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Our current understanding of strangles Part 1: etiology, epidemiology, pathogenesis, clinical signs and diagnosis

Pääntauti, osa 1: etiologia, epidemiologia, patogeneesi, oireet ja diagnoosi

SUMMARY

This is the first paper in a two-part series that reviews strangles. Strangles is a highly contagious bacterial disease of horses involving the upper respiratory tract and the lymph nodes of the head and neck. The disease is caused by Streptococcus equi s*ubspecies* equi *and is arguably the most prevalent infectious equine disease worldwide. Strangles has been recognized for centuries, and veterinary records describing cases of strangles can be found as far back as 1251.* S. equi *is most commonly transmitted via direct contact between infected and susceptible horses and outbreaks are most common in areas with high concentrations of horses and transient populations. Asymptomatic carriers represent an important reservoir of infection in susceptible populations for many months, and in some cases years. Infection is characterized by fever, inappetance, lethargy, bilateral purulent nasal discharge and regional lymph node abscessation. Routine diagnosis requires bacterial culture and/or quantitative PCR from samples obtained from nasal swabs, nasopharyngeal washes or purulent discharges. Identification of asymptomatic carriers requires endoscopic examination of the guttural pouches and submission of washes for bacterial culture and/or qPCR. Serological testing identifies recent exposure to* S. equi *and is useful for screening purposes.*

YHTEENVETO

Tämä on ensimmäinen osa kaksiosaisesta artikkelista, jossa esitellään uusimmat tiedot pääntaudista. Pääntauti on erittäin tarttuva hevosen ylempien hengitysteiden sekä pään ja kaulan imusolmukkeiden bakteeritulehdus. Pääntaudin aiheuttaa Streptococcus equi *-bakteerin alalaji* equi *ja se on todennäköisesti tavallisin hevosen tartuntatauti maailmassa. Tauti on ollut tunnettu vuosisatojen ajan. Eläinlääketieteellisessä kirjallisuudessa se on kuvattu jo vuonna 1251.* S. equi *-bakteeri leviää tavallisesti suorassa kontaktista infektoituneesta terveeseen hevoseen. Tartunnat ovat tavallisia erityisesti tiheissä ja tiuhaan vaihtuvissa populaatioissa. Oireita ovat kuume, huono ruokahalu, bilateraalinen purulentti sierainvuoto ja paiseiden muodostuminen paikallisiin imusolmukkeisiin. Infektoituneiden hevosten diagnoosi ja oireettomien kantajien löytäminen ovat tärkeitä taudin rajoittamiselle. Rutiinidiagnoosi vaatii bakteerin eristämisen ja/tai kvantitatiivisen PRC-kokeen sierainnäytteestä, nasofaryngeaalisesta huuhtelunäytteestä tai märkivästä eritteestä. Oireettomien kantajien toteaminen edellyttää ilmapussien endoskooppista tutkimusta ja niiden huuhtelunäytteen analyysiä bakteriologisesti ja/tai qPCR-testillä.*

ETIOLOGY

Strangles is caused by the obligate β-hemolytic, grampositive cocci *Streptococcus equi* subspecies *equi* aka *S. equi.*[1, 2] Other pathogenic β-hemolytic streptococcal species that belong to the Lancefield group C and should not be confused with *S. equi* include *Streptococcus equi* subspecies *zooepidemicus* and *Streptococcus dysgalactiae* subspecies *equisimilis. S. equi* is in fact a clonal descendent of an ancestral *S. zooepidemicus* strain, with which it shares greater than 98 % DNA homology and therefore expresses many of the same proteins and virulence factors.[3] Virulence factors for *S. equi* include 1) a hyaluronic acid capsule that mediates adherence to host cells in the epithelium of the upper respiratory tract; 2) a number of degradative enzymes including hyaluronidase and cytolytic toxins such as streptolysin-S

like haemolysin that assist in invasion of the respiratory epithelium and 3) major cell wall-associated M-like proteins SeM and SzPSe that are antiphagocytic and prevent bacterial opsonisation, a prerequisite for efficient phagocytosis by neutrophils. Phagocytosis is also inhibited by the hyaluronic acid capsule, demonstrated by the fact that non-encapsulated strains of *S. equi* are less pathogenic in mice and horses.[4]

EPIDEMIOLOGY

Susceptible animals

In strangles outbreaks, the morbidity tends to be very high. All horses (and other equids) are susceptible, although young (one to five years old) and immunocompromised (e.g. aged) horses are more likely to become infected. Mortality is low. About 75 % of horses recover and will develop solid protective immunity that lasts for up to five years.[5] Older horses with residual immunity have limited susceptibility and usually develop a milder form of the disease. Colostral antibodies from mares that have recovered from strangles afford foals protective immunity until weaning.[6] The disease does not usually affect other domestic animals; however there is one report of strangles in a dromedary camel.[7] It is important to note that the disease does carry a very low zoonotic potential, with cases reported in elderly immunocompromised patients.[8]

Transmission

S. equi is transmitted via direct contact between infected and susceptible horses (especially contact with mucopurulent discharges) or indirectly via contact with fomites such as contaminated stables, fences, equipment, water sources, feed, farriers and veterinary equipment.

Source of infection

Infected horses start to shed bacteria two to three days after developing a fever and should be considered a potential source of infection for at least four to six weeks after the clinical signs of strangles have resolved.[6] Older horses with residual immunity tend to develop a milder form of the disease, but are still able to shed virulent bacteria. These atypical cases can be problematic in a herd outbreak and may act as an ongoing source of infection. Another important source of infection is apparently healthy horses that have recovered from

YDINKOHDAT:

- Strangles is caused by the gram-positive cocci *Streptococcus equi* subspecies *equi*.
- *S. equi* is transmitted via contact with infected or asymptomatic carriers or indirectly via contact with fomites.
- Infected horses start to shed bacteria two to three days after developing a fever and may be a source of infection at least four to six weeks after the signs have resolved.
- Typical signs include fever, lethargy, inappetance, mucopurulent nasal discharge and regional lymphnode abscessation.
- Diagnosis is based on culture and/or qPCR of nasopharyngeal swabs or washings.
- Detection of carriers requires bacterial culture and qPCR of guttural pouch washings obtained via endoscopy.
- Serological testing identifies recent exposure to *S.equi* and is useful for screening.

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FIGURE 1 KUVA

Bilateral purulent nasal discharge in a horse with strangles. Purulent discharges contain high numbers of virulent bacteria and direct contact with susceptible horses is the principle mode of disease transmission.

Bilateraalinen purulentti sierainvuoto pääntaudista kärsivällä hevosella. Purulentti erite sisältää paljon virulenttia bakteeria. Herkkien hevosten kontakti on taudin tärkein leviämismuoto.

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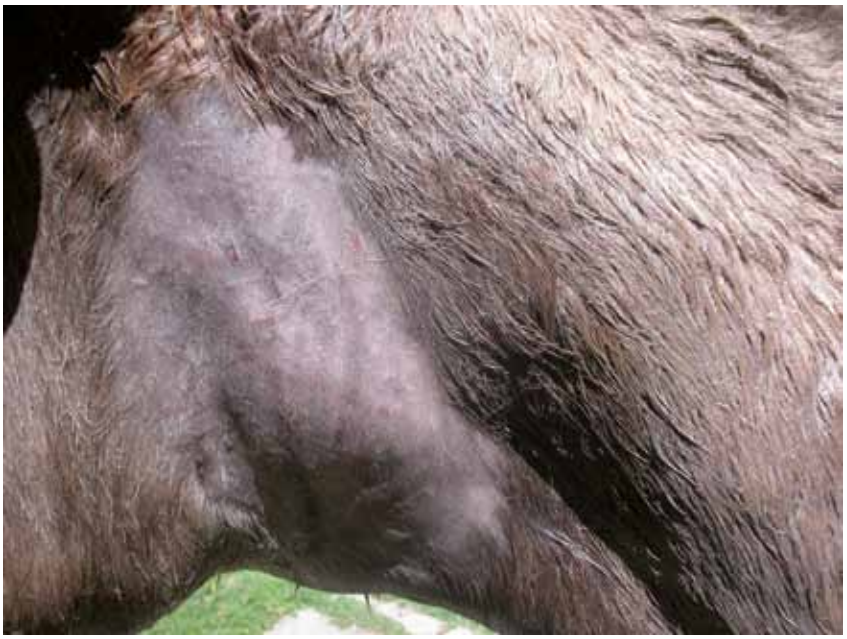

FIGURE 2 KUVA

Enlarged submandibular and retropharyngeal lymph nodes in a horse with strangles. Obvious lymph node swelling and abscessation is usually evident approximately 1–2 weeks after infection.

Pääntaudista kärsivan hevosen suurentuneet leuanalus- ja nieluntakaiset imusolmukkeet. Imusolmukkeiden turvotus ja paiseenmuodostus havaitaan yleensä yhdestä kahteen viikkoa infektion jälkeen.

strangles but continue to harbor the organisms in their guttural pouches (or less commonly their sinuses) and infect other horses via normal nasal secretions.[9] These asymptomatic carriers are very difficult to identify and represent an important reservoir of infection in susceptible populations for many months, and in some cases years.

S. equi is not very persistent in the environment. Recent research indicates that the survivability of *S. equi* on surfaces in the outdoor environment is poor, ranging for 1–3 days,[10] but moist shady environments away from direct sunlight may allow *S. equi* to persist longer. Current recommendations suggest that contaminated paddocks should be rested for at least four weeks to before they can be considered non-infectious.[6, 11]

PATHOGENESIS

The incubation period following exposure is 3–14 days and the severity of disease appears to be directly related to the infective dose.[6] *S. equi* enters the body via the mouth and nostrils and colonizes the epithelial cells and subepithelial follicles of the pharyngeal and tubal tonsils. Translocation of bacteria then occurs to the local lymph nodes that drain the pharyngeal and tonsillar region. A combination of antiphagocytic virulence factors released by the bacteria, including the hyaluronic acid capsule and the major cell wall-associated M-like protein SeM, prevent neutrophils phagocytosing and killing the bacteria, and accumulation of bacteria and large numbers of degenerating neutrophils within the lymph nodes eventually leads to abscess formation and rupture. Strangles primarily involves the guttural pouch and the lymph nodes of the upper respiratory tract. However, haematogenous or lymphatic metastasis to distant locations may occur, resulting in abscess formation in other lymph nodes and organs of the body. This is commonly known as bastard strangles. The first clinical sign of disease is fever, and infected horses usually begin to shed bacteria two to three days later. In most cases, nasal shedding persists for two to three weeks, at which time systemic and mucosal IgGb and IgA immune responses facilitate mucosal clearance of the bacteria.[12]

CLINICAL SIGNS

Horses infected with *S. equi* develop a fever 3–14 days after exposure and will become lethargic, depressed and inappetant.[6] It is important to remember that this may be the only clinical sign that is recognized in atypical cases involving older horses with residual immunity. These horses may be missed if diagnostic sampling is not performed on all exposed horses. Typically however, bilateral mucopurulent nasal discharge (figure 1) and swelling of the lymph nodes of the upper respiratory tract will begin to develop as the disease progresses. The submandibular and retropharyngeal lymph nodes are most commonly affected, but in some cases the other lymph nodes of the rostral neck may also be involved e.g. parotid and cranial cervical lymph nodes. Obvious lymph node swelling and abscessation are usually evident about one to two weeks after infection (figure 2). The first sign of impending lymph node abscessation is diffuse painful submandibular oedema, and serum may be seen oozing from the overlying skin for several

days as the abscesses mature. As the lymph nodes enlarge, affected horses may develop a cough, stridor and dyspnoea due to compression of the larynx and trachea, hence the term 'strangles' (figure 3). Pharyngitis and compression of the oesophagus may cause dysphagia in some cases. Once the abscesses rupture, they drain nonodorous cream-coloured pus. Retropharyngeal lymph nodes may rupture into guttural pouches (figure 4) and cause guttural pouch empyema. These horses will often have a profuse bilateral purulent nasal discharge and will expel large quantities of pus from the mouth and nose when coughing.

After rupture of the abscesses, most horses will make an uneventful recovery. Complications can occur after an acute episode of strangles. Up to 20 % of infected horses may develop some form of complication.[13] The most common complication is guttural pouch empyema and chondroid formation. Less common complications include dysphagia, severe dyspnoea necessitating an emergency tracheotomy, metastatic (bastard) abscessation, purpura haemorrhagica, anaemia, and immune mediated myositis.[13-15]

DIAGNOSIS

Detection of recent S. equi infection

Diagnosis of a recent strangles infection starts with collecting appropriate samples. Nasopharyngeal swabs or washings are collected most commonly, although aspirates and swabs from draining abscesses are also excellent samples if possible. Endoscopic examination and lavage of the guttural pouches is ideal, but probably not necessary for acute cases. Samples should be submitted to the laboratory in a suitable transport media i.e. M-40 transystem swabs (Copan Diagnostics Inc, USA) or Port-A-Cul vials (Becton-Dickinson, USA) for fluid samples.

Nasopharyngeal washes or swabs?

Nasal washes are more effective than swabs in detection of small numbers of S. equi because a greater surface area within the internal nares is sampled. The technique involves instilling about 50 ml of warm normal saline via a 15 cm of soft rubber tubing (or a sterile uterine pipette) inserted to the level of the nasal canthus and collecting the washings1 A practical method of collecting the washings is to place a rectal sleeve over the horses's muzzle following instillation of the saline and collecting the fluid in the fingers of the glove. The volume of one finger of the glove is usually sufficient for bacterial culture and qPCR.

Once the samples have been collected, a gram stain should ideally be performed to identify grampositive cocci in chains (figure 5).

Bacterial culture has been considered the gold standard for diagnosis of strangles and is required for definitive diagnosis.[6] Samples are plated on Columbia CNA or Trypticase soy agar (TSA) with 5 % sheep or horse blood and observed for development of betahemolytic streptococcal colonies which are usually mucoid (figure 6).[6] In addition selective plates can be used, which may facilitate the detection of betahemolytic streptococci especially in specimens that are contaminated with normal flora. Plates should be incubated at least 48–72 hours, preferably in 5 % CO_2 atmosphere, before negative result can be given.

Since *S. equi* will not be present on the nasopharyngeal mucosa until 24 to 48 hours after the onset of fever, false negatives are

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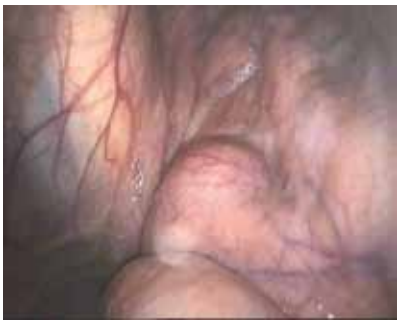

FIGURE 3 KUVA

Endoscopic view of enlarged retropharyngeal lymphnodes in floor of the medial compartment of the guttural pouch. Swelling and abscessation of the retropharyngeal lymph nodes may result in severe nasopharyngeal compression.

Endoskopianäkymä suurentuneisiin retrofaryngeaali-imusolmukkeisiin ilmapussin keskiosan pohjalla. Retrofaryngeaali-imusolmukkeen turvotus ja paiseenmuodostus voi aiheuttaa vakavaa nielun ja sierainten alueen painetta.

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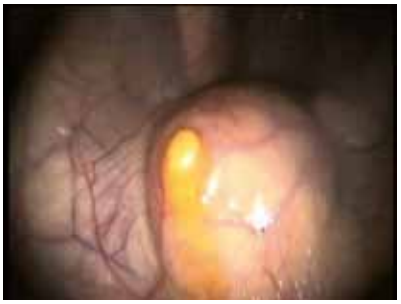

FIGURE 4 KUVA

Endoscopic view of a ruptured retropharyngeal lymphnode in the medial compartment of the guttural pouch. The rupture has caused guttural pouch empyema.

Endoskopianäkymä hajonneeseen nieluntakaiseen imusolmukkeiseen ilmapussin keskiosassa. Ruptura on aiheuttanut ilmapussin täyttymisen mädällä.

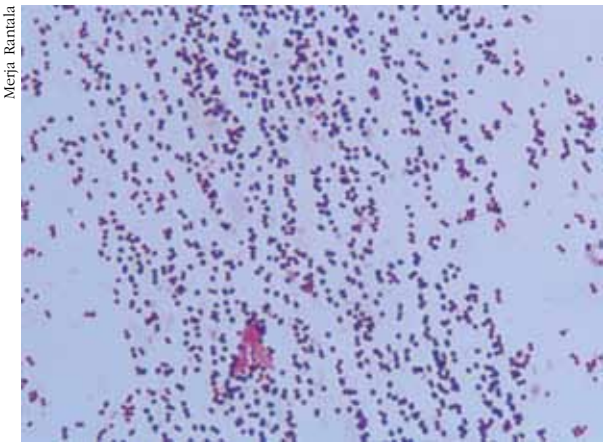

FIGURE 5 KUVA

Gram stain of purulent discharge from a draining abscess caused by S equi. *Notice the grampositive cocci in chains.*

Purulenttia eritettä S. equin *aiheuttamasta tyhjentyvästä paiseesta, gramvärjäys. Kokkeja näkyy grampositiivisina ketjuina.*

FIGURE 6 KUVA

Bacterial cultures of purulent discharge from a draining abscess caused by S. equi *plated on trypticase soy agar with 5 % sheep blood. The mucoid honey-coloured colonies are characteristic for virulent* S. equi.

Purulentin paise-eritteen bakteeriviljely. Tryptikaasi-soija-agarilla, jolla on 5 % lampaanverta, kasvaa S. equi. *Limamaiset, hunajanväriset pesäkkeet ovat tyypillisiä virulentille* S. equi *-bakteerille.*

possible in the early stages of the disease.[2] Furthermore, isolation of β-haemolytic colonies may be confounded by the presence of other bacteria, most notably *Streptococcus equi* subspecies *zooepidemicus* and *Streptococcus dysgalacticae* subspecies *equisimilis*, which may also lead to false negative results.[16] It is important to ensure that the laboratory is aware of the need to differentiate between the β-haemolytic streptococci. Differentiation is done by Lancefield grouping and biochemical tests, including fermentation of different sugars. *S. equi* is unable to ferment lactose and sorbitol.

PCR should be used as an adjunct to bacterial culture for the diagnosis of strangles. The first qPCR test for diagnosis of *S. equi* detected the 5´ region of the SeM gene (the gene for the antiphagocytic M protein of *S. equi*) and was reported to be three times more sensitive than bacterial culture.[17] It was later discovered however, that this region of the SeM gene is highly variable, and was deleted in some strains of *S. equi* isolated from persistently infected carriers,[18] leading to the possibility of false negative results. To overcome this problem and improve the sensitivity of the test, genome sequencing data for *Streptococcus equi* subspecies *zooepidemicus* and subspecies *equi* was used to develop a triplex qPCR assay targeting two genes specific to *S. equi* and a unique base pair control DNA sequence inserted into a pseudogene from a *S. zoopepidemicus* strain (H70).[16] This qPCR assay is able to provide results within two hours, has an overall sensitivity of 93.9 % and a specificity of 96.6 %.[16] Furthermore, it is able to detect *S. equi* at levels below the threshold for bacterial culture, even in the presence of contaminating bacteria.[16] Interpretation of the test should be made with caution, as the qPCR assay detects dead and live bacteria. Because of this, false positives are possible and horses that are qPCR positive should ideally be followed up with culture to confirm active infection.

An SeM-based indirect enzyme-linked immunosorbent assay (iELISA) (IDvet – diagnostic Veterinaire, IDvet) is available for the identification of horses recently exposed to *S. equi*. It detects antibodies to the SeM protein. Unfortunately there is considerable overlap between normal and exposed animals, and cross reactivity with the *S. zooepidemicus* M protein (SzM) has led to false positive results.[19] For these reasons, the SeM-based iELISA test it is currently not considered to be reliable. The test may, however, have some value for determining the need for vaccination, identifying predisposition to purpura haemorrhagica and aiding in diagnosis of metastatic abscessation and purpura haemorrhagica.[6]

To circumvent these problems, the animal health trust (AHT) has

developed a new serological test for identification of horses exposed to *S. equi* using data from the *S. equi* and *S. zooepidemicus* genome sequencing projects. This iELISA detects IgG antibodies to two novel *S. equi*-specific protein fragments (antigens A and C) and reveals recent exposure to *S. equi* with a reported sensitivity of 93.3 % and a specificity of 99.3 %.[20]

Serological testing is used primarily for screening purposes and should not be considered an alternative to conventional diagnostic techniques. Furthermore, seropositive horses do not necessarily pose a risk to other horses, as the test detects exposure, not active infection, and should always be followed up with bacterial culture and PCR to determine the presence of bacteria.

Detection of asymptomatic carriers

After an outbreak of strangles, up to 31 % of horses become asymptomatic carriers.[17] Affected horses do not demonstrate clinical signs of strangles, but continue to harbour bacteria in the guttural pouches for up to 39 months after the initial infection and represent an important reservoir of infection in susceptible populations.[9, 17] Detection of asymptomatic carriers is difficult. Bacteria may be shed intermittently and the sensitivity of bacterial culture from nasopharyngeal swabs can be as low as 45 %.[9] Detection of asymptomatic carriers by bacterial culture and qPCR of guttural pouch washings obtained via endoscopy and lavage is much more sensitive and is currently the diagnostic test of choice to identify subclinical carriers.[6, 17] Because of its sensitivity, qPCR is a very useful adjunct to bacterial culture for detecting asymptomatic carriers and can also be used to determine the success of elimination of *S. equi* from the guttural pouch after treatment. Serological methods are not currently considered useful for detecting strangles carriers.[21]

Where do you send samples if you suspect a case of strangles?
Samples can be sent for bacterial culture at the Keskuslaboratorio, Helsingin yliopisto (merja.rantala@helsinki.fi) or Evira (www.evira.fi/portal/fi/) PCR or iELISA testing for S. equi is not currently available in Finland, and samples should be sent to the Animal Health Trust in the United Kingdom: www.aht.org.uk/skins/Default/pdfs/diag_pricelist.pdf.

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