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Intramuscular administration of two dosage forms of benzylpenicillin in horse - pain assessment

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SUMMARY

In veterinary medicine, penicillin is the most used antibiotic in Sweden. Two dosage forms of penicillin are available in Sweden for use in horses, sodium benzylpenicillin (Na-pc) and procaine benzylpenicillin (proc-pc). Proc-pc is the most used dosage form and is used for intramuscular administration which allows horse owners to treat an animal at home under veterinary instruction. Na-pc is only approved for intravenous use in horses.

Penicillin is normally well tolerated by horses but a serious, sometimes life-threatening, adverse reaction called penicillin shock may occur. Most cases of penicillin shock are believed to be caused by procaine toxicity and the number of adverse reactions could possibly be reduced if proc-pc is replaced by another drug. At the moment, there is no good alternative to proc-pc available. The aim of the study is to see if Na-pc is an alternative to proc-pc for intramuscular administration in horses and to assess pain associated with injection. The study was a randomized, blinded, cross-over study in eight healthy horses. The horses were injected intramuscularly with proc-pc once a day four times and Na-pc twice a day seven times in total. Behavioural studies and clinical evaluation were performed to see if there are any differences in pain associated with injection of Na-pc and proc-pc. The horses were studied for four minutes before and four minutes after the morning injection. The clinical evaluation was performed 24 hours after the last injection. The pain assessment presented in this paper is part of a larger study.

The study shows that after injection with Na-pc horses are more likely to walk and stretch their necks and less likely to rest their necks than after injection of proc-pc. They will also shake their necks more and kick/stamp/scrape with their front legs more. Before injections of Na-pc horses are less likely to rest their necks than before injection of proc-pc, however this difference could not be seen before the first injection. This suggests that the horses might be in pain 12 hours after the previous injection. The clinical examination showed that after repeated injections of Na-pc horses were more swollen at the injection site and had stronger pain reaction than after repeated injections of proc-pc.

The conclusions of the study are that injections of Na-pc causes more pain than proc-pc when administered intramuscularly and that the degree of pain cannot be accepted because of animal welfare concerns. The reason why Na-pc causes more pain could be because of the absence of procaine, which is a local anaesthetic. Another contributing factor to Na-pc causing more pain could be that it was administered twice a day which could result in more muscle soreness than injection once a day. Na-pc cannot be recommended for intramuscular use in horses and the need to find a replacement to proc-pc still remains.

SAMMANFATTNING

Inom veterinärmedicin är penicillin den mest använda antibiotikan i Sverige. Det finns två beredningar av penicillin till häst i Sverige, benzylpenicillinnatrium (Na-pc) och benzylpenicillinprokain (proc-pc). Proc-pc är den vanligaste beredningen och ges intramuskulärt, vilket möjliggör för djurägare att själva administrera läkemedlet efter anvisning från veterinär. Na-pc är endast registrerat för intravenös användning till häst.

Penicillin tolereras oftast väl men det händer att hästar drabbas av allvarliga, ibland livshotande biverkningar, ett tillstånd som kallas penicillinchock. De flesta fallen tros vara orsakade av prokaintoxicitet. Det är troligt att antal biverkningar mot penicillin kan minska om proc-pc kan ersättas av ett annat preparat som inte innehåller prokain. För tillfället finns inga bra alternativ till proc-pc. Syftet med studien var att undersöka om Na-pc kan vara ett alternativ till proc-pc för intramuskulär användning till häst och att utvärdera eventuell smärta i samband med injektion. Studien var en randomiserad, blindad, cross-over-studie som utfördes på åtta friska hästar. Hästarna injicerades intramuskulärt med proc-pc en gång per dag fyra gånger och med Na-pc två gånger dagligen, sju gånger totalt. Beteendestudier och klinisk undersökning av hästarna utfördes för att utvärdera om det är någon skillnad i smärta orsakad av Na-pc och proc-pc. Beteendestudierna utfördes före och efter varje morgoninjektion, varje observationsperiod var fyra minuter lång. Den kliniska undersökningen utfördes ett dygn efter den sista injektionen. Det här arbetet är en del av en större studie.

Studien visar att hästar som injiceras med Na-pc går mer, sträcker på halsen mer och vilar halsen mindre än efter injektion av proc-pc. De skakar också mer på halsen och sparkar/stampar/skrapar med frambenen mer. Innan injektion av Na-pc är hästarna också mindre benägna att vila halsen än innan injektion av proc-pc, denna skillnad kunde dock inte ses innan den första injektionen. Det indikerar att hästarna var smärtpåverkade även 12 timmar efter föregående injektion. Den kliniska undersökningen visade att efter upprepade injektioner med Na-pc blir hästar mer blir mer svullna och palpationsömma än när de fått proc-pc.

Slutsatserna från studien är att injektioner av Na-pc orsakar mer smärta än proc-pc vid intramuskulär användning och att graden av smärta inte kan accepteras med hänsyn till djurskyddsaspekter. Anledningen till att Na-pc orsakar mer smärta kan vara att beredningen saknar prokain som är ett lokalbedövningsmedel. En annan bidragande orsak kan vara att Na-pc injicerades två gånger om dagen vilket kan orsaka mer ömhet än injektion en gång per dag. Behovet att hitta ett lämpligt alternativ till proc-pc är stort men Na-pc kan inte rekommenderas för intramuskulär användning till häst.

INTRODUCTION

In veterinary medicine, penicillin is the most used antibiotic in Sweden (SVARM, 2009). It is one of the most used antimicrobials for treating infections in horses and is effective against the most common horse pathogens. It should, when possible, be the drug of choice when treating infections, because the antimicrobial spectrum is narrow and the impact on resistance is small. Penicillin is normally well tolerated by horses, however an adverse reaction, so called penicillin shock, sometimes occurs in horses (Marshall, 1980; Allpress and Heathcote, 1986; Nielsen *et al*., 1988; Olsén *et al.*, 2007). The clinical signs associated with the adverse reactions can be severe, and death following injection has been reported.

In Sweden, two dosage forms of penicillin are available for use in horses; procaine benzylpenicillin (proc-pc) and sodium benzylpenicillin (Na-pc) (Fass® vet., 2010). Proc-pc is the most used dosage form and is administered intramuscularly, which allows for horse owners to treat the horse at home under veterinary instruction. Na-pc is only approved for intravenous use in horses and can therefore only be administered under supervision by a veterinarian, which limits the use of the drug.

Adverse reactions occur more often with proc-pc than with Na-pc and are in most cases believed to be caused by procaine toxicity (Marshall, 1980; Nielsen *et al.*, 1988; Olsén *et al.*, 2007). In Sweden, nine cases of adverse reactions to proc-pc and one to Na-pc were reported to the Medical Products Agency during 2007 and 2008 (Tjälve, 2009). Three of the horses receiving proc-pc and the one receiving Na-pc died. It is likely that in most instances adverse reactions of a more serious nature are reported and that the real incidence is higher than that suggested from the number of veterinary reports (Olsén *et al.*, 2007).

There is no available data on the amount of penicillin, or indeed the different dosage forms, used in horses. This is because the preparations used in horses are registered for other species and only data for the total amount sold is available. The incidence of adverse reaction to different dosage forms of penicillin can therefore not be calculated.

Objective

Because of the severe adverse reactions, which are mainly associated with administration of proc-pc, it is desirable to find an alternative to the drug. The only other available antimicrobial in Sweden that allows horse owners to treat a horse at home is trimethoprim-sulphonamides, for oral use (Fass® vet., 2010). Trimethoprim-sulphonamides have wider spectrum of antibacterial activity than penicillin (SVARM, 2009). The effect on the animal´s normal bacterial gut flora is greater when using an antimicrobial with wider spectrum which increases the risk of developing resistant strains of bacteria. Due to this effect, trimethoprim-sulphonamides are not favourable to use when treating infections caused by pathogens sensitive to penicillin.

At the moments there is no good alternative to proc-pc. There is a need to find a drug that can be administered by horse owners, has a narrow spectrum of antibacterial activity and causes few adverse reactions. Na-pc does not contain any procaine. It is therefore likely that any adverse reactions could be reduced if the drug may replace proc-pc for intramuscular use in horses. This dosage form is already approved for intramuscular use in cattle and swine but not in horses. A pilot study by Lindberg (2009) indicated that Na-pc can be administered in the muscle twice a day and therapeutic blood levels against the most common horse pathogens can be maintained. This study is a continuation of Lindberg's work.

The aim of this study is to evaluate if Na-pc can replace proc-pc for intramuscular use in horses and to assess pain associated with administration of the different dosage forms. Behavioural studies and clinical examination were performed to evaluate the degree of pain caused by the different dosage forms. The work presented in this paper is part of a larger study.

LITERATURE REVIEW

Na-pc is a highly water-soluble salt and the solution is approved for intravenous use in horses and for intravenous and intramuscular use in cattle and swine (Fass® vet., 2010). The half-life of Na-pc is short and it is recommended that it is administered every 6 to 12 hours (Horspool and McKellar, 1995; Fass® vet., 2010).

Proc-pc is a salt with low water solubility that is used for intramuscular injection. When injecting proc-pc into the muscle, the salt must dissolve to free procaine and benzylpenicillin before it can be absorbed into the bloodstream (Uboh *et al.,* 2000; Olsén *et al.,* 2007). The salt acts as a depot of the drug, making the absorption of free benzylpenicillin into the bloodstream slow, and a therapeutic effect can be maintained for at least 24 hours.

There is no penicillin available in Sweden for oral use in horses. The risk of developing antibiotic associated diarrhoea is high in horses treated orally with penicillin and it is not a recommended treatment regime (Horspool and McKellar, 1995). Furthermore, most penicillins are sensitive to gastric acid and the systemic availability is low after oral administration.

Adverse reactions – penicillin shock

Adverse reactions to penicillin treatment are probably caused by different mechanisms (Nielsen *et al.,* 1988; Chapman *et al.,* 1992; Olsén *et al.,* 2007). Most of the reactions are most likely due to procaine toxicity but some might have other causes. Formation of vascular emboli after unintentional intravenous injection of proc-pc, leading to pulmonary emboli and severe respiratory distress, has been suggested as a cause of penicillin shock. However, the observed clinical signs and pathological findings in horses that suffer from penicillin shock are not consistent with this theory (Nielsen *et al.,* 1988; Olsén e*t al.,* 2007).

Severe adverse reactions and even death have been reported in horses treated with water-soluble penicillins that do not contain procaine (Olsén *at al.,* 2007; Tjälve, 2009). This implies that not all adverse reactions are due to procaine toxicity. Hypersensitivity/allergy reactions to penicillin, or other compounds in the drug, can be the mechanism behind some cases of penicillin shock (Nielsen *et al.,* 1988; Olsén *et al.,* 2007). Allergies to penicillin account for many drug-induced anaphylactic reactions in humans and it is believed that penicillin can cause allergy in horses (Davis, 1984; Davis, 1987). Anaphylaxis is a type of severe allergic reaction which can be life-threatening. Some of the typical symptoms are hypotension, bronchospasm and cardiac dysrhythmia. Urticaria and pruritus have also been seen in horses treated with penicillin and are believed to be caused by an allergic reaction (Olsén *et al.,* 2007; Tjälve, 2009).

Even though allergy might be the cause of some adverse reactions seen in horses treated with penicillin, most cases are not consistent with hypersensitivity reactions (Nielsen *et al.,* 1988; Olsén e*t al.,* 2007). Several horses that have suffered from a penicillin shock have been treated again with proc-pc or a water-soluble benzylpenicillin without showing any symptoms of adverse reactions. One of the features of allergy is that the same, or sometimes worse, reaction will occur if the animal is exposed to the allergen again (Davis, 1987).

Penicillin shock can occur after the first injection given to a horse, or after a number of injections which might not have caused a reaction previously (Nielsen *et al.*, 1988). In a study by Olsén *et al.* (2007), the authors found that in 59 horses suffering from an adverse reaction to procaine, sodium, or potassium benzylpenicillin, a majority of the horses had been treated with penicillin on a prior occasion.

Clinical signs

The clinical signs of penicillin shock include behavioural and locomotoral changes, suggesting a central nervous involvement (Allpress and Heathcote, 1986; Nielsen *et al.*, 1988; Olsén e*t al.*, 2007; Tjälve, 2009). Symptoms can be of a more mild nature, like shivering and restlessness, or severe like ataxia, startled behaviour, galloping and rearing. Severely affected horses might collapse, seriously hurt themselves or die and there is also a risk that the person handling the horse might get injured. The onset of clinical symptoms is often rapid, occurring during or immediately after the injection.

Procaine toxicity

Most adverse reactions in horses treated with penicillin are likely due to procaine toxicity. Procaine is a local anaesthetic and was developed as a synthetic substitute for cocaine (Rang *et al.*, 2003). Adverse reactions to proc-pc have also been reported in humans and are believed to be caused by procaine toxicity (Björnberg and Selstam, 1960; Downham *et al.* 1978). Some of the observed symptoms are muscle tremor, hallucination and fear of death.

In studies by Tobin e*t al.* (1977) and Chapman *et al.* (1992), procaine was injected intravenously in horses. After the injections, the horses showed behavioural excitation and locomotoral changes similar to those seen in horses suffering from penicillin shock. The dose of procaine in these studies was 2.5-10 mg/kg body weight (b.w). The recommended dose of proc-pc is 20 mg/kg b.w., making the dose of procaine 8.3 mg/kg b.w. Tobin e*t al.* (1977) showed that horses are a lot more sensitive to procaine than humans and that central nervous excitation could be seen in horses with plasma levels one twentieth of that in humans with symptoms of central nervous stimulation.

It is believed that procaine toxicity occurs when proc-pc enters the circulatory system rapidly (Chapman e*t al.*, 1992; Olsén *et al.*, 2007). This could happen if proc-pc is accidently injected intravenously. The salt will then dissolve quickly into free procaine and benzylpenicillin and the amount of free procaine in the circulation will be high. Repeated injections leading to inflammation and increased vascularization might increase the uptake of procaine into the circulation and also the risk of unintentional intravascular injection.

When procaine enters the circulatory system it is hydrolysed to non-toxic metabolites by plasma esterases (Tobin *et al.*, 1976). If large amounts of procaine enters the circulatory system the hydrolysing capacity of the plasma esterases might be exceeded, which could lead to procaine toxicity. Olsén e*t al.* (2007) showed that horses that had reacted to treatment with proc-pc had lower plasma esterase activity than a control group, and suggested it to be a risk factor for penicillin shock.

Local anaesthetic activity of procaine

Procaine has a weak local anaesthetic activity with short duration (Kushembuck *et al.*, 2007). It has been used to reduce pain associated with minor surgeries, to produce nerve blockades and to mask pain in race horses (Tobin *et al.*, 1977; Kushembuck *et al.*, 2007.) Possibly, procaine decreases the pain associated with injecting large volumes of proc-pc into the muscle

of horses (Tobin *et al*., 1977, Uboh *et al*., 2000). Studies have showed that injection of proc-pc causes significantly less pain in humans than injection of benzylpenicillin without procaine (Harari *et al.,* 1988; Bycroft *et al.,* 2000). In a study by Harari *et al. (*1988), adults were injected with benzylpenicillin in one buttock and proc-pc in the other buttock. A significant majority of the people found that benzylpenicillin caused more pain than proc-pc immediately after the injection, later in the day and the day after the injection.

Pain and behaviour

When evaluating a new drug, or a new way of administering a drug, it is important to assess the potential pain associated with use of it. Furthermore, reliable ways to evaluate pain must be used. Changes in physiological and behavioural factors can be studied for this purpose and behaviour is considered one of the most useful tools to evaluate pain in animals (Hansen, 1997; Molony and Kent, 1997; Anil *et al.,* 2002).

There are several difficulties that have to be considered when studying animal behaviour as a way to assess pain. Different species, breeds and individuals react differently to pain and the manifested behaviour varies (Hansen, 1997). The expressed behaviour may also be affected by the animal's environment. Seriously injured or depressed animals might not be able to express pain. Different observers might also evaluate the same animal differently. However, even though studying behaviour is a subjective method, it is a very useful way to assess pain in animals. In general, it is not so difficult to assess if an animal is in pain but it is hard to evaluate the degree of pain (Anil *et al.*, 2002).

One way to make behaviour studies more objective and to limit bias is to use observer-based pain rating scales (Hansen, 1997). These scales are based on the absence or presence of defined pain-associated behaviours and to what degree they are expressed. Another method is to study an animal´s observable behavioural traits and the frequency of them. This method can be useful to evaluate pain in one animal at different times, for example before and after analgesia.

There are many ways an animal may react to painful stimuli (Molony and Kent, 1997; Anil *et al.*, 2002). Some of these are loss of normal behaviour, change in body posture, excessive or depressed activity, lethargy, anxiety and vocalization. In an article by Ashley *et al.* (2005), the authors comment on the fact that there is limited research on pain associated behaviour in horses compared to other species. However, the authors state that horses change their behaviour when they are in acute pain and some examples of behavioural indicators of pain are restlessness, anxiety, agitation and aggression.

MATERIAL AND METHODS

Study design

Eight healthy Standardbred trotters, seven mares and one gelding, 7-17 years old, were used in the study. The horses weighed between 471 and 581 kg. The study was performed in 2009 during one week in spring and one week in autumn and was approved by the Animal Ethics Committee, Uppsala, Sweden (C8-88). The study was a randomized, blinded cross-over study. In the spring four of the horses, which were randomly chosen from the group, were given Na-pc and the other four proc-pc. In the autumn the horses were given the other drug preparation. The time between the two periods was 19 weeks.

Na-pc (Geepenil® vet., Orion Pharma, Sollentuna Sverige, 24 g, injection concentration 300 mg/ml) was given in a dose of 15 mg/kg b.w. seven times with 12 hour intervals. Proc-pc

(Penovet® vet. Boehringer Ingelheim, Köpenhamn, Danmark, 300 mg/ml*)* was given in a dose of 21 mg/kg b.w. four times with 24 hour intervals. The horses were injected in the musculature on the neck, the side changing at every injection (figure 1). The drug administration was done in the horses´ stalls.

The horses were placed in individual stalls on straw and they had daily access to a small paddock outside. They were fed hay and oats, and had free access to water. The first feed in the morning was given after the behavioural studies were completed.

In addition to the behavioural studies and the clinical examination presented in this paper, blood samples were taken frequently and the horses were examined with pressure algometry and thermography.

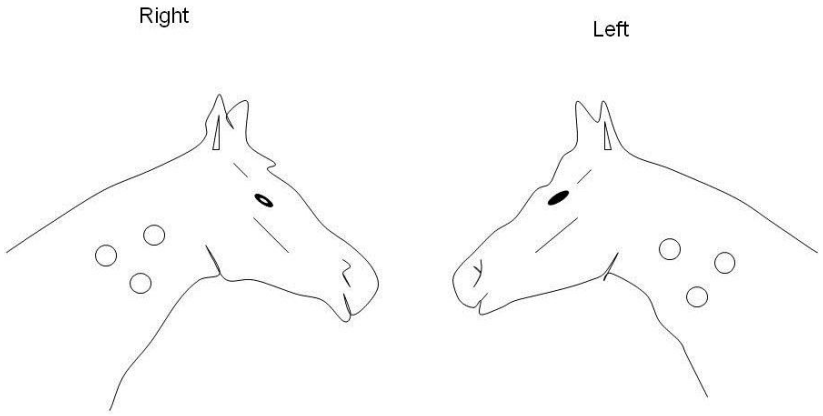

Figure 1. The horses were injected in the musculature at three different sites on the right and left side of the neck.

Behavioural studies

The behavioural studies were performed by two people who were blinded to the dosage form. Each observation period was performed by one observer who was chosen randomly. Every horse was observed from outside the stall during a four minute period before and after the morning injection. The horses that received Na-pc were also injected at night but no behavioural studies were conducted at night. The observation before the morning injection was performed within a 30 minute period before the horse was injected. When the horse was injected, the observer stood with their back to the horse so as not to see which formula the horse was injected with. As soon as the horse was injected, the person who injected the horse made a signal and the observation started. At 12 observation occasions; every 20 seconds during the four minute period, it was noted if the horse was walking or standing and if the horse was eating or made any jaw movements. The position of the horse´s neck, as well as whether it had muscle fasciculation, was also noted. A timer that beeped every 20 seconds was used for this purpose. Furthermore, discrete (frequency) behavioural traits that the horse performed were noted during the observation period. A protocol was used to record all the observations (see appendix 1).

Some comments on the protocol:

"Resting neck" was defined as normal placement of the neck in a resting position and with no movement of the neck. "Eating" was noted as an ongoing behavioural trait, however if a horse just picked some hay without continuing to eat, this was noted under discrete behaviour as "hay pick". "Chewing" was defined as biting at material in the stall.

Clinical evaluation

The clinical evaluation was performed 24 hours after the last injection. Every horse was examined by two people that were blinded to the dosage form. An area on the horse necks around the injection site was estimated and the degree of swelling and pain in this area was recorded (see figure 1). A protocol with a four grade scale (0-3) was used for this purpose (see appendix 2). Because the protocol was not strictly used and marks were made in between the grades the four grade scale was converted to a six grade scale. The scale is presented below.

The expansion of the swelling in the area on the horse neck:
0: no swelling
1: < 1/3
2: 1/3
3: > 1/3 and < 2/3
4: 2/3
5: > 2/3

The height of the highest point of the swelling:
0: 0 cm
1: < 1 cm
2: 1 cm
3: > 1 cm and < 2 cm
4: 2 cm
5: > 2 cm

The pain reaction in the horses was noted while pressing at the site of injection with a constant pressure and graded in a six grade scale:
0: No reaction
1: Slight reaction
2: Slight to moderate reaction
3: Moderate reaction
4: Moderate to strong reaction
5: Strong reaction

Statistical analysis

Behavioural studies

The results from the behavioural studies were analysed statistically with SAS software (SAS Institute version 9.2). Two versions of generalized linear mixed models were used, one for the number of times out of 12 a certain behavioural trait was observed (ongoing behaviour) and the other when the number of times during four minutes a behavioural trait was observed (frequency behaviour). The models were designed to see if there was any significant difference between dosage form, observations made before and after injection, individual horses, season and day. The significance level was set to $p < 0.05$. All observed behavioural traits were first tested with these models, before the effects that were not significant were

removed from the models, and the results were analysed again to give more exact results. Behavioural traits that were not observed to an adequate extent, or which were observed exclusively in only one or a few of the horses, were not analysed.

For the behavioural trait "resting neck" the average before the first injection was calculated in Microsoft Office Excel 2007 and Student's paired t-test was used to compare the difference before the first injection.

Ongoing behavioural traits

Four behavioural traits were analysed, being "walking", "resting neck", "stretched neck" and "jaw movements". A binomial distribution model was used as these behavioural traits were observed as the number of times out of 12. This model was used for estimating the odds *o* for observing the behavioural trait before and after injection with Na-pc and proc-pc, respectively. The odds correspond to the probability for something to happen in comparison to not happen. The model also was used to estimate the odds ratios of Na-pc versus proc-pc for injections before and after, respectively. The probability *p* for a horse to exhibit a behavioural trait was calculated according to the formula $p = o / (1+o)$. The reason for using a model with odds instead of probability was that odds range from 0 to infinity which makes it easier to take into account the effects of horses, seasonal changes, day and similar variables compared to probability that ranges from 0 to 1.

The model could not compare behavioural traits if they were not performed to an adequate extent, therefore chewing and fasciculation were not analysed. "Eating" could not be analysed since the horses that were injected with Na-pc did not eat at all after being injected. "Stretched neck" and "jaw movements" were not seen before injection during most of the observation period. As a result, a reduced model which only compared behavioural traits after injections was used for these variables.

Frequency behavioural traits

The behavioural traits "neck shake", "front leg" and "hay pick" were analysed using a Poisson distribution model, which expresses the number of events occurring in a period of time. A Poisson distribution model was used as the frequency behavioural traits could be observed unlimited times in the observation period compared to the ongoing behavioural traits that were observed a number of times out of 12. No other frequency behaviours were tested using this model since they were not observed to a sufficient extent to be analysed.

Clinical evaluation

The average and standard deviation for the swelling and pain in the different groups of horses were calculated using Microsoft Office Excel 2007. The statistical analysis was performed using Student's paired t-test. The significance level was set to $p < 0.05$. Analyses (Student´s paired t-test) were performed to see if the data differed significantly between the two persons performing the clinical evaluation.

RESULTS

One horse showed so much irritation after being injected with Na-pc that it was decided to discontinue injecting that horse after the fifth injection. The data that is available from this horse has been included in the analysis.

When all the data from the behavioural studies was collated, "walking steadily" and "walking restlessly" were put together as one behavioural trait. This was because "walking restlessly" was only noted by one of the observers and it was decided that it was too subjective to be a

stand-alone behavioural trait. Kicking, stamping and scraping with the front leg were summarized collectively as the behaviour "front leg". Standing was not analysed given that the horses were standing when they were not walking, and no horse swayed while standing.

Behavioural studies

After being injected with Na-pc the horses walked more, stretched their necks more and rested their necks less than after injection with proc-pc. The horses also shook their necks more and kicked/scraped/stamped with the front leg more.

Ongoing behavioural traits

The probability that a horse is going to exhibit a certain behavioural trait at any given moment before or after injection with Na-pc and proc-pc is presented in figure 2.

Walking

After injection with Na-pc the probability of walking is greater than after injection of proc-pc ($p = 0.0026$), 0.19 compared to 0.06. The odds that a horse will walk after injection of Na-pc are 3.5 times higher than after injection of proc-pc (odds ratio; 95% confidence interval 1.6 to 7.7).

The probability that a horse will walk before injection is the same irrespective of whether the horse is injected with Na-pc or proc-pc ($p = 0.5924$).

Neck position

Horses are more likely to stretch their necks ($p < 0.0001$) and less likely to rest their necks ($p < 0.0001$) after receiving Na-pc than after being injected with proc-pc. The odds that a horse will rest its neck after injection of proc-pc are 17.2 times higher than after injection of Na-pc (odds ratio; 95% confidence interval 7.7 to 38.4). The odds that a horse will stretch its neck after injection of Na-pc is 57.0 times higher than after being injected with proc-pc (95 % confidence interval 12.2- 266.5).

According to the results, the probability that a horse is going to rest its neck is higher before receiving proc-pc than Na-pc ($p = 0.0052$). According to a Student's paired t-test, this difference could not be seen before the first injection ($p = 0.1729$).

Jaw movements

There is no significant difference in making jaw movements between horses injected with Na-pc and horses injected with proc-pc ($p = 0.1729$).

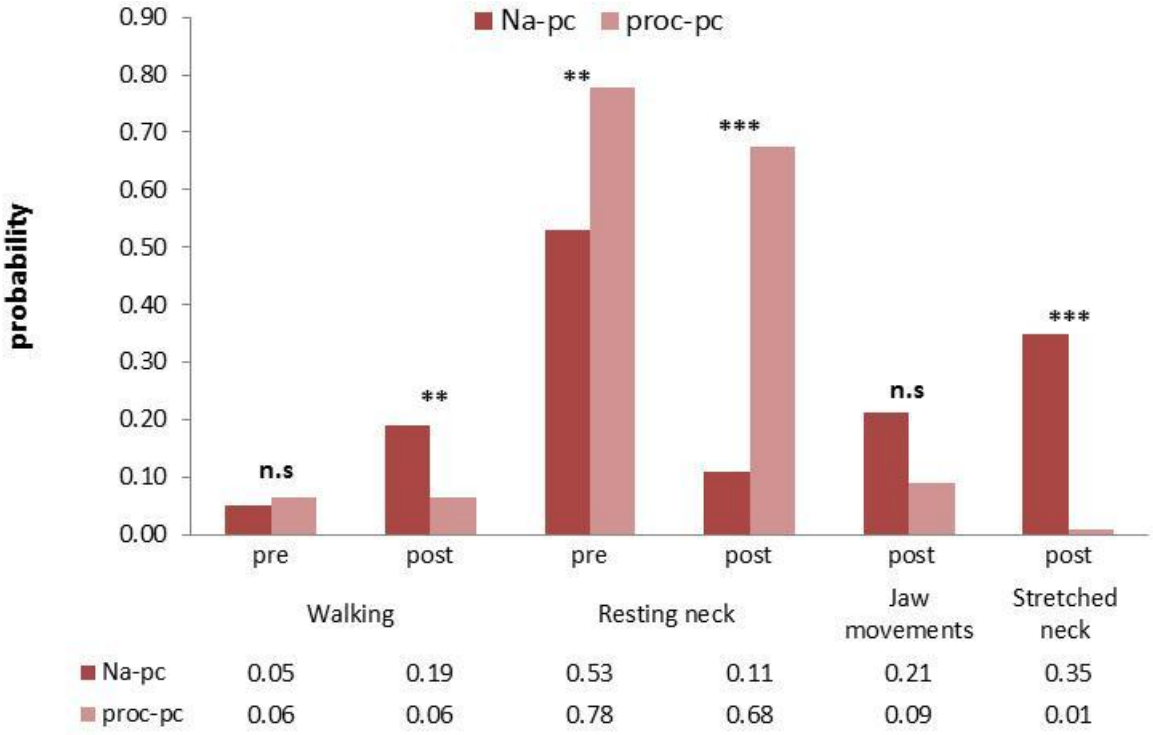

*Figure 2. The probablity (p) of walking, resting neck, stretching neck and making jaw movements in horses before (pre) and after (post) injection with Na-pc and proc-pc. For the behavioural traits walking and resting neck pre and post results are presented. For jaw movements and stretched neck only post results are presented. The level of significance is showned above the columns: n.s (not significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). After injection of Na-pc horses are more likely to walk and stretch their necks and less likely to rest their necks than after injection of proc-pc. The difference in making jaw movements after injection is not significant. Before injection with Na-pc horses are less likely to rest their necks than before injection with proc-pc. This difference could not be seen before the first injection.*

Frequency behavioural traits

The estimated number of times a horse will shake its neck, kick/stamp/scrape with its front leg and pick hay, before and after injection with Na-pc and proc-pc during a 4 minute period is presented in the figure 3.

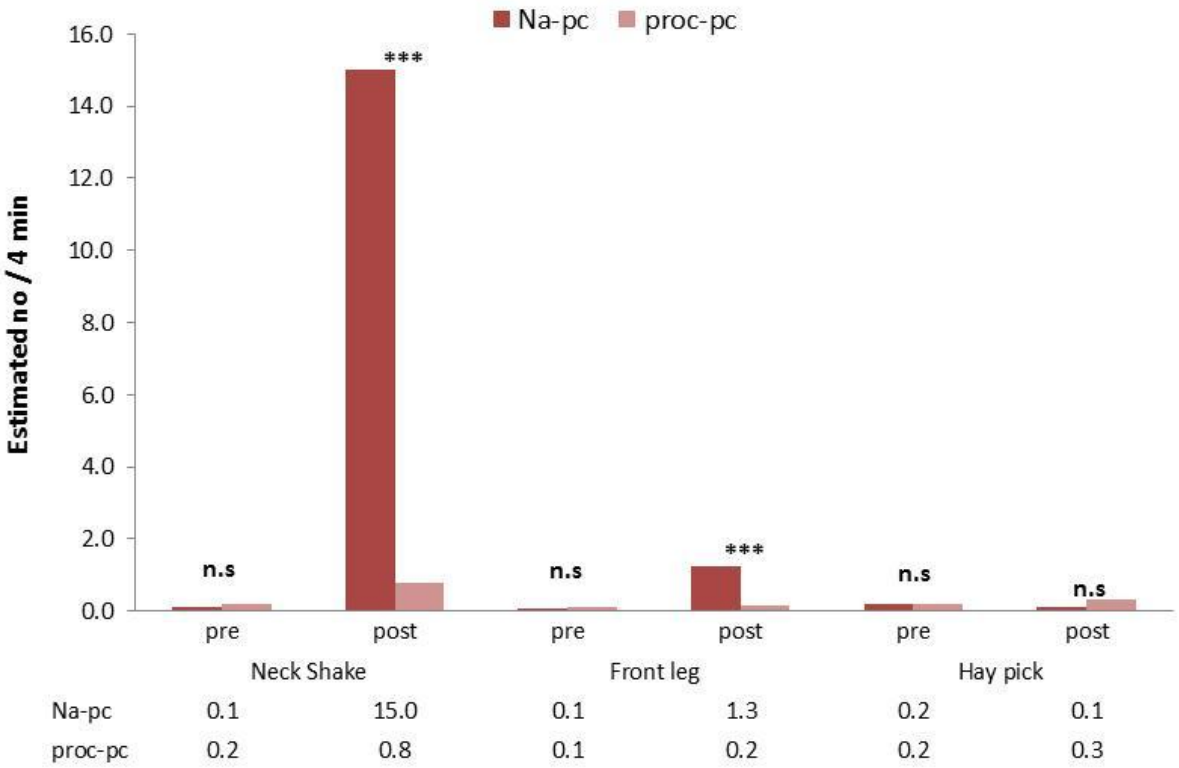

*Figure 3. The estimated number of times during 4 minutes a horse will shake its neck, kick/stamp/scrape with its front leg and pick hay, before (pre) and after (post) injection with Na-pc and proc-pc. The level of significance is shown above the columns: n.s (not significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). After injection of Na-pc horses will shake their necks and kick/stamp/scrape with their front legs more than after injection of proc-pc. The differences in the observed number of hay picks between horses injected with Na-pc and proc-pc respectively are not significant. Before injection the behaviours presented in the figure will be performed to the same extent irrespective of whether the horse will be injected with Na-pc or proc-pc.*

As indicated in figure 3, a horse will shake its neck 19.7 times more after injection of Na-pc than after being injected with proc-pc ($p < 0.0001$). Horses are also more likely to kick/stamp/scrape with their front legs ($p = 0.0001$). The difference in the observed number of hay picks between horses injected with Na-pc and proc-pc is not significant ($p = 0.1706$). Before injection horses will shake their neck, kick/stamp/scrape with their front leg and pick hay to the same extent whether they will be injected with Na-pc and proc-pc ($p = 0.3357$; 0.6268; 0.9910).

Other observations

After injection of Na-pc one horse showed adverse reaction so apparent that it was decided to stop injecting this horse. Behavioural traits observed in this horse after being injected with Na-pc included rearing, kicking, neck shaking, snorting, stretched neck and jaw movement. By comparison, after being injected with proc-pc, this same horse stood still for most of the observation period.

In one horse muscle fasciculation was observed after injection. When injected with Na-pc, fasciculation was observed 20% of the observation period compared to after injection with proc-pc, where it was only observed 2% of the time. Another horse was observed flehmening a total of 35 times after being injected with Na-pc but only twice after being injected with proc-pc. No horses were observed eating after being injected with Na-pc, whereas horses ate 8% of the time after being injected with proc-pc.

Kicking with hind leg was not seen frequently, however one horse was observed kicking a total of 16 times after being injected with Na-pc compared to no kicking after being injected with proc-pc.

Other behavioural traits that were observed (but not frequently and therefore not analysed) were biting, snorting, yawning, neighing, sighing, oestrus behaviour, shifting weight, wagging, scratching, mouth scratching, nodding, tail wagging, urinating, defecating, tail lifting, drinking, hyperventilating, stretching, stamping, rearing, attempting to lie down, hind leg lifting, changing position and pushing at the manger.

No horses showed any signs of penicillin shock (falling and rushing around). No horses were observed swaying when they walked or while standing.

Clinical evaluation

The data from did not differ between the two people performing the clinical examination (p = 0.48). The average degree of pain and swelling at the injection site differed between the horses that received Na-pc and the horses that received proc-pc. The reactions were stronger in horses that received Na-pc, as demonstrated in figure 4.

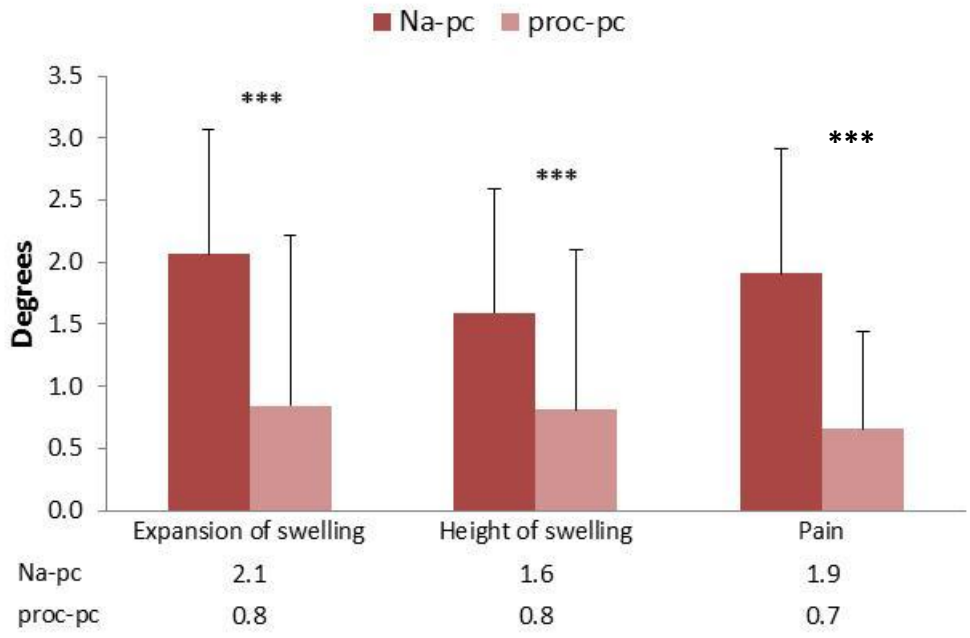

*Figure 4. The average degree and standard deviation of swelling and pain reaction on a five grade scale, 24 hours after the last injection of Na-pc and proc-pc respectively. An area around the injectiom site was marked on the horse necks and the expansion of the swelling was estimated on a scale from 0-5. 0 = no swelling, 5 = > 2/3 of the area swollen. The height of the swelling was also estimated, 0 = no swelling, 5 = > 2 cm. The pain reaction was noted while pressing at the injection site. 0 = no reaction, 5= strong reaction. Both the degree of swelling and that of pain were significantly greater after injection of Na-pc compared to that of of proc-pc. The level of significance is shown above the columns: n.s (not significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).*

As indicated in figure 4, the pain reaction was 2.9 times stronger in horses that received Na-pc than in horses that were injected with proc-pc ($p < 0.0001$).

The site of injection was more swollen in horses that received Na-pc than in horses that received proc-pc ($p < 0.0001$). Both the expansion and height of swelling were greater in horses that received Na-pc. The expansion of swelling in the horses was 2.4 times greater after injection of Na-pc than in those injected with proc-pc ($p < 0.0001$). The height of the swelling was 2.0 times greater after the injection of Na-pc than of proc-pc ($p = 0.0003$).

DISCUSSION

After injections of Na-pc, the horses showed behavioural changes indicating that they were in more pain than after injections of proc-pc. Because of the limited research on pain associated behaviour in horses, it is hard to say exactly what pain associated behaviour is. This study shows that, after injection of Na-pc, horses are more likely to walk and stretch their neck, and less likely to rest their neck than after injection of proc-pc. A horse injected with Na-pc will also shake its neck more and kick, stamp or scrape more with its front leg. These results indicate that intramuscular injection of Na-pc causes more pain than injection of proc-pc. This is supported by the observation that, after injection of Na-pc, a horse will on average shake its neck 15.0 times during a 4 minute period compared to 0.8 times after injection of proc-pc. This has to be considered a major difference. Observations of other behavioural traits that were not statistically analysed support this theory; fasciculation, kicking and flehmening were seen more after administration of Na-pc and no horses ate after injection of Na-pc. One horse showed behavioural changes indicating that it was in a so much pain after injection of Na-pc that continuing with injections could not be done because of welfare concerns.

In this study, the horses were only studied for a period of 4 minutes post injections. It would have been interesting to see how long the effects on behaviour lasted, so as to get a better idea of how long the horses were in pain. According to the results, horses are less likely to rest their neck before injection of Na-pc than of proc-pc, which indicates that horses may still be in pain 12 hours after the previous injection. This difference could not be seen before the first injection, but it should be mentioned that the statistical method of testing the significance was not the same. These results are consistent with the results in the study by Harari *et al.* (1988) where the majority of the people had pain at the injection site later in the day and 24 hours after the injection of Na-pc.

The clinical evaluation showed that horses were more swollen and showed more pain during palpation after injection of Na-pc. The estimated pain reaction however was still only classified as "slight to moderate" after injection of Na-pc, compared to "slight" after proc-pc. The time between the two periods was 19 weeks which should be sufficient time for any inflammatory reactions to heal.

To my knowledge, pain assessment has not been performed in horses after intramuscular injection of Na-pc. In this study, pain associated with the injections was assessed by the use of behavioural studies and clinical examination. Even though these methods are not perfect for evaluating pain in animals, they remain the most used methods. Noticing changes in behaviour is often the first way an owner or a veterinarian will realise that an animal is in pain. Clinical evaluation, although subjective, is a standard method veterinarians use to evaluate inflammation and pain in animals. Because of the common use of this method, it was considered to be an important evaluative tool in this project. The clinical examination was performed by myself and a veterinarian, and it is important that the person who performs a clinical evaluation has knowledge about the animal´s physiology and behaviour. A significance test showed that the evaluation between the two examiners did not differ, which implies that, even though it is a subjective method it can still be used to evaluate pain and inflammation.

The reason why intramuscular injection of Na-pc causes more pain than proc-pc can be due to its absence of procaine. The local anaesthetic effect of procaine can be assumed to be of importance to reduce pain at the injection site. Another contributing factor to Na-pc causing more pain and swelling could be that Na-pc was given twice a day, which could result in more muscle soreness. The results are similar to those seen in human studies. In the study by Harari *et al.* (1998), a significant majority of people receiving Na-pc and proc-pc found injection of Na-pc to be more painful than injection of proc-pc. It is likely that animals, like humans, would experience more pain and for a longer period of time when injected with Na-pc than with proc-pc. Na-pc is approved for intramuscular use in cattle and swine and although not investigated, it is likely that it causes pain also in these species.

A horse that has to be injected twice a day with a substance causing a lot of pain can become very difficult to handle. The horses in this study were owned by the university, and have been in similar projects before. It cannot be excluded that other horses, not so used to injections, may show a stronger reaction than the horses in this study. The opposite situation might also be possible; the horses in this study might show a stronger reaction than the average horse because with repeated injections they might become more sensitive to injection. This has to be considered less likely.

Na-pc does not seem to be a good alternative to proc-pc for intramuscular administration in horses. The only alternate drug to proc-pc that allows horse owners to continue treatment at

home is trimethoprim – sulphonamides for oral use. It is my experience that some horse owners request trimethoprim – sulphonamides instead of proc-pc because of the fear that their horse will suffer from penicillin shock. This is undesirable for several reasons. Most horse pathogens are sensitive to penicillin but resistance to trimethoprim-sulphonamides are common (SVARM, 2009). Trimethoprim – sulphonamides also have a wider spectrum of antibacterial activity which might increase the risk of the development of resistant bacterial strains. Furthermore, the risk of developing antibiotic-associated diarrhoea in horses is greater with oral administration of an antibiotic than with parenteral administration. Trimethoprim-sulphonamides cannot be considered a good alternative to proc-pc.

If the pain horses experience when given Na-pc could be reduced, this dosage form could possibly become an alternative. The additive of another local anaesthetic that does not have the same toxic effect of procaine, for example a small dose of lidocaine, may be an alternative. Amir *et al.* (1998) showed that diluting benzathine benzylpenicillin in lidocaine significantly reduces the pain of injection in children. The use of lidocaine did not affect the penicillin concentration in body fluids. This dosage form would have to be evaluated in horses before it could be considered as an alternative.

CONCLUSION

Na-pc cannot be recommended for intramuscular use in horses as an alternative to proc-pc. The dosage form causes a degree of pain that cannot be accepted due to animal welfare concerns. Furthermore, a course of treatment that consists of injecting a substance which causes a lot of pain twice a day would probably be difficult to continue. It is still of great importance to find an alternative to proc-pc in horses to reduce the risk of adverse reaction.

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APPENDIX

Appendix 1

Protocol, behavioural studies

Horse: Date: Administration nr: BEFORE/ AFTER

Person: Comments:

Ongoing behaviour		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Walking	Steadily												
	Restlessly												
Standing	Steadily												
	Swaying												
Posistion head and neck	Stretched neck (upp, down, forward)												
	Resting neck												
Jaw movements													
Eating													
Chewing													
Fasciculation													

Discrete behaviour (Behavioural traits that are noted every times they are observed)

Falling*	Rushing around*	Bite	Hay pick	Tail wag	Flehmening	Neck shake	Other

The horse shall be observed for 4 min before and immediate after injection.
Every 20 s during 4 min ongoing behaviour is registered.
* In case that the horse will suffer from pencillin shock

Appendix 2

Pc-study 2009

Evaluation of the site of injection 24 hours after last injection

Horse: Date: Time: Person:

	Left				Right			
Degree	0	1	2	3	0	1	2	3
Swelling, A								
Swelling, B								
Pain at palp.								

A: The expansion of the swelling. **0**= 0% **1** < 33% **2** < 67% **3** > 67%

B: The height of the swelling. **0**= 0 cm **1** < 1 cm **2** > 1 cm **3** > 2 cm

Pain at palpation, constant pressure

0= No reaction

1= Slight reaction

2= Moderate reaction

3= Strong reaction