). According to current concepts 75% of horses surviving the disease become immune for more than five years (Todd, 1910) but 25 % are susceptible again after only months of time (Timoney, 2004).

Strangles seems to be more likely to be diagnosed in the spring than in the summer (Duffee *et al.*, 2015).

Transmission

The bacterium is transmitted directly via nasal discharge or via abscess secrete from infected horses. Indirect transmission occurs through contact with contaminated fomites, environment and people. According to the ACVIM Consensus Statement, nasal shedding of *S. equi* normally begin 24 to 48 hours after the onset of fever and persists for 2 to 3 weeks in most animals, (Sweeney *et al.*, 2005) but not all *S. equi* infected horses seem to acquire fever. Tscheschlok *et*

al. (2017) reports that 12 of 14 culture positive horses did not have fever at the time of sampling and 6 of these did not acquire clinical strangles throughout the outbreak (Tscheschlok *et al.*, 2017). This is in line with a study were only 78 of 108 strangles infected horses had pyrexia (Duffee *et al.,* 2015).

Some infected horses become chronic/persistent carriers of *S. equi.* These horses harbor *S. equi,* most commonly in the guttural pouches, for a long time without showing clinical signs. One study by Newton *et al.* (1997), showed that the organism could be detected in guttural pouch content, of subclinically infected horses, for at least 39 months. Nasal swab samples from horses in the study were intermittently negative (Newton *et al.,* 1997). There is some evidence that these chronically infected horses present a risk of spreading strangles but exactly how extensive this risk is, is not known (Todd, 1910; Newton *et al.,* 1997; Waller, 2014). Newton *et al.* (2000) analysed nasal lavage samples from horses from three outbreaks that had recovered from strangles with PCR or culture, and found the incidence of chronic carriage to be between 9 and 44% (Newton *et al*., 2000). New knowledge about diagnostics may make it easier to diagnose chronic carriers in the future (Newton *et al.,* 1997; Lindahl *et al.,* 2013).

Survival in the environment

There is limited information about the survival of *S. equi* in the environment and how this affects the spreading of the disease. Weese *et al.* (2009) conclude that survival of the bacteria outdoors in late summer conditions is short, in general under three days. In their study, rain, surface type or temperature did not affect the survival of the bacteria. Sun light on the other hand seemed to decrease the survival time (Weese *et al.,* 2009). Jorm (1992) showed that *S. equi* could survive indoors for 63 days on wood at 2°C (relative humidity at 32 %) and 48 days on glass or wood at 20°C (relative humidity at 49 %). However, there are many factors, not taken into consideration in mentioned studies (Jorm, 1992; Weese *et al.,* 2009) that potentially could affect the survival time.

Diagnostics

Research about the *S. equi* genome, has made it possible to develop more sensitive and specific tests that makes it easier to diagnose horses with strangles. Commonly used diagnostic methods are PCR, bacterial culture and antibody ELISA. Currently, much focus is put on finding diagnostic method for identification of chronic carriers (Waller, 2014).

PCR

There are PCR-methods for detection of *S. equi* and for *S. zooepidemicus.* Real-time/quantitative PCR (qPCR) is shown to be a more sensitive method to detect *S. equi* than culturing (Båverud, Johansson & Aspan, 2007; Webb *et al.,* 2012; Lindahl *et al.,* 2013). Webb *et al.* (2012) showed that a triple qPCR method (targeting the genes eqbE and SEQ2190) had a sensitivity and specificity of 93,9% respective 96,9% (information about clinical stage of sampled horses and sample methods were not reported) and Lindahl et al. (2013) demonstrated that a nasal lavage samples analysed with a qPCR (targeting the genes sodA and seeI) could detect *S. equi* in over 90% of horses with clinical signs of strangles. However, Tscheschlok et al. (2017) reports of an outbreak where only 20 of 34 horses (59%) with clinical strangles had qPCR positive nasal swab samples (Tscheschlok *et al.,* 2017). Nasal lavage samples yielded

5% more positive results than nasal swab samples in a study with 57 horses (Lindahl *et al.*, 2013). Nasal samples are reported to be negative up to 24 to 48 hours after the first fever (Sweeney *et al*., 2005). Samples taken directly from abscesses and samples from guttural pouches can also be analysed (Lindahl *et al.*, 2013).

Bacterial culture

Traditional bacterial culture is, compared to PCR-analysis, considerably less sensitive but can detect other bacteria than *S. equi* and *S. zooepidemicus* (Sweeney *et al.*, 2005; Lindahl *et al.*, 2013). Such bacteria can be *Actinobacillus* spp., *Bordetella bronchiseptica* and *Rhodococcus equi* (SVA, 2017b). According to Webb *et al.* (2012) the sensitivity was only 60,3% for culturing. Specificity was however as high as 100% (Webb *et al.*, 2012). Culturing is more time consuming than PCR. Sample types used for culture are the same as for PCR-analysis (SVA, 2017a; Waller, 2014).

Serology

Serological analyses for detection of *S. equi* antibodies is used for diagnostics and for disease surveillance. One serology method used today is the enzyme-linked immunosorbent assay (ELISA). Animal Health Trust (AHT) has developed a combined iELISA-test that examines two surface antigens (antigen A and antigen C) which both are unique for *S. equi* (Knowles, 2011). Antigen A consists of recombinant N-terminal protein fragments of the SEQ2190 sequence and antigen C is a part of the SeM protein (Robinson *et al.*, 2013). Robinson at al. (2013) found that the sensitivity and specificity for this test is 93,3% respective 99,3%. Another ELISA-method from ID vet is solely based on the SeM-antigen and has a sensitivity of 89,9% and a specificity of 77,0% (Robinson *et al.*, 2013). Negative pair samples analysed with the combined iELISA, taken with an interval of 10-14 days, should allow exclusion of past and recent infection (Knowles, 2011; SVA, 2017).

Hygiene measures

Hygiene measures are important to reduce the spread of disease and to avoid reinfection. Persons handling infected horses should use protecting clothing and dedicated equipment should be used to infected horses. It is preferable if different people handle infected and susceptible horses but in cases when this is not possible, susceptible horses should be handled before infected ones. There are different recommendations regarding how long horses, in stables with demonstrated strangles, should be isolated. According to Swedish trotting (2017), horses shall be isolated at least until all horses have been free from clinical signs for 20 days (Swedish trotting, 2017). SVA (2017b) recommend an isolation time for 4 to 6 weeks (SVA, 2017b).

During an outbreak, cleaning of the environment is important to reduce the amount of environmental contamination. This should be followed by more extensive cleaning after the outbreak. Removing of all organic material should be done before application of suitable disinfection. Repeated rounds of disinfection are preferable. Resting of pastures from animals for about 4 weeks is also recommended (Sweeney *et al.*, 2005).

Prevention

Isolation of new horses, before contact with a new population, reduce the risk of spreading strangles. Sweeney *et al.* (2005) recommend isolation for three weeks before introduction to other horses. It is also preferable if new horses are screened for *S. equi* by repeated nasopharyngeal swabs or lavages or by samples taken from the guttural pouches. During the isolation time, hygiene is important to decrease the risk for indirect contamination. Daily control of rectal temperatures, nasal discharge and cough in newly arrived horses can make it possible to isolate sick horses in an early stage of disease before they start to shed the disease (Sweeney *et al.*, 2005; Waller, 2014).

Vaccines

Researchers have for a long time tried to develop an effective vaccine against strangles. It is a big challenge to produce an effective vaccine without adverse effects. Examples of adverse effects associated with earlier and present vaccines are nasal discharge, lymphadenopathy, submandibular abscesses, purpura haemorrhagica and severe injection site reactions (Waller, 2014).

In Europe, Equilis StrepE (MSD Animal Health) is currently the only available vaccine against *S. equi* (Waller, 2014). This vaccine is however not licensed in Sweden (SVA, 2017b). It is a live attenuated vaccine from the TW928 strain on which a deletion of the aroAgene has been done. Intramuscular injection gave 100% immunity in one study but was associated with severe injection reactions. Pinnacle ® i.m. (Zoetis US) is an intranasal vaccine used in USA. It is live attenuated, acapsular and has been treated with nitroguanidine causing chemical mutagenesis. Because of earlier mentioned adverse effects it is not licensed in Europe. There are also different cell free extract vaccines (Equivac S by Zoetis, Strepguard by MSD Animal Health and Strepvax II by Boehringer Ingelheim) with unclear efficacy (Waller, 2014). New vaccines are under development. One example is a vaccine targeting 6-poly-N-acetyl glucosamine (PNAG) that appears to partly protect horses against *Rhodococcus equi*. A study on sera from strangles-infected horses indicates that the vaccine also could be effective against *S. equi* (Cohen *et al*., 2017). Strangvac (Intervet) is a subunit vaccine under development that is composed of recombinant polypeptides (CCE, Eq85, and IdeE). Clinical trials show that intramuscular injections may protect against and reduce clinical signs of *S. equi* (Flock *et al.*, 2017). Researchers are also working on a vaccine based on a modified superantigen (SzeQ). This superantigen normally leads to a misdirection of the immune response (Waller *et al.*, 2017).

There is a lack of vaccines that makes it possible to differentiate between infected/previously infected and vaccinated animals (so called DIVA-vaccines). A DIVA-vaccine would be necessary for effective serological disease control and eventual eradication of disease is areas with vaccinated horses (Newton, Robinson & Waller, 2016). Newton *et al.* (2017) investigated Equilis streptE (MSD Animal Health) and found that the vaccine contributed to seropositivity in vaccinated horses when analysed with iELISA (Newton *et al.*, 2017). El-Hage *et al.* (2017) suggest that there is a potential for DIVA in a cell free extract vaccine (Equivac, Zoetis), using iELISA. According to their study, the vaccine would not greatly interact with detection of cases of strangles but they assert that a larger study is needed for confirmation of this (El-Hage *et al.*, 2017).

Treatment

Treatment recommendations for horses with strangles are today inconsistent. Many horses recover spontaneously but some need medical treatment. Surgical drainage and flushing of mature abscesses can fasten the course of the disease. Fluid therapy and intensive care can be necessary in severely affected horses and tracheotomy may be essential and lifesaving in horses with severe dyspnea (Reed, Bayly & Sellon, 1998).

Anti-inflammatory treatment

Anti-inflammatory medication, as NSAIDs, can be used as treatment of horses with strangles as it reduces the fever, lessens the pharyngeal discomfort and improves the demeanor. Horses with purpura haemorrhagica may need treatment with corticosteroids as dexamethasone (Yelle, 1987; Reed, Bayly & Sellon, 1998).

Chronic carriers

To reduce the risk of spreading strangles treatment of chronic carriers can be necessary. Common treatment of these horses includes flushing of the guttural pouches with saline. Local and systemic treatment with antibiotic and local treatment with Acetylcysteine to resolve empyema and chondroids has been described. Destruction or removing of chondroids in the guttural pouches can in some cases be necessary. Removal of the chondroids is usually carried out with endoscopically guided instruments. Surgical removal, that before was common, seems only to be necessary in certain cases (Newton, Wood & Chanter, 1997; Verheyen *et al.*, 2000). According to a study by Verheyen *et al.* (2000), a combination of these treatment methods showed varying results. Of 14 horses with varying clinical signs, 5 only required removal of inflammatory material from the guttural pouches and systemic treatment with sulphonamide for three weeks while 4 of 14 needed further removal of inflammatory content as well as topical and systemical treatment with sulphonamide. The remaining 5 horses also needed topical and systemic treatment with penicillin or ceftiofur before they were counted as free from chronic infection. Most horses required repeated rounds of flushing of the guttural pouches. The authors point out that future controlled studies of treatment of chronic carriers are needed (Verheyen *et al.*, 2000).

Antibiotics

There is a debate regarding the use of antibiotics in horses with strangles. In review articles, books and antibiotic policies it is suggested that horses treated with antibiotics are unlikely to develop immunity to the bacteria and that treatment of subclinically infected horses and horses with abscesses only delay onset of clinical signs. It has also been argued that an impaired immunity could increase the risk of bacteremia, septicemia and metastatic abscessation. Unfortunately, there is currently not enough research that supports or refutes this (Reed, Bayly & Sellon, 1998; Harrington, Sutcliffe & Chanter, 2002; Sweeney *et al.*, 2005; SVS, 2013; Waller, 2014; Duffee *et al.*, 2015).

There are studies supporting a positive effect of antibiotic treatment in horses with strangles. Evers (1968) observed that horses with experimental infection with *S. equi* treated with furaltadone hydrochloride (an antimicrobial metabolite of Nitrofuran) led to a decrease in body

temperature and nasal and ocular discharge. The horses also became more alert and their appetite increased. Horses treated only 24 hours after fever and nasal and ocular discharge first occurred did not develop abscesses. Piché (1984) reports that three stallions with anorexia, fever and depression, treated with intravenous Oxytetracyclin for one week and intramuscular benzylpenicillin procaine for one week or only benzylpenicillin procaine for ten days were free from clinical signs after a few to five days. Additionally, all the foals at the farm were treated with antibiotics prophylactically when their body temperature reached 38,5°C. The treatment was continued until at least one week after the foals were free from fever. All foals developed disease after completed treatment but they did not acquire any abscesses (Piché, 1984). Christmann and Pink (2015) concludes that an antibiotic regime during a large strangles outbreak coincided with reduced incidence and eventually the resolution of the outbreak. This was however only an observational study (Christmann & Pink, 2015). There are also studies indicating that antibiotic treatment improves blood values. Evers (1968) observed a decrease level of leucocytes after early treatment with Furaltadone and Neamat-Allah & El Damaty observed that hematological, biochemical and acid base parameters improved after treatment with procaine penicillin for 10 days (Neamat-Allah & El Damaty, 2016; Evers, 1968).

One hypothesis is that antibiotic treatment prolongs the course of the disease (Sweeney *et al.,* 2005). Piché (1984) conclude that prophylactic treatment, in previously mentioned foals, may have delayed the disease development but also suggests that colostrum intake might have played a role. The treatment program was discontinued after weaning and then all foals contracted the disease (Piché, 1984). In the ACVIM Consensus Statement, by Sweeney *et al.* (2005), it is written that treatment of horses with immature abscesses is thought to prolong the course of disease as it is supposed to prolong enlargement and time to rupture of the abscesses. Surgical draining could therefore be indicated before antibiotic treatment. Something to consider is however that the abscesses may have a honeycomb structure and that surgical draining then only may lead to minimal exudate drainage. In severely affected horses with partial airway obstruction, antibiotics is probably indicated to reduce abscess size (Sweeney *et al.,* 2005).

Yelle (1987) recommend, in a review article, high doses of penicillin procaine for a prolonged period (1 to 6 months) to treat intraabdominal abscesses (Yelle, 1987). Prolonged intravenous antibiotic treatment was by Berlin *et al.* (2013) reported to cure four horses with suspected intraabdominal *S. equi*-abscesses. All horses were treated with high doses of Penicillin (44 000 IU/kg) for over one month of time (Berlin *et al.,* 2013). There is no evidence in the literature that antibiotic treatment increase the risk for metastatic abscessation (Duffee *et al.,* 2015).

Penicillin is the antibiotic of choice against *S. equi*. Streptococcal resistance against penicillin is not documented for any species, in Sweden or in other countries (Sweeney *et al.,* 2005; SVS, 2013; Waller, 2014; MPA, 2015). There is no documentation about *S. equi* and resistance in Sweden but for *S. zooepidemicus* no isolates were found resistant to penicillin and 6% were resistant to trimethoprim sulphamethoxazole in 2016 (Public Health Agency of Sweden and National Veterinary Institute, 2017). There are a few studies from other countries, covering the susceptibility of antibiotics to different streptococci. *S. equi* resistance to gentamicin (51,9%), trimethoprim sulfamethoxazole (25,9%), enrofloxacin (18,5%), doxycyclines (4,2%) and

tetracyclins (4,2%) was observed in one retrospective study with cases from the Royal Veterinary College Diagnostic Laboratory from 1999 to 2012. The bacteria were in the same study susceptible to both ceftiofur, penicillin and oxytetracyclin in all examined cases (Johns & Adams, 2014). Another similar study was done on cases from 2000 to 2010, at the University of Kentucky Veterinary Diagnostic Laboratory. *S. equi* was in this study 91,3% resistant to Sulfa drugs, 42% to trimethoprim and sulfamethoxazole, 13,2% to novobiocin, 10,5% to gentamycin and 0,9% to tetracycline. No resistance was reported to penicillin but 1,3% of the bacteria had only an intermediate susceptibility (Erol *et al.*, 2012).

Limited research is done on the susceptibility of different antibiotics to strangles in vivo in horses with or without abscesses. Ensink, Smit & Duijkeren (2003) showed that trimethoprim sulfamethoxazole did not eliminate bacteria in horses with *S. zooepidemicus* implanted subcutaneously in tissue chambers. In seven of eight horses, no bacteria were however demonstrated in tissue chambers after treatment with penicillin. The authors states that a probable reason to that trimethoprim sulfamethoxazole is less effective is an inhibited action of the antibiotic in purulent material but other factors may also have played a role (Ensink, Smit & Duijkeren, 2003). No similar study is done on *S. equi*. Christmann and Pink (2015) reports about an outbreak where ceftiofur seemed to have an effect against *S. equi* in vivo (Christmann & Pink, 2015).

In Sweden, there is no specific recommended dose of benzylpenicillin procaine for treatment of strangles. Commonly used doses of benzylpenicillin procaine, independent on type of infection, are around 25 mg/kg (corresponds to 25 000 IU/Kg) every 12th or 24th hour (MPA, 2015). MPA (2015) suggest that the dose of 20 mg/kg every once a day should be enough for the majority of streptococcal infections according to PK/PD-data (MPA, 2105). Timoney (2015) and Radostits (2007) recommended benzylpenicillin procaine doses of 22 000 IU/kg every 12th hour as treatment of horses with strangles (Radostits, 2007; Timoney, 2015). Ensink, Smit & Duijkeren (2003) considered the dose 20 000 IU/kg penicillin procaine once a day to be sufficient for treatment of horses with *S. zooepidemicus* implanted subcutaneously in tissue chambers and mean that the dose regime on 20 000 IU/kg twice daily is unnecessary. This was based on a study showing that 12 000 IU/kg was enough to reach MIC for both *S. zooepidemicus* and *S. equi* (Ensink *et al.*, 1993). According to Ensink *et al.* (1993), MIC were the same for *S. zooepidemicus* and *S. equi* in an *in vitro*-examination. In the earlier mentioned outbreak, reported by Piché (1984), benzathine penicillin at a dose of 30 000 IU/kg was given to all foals twice a day while affected horses with ruptured abscesses were given benzylpenicillin procaine at the dose 25 000 IU/kg twice a day (Piché, 1984). In the outbreak reported by Christmann and Pink (2015), the penicillin procaine dose was 20 000 IU/kg twice a day. Regarding intravenous treatment, MPA (2015) recommend benzylpenicillin sodium at 10 mg/kg (corresponds to approximately 17 000 IU/kg) every 12th hour for treatment of streptococcal infections but they also state that there is no proof for effective treatment in cases with abscesses present (MPA, 2015). Radostits (2007) recommends intravenous treatment with potassium or sodium benzylpenicillin at a dose of 22000 IU/kg every 6 hour (Radostits, 2007).

Antibiotics and relationship to development of antibodies

Sweeney *et al.* (2005) referring to Piché (1984), states that antibiotic treatment in horses with early strangles may be curative but also will inhibit synthesis of protecting antibodies (Sweeney *et al.*, 2005). Piché (1984) reports about the previously mentioned prophylactic treatment regime for foals. The foals were the only animals that during the outbreak that were treated in an early stage of disease (prophylactic treatment initiated when the body temperature reached 38,5°C). All foals acquired strangles subsequent to completion of the treatment regime but they did not develop any abscesses. The outbreak of strangles was almost over when the treatment program ended and the reduced degree of environmental contamination and thereby challenge dose may have contributed to reduced severity of the infection. Piché (1984) also comments that it is possible that the foals, during treatment, were sufficiently antigenically stimulated to produce some circulating antibodies that later reduced the severity of clinical disease (Piché, 1984). Eventual impaired development of immunity is hypothesized to be caused by altered protein synthesis of the bacteria because of antibiotic treatment or by acting of antibiotics on the bacterial cell wall, inhibiting cells from forming antibodies (Sweeney *et al.*, 2005). Few reports, except the one by Piché (1984) include information about the rate of reinfection in antibiotic treated horses with strangles. Duffé et al. (2015) reports about recurring strangles in two cases (3%) of a total of 63 antibiotic treated strangles infected horses, treated with trimethoprim-sulfamethoxazole and oxytetracycline respectively. Unfortunately, time or duration for treatment was not recorded in the study (Duffé *et al.*, 2015).

Future challenges

There are several questions about strangles that are not yet answered and a lot of statements without support in the literature. Several fields need more research. For example, there is no effective and secure vaccine which makes it possible to distinguish between vaccinated and infected horses (Waller, 2014), there is a lack of information about the survival of *S. equi* in the environment and there are still many unanswered questions regarding chronic carriers of strangles. In addition to this, there is no research about how antibiotic treatment affects immunity and disease duration. The aim with this project is to look closer into how treatment with antibiotics affect the immunity.

MATERIALS AND METHODS

Description of the outbreak

Information and data from an outbreak of strangles on a Swedish Icelandic horse farm 2015 was used in this project. The index-case was a horse returning to the farm after rehabilitation the 12th of April 2015. The 22nd of April it developed nasal discharge and fever. Samples were taken for suspected strangles and the diagnosis was confirmed the 24th of May. The following week more horses showed clinical signs of disease. Initially, affected horses were isolated but as the outbreak proceeded this measure was no longer possible. Most horses started to show clinical signs in the end of April/beginning of May. While severity of clinical signs varied between horses all but one horse had fever at some point. However, given that this was a natural field outbreak detailed information about clinical signs was not available for all horses. By the end of September, all horses were clinically healthy from strangles. At that time some horses were still positive on nasal lavage samples and/or guttural pouch samples and thus were treated

for carriage of *S. equi*. Subsequent sampling and endoscopies were done during late 2015 and 2016 and by the last sampling occasion that took place the 22nd of August 2016, every horse had been tested negative on at least one occasion.

At the beginning of the outbreak there were 43 adult horses at the farm. Two horses were euthanized in May 2015, before the sampling period started, because of development of clinical signs of strangles in combination with advanced age and poor dentition. One horse was euthanized in November 2015 because of laminitis and one in December 2015 because of peritonitis. One horse moved from the farm in December 2015 after it was tested free from *S. equi*. Two new horses arrived at the farm in 2016, they did not acquire clinical signs of strangles.

No horse had been vaccinated against *S. equi.*

Examinations and sampling

During the outbreak, samplings and clinical examinations of the horses were done on several occasions by the district veterinarians of the area, and by veterinarians at Swedish university of agriculture (SLU) and UDS horse clinic (Figure 1). Rectal temperatures were documented by the horse owners for most horses daily during the early period of the outbreak (24th of April to 20th of May). Horses with rectal temperatures over 38,2ºC were deemed as febrile.

The first cases were diagnosed by samples taken by the district veterinarians. Following nasal lavage samples were taken by a veterinarian at SLU, over a period of 14 months, and analysed by culturing and qPCR. Samples for qPCR and culturing were also taken from the guttural pouches on 6 occasions. All horses were not sampled on every occasion. Horses positive on qPCR or culture, on either a nasal lavage sample or a guttural pouch sample, in the beginning of March 2016 were classified as chronic carriers of *S. equi*.

Serological blood samples were collected on seven occasions over 10 months. The samples were analysed for the previously described antigen A and antigen C (iELISA from AHT). Horses with iELISA od values (optical density at 450 nm) for respective antigen over or equal to 0,5 were classified as seropositive.

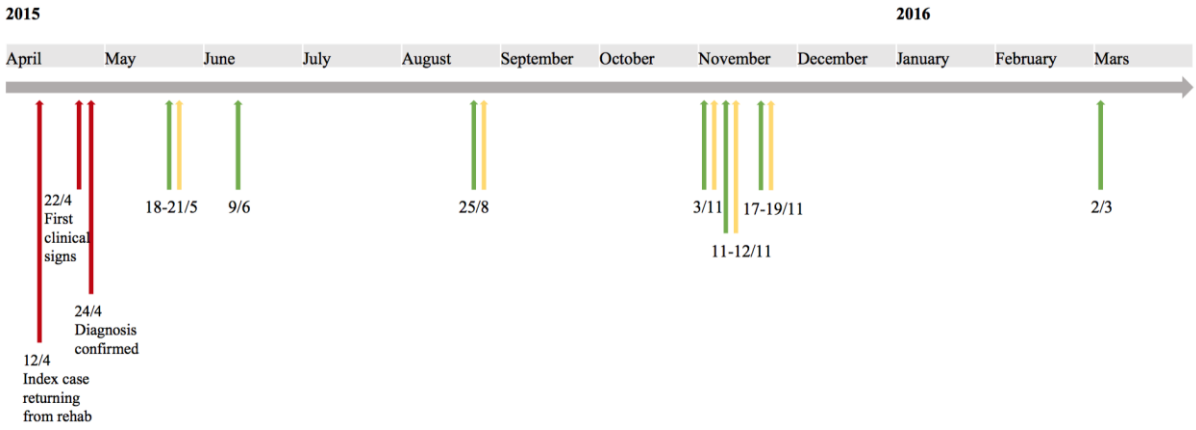

Figure 1. *Time line from the beginning of the outbreak in April 2015 until March 2016. Green arrows represent serology sampling occasions and yellow arrows represents occasions for nasal lavage sampling and clinical examinations. Guttural pouch sampling occasions are not included in the time line. Examinations and sampling occasions carried out by the district veterinarians and UDS are not shown.*

Treatment

Twenty-two horses were treated with antibiotics at least once during the outbreak. According to the district veterinarians, only severely affected horses were treated with antibiotics during the initial period of the outbreak. Reasons for antibiotic treatment in the acute phase were mainly decreased appetite, high and/or persistent fever and decreased demeanor.

The most commonly used antibiotic was Benzylpenicillin Procaine given intramuscularly. The Benzylpenicillin Procaine[1] dose was around 20 mg/kg. Most horses were treated once a day but some horses got Benzylpenicillin Procaine twice a day. Some horses were referred to UDS horse clinic and were treated with Benzylpenicillin sodium[2] intravenously. Two horses were treated with Sulfadiazine and Trimethoprim[3]. These were not treated during 2015. Duration of antibiotic treatment varied (table 1).

Totally 14 of 38 horses (37%) were identified as chronic carriers; horses qPCR positive on either a nasal lavage sample or a guttural pouch sample in Mars 2016, about 11 months after the beginning of the outbreak (table 6). Five of these were also positive on culture. Treatment of these chronic carriers included flushing of the guttural pouches and systemic and/or topical antibiotic treatment. Some of these horses had also been treated during the acute phase of the outbreak.

Sampled horses from the outbreak were put into three groups (table 1) based on time of antibiotic treatment in relation to appearance of clinical signs of strangles for two of the groups. The third group included those horses that developed strangles but were not administered any antibiotics during acute illness. Six horses (group 1) were treated with antibiotics between 3 and 16 days after the first horse showed signs of fever (>38,2ºC) and nasal discharge. These horses were considered to have been treated early during the outbreak. No horse got earlier treatment. Horses in the second group were treated with antibiotics in 2015 but later than 16 days after the first horse started to show clinical signs of disease. One horse in this group was treated with antibiotics because of a suspected intraabdominal *S. equi* abscess (table 2, horse nr. 11) and another (table 2, horse nr. 2) was treated because of a wound injury. Time from first clinical signs (body temperature > 38,2°C) for respective horse, to antibiotic treatment is shown in table 2.

Table 1. *Definition of group 1, 2 and 3 and the amount of horses (N) in each group*

Group 1 (N=6)	Horses treated with antibiotics within 16 days after the first horse started showing clinical signs (rectal temperature > 38,2ºC).
Group 2 (N=6)	Horses treated with antibiotics between 16 days and 22 days after the first horse started showing clinical signs (rectal temperature > 38,2ºC).
Group 3 (N=29)	Horses not treated with antibiotics during the acute period.

[1] Ethacilin vet., Wim de Körverstraat 35, P.O. Box 31, NL-5830 AN Boxmeer, the Netherlands

[2] Benzylpenicillin Panpharma, Z.I. du Clairy-Luitré, 35133, Fougères, France

[3] Hippotrim vet., Arne Jacobsens Allé 13, 2300 Köpenhamn S, Denmark

Duration of antibiotic treatment (table 2) in group 1 varied between 7 and 18-20 days (unsure information regarding treatment duration for horse 3). Three of these horses were partly treated with intravenous Benzylpenicillin sodium for 4 to 9 days. Horses in group 2 were treated between 5 and 54 days, 3 of them partly with Benzylpenicillin sodium intravenously.

Table 2. *Antibiotic treatment (Benzylpenicillin procaine and/or Benzylpenicillin sodium) for the horses in group 1 and 2 (treatment duration and x times a day) and time from first clinical signs (for the horse that first showed clinical signs and for the horse in question) to antibiotic treatment. Benzylpenicillin sodium were not used at the same time as benzylpenicillin sodium but before or after the period of benzylpenicillin sodium treatment. Eventual additional treatment during 2016 is not included*

Group	*Horse nr.*	*Benzylpenicillin procaine*	*Benzylpenicillin sodium*	*Time from that the first horse started showing clinical signs to antibiotic treatment*	*Time from first clinical sign for the individual horse to antibiotic treatment*
Group 1	1	8 days x 1	-	7 days	3 days
	2	7 days x 1	-	12 days	10 days
	3	1 day x 1 + 8-10 days x 2	9 days x 3	15 days	8 days
	4	7-10 days x 1-2	5 days x 3	16 days	16 days
	5	1 day x 1 + 10 days x 2	4 days x 3	16 days	11 days
	6	7 days x 1	-	16 days	9 days
Group 2	7	10 days x 1	-	22 days	7 days
	8	8 days x 1-2	5 days x 3	22 days	6 days
	9	14 days x 1 + 3 days x 2	15 days x 4	43 days	1-2 months
	10	10 days x 1	-	49 days	26 days
	11	4 days x 1 + 10 days x 1	40 days x 4	60 days	33 days
	12	5 days	-	61 days	43 days

Of 35 horses, 32 were on one or more occasions treated with Meloxicam[4]. Some horses were administered Flunixin meglumine[5] intravenously during their stay at the UDS horse clinic. The dose for Meloxicam were 0,6 mg/kg and for Flunixin meglumine 1,1 mg/ml once a day. Treatment duration varied between horses.

The horses in the three groups were between 7 and 32 years old and there was no significant difference in age between the groups.

[4] Metacam for horses, 55216 Ingelheim/Rhein, Tyskland

[5] Flunixin N-vet, Uppsala Science Park, 751 83, Uppsala, Sweden

Statistical analysis

Serological values, PCR results, rectal temperatures, antibiotic treatments and other information from veterinary journals (District veterinarians and UDS) and horse owners were compiled in an excel document.

Descriptive statistics were recorded for the raw and graded (negative versus suspicious versus positive) serological response from the horses in group 1, 2 and 3 and groups response in relation to antibiotic treatment compared by the Fishers exact test.

RESULTS

All horses had qPCR positive nasal lavage samples on either the first (39/41 positive horses) or the second sampling occasion. Most of the horses (29/40) also had positive culture results during the first sampling occasion. All horses showed at some point positive results on qPCR to *S. equi*.

All sampled horses had positive iELISA values for antigen A at some point. On the other hand 3 of 41 horses did not have values over or equal to 0,5 for antigen C at any occasion. Antigen C values were negative in a higher frequency than Antigen A values. The following diagrams (diagram 1, 2 and 3) show the percentage of iELISA positive horses from the three groups. The diagrams are based on values shown in table 3, 4 and 5.

On the first sampling occasion, about one month after the beginning of the outbreak 44% of the horses were positive for both antigen A and C (diagram 1, table 3). No significant difference between the groups was observed. About 3 weeks later the proportion of positive horses had increased but there was still no significant difference between the different groups. However, by the end of August the biggest difference between groups is observed with significant difference over time between group 1 and 2 where the percentage positive horses in group 2 was 83% compared to 0% in group 1 (P= 0.015). In March 2017, a decrease in positive horses was seen also in group 2 but this was not statistically significant.

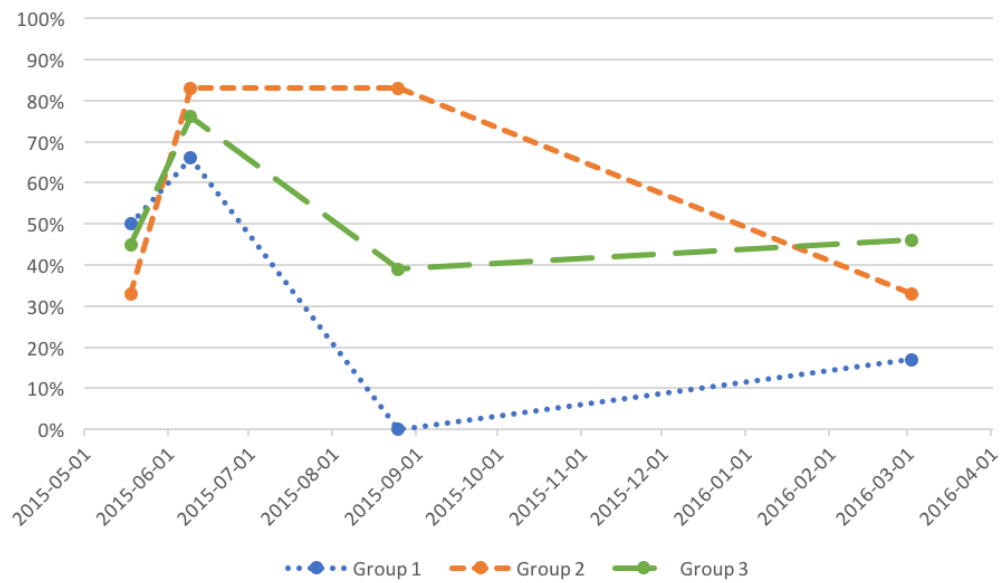

Diagram 1. *Percent horses positive for both antigen A and antigen C over time.*

Table 3. *a. Amount of horses positive for both antigen A and C on different occasions. b. P-values achieved when comparing the groups with Fisher's exact test*

a.

Group	*2015-05-18*	*2015-06-09*	*2015-08-25*	*2016-03-02*
Group 1	3/6 (50%)	4/6 (66%)	0/6 (0%)	1/6 (17%)
Group 2	2/6 (33%)	5/6 (83%)	5/6 (83%)	2/6 (33%)
Group 3	13/29 (45%)	22/29 (76%)	11/28 (39%)	12/26 (46%)

b.

Groups compared	*2015-05-18*	*2015-06-09*	*2015-08-25*	*2016-03-02*
1 and 3	1,0000	0,6353	0,1454	0,3606
1 and 2	1,0000	1,0000	0,0152	1,0000
2 and 3	0,6804	1,0000	0,0782	0,6722

Most horses (90%) were on the first sampling occasion positive for antigen A (diagram 2, table 4). Group 2 and group 3 are relatively stable over time while a decrease in positive horses are observed in group 1. A significant difference is seen between group 1 and 3 in the end of August (*P*=0,0305) and in March 2016 (P=0,0063). A significant difference is also observed between and group 1 and 2 (*P*=0,0152) in March 2016.

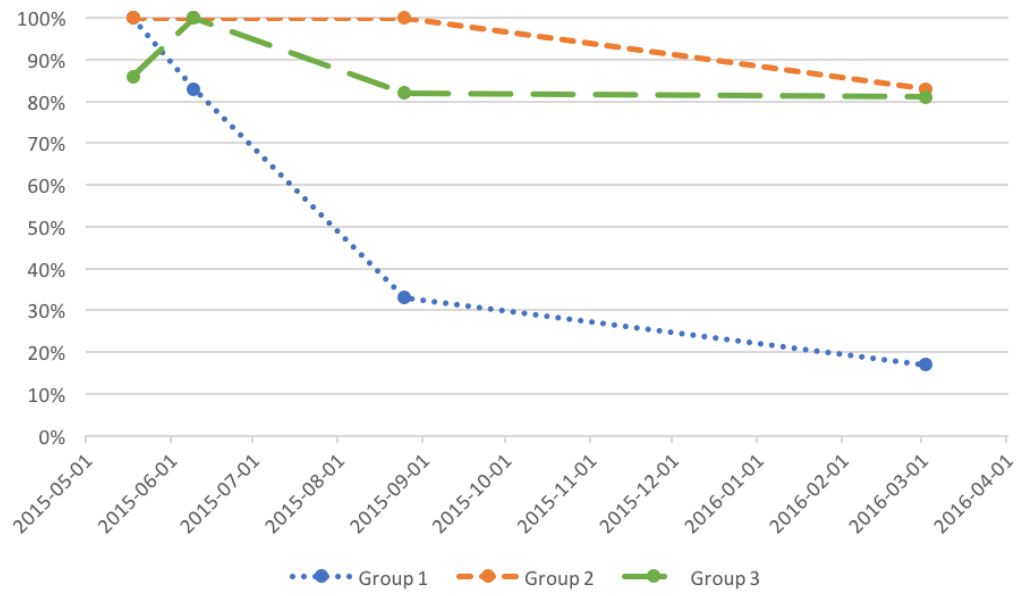

Diagram 2. *Percent horses positive for antigen A over time.*

Table 4. *a. Amount of horses positive for antigen A on different occasions. b. P-values achieved when comparing the groups with Fisher's exact test*

a.

Group	*2015-05-18*	*2015-06-09*	*2015-08-25*	*2016-03-02*
Group 1	6/6 (100%)	5/6 (83%)	2/6 (33%)	1/6 (17%)
Group 2	6/6 (100%)	6/6 (100%)	6/6 (100%)	5/6 (83%)
Group 3	25/29 (86%)	29/29 (100%)	23/28 (82%)	21/26 (81%)

b.

Groups compared	*2015-05-18*	*2015-06-09*	*2015-08-25*	*2016-03-02*
1 and 3	1,0000	0,1714	0,0305	0,0063
1 and 2	1,0000	1,0000	0,0606	0,0152
2 and 3	1,0000	1,0000	0,5585	1,0000

For Antigen C (diagram 3, table 5), the percentage positive horses was in average 44% on the first sampling occasion. In August 2015, the amount of positive horses had decreased in group 1 and 3. No significant decrease was observed between the groups at this time. In March 2016, the percentage positive horses was similar in all groups.

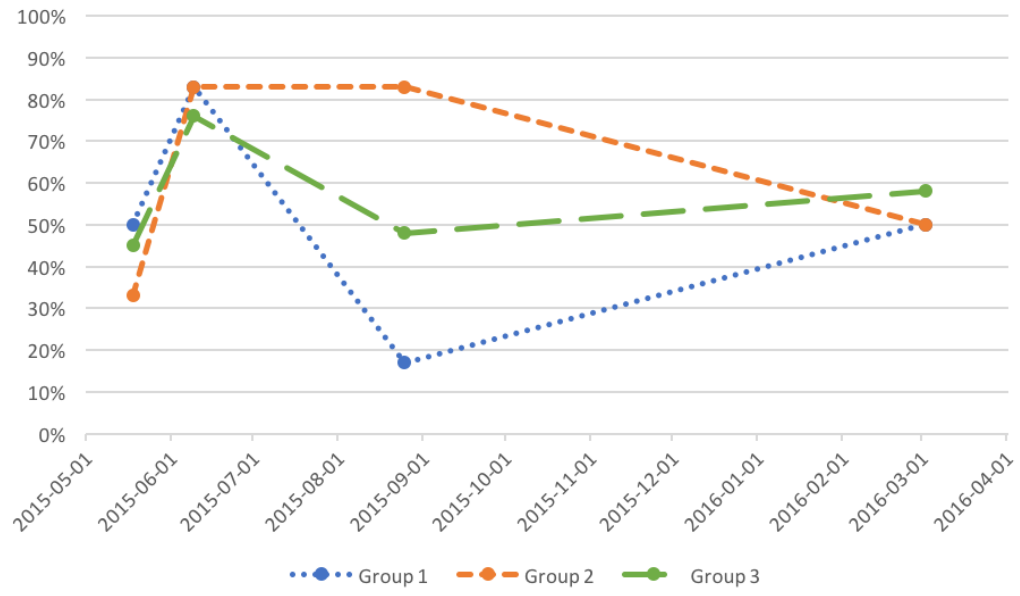

Diagram 3. *Percent horses positive for antigen C over time.*

Table 5**.** *a. Amount of horses positive for antigen C on different occasions. b. P-values achieved when comparing the groups with Fisher's exact test*

a.

Group	*2015-05-18*	*2015-06-09*	*2015-08-25*	*2016-03-02*
Group 1	3/6 (50%)	5/6 (83%)	1/6 (17%)	3/6 (50%)
Group 2	2/6 (33%)	5/6 (83%)	5/6 (83%)	3/6 (50%)
Group 3	13/29 (45%)	22/29 (76%)	11/28 (39%)	12/26 (46%)

b.

Groups compared	*2015-05-18*	*2015-06-09*	*2015-08-25*	*2016-03-02*
1 and 3	1.0000	1.0000	0.3891	1.0000
1 and 2	1.0000	1.0000	0.0801	1.0000
2 and 3	0.6804	1.0000	0,0782	1.0000

DISCUSSION

Among many veterinarians, it is commonly presumed that treatment with antibiotics in horses with strangles may lead to a lack of antibodies and a risk of reinfection. However, in searching the literature, there is little information to support this concept. The aim with this study was to look closer into the subject and examine whether antibiotics in horses affects the serological antibody response.

All horses in this study were at some point positive on nasal lavage samples analysed by qPCR and were in other words exposed for antigenic stimuli. During the first sampling occasion 39 of 41 horses (95%) were positive on qPCR while only 29 of 40 (72,5%) were positive on culture. This strengthens the fact that qPCR is a more sensitive method for diagnosing strangles in horses than culture (Båverud, Johansson & Aspan, 2007; Webb *et al.,* 2012; Lindahl *et al.,* 2013). Horses in this project were continuously positive on PCR even if the number of positive horses decreased. For example on the 21th of May 95% of the horses were PCR positive and the 25th of August 26% were still PCR positive. Additionally, there was a recrudescence of PCR positivity in November 2015 (not shown in the results). As the horses were not isolated from each other, it was assumed that horses comingling may have been antigenically stimulated and thus affecting the antibody response in a manner of vaccine booster effect. As this often is the case in natural outbreaks, the results are of interest but difficult to explain in this work.

Two antigens were examined when using the AHT's iELISA serology test. The test is mainly constructed as a diagnostic device. Looking at the percentage of horses that were positive for both antigens there was mainly a difference in august 2015 between 1 and 2 (P=0.0152). Positive serology samples for both antigen may indicate a good immune response. Even if there was a difference between A significant difference between group 1 and 2 indicate a difference between earlier and later treated horses but it is notable that a significant difference was not

observed at the first two sampling occasions, closest after time of treatment. Additionally, there is no difference between group 1 and 3.

Antigen C consists of a part the SeM protein. This protein is an important antiphagocytic virulence factor of *S. equi* and a loss of the SeM expression lead to loss of full virulence of the bacteria (Hoffman, 1991; Hamlen, Timoney & Bell, 1994; Harrington, Sutcliffe & Chanter, 2002; Timoney, 2004). Hamlen, Timoney and Bell (1994) showed that protection against strangles in foals were associated with high ELISA values of SeM IgG while it in other studies have been observed that resistance against *S. equi* in infected or vaccinated horses was independent of bactericidal levels of SeM antibodies in serum (Galan & Timoney, 1984; Timoney & Eggers. 1985). Although sensitivity and specificity combining antigen A and C, was 93,3% respective 99,3% in a study (Robinson *et al.*, 2013), the sensitivity and specificity for only antigen C (a part of SeM) was 59,6% respective 100% with an 0,5 od value as breakpoint. In a recent study by Tscheschlok et al. (2017), only 7 of 112 horses in a natural outbreak of strangle, had been seropositive for antigen C on at least one sampling occasions, 19 weeks after the outbreak resolution (opposed to antigen A, where 91 of 112 horses had been seropositive on at least one occasion). The morbidity was in that outbreak was only 53% and only 34 of 112 horses had clinical strangles. The authors discuss that a reason for this may be a less pathogen strain of *S. equi*, possibly because of an observed deletion of the gene SEQ_0402. There were however no changes in the SeM-gene. It is also possible that the low morbidity was due to a low infectious dose (Tscheschlok *et al*., 2017). Mentioned studies indicate that it is not obvious that high levels of antibodies against SeM/antigen C in sera is correlated to protection against *S. equi*. Thus the lack of robust seroconversion and retention of antibodies to Antigen C in this study raise questions regarding the sensitivity and specificity of the ELISA used that relies of the Antigen C as a complement for the testing (Robinson *et al.* 2013).

In this study, the proportion horses positive for antigen C was lower than the proportion positive for antigen A but only 3 of 40 horses had negative serology samples for the antigen over the whole sampling period. A non-significant difference between the groups were for antigen C observed in September 2015. As there were no significant differences and hesitant correlations between for seropositivity for antigen C and protection against strangles, these results do not support the hypothesis that antibiotics affect the development of antibodies in horses with early strangles.

Information about the antigen C (recombinant N-terminal protein fragments of the EQ2190 sequence) in the literature is lacking. EQ2190 is a sequence encoding for a putative sortage-processed protein and it was identified in all examined strains in a study by Webb et al. (2015). Equal to antigen C, antigen A is an antigen unique for *S. equi*. Sensitivity for only antigen A was according to Robinson at al. (2013), 74,2% and specificity 99,3%, using an od value of 0,5 as breakpoint. This higher sensitivity is in line with the outbreak described by Tscheschlok *et al.* (2017), where 91 of 112 horses were seropositive for antigen A. The higher prevalence of seropositivity compared to antigen C may possibly make antigen A a better measure of the antibody response but more information is needed to say whether there is any correlation between protection against *S. equi* and seropositivity for antigen A.

All horses had positive serology samples for antigen A during the first or second sampling occasion. At the first sampling occasion, most of the horses were positive. Antibodies against antigen A seem thereby to be more prevalent than antibodies against antigen C. Over time, the percentage positive horses for Antigen A decreased more in group 1 than in group 2 and 3. This is the only result that at some point may support the statement that treatment with antibiotics affect the development of protecting antibodies (Reed, Bayly & Sellon, 1998; Harrington, Sutcliffe & Chanter, 2002; Sweeney *et al.*, 2005; SVS, 2013; Waller, 2014). Significant differences were however only observed in March 2016 (P=0,0152), when comparing group 1 and 2 and in August 2015 (P=0,0305) and March 2016 (P=0,0063) when comparing group 1 and 3. No significant differences were observed between the groups during the first two sampling occasions, closest to the time of treatment.

There is research indicating that local immunity in the nasal mucosa is important for full protection against *S. equi* (Galan & Timoney, 1985; Sheoran, 1997). Sheoran *et al.* (1997) observed a strong local mucosal antibody response against SeM in infected horses but not in horses vaccinated with an intramuscularly administered SeM vaccine (Sheoran *et al.*, 1997). Serum and mucosal antibodies seems to react to different parts of SeM and not all parts of the SeM seems to stimulate lymphocytes (Timoney *et al.*, 2010). This suggests that different antigens stimulate the immune responses in different ways. During infection, all parts of *S. equi* are present and it is probable that all parts of the immune response are stimulated. The iELISA only gives a measure of the levels of antibodies against two antigens and does not give any information about local immunity or the cell mediated immune response. What also is discussible is which levels of antibodies that should be considered as protective and what should be considered as seropositivity.

The hypothesis about an impaired development of immunity is directed to horses treated with antibiotics in an early stage of disease (Reed, Bayly & Sellon, 1998; Harrington, Sutcliffe & Chanter, 2002; Sweeney *et al.*, 2005; SVS, 2013; Waller, 2014). The hypothesis is based on a study by Piché (1984) where foals on a farm were treated with antibiotics prophylactically when they reached a body temperature of 38,5ºC. Opposed to foals in the study by Piché (1984), the horses in this study were not treated until they showed severe clinical signs. According to AHT (n.d.), it takes approximately 2 weeks after infection before enough antibodies for protection can be found in sera. With an incubation time of 3 to14 days (Sweeney *et al.*, 2005), the antibiotic treated horses were probably exposed to antigen before they started to show clinical signs. It is therefore probable that the antibiotic treated horses in the present study already had developed some amount of antibodies before the treatment started. As the horses in group 1 were the earliest treated horses, they were however considered to be least likely to have developed a higher level of antibodies and how these horses were treated is more compatible with the reality and a restrictive usage of antibiotics than if they would have been treated at an earlier stage of disease.

In the current study, horses were divided into groups based on at which time after the first horse started to show clinical signs, they were treated with antibiotics. It is possible that another group constellation would have given other results, especially as the early treated horses during this outbreak were few. With only 6 horses in group 1 and 2 it is hard to draw any real conclusions.

None of the horses at the farm showed clinical signs of reinfection and this may indicate that all horses had enough immunological response for protection against circulating antigen. This low frequency of reinfection is similar to what is reported in the study by Duffee et al. (2015) where only two of 63 horses had recurring strangles. That the frequency was higher in the outbreak reported by Piché (1984) may be due to earlier treatment or due to differences in the study populations as all horses referred to in the study by Piché (1984) were foals. It is however not known what would have happened if the horses in the present study would have been exposed to a clinically infected horse, possibly with a higher excretion of antigen.

It should be taken in consideration that biological factors, as differences in immune response between horses, may have affected the results. According to the district veterinarians, only severely affected horses were treated with antibiotics. This implies that horses in group 1 and 2 had a higher clinical scoring than horses in group 3. One hypothesis is that the horses in group 1 had a lower immune defense from the beginning which may have affected the serological values. It is hard to confirm a difference in clinical scoring between the groups as there is not enough clinical information about all horses. Exact information about disease duration and clinical signs for each horse would have greatly assisted interpretation and grouping of treatments.

Duration and type of penicillin varied somewhat between the horses in group 1 and 2. Given the high sensitivity of *S. equi* to penicillin an influence on eventual decreased development of antibodies is not probable. However, it is possible that treatment duration and penicillin dose has an impact on the effectiveness when treating for example horses with abscesses.

Treatment with anti-inflammatory medicines was common during the outbreak. When analysing the immunity there were no consideration taken to treatment with anti-inflammatory medicines. This does not exclude the possibility that anti-inflammatory treatment may affect immunity to *S. equi*. There is however no evidence that this would be the case.

The sampling occasions from November 2015 were not included in the results. During these sampling occasions, there were a rise in horses positive on qPCR (nasal lavage samples) and seropositive horses. Antibiotic treated horses were not PCR-positive in a higher degree than non-antibiotic treated horses. The reason for what caused the immunostimulation in November 2015 is not known. This is emphasizing the difficulty of doing research on real cases and outbreaks. It is probable that what happened in November 2015 has affected the serology results in March 2016.

It is possible that treatment with antibiotics can prolong the course of disease in horses with abscesses. Long-term treatment has in some cases been proved to cure horses with intraabdominal abscesses (Berlin *et al.,* 2013). If treatment is discontinued before the abscess is totally healed it is possible that it will start growing again until it ruptures. During this outbreak, one gelding with an ultrasonically diagnosed abscess in the abdomen of about 7 cm, were treated for 54 days with Penicillin (table 2, horse number 11). The abscess decreased in size during the treatment period and was no longer visible when the treatment was discontinued. Unfortunately, long term treatment with Penicillin is hard to acquire outside a hospital, making this treatment quite expensive.

After this study, it is not possible to say whether the hypothesis that early antibiotic treatment in horses in strangles affect the development of antibodies is true or not. There is lot of factors that are unsure, making it impossible to draw any conclusions. Whether antibiotics affect the immune response or not, it is always important to be restrictive with antibiotics. Streptococcal resistance against Penicillin is not reported but it is possible that the situation will be different in the future (Sweeney *et al.*, 2005; SVS, 2013; Waller, 2014; MPA, 2015). Resistance to other antibiotics against *S. equi* has been observed. Treatment with Penicillin is in many cases a challenge as it must be administered either intramuscularly or intravenously and there is also a risk for shocking with severe consequences. For these reasons, veterinarians may be tempted to choose other antibiotics that may have a higher risk for development of resistance and that may be less effective. Avoiding antibiotic treatment is advantageous as a part of a generally restrictive use of antibiotics. In spite of this, penicillin treatment should be considered to severely affected horses, to horses with prolonged clinical signs and in some cases when bastard strangles is suspected. To decrease spreading of disease, treatment could also be adequate in horses diagnosed as chronic carriers. For more evidence based recommendations, more research is however needed.

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