

Final report

Pastern dermatitis in Dole and North Swedish Draught horses: occurrence, diagnostics and treatment

(Project title in Swedish: Mugg och rasp på dölehästar och nordsvenska brukshästar: förekomst, diagnostik och behandling)

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Part 1: Detailed summary

Projektets första delmål var att studera förekomst och karaktär vid hudlidandena mugg och rasp på benen hos de skandinaviska hästraserna dölehäst och nordsvensk brukshäst. Eftersom hudparasiten, kvalstret *Chorioptes bovis* som orsakar benskabba ofta förekommer vid dessa tillstånd, men kan vara svår att finna vid traditionell mikroskopidiagnostik, var ett annat delmål att ta fram och utvärdera en ny, känsligare analysmetod, som använder polymeraskedjereaktion (PCR) för att spåra parasitens arvs massa (DNA). Ytterligare ett mål var att utvärdera effekten av tillgängliga antiparasitära behandlingsmetoder.

För att karakterisera sjukdomen genomfördes en enkätstudie bland ägare till dölehästar, nordsvenska brukshästar och andra hästar med mugg och rasp. Därtill genomfördes kliniska undersökningar av 103 hästar med olika grad av hudproblem. Från dessa analyserades hudprover för bakterier, svamp och *C. bovis*.

Enkätstudien med över 1500 svar visade att mugg och rasp var ganska vanligt förekommande lidanden året runt, men främst på vintern, och att de orsakar en betydande nedsättning av den upplevda livskvaliteten både hos drabbade hästar och deras ägare. Med hjälp av den kliniska studien kunde de vanligaste skadorna beskrivas liksom de vanligaste utbredningsområdena på benen. Ett intressant fynd var att nästan hälften av hästarna med mugg/rasp led av så kallad "mallenders/sallenders", vilket är områden med torr och förtjockad, fjällande hud i böjveckan vid carpus (framknä) och hasleder, eller kroniskt progressivt lymfödem (CPL) som ger kraftigt förtjockade hudveck. utformades med hjälp av publicerade gensekvenser och referensmaterial. Den nya PCR-analysen för *C. bovis* togs fram och validerades med hjälp av publicerade

gensekvenser och referensmaterial. Metodens styrkor är dess snabbhet och förmågan att detektera mycket små mängder av smittämnet i fråga, så det behöver inte vara hela kvalsterdjur med i provet, som vid mikroskopi.

I studierna av *Chorioptes bovis* som bakomliggande orsak, togs även den bästa provtagningsmetoden fram, så att den bästa metoden kunde appliceras i den kliniska delen. Benskabb kunde konfirmeras med PCR (den känsligare metoden) hos 39% av de undersökta hästarna, liksom hos över hälften av testade kontakthästar till dessa. Klåda och hudförändringar förekom på 80,5 respektive 90,5% av hästarna med benskabb. De mikrobiologiska odlingarna visade att det förekommer sekundära bakteriella infektioner med bland annat *Staphylococcus aureus* och *Streptococcus dysgalactia equisimilis* vid mugg/rasp, men att svampinfektioner var mer sällsynta. Genomförda behandlingar mot benskabb var i de flesta fall framgångsrika, även om DNA från *Chorioptes* ännu kunde påvisas i en del prover under en tid efter behandling.

Studiens resultat är relevanta för hela hästsektorn eftersom de ökar kunskapen och medvetenheten om problemen med mugg, rasp och benskabb, och presenterar för första gången i världen en ny och effektiv, känslig PCR-metod för att påvisa den mest betydande bakomliggande orsaken, *C. bovis*. Rekommendationerna efter denna studie är att analys av *Chorioptes bovis*, som orsakar benskabb, alltid ska ingå i den kliniska undersökningen av mugg och rasp, och att kontakthästar, som lever tillsammans med hästar med benskabb, också ska behandlas för att stoppa möjliga smittkedjor i besättningen. Ytterligare studier behöver göras för att finna optimala behandlingar mot *C. bovis*, “mallenders”, “sallenders” och CPL.

Part 2: Main report (max. 10 pages)

Introduction

The painful, crusty and sore-causing inflammation of the skin associated to pastern dermatitis (“mugg” and “rasp” in Swedish) and the powerful and insufferable itch associated to leg mange caused by *Chorioptes bovis* (one of several mange-mites) are undesirable animal welfare problems. These diseases can last for weeks, sometimes months or become chronic over years and many horses experience these conditions several times during their lives. In addition, leg mange is contagious and optimal control is poorly investigated. Pastern dermatitis is threatening Nordic horse breeders through reduced animal welfare and risk of interruption in operations. The various conditions are well known in several horse breeds, including the Norwegian Døla horse (“Dølahest”), the North Swedish horse (“nordsvensk brukshäst”) and coldblooded trotting horse, but an accurate estimation of these problems is still not available. For a better welfare of horses and for the benefit of the horse industry, it is important to find the right tools to control pastern dermatitis and leg mange through several research actions.

The overall aim for this project was to improve the possibilities for an effective diagnosis and treatment of pastern dermatitis in Døla horses and North Swedish horses, specifically:

- to gain deeper knowledge about the occurrence and etiology of pastern dermatitis and associated conditions within the breeds Døla horse and North Swedish horse
- to provide a sensitive, reliable and rapid method for diagnosing the occurrence of *Chorioptes* mites, leg mange
- to reduce the suffering and contagious spread of pastern dermatitis

The project consisted of five parts:

1. Investigation of the occurrence of pastern dermatitis in Norway and Sweden
2. Development of a real-time PCR analysis method for detection of *Chorioptes bovis*
3. Comparative studies of diagnostic methods for detecting *Chorioptes bovis*
4. Characterization of pastern dermatitis in Døla and North Swedish working horses and other breeds
5. Effect of different protocols for treatment of chorioptic mange

Material and methods

1. Investigation of the occurrence of pastern dermatitis in Norway and Sweden

A web-based questionnaire survey (Questback Easyresearch) about pastern dermatitis, leg mange and skin health of horse legs was distributed to horse owners in Norway and Sweden in 2018. Invitations to the survey were distributed via e-mail to members of “Landslaget for dølahest” in Norway and the association of North Swedish working horses’ owners in Sweden. The invitation and a link were also available on SVA’s webpage, in social media from SVA and Hästsverige web platform, as well as in local horse-dedicated groups on Facebook and with targeted advertisements for horse owners in Sweden and Norway on Facebook. A veterinary student, Karin Juto, processed data from the survey as part of her degree project.

2. Development of a real-time PCR analysis method for detection of *Chorioptes bovis* mites

Primer and probe design for the real-time PCR assay

PCR and Sanger sequencing of the mitochondrial COI-gene of *Chorioptes bovis* mites from four different horses were done by using primers COIF and COIR from Gu et al (2004). The sequences were aligned together with Genbank sequence KF891935.1. Primer and probe design for a Taqman assay was performed using the oligo design software Allele ID 7 (Premier Biosoft, Palo Alto, CA, USA), including ambiguous bases according to the variation detected in the alignment.

Optimization of sampling and DNA extraction method

Four horses previously determined as positive for *Chorioptes bovis* and eight determined as negative by microscopy were sampled for PCR in four different ways: 1) scraping a shaved area with a dull knife and collecting the sample in a jar, 2) swabbing using eSwab® (Copan, Murrieta, Ca, USA), 3) swabbing using a dry cotton swab and 4) swabbing using an eSwab® placed in a tube containing 2 mm zirconium beads for homogenization before DNA extraction. One of the microscopy positive horses was sampled one more time 6 weeks after treatment (at that point *Chorioptes* could not be found at microscopy). The collected samples were pre-treated in the following ways: 1) The jar was swabbed with a cotton swab whereafter the swab was placed in a 2 ml tube containing 850 µl TE-buffer pH 8 and incubated at 99°C for ten minutes followed by short vortexing. 2 and 3) The eSwab/cotton swab was placed in a 2 ml tube containing 850µl TE buffer and then vortexed. 4) 850 µl TE buffer was added to the eSwab which was then homogenized at 6.5 m/s for one minute in an MP-Bio Fastprep homogenizer (MP Biomedicals, Irvine, CA, USA). DNA extraction was performed in an IndiMag instrument (Indical, Leipzig, Germany) using the IndiMag Pathogen extraction kit according to instructions.

Minor groove binder (MGB) hydrolysis probe real time PCR and definition of a positive test result

The mastermix consisted of 7.5 µl Toughmix supermix (Quanta Bio, Beverly, MA, USA), 400 nM of each primer ChorioptesF and ChorioptesR (Integrated DNA Technologies, Coralville, IA, USA) and 133 nM of the hydrolysis probe ChorioptesMGBP labelled FAM-MGBNFQ (Thermofisher Scientific, Waltham, MA, USA). Reactions were run in a total volume of 15 µl of which 2 µl was template DNA retrieved from the DNA extraction. Samples were run in duplicate using an Applied Biosystems 7500 Fast instrument (Life Technologies, USA) in fast mode (ramp rate 5 °C per second). The reaction was initiated by 2 minutes of initial denaturation at 95°C followed by 45 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Samples were considered positive if the reaction curve exhibited the characteristic exponential curve shape and reached above the selected threshold based on the baselines in the samples and controls.

Testing specificity of the PCR

To test the specificity of the assay, nine species of mites not associated with chorioptic mange in horses were tested: *Tyrophagus putrescentiae*, *Acarus siro*, *Dermatophagoides farinae*, *Euroglyphus maynei*, *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Lepidoglyphus destructor*, *Chortoglyphus arcuatus* and *Glycyphagus domesticus*. One mite of each species was extracted using the same instrument and kit as previous samples. Although not available for testing, mismatches in primer and probe sequences suggest that the assay will not pick up for example *C. sweatmani* or any of the other related species for which COXI sequences are available.

3. Comparative studies of diagnostic methods for detecting *Chorioptes* mites

To evaluate the best combination of sampling and detection method for *Chorioptes* mites, two sampling methods (skin scraping and tape preparation) were performed in horses from farms with a history of equine leg skin problems (n=106). On these farms, samples from some horses with no suspect of leg mange were also collected; these horses were defined as “contact horses” (n=29). Two detection methods were then used in three different combinations: PCR for the skin scrapings, and direct microscopy of skin scrapings and tape preparations respectively.

4. Characterization of pastern dermatitis in Døla and North Swedish working horses and other breeds

A field sampling and clinical study (responsible: Kerstin Bergvall) was performed by five veterinarians in 103 horses (50% mares, 40% geldings, 10% stallions) of different ages and with different geographical locations in Sweden (Uppsala, Gothenburg, Småland province) and Norway. Among those, 60 (58.3%) were North Swedish Draught (NSD, 36%) and Døla (23%) horses. Horses were recruited by information sent out to the breed associations (NSB, Døla horse). Also NSB, Døla and other heavy feathered horses visiting the participating clinics (Uppsala, Gothenburg or Oslo) for any reason, were included. Other breeds included were then Friesian, Tinker/Gypsy cob, Belgian draught, Shire and others. The distal leg cutaneous health was clinically examined in each horse.

An Equine Leg Dermatitis Score (ELDS) protocol was developed for each of nine different types of lesions (alopecia, scaling, crusts, seborrheic callosity, erosions, swelling, skin folds, nodules, and erythema) for 10 different specific areas per leg. The degree of pain was noted, and the owner indicated the degree of itch on a visual analog scale. The maximal total ELDS score per leg was 85, and the maximal pain and itching score was 10 for each.

For detection of *Chorioptes* leg mites, skin scraping of shaved areas with a dull knife to collect material in a jar for PCR testing, scraping with a dull knife and mineral oil to collect material to a glass slide for direct microscopy and tape preparation were performed and subject to direct microscopy and PCR analysis in 106 horses (horses used to characterize leg dermatitis plus horses (n=3) from farms with suspected or verified chorioptic mange). Skin biopsies were collected from two horses with hyperkeratotic fissuring in carpal or tarsal flex areas (“sallenders” or “mallenders”).

In addition to the above study population, control skin samples from the same body region for histopathology were collected from 5 dermatologically healthy horses of feathered breeds and 1 warmblood horse which were admitted for necropsy for any reason at the Swedish University of Agricultural Sciences (SLU).

A total of 53 samples from 43 horses with pastern dermatitis were received for mycological and bacteriological examinations. These samples were swabs, skin scrapings or brush samples. Bacteriological analyses were performed on all samples, and all but one were analysed mycologically.

All bacteriological samples were inoculated on two Blood Agar (BA) plates and one Eosin Methylene Blue Agar (EMB). One BA plate was co-inoculated with *Staphylococcus aureus* and incubated in 5% CO₂ atmosphere, the other BA plate was incubated anaerobically. The EMB plate was incubated in normal atmosphere, all three plates at 37 ° ± 1 °C for 16 - 24 h. The bacterial growth was characterized and representative colonies sub-cultured and identified. Samples of hair and crusts were inoculated on Sabouraud (SAB) and Mycobiotic agar (MY) plates for mycological examinations. SAB and MY were incubated in plastic bags for 30 days at 30° ± 1°C and 37° ± 1°C in the dark, the latter in bags containing filter paper soaked in sterile water in order to secure sufficient humidity. The bags were opened for aeration and inspection

of the plates at least twice a week. Representative colonies were sub-cultured and identified based on macroscopic and microscopic morphology according to accepted keys. Fungi that failed to produce distinct features for reliable identification were identified by ITS sequencing.

5. Effect of different protocols for treatment of *Chorioptes*

Information regarding long-term outcome was available in 18 horses with chorioptic mange infestation from the study population in part 4. End result was collected at 11-24 months after diagnosis, by interviews with owners, and follow-up clinical examinations and testing for *Chorioptes* when possible.

Results and discussion

1. Investigation of the occurrence of pastern dermatitis in Norway and Sweden

Most replies (n=1549) in the survey regarded North Swedish working horses, who have strongly feathered legs. Box or loose operations were the most common housing system and the type of bedding material used varied. Almost all the horses were out on pasture during the summer. About half of the horse farms experienced skin problems on the legs of their horses. Often, there were only a few horses in each farm that had skin problems. Of these, most had recurring or constant problems. Variations in this condition over time were common. Autumn and winter were often reported as the worst periods. More than half of the horses having leg skin problems showed signs in two or more legs, and most often on the hind legs. No significant correlation was found between the leg pigmentation and skin problems, even if white legs were more affected. Sores, itchy and thickened skin were the most common clinical signs. More than one third of the horses were diagnosed with leg mange. Often, both affected horses and healthy herd mates had been treated against leg mange. The quality of life was assessed as deteriorated in half of the affected horses. Some comments were: "Seems very difficult and irritating to the horse", "Extremely stressed and kicked holes in the walls, tired because he could not sleep, showed a very unusual behavior for several months", "Very difficult with the concentration due to itching". The horse owners' quality of life when the horse was affected by leg skin problems was experienced as impaired by over half of them: "time-consuming and laborious when the horse is suffering", "There is a lot of work to keep your horse's legs healthy when it has pastern dermatitis" and "Last winter's allergy with hair loss and fanatic itching was driving us crazy" were some illustrative comments. Over half of the responders thought it was difficult to get the right help for their horse. Many commented that the problem does not seem to be taken seriously by the veterinarian or by the surrounding community, and that the recommended treatment had been insufficient.

2. Development of a real-time PCR analysis method for detection of *Chorioptes* mites

The results from microscopy of the samples used for optimization of sampling were in general congruent with the PCR results, except in one case. The horse positive for *Chorioptes* sampled before and six weeks after treatment was PCR-positive also after treatment using sampling method 1), 2) and 4) as described in section 2 of materials and methods (although Ct-values were higher). Results between sampling methods were similar, average Ct-values of the methods differing less than 1.5. None of the other species of mites tested using the assay gave reaction curves.

Since the different methods of sampling gave similar results, we chose to use method 1 (scraping a shaved area with a dull knife, collecting in a jar). Veterinarians and even some horse owners are familiar with this method, and no dedicated sampling material is needed.

The designed PCR assay detects *Chorioptes bovis* while being specific, not detecting the nine other tested species. Although the samples from the previously positive horse sampled six weeks after treatment, negative using microscopy, were PCR positive (they could be considered as false positive according to both treatment status and microscopy results), this can be explained by residual DNA remaining in the skin and coat of the hoof. In essence, the assay is specific to the genetic material of the intended species but can generate false positive results in previously infected horses because of residual DNA. We suggest PCR-testing after treatment of infected horses should be done no sooner than three months after completed treatment.

3. Comparative studies of diagnostic methods for detecting *Chorioptes* mites

Chorioptes was detected in 41 out of 106 sampled horses (38,7%) by at least one analysis method.

The sensitivity for PCR of skin scrapings, microscopy of skin scrapes and microscopy of tape preparations were evaluated in 40 of the *Chorioptes*-positive horses with a full set of samples. PCR analysis of skin scrapings were positive in 40/40 horses, microscopic analysis of skin scrapings was positive in 25/40 horses (65.5% sensitivity, 34.5% false negative) and microscopic analysis of tape preparations was positive in 18/40 horses (45% sensitivity, 55% false negative).

PCR testing for *Chorioptes* was negative in 65 horses, of which 59 horses were tested both by PCR and direct microscopy. Of these 59 horses, all were also negative on direct microscopy of skin scraping or tape preparation. In one horse with pruritus and skin lesions, that was negative for *Chorioptes* on all tests, it was reported that all clinical signs resolved after treatment with the anti-parasitic drug permethrin, although no ectoparasites had been demonstrated macro- or microscopically. It is possible that this horse represents a case of *Chorioptes* infestation with false negative test results, or that the horse was infected with undetected ectoparasites other than *Chorioptes*. Another horse with prominent leg pruritus, had an infestation of *Bovicola equi*, with massive numbers of lice on the legs.

Of the 29 contact horses, i.e. with known contact with a confirmed *Chorioptes* infested horse, 67% were positive for *Chorioptes*.

4. Characterization of pastern dermatitis in Døla and North Swedish working horses and other breeds

For the 103 examined horses of all breeds the highest score for skin lesions was on the palmar aspect of the cannon bones, followed by the palmar aspect of the pastern region and the flex region of the carpus and hock. Regarding type of lesion, the highest score was recorded for scaling, followed by crusts, skin folding and hyperkeratotic fissuring. There were three times higher scoring for lesions in the flex area (carpus) of the front legs as compared to the flex area of the hocks in the hind legs. For lesions on the palmar/plantar cannon bones, the scores in the hindlegs were 1.6 higher compared to the front legs. Scoring for skin folding were similar in front and hind legs. Pruritus (itching) was reported in 46% and pain in 28% of all the horses. Among the examined NSD and Døla horses, 65% were affected with skin lesions, whereas 35% exhibited no clinical lesions. Lesion type and distribution were similar in NSD and Døla horses as compared to the other types of horses. Hyperkeratotic fissuring in carpal or tarsal flex areas (“sallenders” or “mallenders”) was recorded in 46.7% of the NSD and Døla horses. Clinical signs compatible with chronic progressive lymph oedema (CPL) was recorded in 46,7% of the horses of these breeds.

Diagnostic work up for *Chorioptes* infestation was carried out in 106 horses. In 41/106 horses (38.7%) the diagnosis of leg mange was confirmed by at least one test method. Of 29 horses with known contact with a *Chorioptes* infested horse, 67% were positive for *Chorioptes*.

In the 41 horses diagnosed with Chorioptic infestation, skin lesions were observed as follows: pastern area, 73%; in three areas (pastern, cannon bone and carpal/hock flex area), 63%; and 9.7% lacked any apparent clinical lesion. Pruritus was reported in 80,5% and was absent in 19,5% of the horses positive for *Chorioptes*, and pruritus scores varied between 0-9 (PVAS 0-10; mean 3.5; median 3). In the 65 horses that tested negative for *Chorioptes* on all tests, pruritus was recorded in 30.8% and pruritus and skin lesions in 21.5%. The interval in pruritus score was 0-6 (mean 1.8; median 0) in horses that tested negative for *Chorioptes*.

The results from skin biopsies in healthy horses and horses with “mallenders” or “sallenders” were not finished at the time for this report.

Rich amounts of *Staphylococcus aureus* were detected from 22 of the 43 horses (51%), and *Streptococcus dysgalactia equisimilis* were detected from 10 horses, in 8 cases in combination with *S. aureus*. None of the *S. aureus* strains were methicillin resistant. Yeasts were detected from 20 of the 42 horses (48%). A variety of mould species were detected from 29 horses (69%) while no fungi were detected from three horses. Fungi responsible for skin diseases (i.e. dermatophytes) were not detected in the analyzed samples.

Draught horses and horses with heavy feathering of distal legs have been identified to carry a higher risk for pastern dermatitis (Scott and Miller, 2011; Yu, 2013). This is the first detailed study of leg dermatitis, “mallenders”, “sallenders”, CPL and the occurrence of the leg mange (chorioptic mange) in feathered horses in Scandinavia, with a focus on Nordic origin breeds such as NSD and Døla horse. The distribution of lesions was similar in the Nordic breed types and feathered horses of other breeds. The prevalence of pastern dermatitis in Nordic draught horses (NSB and Døla) has not been evaluated earlier. In this material 65% were affected.

This is higher as compared to a study by Frederici et al 2015, looking at the draught breed Frieberger (Frances-Montagnes) horse, where 12,5% were affected. On the other hand, in a study by Wallraf (2004) the prevalence varied between 47.5-96.1% depending on breed. The Frieberger horse does typically not have heavy leg feathering, and less than the NSB and Døla horse, whereas the two breeds with the highest prevalence in the study by Wallraf have prominent leg feathering. Out of the horses affected by the disease, half of them had itching and in one third of them the lesions were painful. *Chorioptes* mange was part of the problem in many horses, often characterized by itching behaviour but in 19.5% and 9.7% of *Chorioptes* positive horses, respectively, neither pruritus nor skin lesions were noticed. This makes necessary to have in place a correct diagnostic protocol and an effective and safe treatment protocol. Bacterial infections might develop in pastern dermatitis areas, while mycological infections seem to be a minor problem. The prevalence of the combination of *Staphylococcus aureus* and B-haemolytic streptococci is consistent with previous reports (Sangiorgio et al., 2021). *S. aureus* is a serious pathogen in humans and animals, causing a broad spectre of diseases, including infections in soft tissue, joints and skin (Burton et al., 2008). The normal habitat of *Streptococcus dysgalactiae* subsp. *equisimilis* in the horse appears to be the skin and mucous membrane (Preziuso et al., 2010). The interest in these bacteria has increased due to isolation from cases of strangles-like disease in absence of *Streptococcus equi* infection (Preziuso et al., 2010). Furthermore, *S. equisimilis* is an emerging human pathogen responsible for a variety of clinical syndromes, including primary bacteraemia, pneumonia, endocarditis, arthritis, and streptococcal toxic shock syndrome (Oppegaard et al., 2017). The relatively high prevalence of potentially human pathogenic bacteria should be considered when the owners are treating horses with dermatitis.

5. Effect of different protocols for treatment of *Chorioptes*

Seventeen out of 18 horses were reported to be in complete remission at the last follow up (11 months - >2 years). The horses had been treated with phoxim, lime sulphur, fipronil, permethrin or eprinomectin. Three horses were not PCR re-tested.

In 12 horses a negative PCR analysis for *Chorioptes* was achieved >4 months after treatment. Three other horses were tested between six weeks and two months after treatment (phoxim, topical application) and were all *Chorioptes* PCR positive. No further treatment was instituted as they did not show further clinical signs. These three horses were still clinically in complete remission at 1-1.5 year follow up, and one was also re-tested with PCR with a negative test result. Another horse was PCR positive > 4 months after treatment and still exhibited clinical signs. This was the only case where treatment had not addressed contact horses and environment.

The outcome in the studied case series shows that *Chorioptes* mange in feathered horses can be successfully treated and the prognosis for remission is good. The positive PCR results at follow up of some clinically healthy horses may be explained by remaining nucleic acids (DNA) from dead mites or their contaminating remnants on the skin, and it is advised that early follow up sampling giving positive PCR results should be interpreted with caution. The infestation of one horse some months after treatment is suggested to be a result of re-infection, due to failure of sanitizing the environment and contact horses from mites. This illustrates the importance of such measures.

Pastern dermatitis is a relevant but underinvestigated problem in Nordic horse breeds, causing discomfort and reduced quality of life of both horses and their owners. The diagnostic process is complex and can lead to misdiagnosis if using traditional diagnostic techniques. By using a panel of bacteriological, mycological, histopathological and parasitological methods in the present study, we could demonstrate that “mugg and rasp” are often associated with *Chorioptes* infestation. The molecular method (PCR) developed in the project for *Chorioptes* detection was sensitive and specific and was also adapted to a simple sample collection method, which makes it very useful for both horse owners and veterinary practitioners.

Other skin alterations like “mallenders”, ”sallenders” and chronic progressive lymph oedema were present in horses belonging to the studied breeds showing clinical signs of pastern dermatitis; efficacious treatments for these diseases deserve further studies.

The preliminary results of treatments against ectoparasites are promising but more studies are needed to find more effective methods.

The results indicate that *Staphylococcus aureus* and *Streptococcus dysgalactia equisimilis* are the most commonly involved micro-organisms in “mugg” and “rasp” pastern dermatitis, while mycological infections seem to be a minor problem.

Relevance for the practical horse sector incl. recommendations

A new sensitive and reliable diagnostic tool (PCR for the detection of *C. bovis*) has been developed and since June, 2021, is available and offered to both horse owners and veterinary practitioners, since the validated sampling method is non-invasiv. This can help to identify those horses that are suffering from chorioptic mange and that can also be a source of infection for other horses. The clinical and aetiological characterization of pastern dermatitis has been implemented through an international and interdisciplinary collaboration. *C. bovis* mites are often responsible for the clinical picture, while other etiological agents seem to play a secondary role in the pathogenesis of the disease. The available information about the most common body region affected, as well as the identification of the most common causes of the disease make it easier to both horse owners and veterinarians to suspect the presence of the disease, so that a quicker diagnosis and treatment can be achieved. As a result of the study it

has been possible to establish new recommendations to the owners and to the veterinarians: it is very important to include always *C. bovis* within differential diagnosis protocol and whenever the mite is detected it is mandatory to treat against the parasites not only the infested individual but also the horses that have been in contact with her/him. Also, the hygienization of the environment and the diagnostic follow-up after the treatment are very important. When using PCR as a diagnostic method, it must anyway be considered that some DNA traces from mites can be present some weeks after a successful treatment and could lead to a false positive result. Further investigations are needed to i) study the molecular epidemiology of chorioptic mites (apparently only *C. bovis* out of six *Chorioptes* species can infest horses but the available data are still limited) and ii) study innovative alternatives to miticidal treatment by testing on horse substances that are nowadays only available for dogs and cats.

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Part 3: Result dissemination

Scientific publications, manuscript	Isaksson M, Grandi G, Skaar I, Osterman-Lind E, Bornstein S, Bergvall K. Development of a qPCR for the detection of Chorioptes spp in equine skin scrape samples (manuscript in preparation)
	Bergvall K, Isaksson M, Grandi G, Skaar I, Osterman-Lind E, Skaar I. Characterisation of equine pastern dermatitis (greasy heel, mud fever) in Nordic breeds Døla and North Swedish coldblooded horse (manuscript in preparation)
Conference publications/ presentations	<i>Presentation of project's results. Most 2020 events have been canceled or delayed because of COVID pandemic</i>
	Italian society for parasitology (SoIPa), online conference, Teramo, 2021 (Characterisation of equine pastern dermatitis (greasy heel, mud fever) in Nordic breeds Døla and North Swedish coldblooded horse and Development of a qPCR for the detection of Chorioptes bovis in equine skin scrapings, poster presentation, see attached posters below)
	European congress of veterinary dermatology 2021, online conference (Development of a qPCR for the detection of Chorioptes spp in equine skin scrapings, oral short communication, see abstract below)
Other publications, media etc.	<i>Informative webpages on leg/pastern dermatitis on the following websites (upcoming):</i>
	www.sva.se
	www.hästsverige.se
	www.vetinst.no
Oral communication, to horse sector, students etc.	<i>Presentation of project's results</i>
	Hippocampusdagen 2021 (presented on 5th November, 2021 by G. Grandi and G. Gröndahl, see abstract below)
	Nordsvenska brukshästen and Døla häst breeders' associations (time to be defined)
	Svensk Frieserhästförening (planned in 2021, compatibly with COVID pandemic)
	Tinkersällskapet (planned in 2021, compatibly with COVID pandemic)
Student theses	Karin Juto (Veterinary program, supervisors Giulio Grandi, Gittan Gröndahl), "Svenska och norska hästar med mugg och rasp: en deskriptiv studie". 2019, Uppsala: SLU, Dept. of Clinical Sciences, https://stud.epsilon.slu.se/14905/

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Characterisation of equine pastern dermatitis (greasy heel, mud fever) in Nordic breeds Døla and North Swedish coldblooded horse

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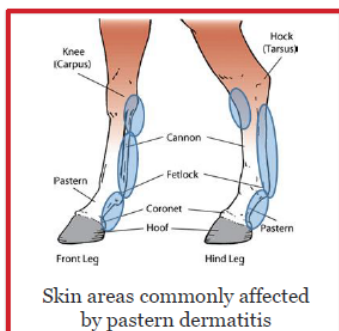
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INTRODUCTION. Pastern dermatitis and leg mange caused by *Chorioptes bovis* are undesirable and often chronic animal welfare problems in horses. The overall aim for this study was to gain deeper knowledge about the occurrence and etiology of pastern dermatitis and associated conditions in horses.

MATERIALS AND METHODS.

Chorioptes mites were detected from skin scrapings with PCR method and direct microscopy (n=106). The distal legs were examined in n=103 horses (58% belonging to North Swedish Draught and Døla horses) in Sweden and Norway using an Equine Leg Dermatitis Score (ELDS) protocol (Fig. 1). A total of 53 samples from 43 horses with pastern dermatitis were examined for bacteria and fungi.



Legs:	HF	VF	HB	VB						
Lesion	Carpus/hock flex	MC/MT rostrat	MC/MT lat/med	MC/MT palmar	Pastern rostrat	Pastern lat/med	Pastern palmar	Coronary band	Chewnuts	Fetel
Alopecia										
Scaling										
Seborrheic callosity										
Crusts										
Erosions										
Erythema										
Swelling										
Skin folding										
Nodules										
Subtotal										
Pain (0-10)										
Pruritus (0-10)										
Total										
Colour (0/5)										

Fig. 1: ELDS – each leg was divided in 10 distinct areas & scored 0-5 for different types of lesions (alopecia, scaling, crusts, seborrheic callosity, erosions, swelling, skin folds, nodules and erythema). Pain & pruritus was graded 0-10.

RESULTS AND CONCLUSIONS. Pastern dermatitis was detected in 65% of horses. Highest score for skin lesions was reached on the palmar aspect of the cannon bones, followed by the palmar aspect of the pastern region and the flex region of the carpus and hock.

Clinical signs compatible with keratinization defects “sallenders & mallenders” were recorded in 50/103 (50%) of the horses and 51/103 (50%) were affected with lesions typical for Chronic Progressive Lymphedema (CPL).

Pruritus (itching) was reported in 46% and pain in 28% of all the horses.

In 41/106 horses (38.7%) chorioptic mange was diagnosed by at least one test method. *Staphylococcus aureus* and *Streptococcus dysgalactia equisimilis* were detected in 51% and 23% of the examined horses respectively; yeasts and molds were detected in 48% and 69% of horses respectively, while no dermatophytes were detected.

Chorioptic mange was part of the problem in many horses, often characterized by itching behaviour and skin lesions but in 19.5% and 9.7% of *Chorioptes*-positive horses, respectively, neither pruritus nor skin lesions were noticed.

This makes necessary having in place a correct diagnostic protocol and an effective and safe treatment protocol. Bacterial infections might develop in pastern dermatitis areas, while mycological infections seem to be a minor problem.



a. Seborrheic, horizontal callosity (photo K. Bergvall)



b. Skin folding & nodules typical for CPL (photo C. Petterson)

Source of funding: Swedish-Norwegian Foundation for Equine Research

Development of a qPCR for the detection of *Chorioptes bovis* in equine skin scrapings

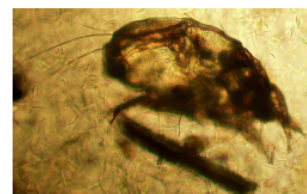
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INTRODUCTION. Equine leg mange caused by *Chorioptes bovis*, often associated to pruritic pastern dermatitis, is a recognized welfare problem in horses. Diagnosis by direct microscopy can be challenging and frustrating in field practice. Our aim was to develop a reliable, molecular tool for the detection of *C. bovis* DNA in skin scrapings.

MATERIALS AND METHODS. A new set of primers and a Taqman probe were designed within the mitochondrial Cytochrome c Oxidase (COI) gene sequence of *C. bovis*. Before designing the Real-time PCR system, a qualitative PCR was first performed using generic COI primers (Gu et al., 2014 Parasites & Vectors 7:340; Fig. 1). DNA was extracted from *C. bovis*-positive (n=4) and *C. bovis*-negative (n=8) horses sampled with different techniques; mites were detected at microscopy (Figs. 2 and 3). PCR analysis was therefore run using 400 nM of each primer and 133 nM of the hydrolysis probe labelled with FAM-MGBNFQ (2 minutes of initial denaturation at 95 °C followed by 45 cycles of 95 °C for 5 seconds and 60 °C for 30 seconds). Samples were considered positive if the reaction curve (Fig. 4) exhibited the characteristic exponential shape and reached above the selected threshold based on the baselines in the samples and controls. To test the specificity of the assay, nine species of environmental mites were tested, all with negative results.

RESULTS AND CONCLUSIONS. The results from microscopy of the samples used for optimization of sampling were in general congruent with the PCR results except in one case when after acaricidal treatment a horse was still positive at PCR but negative at microscopy, suggesting residual DNA from *C. bovis* was still present in the sample. While our PCR method showed good specificity and sensitivity, it still needs to be tested on larger sample batches and the timing for post treatment follow-up analyses has to be set up.



Figs. 2 and 3: *Chorioptes* mites observed in skin scrapings (40x magnification).

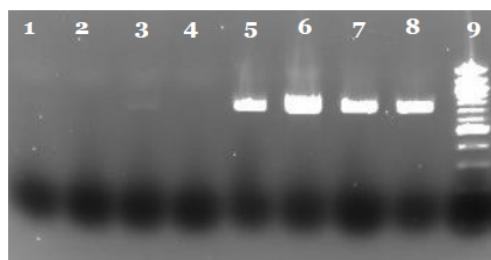


Fig. 1: qualitative PCR for mitochondrial Cytochrome c Oxidase (COI) gene target. 1-4: DNA from one clinical sample, a weak positive signal (n. 3) can be seen and has been sequenced. 5-7: DNA from another clinical sample; n. 5 DNA from a *Chorioptes* mite, n. 6-7 DNA from skin scraping. 8: positive control from a previously sequenced sample. 9: DNA ladder (100 bp).

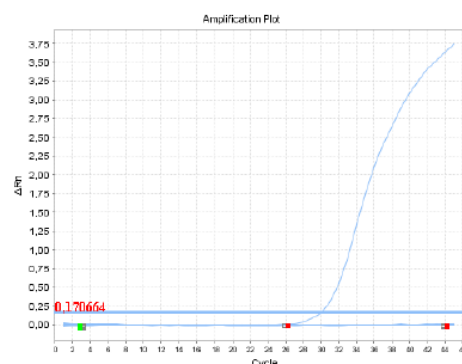


Fig. 4: amplification curve obtained from one of the *Chorioptes*-positive samples.

Source of funding: Swedish-Norwegian Foundation for Equine Research



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences



Veterinærinstituttet
Norwegian Veterinary Institute



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Abstract European Conference of Veterinary Dermatology 2021

Development of a qPCR for the detection of *Chorioptes* spp in equine skin scrapings

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Equine leg mange caused by *Chorioptes bovis*, often associated to pruritic pastern dermatitis, is a recognized welfare problem in horses. Diagnosis by direct microscopy can be a challenge in field practice. Our aim was to develop a reliable, molecular tool for the detection of *C. bovis* DNA in skin scrapings. A new set of primers and Taqman probe were designed within the mitochondrial COI-gene sequence of *C. bovis*. DNA was extracted from four *C. bovis* positive and eight *C. bovis*-negative horses at microscopy sampled with different techniques. PCR analysis was run using 400 nM of each primer and 133 nM of the hydrolysis probe labelled with FAM-MGBNFQ (2 minutes of initial denaturation at 95°C followed by 45 cycles of 95°C for 5 seconds and 60°C for 30 seconds). Samples were considered positive if the reaction curve exhibited the characteristic exponential shape and reached above the selected threshold based on the baselines in the samples and controls. To test the specificity of the assay, nine species of environmental mites were tested, all with negative results. The results from microscopy of the samples used for optimization of sampling were in general congruent with the PCR results, except in one case, when after acaricidal treatment a horse was still positive at PCR but negative at microscopy. While our PCR method showed a good specificity and sensitivity, it still needs to be tested on larger sample batches and the timing for post treatment follow-up analyses has to be set up.

Source of funding: Swedish-Norwegian Foundation for Equine Research

Conflicts of interest: None declared

Abstract Hippocampusdagen 2021:

Mugg och rasp: en hudproblematik hos bland annat dölehäst och nordsvensk brukshäst

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Hudlidandena mugg och rasp är ett viktigt problem på benen hos de skandinaviska hästraserna dölehäst och nordsvensk brukshäst och hos andra raser med kraftigt hovskägg på benen, dvs kallblodhästar. För att karakterisera sjukdomen genomfördes en enkätstudie bland ägare till dölehästar, nordsvenska brukshästar och andra hästar med mugg och rasp. Därtill genomfördes kliniska undersökningar av hästar (n=103) med olika grad av hudproblem. Från dessa analyserades hudprover för bakterier, svamp och *Chorioptes bovis*. Detta är en kvalsterart som orsakar benskabb och ofta förekommer vid mugg och rasp. Enkätstudien med över 1500 svar visade att mugg och rasp är ganska vanligt förekommande lidanden året runt, men främst på vintern, och att det orsakar en betydande nedsättning av den upplevda livskvaliteten både hos drabbade hästar och deras ägare. De vanligaste skadorna liksom de vanligaste utbredningsområdena på benen kunde beskrivas med hjälp av den kliniska studien. Nästan hälften av hästarna med mugg/rasp led av så kallad ”mallenders/sallenders”, vilket är områden med torr och förtjockad, fjällande hud i böjveckan vid carpus (framknä) och hasleder, eller kroniskt progressivt lymfödem (CPL) som ger kraftigt förtjockade hudveck. Då kan det vara svårt att finna *C. bovis* vid traditionell mikroskopidiagnostik, en ny PCR-analys för *C. bovis* och även den bästa provtagningsmetoden togs fram och validerades med hjälp av publicerade gensekvenser och referensmaterial. Metodens styrkor är dess snabbhet och förmågan att detektera mycket små mängder av smittämnet i fråga, så det behöver inte vara hela kvalsterdjur

med i provet, som vid mikroskopi. Benskabb kunde konfirmeras med PCR (den känsligare metoden) hos 39% av de undersökta hästarna, liksom hos över hälften av testade kontakthästar till dessa. Klåda och hudförändringar förekom på 80,5 respektive 90,5% av hästarna med benskabb. De mikrobiologiska odlingarna visade att det förekommer sekundära bakteriella infektioner med bland annat *Staphylococcus aureus* och *Streptococcus dysgalactia equisimilis* vid mugg/rasp, men att svampinfektioner var mer sällsynta. Genomförda behandlingar mot benskabb var i de flesta fall framgångsrika, även om DNA från *Chorioptes* ännu kunde påvisas i en del prover under en tid efter behandling. Rekommendationerna efter denna studie är att analys av *Chorioptes bovis*, som orsakar benskabb, alltid ska ingå i den kliniska undersökningen av mugg och rasp, och att kontakthästar, som lever tillsammans med hästar med benskabb, också ska behandlas för att stoppa möjliga smittkedjor i besättningen. Ytterligare studier behöver göras för att finna optimala behandlingar mot *C. bovis*, “mallenders”, “sallenders” och CPL.