COMPARISON OF ARTHROSCOPIC LAVAGE, NEEDLE LAVAGE, AND LAVAGE VOLUME ON THE RECOVERY OF COLORED MICROSPHERES FROM THE TARSOCRURAL JOINT OF THE HORSE

by

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Abstract

Comparison of arthroscopic lavage, needle lavage, and lavage volume on the recovery of colored microspheres from the tarsocrural joint of the horse

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Objectives: To quantify recovery of colored microspheres from cadaver tarsocrural joints via arthroscopic or needle lavage, and to compare recovery for 1-5L of lavage fluid.

Study design: Randomized experimental trial.

Methods: 8 adult Quarter Horse cadavers had 1.5 million 15μm, colored microspheres injected into each tarsocrural joint. Each joint was randomly assigned to receive lavage with an arthroscope and egress cannula (group A) or three (1 ingress, 2 egress) 14 gauge needles (group N) with 5L 0.9% NaCl. The egress fluid from each liter of lavage was collected separately, and the number of microspheres present in each recovered liter was determined via spectrophotometry.

Results: A significant interaction ($p<0.01$) was present between treatment group and liter. The number of microspheres recovered in the first liter of lavage fluid was significantly higher in the needle group than in the arthroscope group ($p<0.01$). For both groups the number of microspheres recovered in the first liter of lavage fluid represented a majority of the total microspheres collected, and was significantly different from the subsequent liters collected ($p<0.01$). The number of microspheres recovered did not differ between liters 2, 3, 4, and 5, within or between treatment groups.

Conclusions: In this model, tarsocrural lavage with three 14-gauge needles was more effective at removing colored microspheres from the joint than arthroscopic lavage, suggesting the number or placement of portals present may be more important than portal size and flow rate. No difference in microsphere recovery was seen with lavage volumes greater than 1L.

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Chapter 1 - Introduction

Septic arthritis is a common disease of horses, which can be life-threatening without aggressive treatment.[1,2] Treatment of horses with septic arthritis involves analgesic, anti-inflammatory, local and systemic antibiotic therapies, and thorough joint lavage. Normal nucleated cell counts in the equine joint are less than 1,000 cells/μL, but during sepsis, nucleated cell counts of 50,000-100,000 cells/μL may be reached[3]. When neutrophils degranulate, collagenases and matrix metalloproteinases are released,[4-6] which degrade the collagen framework of articular cartilage. Over time, these enzymes, bacterial toxins,[7] and synovial fluid changes[8] can permanently damage cartilage, such that lameness may be irreversible even if the septic process is halted.[9]

Thorough lavage of an infected joint is utilized to remove granulocyte enzymes, debris, and microorganisms. Lavage in horses can be performed arthroscopically,[10] through large gauge needles,[11] or via arthrotomy.[12] Arthroscopic lavage is generally advocated as being more effective than needle lavage,[9,10,13,14] however there is no data that directly compares the two methods.

Needle lavage has been shown as effective in the treatment of septic arthritis in a group of affected horses.[11] In another multi-year retrospective study of septic arthritis, most horses seen early in the study period were treated with needle lavage and those later in the study period were treated with arthroscopic lavage. No difference in survival was observed between these groups.[15] The purpose of that study was not to compare treatment methods, but the efficacy of needle lavage was supported. Joint lavage with needles requires no special equipment, and may be performed in the standing patient. This reduces treatment costs and is within the capabilities

of most equine practitioners. Arthroscopic evaluation and debridement can be necessary in chronic cases or when foreign body contamination is likely,[10,11,16,17] but needle lavage may be as effective in treating the acute stage of septic arthritis.

Lavage with fluid volumes ranging from 2L to 6L has been recommended,[2,18-21] while others simply recommend thorough lavage with large volumes.[9,16,22,23] These recommendations are based on clinical impressions and previous practice, but no data is available to determine what volume is considered adequate.

Colored polysterene microspheres have been used as markers to quantify tissue perfusion after systemic injection.[24-29] After collection, the dye in each microsphere is released using specific solvents and measured using spectrophotometry. Although not used as such previously, these 15µm microspheres approximate granulocyte size, making them a useful marker to measure efficacy of joint lavage in removing particulate matter.

The objective of the present study is to compare the efficacy of lavage of the tarsocrural joint in recently euthanized horses using two commonly used clinical methods. Group A uses an arthroscope for ingress and one dorsolateral egress cannula and Group N uses three 14-gauge needles: a dorsomedial ingress needle and two egress portals placed dorso- and plantarolateral. Additionally, the effect of increasing volumes (1L to 5L of 0.9% NaCl) will be determined. We hypothesize that removal of microspheres between the two treatment groups will be similar, and that no significant microsphere recovery will occur after 2L of lavage fluid.

Chapter 2 - Materials and Methods

Eight Quarter Horses, aged 3-21 years (mean 9.8 +/- 6.6 years), weighing 422-535kg (mean 485 +/- 43kg), being euthanized for reasons other than musculoskeletal disease, were used for the study. Horses were euthanized with an overdose of barbiturate, and all procedures were performed immediately following euthanasia. For each horse, each tarsocrural joint was randomly assigned, via coin toss, to be lavaged using three 14-gauge needles (group N) or an arthroscope and egress cannula (group A). Order of treatment was also randomly assigned via coin toss. Horses were placed in dorsal recumbency, and 1.5 million 15μm, polystyrene yellow microspheres (Dye Trak, Triton Technology Inc., San Diego, CA) were injected intra-articularly followed by 30mL 0.05% polyoxyethylenesorbitan monooleate (Tween 80, Croda International Plc, East Yorkshire, England) to disperse and prevent clumping of the microspheres. The limb was maximally flexed and extended for 5 minutes to further disperse the microspheres in the joint prior to lavage.

Joint Lavage

For group N, 14 gauge, 3.81 cm needles were placed centrally in the dorsomedial, dorsolateral, and plantarolateral pouches of the tarsocrural joint, with the dorsomedial needle acting as the ingress portal (Fig 1A). In group A, an 5.5mm diameter arthroscopic cannula and obturator (Karl Storz Veterinary Endoscopy, Goleta, CA) were placed in the dorsomedial pouch of the tarsocrural joint in a routine manner (Fig 1B),[30] and the obturator was replaced with 30° 4mm diameter arthroscope (Karl Storz Veterinary Endoscopy). A 3.2mm diameter egress cannula (Karl Storz Veterinary Endscopy) was placed in the dorsolateral pouch of the joint. All accessible portions of the tarsocrural joint were examined throughout arthroscopic lavage.[30] One-

liter bags of 0.9% NaCl were used for lavage, through a flow-controlled infusion pump. Fluid flow was maintained such that there was steady flow of fluid through egress portals without excessive joint distension or extravasation. Flow rate was recorded for each joint. Egress fluid was collected using open-mouthed glass containers held directly below each egress portal, such that egress flow was not affected. All egress fluid was recovered, and each liter of lavage fluid was collected separately. Immediately after collection, 5mL of a detergent, 10% polyoxyethylenesorbitan monooleate (Croda International Plc), was added to each liter of recovered fluid, resulting in a 0.05% solution, to prevent clumping of the recovered microspheres. As a control to quantify any loss of microspheres during processing, $1x10^5$ 15µm blue polystyrene microspheres (Triton Technologies) were added to each liter of recovered lavage fluid and measured separately from the yellow microspheres.

Sample Processing

A stir bar was placed in each collected liter of lavage fluid and the contents were pipetted into 50mL conical polypropylene centrifuge tubes. The tubes were centrifuged at 1500g for 5 minutes, and the supernatant was aspirated to a safe level above the microsphere pellet. The microsphere pellet of four 50mL tubes was combined into one 50mL tube. Each emptied tube was rinsed with 100% ethanol, which was added to the combined contents. These tubes were centrifuged at 1500g for 5 minutes, the supernatant was aspirated to safe a level above the microsphere pellet, and the contents of each 50mL tube was transferred to a 15mL conical polypropylene centrifuge tube. The empty 50mL tube was rinsed with 100% ethanol that was added to the 15mL tube. All 15mL tubes were filled to 10mL with 100% ethanol, centrifuged at 1500g for 5 minutes, and stored at 4°C until all samples were collected for final analysis.

When all samples were collected, the supernatant above the microsphere pellet in the 15mL centrifuge tubes was aspirated to a safe level and the remaining ethanol was allowed to evaporate. In more concentrated samples, a yellow tint could be seen in the supernatant. For these samples the entire 10mL of ethanol was allowed to evaporate, leaving the prematurely eluted dye in the centrifuge tube. When all samples were dry, 150µL of n,n-dimethylformamide (Sigma-Aldrich, St. Louis, MO) was added to each centrifuge tube to release the dye from the microspheres. The 15mL centrifuge tubes were vortex-mixed then centrifuged at 1500g for 5 minutes.

Sample Analysis

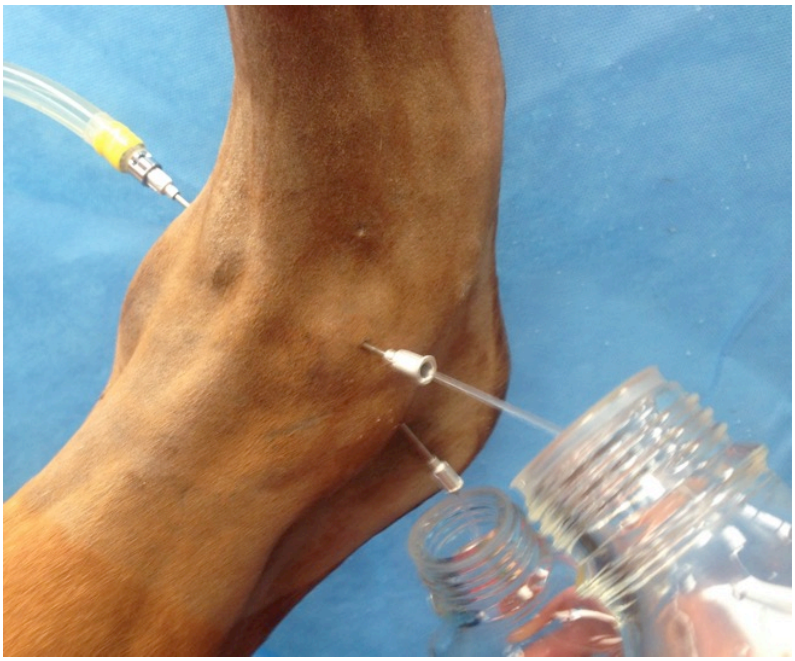
A UV-Vis spectrophotometer (SmartSpec 3000, Bio-Rad Laboratories, Inc, Hercules, CA) was used to measure peak absorbance at 448nm for the yellow microspheres and 672nm for the blue microspheres, using n,n-dimethylformamide (Sigma-Aldrich) as a blank. 100µL of solution from each sample was transferred to a microcuvette for measurement. Samples measuring over 1.3 absorbance units (AU) were diluted to remain in the linear region of the spectrophotometer. Standard curves for the yellow and blue microspheres were created. Samples below 0.07 AU were recorded as 0.07AU, as this was the lower limit of detection as determined by the standard curve. Any sample with a negative AU was recorded as 0.0 AU. Total microsphere numbers recovered per liter of collected egress fluid were calculated and totaled.

Data Analysis

Generalized linear models were used to determine potential associations between the number of yellow microspheres recovered with the treatment (N or A), the liter of fluid administered (1-5) and the potential interaction between treatment and liter number. Blue

microsphere recovery and lavage flow rate were also compared between treatment groups. All models included an effect to account for repeated measures from an individual horse. Results are reported as least square means +/- standard error. Statistical significance was set at $p<0.05$.

Figure 2.1 Group N



Portal placement and collection method for the needle lavage group. An ingress portal was placed in the dorsomedial joint pouch, and egress needles were placed in the dorsolateral and plantarolateral joint pouches.

Figure 2.2 Group A

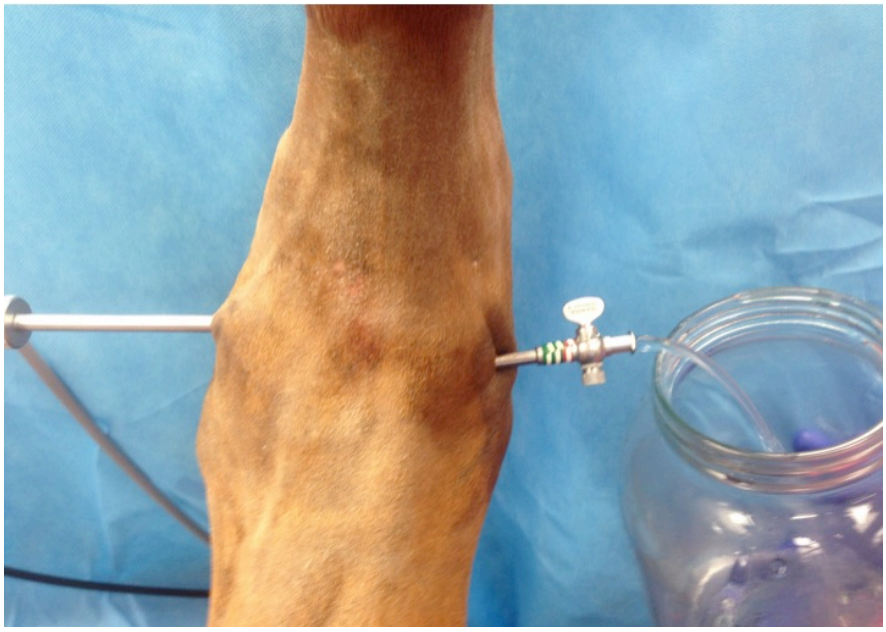


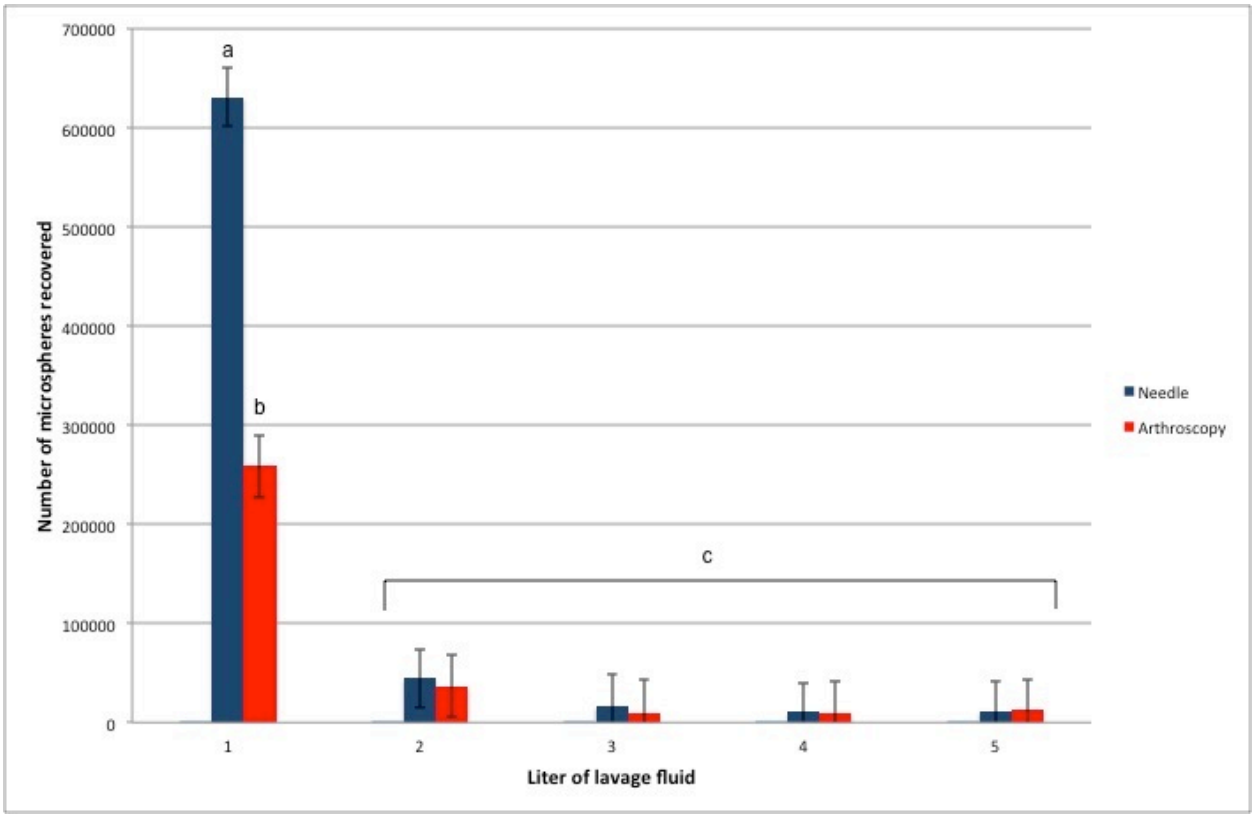
Figure 2.2: Portal placement and collection method for the arthroscopic lavage group. The arthroscope and ingress cannula were placed in the dorsomedial joint pouch, and an egress cannula was placed in the dorsolateral joint pouch.

Chapter 3 - Results

A significant interaction was detected between treatment group and liter of lavage fluid. Significantly more ($p<0.01$) yellow microspheres were recovered in the first liter from tarsocrural joints in group N than those in group A (Figure 3.1). Group N recovered 630,866 +/- 29,334 (mean +/- SE) yellow microspheres and group A recovered 258,872 +/- 31,443 (mean +/- SE). Also, significantly more microspheres were recovered in the first liter from both treatment groups than from subsequent liters ($p<0.01$). There was no significant difference in microsphere recovery within or between treatment groups for liters after the first (Figure 3.1). In the needle lavage group, 88% of the total recovered microspheres were present in the first liter, 6% in the second, and 3%, 1%, and 2% in liters 3, 4, and 5 respectively. In the arthroscopic lavage group, 79% of total microspheres recovered were in the first liter, then 11%, 3%, 3%, and 4% for liters 2, 3, 4, and 5 respectively.

Lavage flow rate was higher for Group A at 0.57L/min (+/- 0.02 SE) than Group N at 0.36L/min (+/- 0.02 SE; $p<0.01$). Of the $1x10^5$ blue microspheres added to each liter sample after egress fluid collection, a mean of 55,793 (+/- 7,912 SE) and 59,458 (+/- 7,626 SE) microspheres were measured for Group A and Group N respectively. This difference was not significant ($p=0.39$).

Figure 3.1 Yellow Microsphere Recovery



Mean (+/- SE) number of microspheres recovered per liter of lavage fluid for the needle lavage group and the arthroscopic lavage group. Significant differences ($p<0.05$) between treatment groups and liter are depicted with differing letters.

Chapter 4 - Discussion

The present study showed that needle lavage with three 14-gauge needles, with dorsal and plantar egress, was more effective at removing colored microspheres from the tarsocrural joints of horse cadavers than an arthroscope and dorsal egress cannula, and that the majority of the microspheres were removed in the first liter of lavage fluid. It has been stated that joint lavage with large gauge needles is not as effective as using arthroscopic cannulas,[13,31] as arthroscopic lavage has the advantage of larger diameter cannulas, higher flow rate, and the ability to provide targeted lavage. Experimental evaluation of various treatments for septic arthritis in the horse has been performed,[32-35] but evaluation of needle lavage and arthroscopic lavage in published data is limited to retrospective studies.[10,11,15,36,37] Reported survival is comparable in adult horses treated with needle lavage, at 81[11] and 84%[15], to those treated with arthroscopic lavage, at 86%[15] and 89%.[10] Variability within and between these studies prevents direct comparison of lavage technique, but both methods prove effective.

The objective of this study was to compare the techniques of needle and arthroscopic lavage as performed clinically by many equine surgeons. Needle lavage in the tarsocrural joint is often performed with dorsomedial and plantarolateral portals,[33,38,39] with the addition of a third needle reported in clinical cases.[11] Arthroscopy of the tarsocrural joint is commonly performed with a dorsomedial arthroscope and dorsolateral instrument and egress portal.[30] The use of every available portal has been recommended to achieve the most complete evaluation of a septic joint,[40] but description of portal placement for joint lavage in retrospective studies and review articles is not provided.[1,2,10,11,14-17,21,36,40] Dorsomedial and dorsolateral portal locations, however, have been described for lavage of tarsocrural joints during experimental treatment of

clinical and induced cases of septic arthritis,[35,41] which mimics the investigators' clinical practice.

As expected, group A was able to attain higher lavage flow rates than group N, likely due to the larger cannulas used. In this model, though, it appears the addition of a second, plantar egress portal was beneficial in removing colored microspheres from the tarsocrural joint. It cannot be determined by this study design whether it is the number of portals, placement of portals, or a combination of the two that increased microsphere removal. Further studies are necessary to evaluate the effects of portal size, number, and location on lavage efficacy.

Arthroscopy allows evaluation of articular cartilage and synovium, removal of debris, and debridement of fibrin clots and pannus, that may occlude needles in more chronic cases of joint sepsis.[10,11,16,17] These factors were not evaluated in the present study, as the investigators were solely comparing the mechanical efficacy of the lavage itself. The benefits of arthroscopic lavage in the treatment of septic arthritis are not in question, however the current study highlights the utility of lavage with large gauge needles.

Current recommendations for the treatment of septic arthritis in horses include lavage with a range of 2L to 6L of sterile fluid[2,18-21] or simply large volumes of fluid,[9,16,22,23] but evaluation of effective lavage volume has not been performed. Comparison of lavage volume was performed in humans with osteoarthritis or rheumatoid arthritis; lavage with 3-10L was more efficacious in improving pain and function than lavage with 0.25-1.0L.[42-44] No difference was detected at volumes greater than 3L. Though the disease process differs from septic arthritis, these studies demonstrate a practical limit to the benefit obtained from increasing lavage volume.

The results of the present study show that recovery of microspheres decreases markedly after 1L of lavage. Between 79-88% of recovered microspheres were present in the first liter of lavage fluid, with very little additional microspheres recovered in subsequent liters of lavage. With arthroscopic lavage, the use of multiple liters of fluid is fast and easily performed during evaluation and debridement in the joint. Lavage flow rate is lower during needle lavage, meaning each additional liter of fluid used increases time of treatment. This can be important if lavage is being performed standing, where patient compliance must be taken into account. It must be noted that in cases of joint sepsis, where inflamed synovium and fibrin can be present, increased volumes may be necessary for thorough debridement. However, for removal of microscopic particulate matter, 1L to 2L lavage is likely sufficient.

Colored microspheres have been used in many tissue perfusion studies.[24-29] The microspheres are 15μm in diameter, slightly larger than a granulocyte, and should provide a good estimation of joint lavage efficacy, acting similar to particulate matter or white blood cells. Measurement of the known quantity of blue microspheres added to the lavage fluid after recovery indicated a 40-45% loss of microspheres during laboratory processing. The reason for this is unknown. It is possible that some microspheres were lost during removal of supernatant from centrifuged tubes, although care was taken to avoid disturbing the microsphere pellets. Also, elution of dye in stored samples, as visibly occurred with high concentrations of yellow microspheres, may have occurred to some extent in all samples. While a visible color change in the ethanol used to store the microspheres prior to analysis was not seen in most samples, it is possible enough dye was lost to alter the spectrophotometer measurements. Although a large percentage of microspheres were lost through processing, the fact that this loss was not different

between treatment groups still allows comparison of lavage efficacy between arthroscopic and needle lavage.

Limitations of the study include the use of clinically normal tarsocrural joints and the unknown behavior of microspheres once injected into the joint. Horses with septic arthritis may have proliferative synovium and fibrin accumulation that will likely affect lavage efficacy. These changes may affect arthroscopic and needle lavage to different degrees.

In this study, tarsocrural joint lavage using 14-gauge needles, with two egress needles, was more effective at removing colored microspheres in a normal cadaveric joint than arthroscopic lavage with a single dorsal egress cannula. The increased number of portals or the placement of a plantar portal in Group N may be the reasons for greater microsphere removal. If so, adding portals and placing at least one plantar can be performed easily during arthroscopic lavage. Also, there was no significant change in recovery of microspheres after 1 liter of lavage in normal tarsocrural joints.

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