Clinical management of infectious arthritis in growing swine: Tools for diagnosis and treatment implementation in the field setting

Paisley Canning

Iowa State University
Clinical management of infectious arthritis in growing swine: Tools for diagnosis and treatment implementation in the field setting

by

Paisley Canning

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Veterinary Microbiology (Preventative Veterinary Medicine)

Program of Study Committee:
Alejandro Ramirez, Major Professor
Locke Karriker
Phillip Gauger
Johann Coetzee
Daniel Correia-Lima-Linhares

The student author and the program of study committee are solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2017

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ACKNOWLEDGMENTS

To Dr. Karriker and Dr. Ramirez, you are phenomenal mentors, teachers, and leaders within SMEC, ISU, and AASV. At my best and worst, you consistently supported me, pushed me to think critically, cultivated my confidence and always reminded me to be practical. It has been a privilege to work with you and I look up to you both. I am joyful and thankful that you took a chance on a wacky Canadian and brought her aboard at VDPAM. I wholeheartedly thank you for this invaluable experience and opportunity at SMEC.

To my committee members Dr. Coetzee, Dr. Linhares, and Dr. Gauger, thank you for your mentorship and support. It has been such a pleasure working with you all, I could not have asked for a better committee. I appreciate all your input and technical assistance with the projects, especially with the live animal work (opening up joints at 5 am), to the write up of the manuscripts. You have had a meaningful and positive impact on the work done here and my experience at SMEC. Thank you!

I would like to thank Kristin Skoland and Jessica Bates for their support, blood, and sweat (but no tears!) in taking this thesis from a series of protocols to fully realized projects. Without you, I would still be installing those waterers at SNF, literally and metaphorically! Thank you from the bottom of my heart for your friendship and all the good times in barns, cubicles and Dodge caravans all over the Midwest for the last 3 years.

To the SMEC staff: Justin Brown, Anna Forseth, Kris Hayman, Heather Kittrell, Mary Breuer, Paul Thomas, Josh Ellingson, and our fabulous interns (especially Chelsea Ruston, Nicole Hershberger, Victoria Thompson, and Katie O’Brien) who contributed so much to these projects and shared teaching, research, and clinical service work to allow me
time to prepare my thesis. Thank you for your commitment to SMEC and the success of the
team. It has been a blast working with you. Keep it yawl.

The projects completed in this dissertation were funded through SMEC, Pharmgate,
ECO, PIC, the Iowa Pork Producers Association, and the National Pork Board. Thank you to
these organizations. To Pharmgate and ECO, the fellowship program has been extremely
formative and I have deep gratitude for your support of this program. Thank you, especially
to Ron Kaptur and Dan Rosener from Pharmgate and the Pharmgate team for welcoming me
into their group and supporting the research process. Liz and Graham at ECO, thank you for
your support, guidance, and technical input on the projects. We also worked with several
production companies and I would like to extend a thank you to them for hosting us for these
projects.

To ISU Field Services and VDPAM admin staff, thank you! Especially to Tiffany
Magstadt and Erica Hellmich for their invaluable contributions to the work done in the
dissertation and putting up with orders for weird and hard to find cylinders, catheters, tubes,
syringes and the like. I would like to thank ISU Farms managers (Jeff, Ben, Karli, Gary, and
Trey), Animal Science faculty and LAR for bearing with my SMEC schedule and supporting
the development of my skills as a clinician, especially Trey Faaborg and the team at Swine
Nutrition Farm which is where a lot of this research was performed.

To the PhAST lab (Dr. Wulf, Dr. Rajewski, and Jackie Peterson), ISU VDL (Dr.
Gauger, Dr. Madson, Dr. Schwartz, Dr. Main, Dr. Halbur, Dr. Arruda, Dr. Clavijo, Dr. Ross,
Dr. Krull, histology section, molecular and bacteriology sections, and VDL necropsy floor
technicians: Bonnie, Jerry, and Kevin) and ISU Clinical Pathology (Dr. Austin Viall, and
Phyllis Fisher), there is simply no other veterinary diagnostic lab like what we have here are
ISU. You are in a league of your own for excellence and professionalism. I am so fortunate to have been able to rely on ISU VDL for the diagnostic testing for these projects. Access to high quality diagnostic tests, equipment and expert staff has been critical and I thank you for all your hard work in the development and execution of these projects.

Thank you to the Canadian consortium for helping me stay connected to the Ontario swine industry.

To Marisa, Josh, Pam and Ru: You give me life! Now let’s get sickening…..

For the last 3.5 years while I have been in Iowa, it has been tough to be so far from family. But during this process, my family and I have discovered the incredible charm of the Midwest. It has been a great experience. To my mom, dad, Bobbie, Judy, Unkie, Aunt Marion, Grandpa, Cait, Jackie, Richelle, Kim, Kristin, thank you for your unwavering support and encouragement on this journey. You are everything. I love you all so much. See you soon!
ABSTRACT

Infectious arthritis in growing pigs is considered the most common type of lameness encountered by veterinarians in the field. Efforts to mitigate, treat, and prevent infectious lameness by veterinarians have been problematic as the efficacy of antimicrobials for infectious arthritis is highly variable and diagnostic investigations often fail to yield actionable information about a field case. The goals of this dissertation were to address specific questions within this broad problem from an applied clinical research perspective.

The first research aim was to determine the most common primary diagnoses for lameness cases at a Midwest diagnostic laboratory and to collect descriptive data on infectious arthritis cases. From the retrospective review of lameness cases involving joints and legs at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL), it was reinforced that infectious arthritis was indeed a common diagnosis (about 40% of all lameness diagnostic lab cases) and highlighted that about 20% of lameness cases yielded inconclusive findings. These results directed the research towards the development of a refined joint fluid collection technique and a new diagnostic test to improve the diagnostic process for practitioners. Specifically, various injectable anesthetic protocols were compared in terms of utility for joint fluid collection and samples collected from that study were used to create reference intervals for fluid analysis and cytologic evaluation for swine joint fluid. These reference intervals serve as a core diagnostic tests for arthropathies in other species but did not exist publically previously for swine. Telazol, ketamine, and xylazine (TKX) were the most effective anesthetic combination for joint fluid sample collection and this study yielded sufficient number of high quality samples to create the reference intervals (37 tarsus and 46 carpus samples). With these newly refined tools to diagnosis infectious arthritis, the
final component of the dissertation was to address treatment considerations and determine if a water soluble macrolide (tylvalosin [TVN]) distributes and maintains concentrations in the joint fluid of healthy pigs. Tylvalosin was identified in joint fluid after oral gavage and during oral medication through ad libitum water access. Substantial variation in water disappearance between individual pigs highlighted large dose ranges between pigs. This was an unexpected finding and emphasized some of the challenges of treating groups of sick pigs with water soluble antimicrobials. Together, the studies in this dissertation contribute practical information for swine veterinarians related to the diagnosis and treatment of infectious arthritis in the field.
CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

In recent years, due to the increased focus on animal welfare within the swine industry and the expansion of molecular diagnostic tools, lameness in growing pigs has become a key swine health topic for veterinarians and caretakers. The most common types of lameness in growing pigs are arthropathies, a term which refers to abnormalities related to the joint(s). Within this umbrella term, infectious arthritis associated with bacterial pathogens garners the most focus from veterinarians as it is a common diagnostic finding and water-soluble macrolides are available for treatment.

Despite the availability of diagnostic tests and antimicrobials, veterinarians report that infectious arthritis (IA) and joint-related lameness remain complex health challenges within their clinical practice. More specifically, lameness diagnostic investigations often fail to provide key information needed for clinical management decisions. Additionally, treatment in suspected or confirmed infectious arthritis cases is regularly reported to be unsuccessful. Clearly there are knowledge gaps in our understanding of the diagnosis and treatment of infectious lameness in growing pigs. The body of literature on infectious lameness in swine is relatively small and the scope of the topic is extensive.

The general approach of this dissertation to this broad problem is as follows: a) focus on applied research aims that can impact the clinical management of cases directly; b) review and understand the key etiologies of lameness broadly from diagnostic submissions to a Midwest veterinary diagnostic laboratory; c) develop and refine a new diagnostic assay applicable to common lameness etiologies to supplement and compliment current lameness diagnostic tools; d) review and, if needed, generate basic
pharmacokinetic data for water-soluble macrolide(s) to facilitate treatment decisions and provide more understanding of potential causes of treatment inefficacy in clinical cases. These objectives serve the long-term goal of this dissertation, which is to improve clinical management of infectious lameness cases in the field.

Statement of specific research aims

The specific research aims are: 1) perform a retrospective review of lameness cases associated with joints and legs from the Iowa State University (ISU) Veterinary Diagnostic laboratory (VDL); 2) develop and refine an injectable anesthetic protocol for the collection of antemortem joint fluid samples; 3) create reference intervals for fluid analysis and cytologic evaluation variables for swine joint fluid collected from healthy animals; and 4) determine synovial fluid concentrations of a water-soluble macrolide after gavage and ad libitum oral dosing.

Organization of the dissertation

This dissertation is in the journal print manuscript format. The tables, figures, and references for each chapter are contained within that chapter. Each chapter is a manuscript formatted for a specific target journal. As per formatting guidelines from the ISU Graduate College, the stage of publication and author contributions are listed for each chapter as applicable.

Literature review

Arthritis refers to inflammation of the joint, including its associated synovial fluid and tissue. In IA cases in swine, the appendicular joints are prioritized for diagnostic sampling and as targets of antimicrobial treatment. The appendicular joints are key anatomical regions for IA and understanding their unique structure and physiology is
critical to the development and execution of the specific research aims listed above. This literature review will discuss joint structure and physiology as it is related to the pathogenesis of IA, the development of diagnostic tools for IA, and macrolide pharmacology. There are limited peer reviewed resources available on the swine joint and extrapolations from literature from humans and other species will be included and identified in this review, as applicable.

**Physiological structure of the joint**

The joint is considered a sequestered site within the body due to the tight regulation of compounds, potential pathogens and cells into and out of the joint space. There are two main structures that confer this unique characteristic of articular spaces. First is the synovium tissue lining the joint capsule that offers active and passive mechanisms to restrict entry of foreign molecules and pathogens into the joint. Next is the joint fluid, composed of hyaluronic acid (HA), which is important for nourishment, regulation of inflammation and removal of foreign compounds within the joint.

**Synovium**

The fibrous joint capsule is lined by the synovium which is composed of synoviocytes cells, adipose, and fibrous cells.\(^1\)\(^,\)\(^2\) The synovium is highly vascularized and innervated.\(^1\) For molecules, cells, and bacteria to reach the synovial fluid they must pass through the synovium. This is a two-part process requiring movement out of the synovial capillaries and then diffusion through a dense network of extracellular matrix and synoviocytes.\(^3\) The synoviocytes form several layers between the capillaries and the joint space and are immersed in a dense collagen matrix.\(^3\)\(^,\)\(^4\) Within these layers, synoviocytes are divided generally into two types of cells. There are highly active phagocytic cells
with processes interdigitating between the cells (synovial type A cells) and there are paracrine cells which secrete hyaluronic acid (synovial type B cells). There is no basement membrane between synoviocytes and synovial capillaries and this allows small molecules (<10kda) to pass from the synovial capillaries through fenestrations and then diffuse through the synovium to reach the joint space. This is essentially an ultra-filtrate of the plasma. Larger molecules have reduced endothelial permeability and diffuse at a reduced rate through the synovium.

Despite the limited movement of cells, molecules, and bacteria through this structure, *Mycoplasma* spp. and the infiltration of inflammatory cells have been identified using histology and immunofluorescence in synovium membranes in experimentally infected pigs. The exact mechanism for transport into the synovium and into the synovial fluid is unclear for *Mycoplasma* spp. but may be related to expression of endothelial cell ligand receptors or inflammation-associated fenestrations in the capillary synovium complex allowing migration of *Mycoplasma* spp. out of the capillary. The entry of bacterial pathogens into the joint from the blood stream requires movement through the synovium, thus it is an important physical and immunologically active barrier to joint infections.

**Role of Synovium in Inflammation**

When infectious arthritis is diagnosed in a joint, gross and histologic indications of inflammation are commonly detected. The synovium, when inflamed, may appear edematous, hyperemic, and/or proliferative. A fibrinous membrane and yellow, red or brown discoloration may also be noted visually when assessing inflamed synovium. Increased capillary permeability, translocation of leukocytes into the synovium, and
increased activity of synoviocytes are all documented changes associated with inflammation of the joint. Histologically, the tissue changes with synovitis are generally non-specific and are dynamic during the progression from acute infection to resolution or chronicity. Initially, edema and infiltration of various inflammatory cell types, typically lymphoplasmaicytic or mixed cell infiltration, are observed microscopically. Synovial cell hyperplasia, lymph and capillary dilation, and hypertrophied synovial villi may also be noted. In more chronic cases of synovitis, fibrosis, synovial villi hypertrophy, and mild lymphocyte infiltrates are noted. Histological and gross changes to the synovium have been characterized for various arthropathies in animals and humans for osteoarthritis, osteochondrosis, and Mycoplasma spp. associated arthritis.

Much of the inflammatory changes noted in infectious arthritis cases are mediated by the type A synovial cells which are key phagocytic cells within the region. Synovitis results in the release of inflammatory mediators and catabolic enzymes into the synovial fluid which negatively affects the synthesis of articular cartilage matrix components. The synovium also contains mechanoreceptors, nociceptors, toll-like receptors, and receptors for interleukins, growth factors, and chemokines. The complex microenvironment of the synovium is critical for host defense and initiation or suppression of the immune response within the joint.

There are no pathognomonic synovial lesions associated with IA in pigs but synovial histology is a useful tool to provide context to synovial fluid clinical pathology, polymerase chain reaction (PCR), and culture results. Thus, gross and histological evaluation of the synovium will be a key assessment tool for IA in the retrospective
diagnostic lab case review (specific research aim 1, Chapter 2) and in the creation of reference intervals for clinical pathology variables for healthy swine joint fluid (specific research aim 3, Chapter 4).

**Synovial Fluid**

Synovial fluid is composed of hyaluronic acid secreted by type B synoviocytes. The fluid serves multiple functions in the joint including lubrication, delivery of nutrition to cartilage, removal of waste products, nociception, and regulation of inflammation.\(^1,2\)

The fluid is fairly permeable to small molecules such as amino acids and glucose but it is anionic and traps large molecules (>67 kDa) and cells, including leukocytes.\(^6\) The trapping of leukocytes and regulation of inflammatory cascades is crucial to joint health as inflammatory mediators including cytokines, complement, antibodies and digestive enzymes, damage the cartilage and synovium. If cells, pathogens and foreign molecules do reach the synovial fluid, they can be promptly removed from the joint space by several mechanisms. Molecules are drained out of the joint space by venules and lymphatics thereby making it difficult to achieve and sustain concentrations of exogenous compounds, such as antimicrobials, in the joint.\(^6\)

Synovial fluid is also critical to the pain signaling pathway. The elastoviscostic properties of hyaluronic acid in normal joint fluid adequately transfer force associated with weight bearing and movement to joint tissues without causing physical or mechanical stress to the joint. In joints affected by inflammation, joint fluid viscosity is reduced and HA is degraded from its linear polymer form by catabolic enzymes produced from inflammatory cascades in the synovium.\(^2,6,7,18\) As the synovium capillaries become expanded and leaky due to inflammation, additional macromolecules and cells enter the
synovial fluid changing its pH and volume and, ultimately, the protective biochemical properties of the synovial fluid.\textsuperscript{2,6,7} As a result, there is more transfer of force to the joint tissue. Increased load bearing and fluid volume within the joint sensitize and trigger nociceptive and mechanoreceptors resulting in pain. As well, cartilage integrity suffers with reduced nutrition from the synovial fluid and direct interaction with degradation enzymes associated with inflammation.\textsuperscript{19} HA polymer fragments themselves can directly trigger pro inflammatory cascades and sensitize nociceptors in nerve end terminals in the joint.\textsuperscript{19}

Synovial fluid has also been described as a critical biomarker for joint disease in other species such as cats, dogs, and horses because it is so responsive to changes in joint homestasis.\textsuperscript{20-27} In horses, fluid analysis and cytologic evaluation of synovial fluid is described as the most important diagnostic tool for diagnosis of infectious arthritis.\textsuperscript{27} Based on its use as a diagnostic tool in other species, it appears there are opportunities to improve and expand diagnostic tools for swine arthritis using joint fluid and clinical pathology. Unlike horses and humans, ante mortem joint fluid collection is not a routine swine clinical technique and reference intervals for clinical pathology variables for joint fluid are not available for swine. Thus Chapter 3 (specific research aim 2) describes the refinement of anesthetic protocols for the collection of ante mortem joint fluid samples. Additionally, Chapter 4 (specific research aim 3) details the creation of reference intervals for fluid analysis and cytologic evaluation for joint fluid samples collected from healthy swine.
Treatment of Arthritis: Considerations for Antimicrobial Pharmacology

General Properties of Macrolides

Macrolides are commonly used to treat infectious arthritis in swine due to their pharmacologic properties and practical considerations related to the availability of Federal Drug Administration (FDA) approved formulations for swine. This drug class is composed of weak bases and is considered bacteriostatic due to their ability to inhibit protein synthesis by interfering the 50s ribosome. They have little to no efficacy for anaerobic or aerobic gram-negative bacteria but are useful for gram-positive, *Mycoplasma* spp., *Erysipelothrix* spp., and *Bordatella* spp. infections. Macrolides generally have a large volume of distribution ($V_d$) resulting in high levels of tissue concentrations, particularly in the lungs. Despite the high volume of distribution, there is not a macrolide-specific transport receptor into the joint space through the synovium. Macrolides must use passive diffusion to leave the capillaries and enter the synovial tissue.\(^6\) This drug class is also susceptible to ion trapping as they are weak bases and become trapped in cells that are more acidic than plasma. This characteristic is pronounced in the lysosome and phagolysomes of neutrophils and macrophages. As these cells which contain “trapped” macrolides are inflammatory cells and the organelles are used for digesting phagocytosed pathogens, this intracellular trapping phenomenon could be a critical component to treatment success in inflamed tissues.\(^6,28\)

Conversely, inflammation reduces pH within the joint space, decreases synovial fluid viscosity and increases blood flow to and from the inflamed area. These factors can reduce macrolide activity and expedite the passive removal of the macrolide (and inflammatory cells) from the joint space via lymphatics and small venules.\(^6,28\) Thus
macrolides possess some characteristics that may be both detrimental and beneficial to their efficacy in inflammatory environments, such as an arthritic joint.

Oral bioavailability of macrolides is generally low depending on the formulation and elimination is largely through hepatic metabolism.\textsuperscript{28} Spectrum of activity, half-life, and volume of distribution do vary between specific macrolides and the selection of a specific macrolide over another for use in a clinical case may be critical for success. For example, tilmicosin and tulathromycin have much larger $V_d$ and longer half-lives than tylosin and tylvalosin, but have been reported to have a reduced spectrum against \textit{Mycoplasma} spp. compared to tylosin.\textsuperscript{28}

Specific Macrolides for the Treatment of Infectious Arthritis

Amongst available macrolides licensed by the FDA for swine, tylosin and lincomycin (a lincosamide, but behaves similarly to macrolides) are commonly used to treat IA and are available in water-soluble, injectable, and feed formulations. There are several other macrolides available for swine but only lincomycin and tylosin in injectable formulations have indications related to IA. Tilmicosin and tulathromycin are available in injectable form but are not labeled for the treatment of arthritis or lameness. There are no feed grade macrolides with label indications for lameness or arthritis and extra label use of feed medication is illegal in the United States (US). In 2012, tylvalosin entered the US market as a water-soluble macrolide with zero day withdrawal indicated for proliferative enteropathy associated with \textit{Lawsonia intracellularis}.\textsuperscript{29} Due to the practical advantages of water-soluble products and tylvalosin’s pharmacological attributes as macrolide, it has been used in the field by practitioners for the treatment of arthritis, particularly for cases associated with \textit{Mycoplasma} spp.
Regardless of the antimicrobial selected for treatment, there is limited pharmacokinetic or clinical trial data on the treatment of IA available on Google Scholar, PubMed, and the American Association of Swine Veterinarians (AASV) Swine Information Library. There is one conference proceeding detailing the levels of lincomycin detected in the joint after administration in the feed.\textsuperscript{30} Concentrations of lincomycin in the carpal joint fluid (0.10 µg/mL) were the same for two doses of lincomycin administered in the feed (110 mg/kg and 220 mg/kg).\textsuperscript{30} Beyond this proceeding, there are no other publicly available sources of swine joint pharmacological data for macrolides. This knowledge gap is to the detriment of veterinarians seeking to apply evidence based medicine to their management of IA. Clinical trials and field trials assessing efficacy of specific macrolide products for specific clinical syndromes would be ideal. In the absence of that data, basic pharmacokinetic data such as half-life, volume of distribution, and concentration attained in target tissues for selected pathogens could help inform treatment decisions.

Due to recent practitioner interest in tylvalosin for treatment for arthritis and the relative lack of pharmacologic data on all swine macrolides, a core aim of this dissertation is to determine if tylvalosin distributes to the joint and if so, to further characterize concentrations attained in the joint over the course of administration through the water. Chapters 5 and 6 (specific research aim 4) focus on generating much needed pharmacologic information on a tylvalosin, a macrolide used currently in the field for the treatment of infectious arthritis.
References


CHAPTER 2. RETROSPECTIVE REVIEW OF LAMENESS CASES
ASSOCIATED WITH JOINTS AND LEGS SUBMITTED TO A VETERINARY
DIAGNOSTIC LABORATORY


P. Canning, DVM; Primary researcher, created protocol, completed data collection,
analysis and preparation of the manuscript

N. Hershberger, DVM; Assisted with data collection

E. Mahan-Riggs, DVM; Assisted with data collection

K. Schwartz, DVM, MS; co-advisor on case review methods, data analysis and reviewer
of manuscript

B. Crim; technical support and performed database search for case database

K. Skoland; co-advisor on case review methods, data analysis and reviewer of manuscript

A. Ramirez, DVM, MPH, PhD, Diplomate ACVPM; co-advisor on case review methods,
data analysis and reviewer of manuscript

D. Linhares, DVM, MBA, PhD; co-advisor on case review methods, data analysis and
reviewer of manuscript

P. Gauger, DVM, PhD; co-advisor on case review methods, data analysis and reviewer of
manuscript

L. Karriker, DVM, MS, Diplomate ACVPM; co-advisor on case review methods, data
analysis and reviewer of manuscript

PC, LK, KS, AR, EMR, NH: Swine Medicine Education Center, Iowa State University
College of Veterinary Medicine, Ames, Iowa.

KS, PG: Iowa State University, Veterinary Diagnostic Laboratory, Ames, Iowa.
Summary

Objectives

The objective of this review was to categorize and quantify the most common causes of joint- or leg-associated lameness by summarizing available information from cases presented to the Iowa State University (ISU) Veterinary Diagnostic Laboratory (VDL) between 2010 and 2015.

Materials and Methods

All cases of lameness or locomotor dysfunction in pigs between 7- and 40-weeks-of-age submitted to the ISU VDL between May 1st 2010 and April 30th 2015 were retrieved. After reviewing the cases to meet inclusion criteria such as field cases involving joints and legs, inclusion of tissue samples for histology and age and/or weight criteria, the cases were individually reviewed and assigned a primary and secondary diagnosis. The criteria for the diagnostic categories assigned to the cases are reported in the paper.

Results

There were 464 of 1847 cases retrieve that met the inclusion criteria. The most common primary diagnosis was represented almost equally across four diagnosis categories, each accounting for about 20% of the cases. These four primary diagnoses are MHS, metabolic bone disease, infectious arthritis due to bacterial infection, and lameness with inconclusive findings. There were 23% (108/464) of the cases that had a
secondary diagnosis with metabolic bone disease (29%; 31/108) identified as the most common.

Implications

Accurate diagnosis is key for selection of effective interventions and setting expectations for likelihood of treatment success. This study reinforces the importance of careful clinical examination, proper sampling, and confirming cause(s) with appropriate diagnostic testing for accurate diagnosis of lameness.

Introduction

Joint- and leg-associated lameness in growing pigs is a common diagnostic and therapeutic challenge for swine veterinarians. A diagnostic investigation usually starts when the caretaker identifies lameness in a group of pigs and notifies a veterinarian for an assessment which may involve submission of samples to a veterinary diagnostic laboratory (VDL). In recent years, there has also been a great interest in *Mycoplasma hyosynoviae* (MHS) which is considered one of the major primary causes of joint-associated arthritis, but the organism may only be present transiently within the joint making diagnosis challenging. Inconclusive diagnostic testing increases uncertainty of diagnosis and decreases confidence in specific recommendations for therapy. Lameness submissions that do not generate actionable information or support a specific etiology for the lameness are reported by practitioners, but the frequency of these cases from veterinary diagnostic laboratory submission databases has not been quantified and reported in the peer reviewed literature.

Prudent use with increasing regulation and oversight of antimicrobials in swine herds reinforces the value of an accurate diagnosis to support treatment decisions and to
investigate non-response to treatment. Diagnostic laboratory data is not equivalent to field prevalence; however, it is helpful for swine practitioners to be aware of the spectrum and relative frequency of lameness causes reported by a Midwest US VDL. Insight into capabilities and expectations of laboratory testing can assist veterinarians in proper sampling, test selection, interpretation of results as well as provide insights into the relative importance of specific lameness etiologies for future research priorities. Specifically, insights from a review of submission trends and diagnostic approaches for cases of acute MHS synovitis may inform improvements in sampling and testing for this common etiology of growing pig lameness. Beyond general recommendations in swine texts there is limited published information on submission practices for joint-associated lameness cases.²

The objective of this review was to categorize and quantify the most common causes of joint- or leg- associated lameness by summarizing available information from cases presented to the Iowa State University (ISU) VDL between 2010 and 2015. The second objective of this study was to summarize submission trends and features of those cases with a diagnosis of MHS.

**Methods**

All cases of lameness or locomotor dysfunction in pigs between 7- and 40-weeks-of-age submitted to the ISU VDL between May 1st 2010 and April 30th 2015 were retrieved for review using the ISU VDL laboratory information management system (LIMS). Each individual laboratory accession was considered a single case irrespective of number of samples submitted. The three specific search criteria, selected with aid of VDL diagnosticians and information technology specialists, were: porcine cases, samples
included tissues examined by histopathology and at least one diagnostic code was assigned by a diagnostic pathologist; the 23 diagnostic codes available for lameness include those specific for MHS and *Mycoplasma hyorhinis* (MHR) PCR and/or a *Mycoplasma spp.* culture.

For each qualifying accession (case), the submission form and laboratory report were reviewed and relevant information extracted into a spreadsheet. The information extracted from the LIMS included accession number, date, age, age unit, diagnostic code, diagnostician, histopathology observations and all tests performed with results. The clinic and bill party information was used to remove those cases that were not diagnostic investigations of field cases, such as research and teaching accounts. Client name, submitting veterinarian, premise ID were not extracted from the database to maintain confidentiality and anonymity. Permission was granted from the ISU VDL for this study.

All cases were individually reviewed to ensure each met the inclusion criteria of age/weight (7 to 40 weeks or >16 kg), histopathology performed, and case type (must be a field case – research cases excluded). If the age or weight was not present in the data output from LIMS, the case remained in the database and the original submission sheet was cross referenced for any submitting information that referenced age or weight. If this age/weight data was not included on the submission sheet, the case was removed. Cases must have included joint tissue for histology to be included in this database; cases only involving serum, oral fluids and/or swabs were excluded.

To confirm that all qualifying cases did involve lameness and locomotion dysfunction, additional information from the submission sheet and final report was entered into the spreadsheet. Specifically, the history, submission notes on the submission
sheet, and the final diagnosis and comments from the diagnostician on the final report were entered and evaluated. Specifically, the case had to include terms involving lameness and/or locomotion in the history and that the diagnostic testing completed had to be relevant to locomotion dysfunction, lameness or joint disease. For example, a case may have M. hyorhinis septicemia as a diagnostic code but if the history did not report any information related to lameness and legs or joints were not submitted with the case, then the case was excluded.

After confirming that all cases remaining in the database involved locomotion dysfunction and meet the other inclusion criteria mentioned above, the cases were individually reviewed and assigned a primary and secondary diagnosis. Specific criteria for each diagnosis was created and applied uniformly to the cases (Table 1). Unless specified on Table 1 as “if available,” all criteria listed for a given category must have been satisfied for a given diagnosis to be assigned to a case. Criteria for each diagnosis were determined by peer reviewed literature and with consultation with a diagnostician at the VDL. Secondary diagnosis refers to a diagnostic category that was relevant to the case but was not the main or most acute cause of lameness. This determination was made from comments in the final report by the diagnostician, severity and prevalence of the abnormalities and understanding of the pathophysiology of the given diagnostic category in question.

For the assignment of primary and secondary diagnostic category, the unit of assessment was the entire submission, not each individual pig within a case.
Submitter trend analysis for *Mycoplasma hyosynoviae* cases

Cases that were assigned MHS as the primary diagnosis were then reviewed further to summarize case attributes related to submission habits. Specifically, the following data points were collected and summarized: year, inclusion of a history on submission sheet (Yes/No), inclusion of differential diagnosis in history (Yes/No), number of differential diagnosis included in history, type and number of specimens submitted, number of MHS PCRs performed per case, number of animals from which the submitted specimens were procured, number of diagnostic tests requested, types of diagnostic test requested, results from non-MHS related tests performed, and secondary diagnosis.

Results

Primary and secondary diagnosis for all lameness cases

The results of each step of the case database creation process are presented in Figure 1. In Table 2, the primary and secondary diagnosis associated with each of the 464 lameness cases is summarized. There were 93 (20%) cases assigned MHS as the primary diagnosis as per the case definition criteria in Table 1. The most common primary diagnosis was represented almost equally across four diagnosis categories, each accounting for about 20% of the cases. These four primary diagnoses are MHS, metabolic bone disease, infectious arthritis due to bacterial infection, and lameness with inconclusive findings. There were 23% (108/464) of the cases that had a secondary diagnosis with metabolic bone disease (29%; 31/108) identified as the most common.

Trend analysis for *Mycoplasma hyosynoviae* cases
The number of MHS cases per full calendar year fluctuated between 7 (2011) and 34 (2013). A review of MHS case characteristics determined that the mean age of pigs diagnosed with MHS was 18.3 weeks (range 10 to 32 weeks). On average, MHS cases conducted 2.4 MHS PCRs per case (range 1 to 16). The cycle threshold values for MHS PCR were generally between 30 to 35 (range 20.3 – 42.9) and for cases requesting three or more MHS PCR tests, on average, 50% of the MHS PCRs performed on a case were positive.

An analysis of submission trends revealed that 99% (92/93) of cases included at least one word in the history on the submission form. Seventy-one percent (66/93) of cases listed differential diagnoses with their submission form and of these cases, 80% (53/66) listed multiple possible differential diagnosis. Nine cases (10%) listed MHS as the sole differential. For cases that listed more than one differential, the most common differentials were MHS (62%; 38/53), MHR (42%; 22/53), *Haemophilus parasuis* (42%; 22/53), *Streptococcus suis* (40%; 21/53), and *Erysipelothrix* spp. (36%; 19/53). Nutritional/metabolic, OCD, and trauma were listed 19 (36%; 19/53) times cumulatively.

The majority of submitting veterinarians selected diagnostic testing to be at the discretion of the VDL diagnostician (69%; 64/93) while 15% (14/93) left the test selection portion of the diagnostic form completely blank. Another 15% (15/93) of submitting veterinarians selected at least four unique diagnostic tests for their case.

Sample types submitted included 43% (40/93) of cases with at least one whole leg, 27% (25/93) at least one whole pig, and 25% (23/93) at least one joint swab or fluid. On average 3.7 (range 1 to 14) legs were submitted per case where 90% (36/40) submitted at least two and 45% (18/40) submitted 3 to 8 legs. On average, 4.4 (range: 1 –
25) whole pigs were submitted where 36% (9/25) submitted two whole pigs, 28% (7/25) submitted 3 to 5 pigs, and 24% (7/25) submitted six or more pigs. Considering all sample types, submissions contained samples from 2.9 animals (range 1 to 25).

There were 30/93 (32%) cases in which there were multiple diagnosis, with OCD (27%; 8/30) and non-mycoplasma bacterial (27%; 8/30) being the most common secondary diagnosis.

The most commonly requested test for pathogens other than *Mycoplasma spp.* was aerobic culture and on average each case involved 2.4 cultures (range 1 to 37), of which 78% (171/220 total cultures) returned no significant growth. This does not include *Erysipelothrix* specific cultures. Erysipelas was commonly listed as a differential (36% of cases; 19/53) but none of the cases listed skin lesions as part of the history or gross lesion findings. Almost half of the MHS cases (45/93) cases performed at least one *Erysipelothrix* culture or PCR with each case averaging 2.5 *Erysipelothrix* assays. Of all these cases, however, only one (2%) returned a positive result.

**Discussion**

This review summarizes the most frequently observed lameness diagnostic categories for case submissions involving joints and legs at the ISU VDL between 2010 and 2015. A similar study reported the frequency of diagnosis of arthritis, specifically MHS and MHR cases between 2003 and 2010 at the ISU VDL. There were 431 clinical cases with infectious arthritis during that time period and MHS represented 17% of the arthritis cases. There were more MHR cases identified in that study than reported here, but that study included pigs <7 weeks of age. Findings from the current study are also consistent with another summary of arthritis cases from 2003 to 2014 at the ISU VDL.
This review found that 25% of the cases were idiopathic, 20% were MHS, 24% bacterial and 12% were MHR based on the diagnostician code alone. These results reinforce that many diagnostic investigations do not reveal a clear etiology of the lameness as a consequence of diagnostic testing alone.

Studies aiming to summarize lameness etiologies have been performed in the context of field cases. One Danish study looked at the microbiological causes of lameness in pigs at slaughter in Denmark. *Erysipelothrix rhusiopathiae* and MHS composed about 10% of the cases each, while 70% joints were sterile. Gross changes to the joints were observed with *E. rhusiopathiae* and MHS associated arthritis but gross pathological changes in the sterile joints were non specific. In another Danish slaughter pig study, MHS was isolated from 60% of the pigs with arthritis in three of the five herds. Claw lesions (22%) and severe OCD (10%) made up the second and third most common diagnosis across all herds.

From the results of this analysis and previous studies, it appears that MHS is an important contributor to arthritis, but there are several other important known and unknown etiologies associated with the lameness. In this study, the four diagnostic categories (idiopathic, MHS, metabolic bone disease, and bacterial) composed about 80% of the cases and about a quarter of the cases had at least one other lameness-associated abnormality. This reinforces that lameness is often multifactorial, and that many swine lameness processes may be cumulative creating difficulty in assigning single etiology causation. Additionally, the high rate of idiopathic and non-infectious lameness cases should prompt practitioners to perform complete diagnostic investigations before implementing expensive interventions or antibiotic treatment in the field.
Lameness with inconclusive findings was the most commonly assigned (22% of cases) diagnostic option for this case review. It is possible that submitter bias, behaviors or inattention to details such as animal selection, sample selection, sample handling, and test selection could artificially increase this number. For example, in cases where practitioners submitted one intact joint, it could be possible that with additional specimens, that case could have received a diagnosis.

Conclusions obtained by retrospective analysis of data from a VDL should not be over interpreted and any extrapolation of the findings should be approached carefully. The data from this review is not field prevalence data. For each case, there were multiple sources of bias that make standardization and objective analysis of VDL data very difficult. First, information is limited to the submission sheet, submitted specimens, and tests requested or VDL diagnostician’s decision on testing. Each case did not test for all possible causes of lameness and the case search criterion focused on arthropathies. Since the completion of this analysis, multiple case reports have highlighted neurological and vesicular viral pathogens as important lameness etiologies, which were beyond the scope of this retrospective review at the time.\textsuperscript{9-13} Furthermore, the analysis was focused on infectious arthritis, specifically MHS, and the MHS case definition was targeting acute cases. This review also did not include sows, boars, gilts, suckling, or nursery pigs; all of which contend with diverse lameness challenges.

Additionally, the retrospective case review process involves subjective steps completed by the veterinarian, laboratory technician, diagnostician and case reviewer. For example, a diagnostician may interpret histopathologic findings differently depending
upon their experience, current/popular health priorities within the industry, areas of expertise, and information provided about the case by the submitting veterinarian.

**Implications**

In this study, the four diagnostic categories of idiopathic, MHS, metabolic bone disease, and bacterial composed about 80% of the cases. These four diagnoses have quite different etiologies and pathogenesis and require different intervention strategies. Accurate diagnosis is key for selection of effective interventions and setting expectations for likelihood of treatment success. This study reinforces the importance of careful clinical examination, proper sampling, and confirming cause(s) with appropriate diagnostic testing for accurate diagnosis of lameness.

**Acknowledgements**

Thank you to the ISU VDL staff, including information technology support for their assistance in creating the case database. Thank you to SMEC staff and interns. Thank you to Dr. Bailey Arruda and Dr. Maria Clavijo for input on search strategy.

**Conflict of Interest**

None. National Pork Board, Iowa Pork Producers Association, the Swine Medicine Education Center, and PIC provided funding for this project.

**Disclaimer**

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accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region

References


Table 1. Diagnostic criteria for each diagnostic category applied to cases associated with joint/leg lameness

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Criteria for inclusion into each diagnostic category</th>
</tr>
</thead>
</table>
| **Lameness: abnormal diagnostic testing results with inconclusive findings (Lameness with inconclusive findings)** | - lameness reported by practitioner and/or diagnostician  
- histology of joint revealed mild non-specific changes to the synovial tissue  
- additional testing not performed or results of additional testing were inconclusive or not significant  
- description of inconclusive or nonspecific joint changes included in final report by pathologist [if available]* |
| *Mycoplasma hyosynoviae (MHS)* | - specimens submitted from animals with clinical lameness and/or joint swelling  
- at least one positive MHS polymerase chain reaction (PCR) result on joint fluid or joint tissue  
- histology lesions consistent with MHS as per diagnostian comments in histology report and/or published histology findings associated with experimental and field MHS cases¹,³,⁴ |
| **Metabolic bone disease** | - abnormal results on any calcium, phosphorus, vitamin D assay and/or bone ash/density tests. Cases did not have to run all of these assays to be included in the metabolic bone disease category  
- diagnostian comments that abnormality is contributing to locomotion issues |
| **Infectious (bacterial, non-mycoplasma species)** | - histology on synovium indicative of infectious (non-mycoplasma) process  
- significant findings on culture†  
- gross description of fluid indicative of infection, ie. purulent, serosanguinous [if available]  
- positive PCR results on molecular testing for *Erysipelothrix spp.* or *Haemophilus parasuis* from joint specimens [if available] |
| **Lameness: no abnormal findings** | - Lameness reported by practitioner and/or diagnostian  
- Culture with no significant findings  
- MHS PCR negative  
- Histology of joint revealed no changes to synovial tissue |
| **Osteochondrosis (OCD)** | - Gross or histologically observed cartilage defects in articular cartilage |
Table 1. Diagnostic criteria for each diagnostic category applied to cases associated with joint/leg lameness (continued)

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Criteria for inclusion into each diagnostic category</th>
</tr>
</thead>
</table>
| *Mycoplasma hyorhinis* | • MHR PCR positive or MHR culture positive on joint fluid or joint tissue  
                        • Histological changes to the synovium consistent with MHR  
                        • Systemic gross and histological lesions from other tissues submitted indicative of systemic MHR cases [if available]  
                        • Serosanguinous synovial fluid or fibrin in synovial fluid [if available] |
| Trauma | • Fractures unrelated to abnormal bone histology indicative of metabolic bone disease [if available]  
         [OR]  
         • Bursitis related to physical contact with slats, as associated in diagnostician comments |
| Osteomyelitis | • Bacterial infection of the bone as per gross and/or histological assessment of the bone  
               • Significant findings on culture [if available] |

* [if available] indicates that for some of the cases this information or specimen may not be available, and that relevant tests to this diagnostic category may or may not have been performed.  
† Significant findings refer to growth of a bacterial species associated with arthritis as per the bacteriologist or published literature.
Table 2. Primary and secondary diagnosis for 464 lameness cases in growing pigs from a retrospective case survey at the Iowa State University Veterinary Diagnostic Laboratory between May 2010 and April 2015.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Primary Diagnosis</th>
<th>Secondary Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lameness with inconclusive findings</td>
<td>101 (22%)</td>
<td>27 (25%)</td>
</tr>
<tr>
<td><em>Mycoplasma hyosynoviae</em></td>
<td>93 (20%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Metabolic bone disease</td>
<td>86 (19%)</td>
<td>31 (29%)</td>
</tr>
<tr>
<td>Infectious (bacterial)</td>
<td>81 (18%)</td>
<td>18 (17%)</td>
</tr>
<tr>
<td>Lameness: no abnormal findings</td>
<td>43 (9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Osteochondrosis (OCD)</td>
<td>29 (6%)</td>
<td>18 (17%)</td>
</tr>
<tr>
<td><em>Mycoplasma hyorhinis</em></td>
<td>19 (4%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Trauma</td>
<td>10 (2%)</td>
<td>8 (7%)</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>2 (0.4%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Total</td>
<td><strong>464</strong> (100%)</td>
<td><strong>108</strong> (100%)</td>
</tr>
</tbody>
</table>
CHAPTER 3. SUITABILITY OF FOUR INJECTABLE ANESTHETIC PROTOCOLS FOR PERCUTANEOUS SYNOVIAL FLUID ASPIRATION IN HEALTHY SWINE UNDER FIELD CONDITIONS AND ASSESSMENT OF LAMENESS SEVEN DAYS POST PROCEDURE.

Submitted to JSHAP in July 2017, currently under review

P. Canning, DVM; Primary researcher, created protocol, completed live animal work, data collection, data analysis and preparation of the manuscript

K. O’Brien; assisted with live animal work and data collection

V. Thompson; assisted with live animal work and data collection

D. Madson, DVM, PhD, Diplomate ACVP; co-advisor on protocol and data analysis, and reviewer of manuscript

K. Skoland; co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

A. Ramirez, DVM, MPH, PhD, Diplomate ACVPM; co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

D. Linhares, DVM, MBA, PhD; co-advisor on protocol and data analysis, and reviewer of manuscript

P. Gauger, DVM, PhD; co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

L. Karriker, DVM, MS, Diplomate ACVPM; co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

PC, KOB, VT, KS, LK, AR: Swine Medicine Education Center, Iowa State University College of Veterinary Medicine, Ames, Iowa.
Objective

This study compared the suitability of four anesthetic protocols for percutaneous aspiration of synovial fluid from healthy swine in field conditions. A supplemental objective was to assess the iatrogenic impact of antemortem joint sampling by monitoring lameness, joint swelling and synovial histology for seven days after procedure.

Materials and methods

Twenty-four 80-90 kg finisher pigs were randomly allocated into one of four treatment groups. Each group was given one of four anesthetic combinations intramuscularly: telazol-ketamine-xylazine (TKX), telazol-ketamine-acepromazine (TKA), ketamine-acepromazine with a lidocaine epidural (KAL), or telazol-acepromazine with a lidocaine epidural (TAL). Once anesthetized, synovial fluid was collected aseptically from one carpus and tarsus joint per pig. The anesthetic protocols were evaluated in terms of successful achievement of general anesthesia, time to sternal and time to standing recovery. Joint swelling and lameness assessments were completed on days two, four, and seven post-sampling. On day seven, pigs were euthanized and synovium was collected from each sampled joint for histologic evaluation.

Results

TKX and TAL were the only anesthetic combinations that provided an adequate depth of anesthesia for fluid collection to occur. Mean (standard deviation) time to sternal
for TKX was 125 (26) minutes and for TAL was 198 (28) minutes. There was no evidence of infection post-aspiration in any of the sampled joints.

**Implications**

TKX was the most effective anesthetic protocol for antemortem joint fluid collection. Antemortem joint fluid collection was not associated with significant joint tissue damage and can be a useful diagnostic tool for infectious arthritis.

**Keywords**

swine, joint fluid, telazol, ketamine, xylazine

**Introduction**

Infectious arthritis in swine is an important cause of lameness in growing pigs.\(^1\)

Diagnosis of infectious lameness in pigs can be difficult due to the transient nature of joint pathogens. Diagnostic investigations generally involve post-mortem samples substantially limiting the number of available specimens to submit for testing. An antemortem joint fluid collection technique would offer practitioners additional flexibility to collect diagnostic samples without having to sacrifice animals. A challenge of this technique is achieving sufficient plane of anesthesia for the procedure in the field.

Although there are published recommendations for injectable anesthesia for pigs, these references typically do not state the effectiveness of the protocols for specific procedures, such as percutaneous joint fluid aspiration.\(^2-5\) Additionally, there are no reports on the impact of antemortem joint fluid collection on lameness and synovial damage post-procedure.

The primary objective of this study was to compare the utility of four anesthetic protocols for antemortem joint fluid collection. The protocols were evaluated in terms of
successful achievement of anesthesia, time to recovery, and cost of the protocol. The secondary objective was to assess the iatrogenic impact of percutaneous joint fluid collection by monitoring lameness and joint swelling for seven days post sample collection and assessing histology at day seven.

**Materials and methods**

**Animals, housing, feed, and water**

The trial was approved through the Iowa State University Institutional Animal Care and Use Committee. Prior to the initiation of the trial, pigs were housed in partially slatted pens in groups of 15 to 20 in a feeder to finisher barn with a total group size of approximately 200. At selection for the trial, 80-90 kg pigs were moved from the group housing to individual pens for the procedures described below. Pigs were first assessed while standing and observed while walking to ensure they did not display lameness or swollen joints. Pigs were also given a physical exam by a veterinarian, which included joint palpation, and pigs had to be free of clinical signs to be included in the trial. Once pigs were fully recovered from anesthesia, the pigs were returned to their original group pens.

All pigs were fed *ad libitum* commercial finisher feed without antibiotics for the duration of the trial and had free access to water *ad libitum*. The diets met the National Research Council requirements for swine.⁶ Neither feed nor water were not withheld from pigs prior to anesthesia.

**Treatment allocation**

There were 24 pigs in the trial and the group was a mix of barrows and gilts. After selection, pigs were weighed, ear tagged, and randomly allocated using a random
number table to one of four anesthetic protocols. Generally, for each protocol, an initial minimum dose was given and then additional step dosing was done until anesthesia was sufficient for the procedure or until a priori maximum dose was achieved within 1.5 hours of the initial dose. If the maximum dose was achieved and an insufficient plane of anesthesia was attained for joint fluid collection, the pig was not given any more anesthetic agents and was monitored until recovery.

Group TKX received an initial intramuscular (IM) injection of 4.4 mg/kg telazol (tiletamine HCl and zolazepam HCl injection, Zoetis, Kalamazoo, MI), 2.2 mg/kg ketamine (Zoetis, Kalamazoo, MI), and 4.4 mg/kg xylazine (VetOne/Akorn, Inc. Lake Forest, IL) combined in the same syringe\(^3,5\) with a maximum cumulative dose of 4.4 mg/kg of ketamine and 8.8 mg/kg each of xylazine and telazol.

The TKA group received an initial IM injection of 0.03 mg/kg acepromazine (VetOne/Akorn, Inc. Lake Forest, IL), 2.2 mg/kg of ketamine, and 4.4 mg/kg telazol combined in the same syringe\(^3\) with a maximum cumulative dose of 10 mg/kg telazol, 0.07 mg/kg acepromazine, and 5 mg/kg of ketamine.

Group TAL received an initial IM injection of 0.3 mg/kg acepromazine and 4.4 mg/kg telazol in the same syringe\(^2,3\) with a maximum cumulative dose of 0.5 mg/kg of acepromazine and 11 mg/kg telazol until the pig was in a suitable plane to administer a lumbosacral epidural. The lumbosacral epidural consisted of 2% lidocaine (MWI, Boise, ID) dosed at 2.2 mg/kg, up to maximum of 10 mL per pig.

The KAL group received an IM injection of 0.5 mg/kg acepromazine and 5 mg/kg ketamine mixed in the same syringe\(^3,4\) with a maximum 1.2 mg/kg acepromazine and 33 mg/kg ketamine until a suitable plane to administer lumbosacral epidural\(^2,7\) was reached.
The lumbosacral epidural (see description below) consisted of 2% lidocaine dosed at 2.2 mg/kg, up to a maximum of 10 mL per pig.

Between 5 and 10 minutes after the initial IM injection, pigs were assessed for depth of sedation based on their behavior and reflex responses. To be considered eligible for the joint fluid collection procedure (sufficient anesthesia), the pig must have been recumbent, with a negative palpebral response and negative toe withdrawal response. If these criteria were not met or the pig reacted to the insertion of the needle in the joint, then an additional dose of the applicable protocol was administered. The animal was then left alone, with minimal background noise and reassessed between 5 and 15 minutes later. This was repeated until a suitable depth was attained or max dose was administered.

**Placement of epidural**

For groups TAL and KAL, a lumbosacral epidural was placed using an 18G X 8.9 cm spinal needle (BD™ New Jersey, USA) as described elsewhere.\(^3,8,9\) Briefly, a 25 cm X 25 cm section on midline at the cranial aspect of the tuber coxae was shaved and aseptically prepared for the epidural. The prep consisted of shaving followed by three steps: a chlorhexidine soap scrub, alcohol scrub, and final prep with tincture of chlorhexidine. Steps one and two were repeated three times. To administer the epidural, a veterinarian wore sterile gloves and inserted the epidural needle into the intervertebral disc space between lumbar vertebrae six and sacral vertebrae one. Lidocaine was injected into the spinal canal as previously reported.\(^3,8,9\)
**Collection of synovial fluid**

Under anesthesia, pigs were positioned in dorsal recumbency. One tarsus (all groups) and one carpus (groups TKX and TKA only) was selected for sampling. An aseptic preparation, as described above for epidural injection, was performed on the joints prior to sampling. Sterile 18G X 3.8 cm needles (Monoject BD Bioscience, San Jose, CA) with 12 mL syringes (Monoject BD Bioscience, San Jose, CA) were used for the joint fluid aspirations. Clean gloves and garments were worn. If a needle was inserted into the joint, regardless if fluid was successfully collected, it was recorded and that joint subsequently monitored as described below.

**Anesthesia monitoring**

From the initial IM injection onwards, pigs were monitored closely until recovery. Heart rate, respiratory rate, rectal temperature, and depth of sedation were monitored at least every 15 minutes until the joint aspiration was performed, then monitoring changed to every 30 minutes. Heart rate was assessed using thoracic auscultation and breath rate was counted by observing the rib cage expansion and contraction. Once the pig was in sternal recumbency, vital parameters were recorded hourly and the pig was assessed visually approximately every 30 minutes until it was ambulatory.

During the anesthesia and recovery process, the following data points were recorded: if sufficient plane was achieved for joint fluid aspiration (yes or no), time to joint sampling from first injection, time to sternal recumbency, and ambulation for pigs that reached sufficient plane for sampling.
Procedure for post-procedure observations

After pigs were recovered and ambulatory, they were scored for lameness and joint swelling and then returned to their original pens. On day two, four, and seven post joint aspiration, pigs were re-assessed for lameness and joint swelling.

Lameness scoring

The gait scoring scale used from a published scoring rubric. Pigs were given a lameness score from zero to five and pigs were evaluated while standing and then while ambulating only. Modifications in the scoring system were used as pigs were not evaluated with respect to response to human presence, opening of gate, or interactions with penmates.

Joint enlargement and swelling scoring

Joint swelling scoring was performed as previously described from a published scoring rubric. In short, score 0 was no or slight joint swelling. Score 1 was soft, non-warm swelling of the joint. Score 2 was marked soft, fluctuating enlargement of the joint and surroundings. Score 3 was a firm and warm periarticular swelling. Pigs were assessed visually and joints were palpated before assigning a score. The same individuals performed the joint and lameness scoring for all days of the trial.

Necropsy and sample collection

Seven days after the joint fluid collection all animals were humanely euthanized for necropsy. At necropsy, all carpus and tarsus joints in which a needle penetrated were examined. The articular cartilage, synovial fluid, and synovial tissue were assessed grossly with abnormalities documented. Additional synovial tissue from each of these joints was collected and placed in 10% buffered formalin for histological evaluation. A
systematic evaluation of the internal organs and other appendicular joints was also performed.

**Synovial tissue scoring**

A board certified veterinary pathologist who was blinded to treatment allocation conducted an evaluation of the synovial tissue samples using a scoring rubric modified from Hagedorn-Olsen, et al.\textsuperscript{12} and published in Neto, et al.\textsuperscript{13} The score for each category was summed to create a composite score ranging from 0 to 15. The scoring rubric encompassed two types of categories of assessment: first, categories indicative of active infectious processes such as neutrophil, fibrin, and hemorrhage were scored; and second, categories such as synovial proliferation or alterations, which encompassed noninfectious and chronic changes to the joint.

**Statistical analysis**

Descriptive statistics were prepared using SAS Version 9.1 (SAS Institute, Cary, NC).

**Results**

**Anesthesia protocols**

In Table 1, a comparison of each anesthetic protocol is presented in terms of successfully producing anesthesia to allow for joint aspiration, costs of anesthetic protocol and recovery time. All treatment groups contained at least two pigs that required additional dosing beyond the initial dose. All pigs in the TKX and TAL groups reached a sufficient plane for joint aspiration. The recovery time for all protocols was over three hours. For group KAL, the first three pigs anesthetized received the maximum IM dose without
reaching a sufficient plane to place an epidural or conduct a joint aspiration. As such, the authors opted to remove the remaining three pigs from KAL group in lieu of dosing them.

In group TKA, pig 185 died after reaching sternal recumbency and attempting to stand during the recovery process. Post mortem evaluation revealed pulmonary congestion affecting both lungs and grossly enlarged heart with ventricular dilation. During the monitoring process, pig 185 had a numerically higher heart rate and respiration rate than its cohorts (Table 2).

The heart rate, respiratory rate and rectal temperature of the pigs were measured regularly until the pigs could stand and the mean and range of each parameter is presented in Table 2. Several pigs in each treatment group required rewarming with blankets as their rectal temperature fell below 37°C.

**Lameness and joint swelling**

All pigs had a lameness score of 0 on day 0, 2, 4, and 7. One pig from the TKX group had mild swelling (score 1) on the right carpus on day 4, which had been sampled on day 0. This swelling decreased to score 0 by day 7. A second pig from the same group also had mild swelling (score 1) on day 2 on the left tarsus, which was sampled previously. The score decreased to 0 on days 4 and 7 during the monitoring period. All other pigs received a joint swelling score of zero on both joints for the duration of the seven-day monitoring period.

**Synovial histology**

The synovium histology scoring indicated that all joints received a score of zero on all three categories related to acute inflammation: neutrophils, fibrin, and hemorrhage. There were four tarsus joints, 2 from TKX and 2 from TAL, in which there were mild,
non-specific changes to the synovium suggestive of a chronic, non-infectious process in the joint. Their cumulative synovium score ranged between 3 to 6 out of a maximum possible score of 15. The gross appearance of the synovial fluid and synovium of these joints were within expected values for a normal joint.

Discussion

Telazol, ketamine, and xylazine (TKX) was the only protocol that was consistently suitable for collection of joint fluid from market sized animals. The depth of anesthesia produced by the other protocols was insufficient to inhibit the foot withdrawal reflex, facilitate epidural placement or, in some cases, achieve unconsciousness. None of the pigs in this study were lame post procedure, nor was there evidence of iatrogenic infectious arthritis identified in any of the pigs.

There are several resources available to practitioners that recommend drug combinations, drug dosages and practical tips for in field anesthesia.\textsuperscript{2,5,7,14} These resources provide general descriptions of duration of effect, contraindications, adverse effects, and pharmacology. Absent from these resources is an evaluation of the utility of a specific protocol for a specific procedure in a specific age of pig. Without this information, there is increased reliance on practical experience in lieu of evidence based medicine for selection of anesthetic protocols for use in the field.

Additional considerations for field applications of TKX are that the duration of xylazine is relatively short (10-30 minutes) and xylazine’s analgesic, sedative and muscle relaxation effects are critical to balance the muscle spasticity and rigidity associated with ketamine in combination protocols.\textsuperscript{3,8,15,16} Thus, there is a short window for optimal joint aspiration procedure using TKX protocol and the practitioner needs to monitor the animal
closely so as to not inadvertently miss it and need to re-dose the animal, particularly if sampling more than one pig simultaneously. Performing a foot withdrawal test using a needle is an easy and non-invasive method of assessing the withdrawal reflex and suitability for joint aspiration.

During substantial recovery times as observed in this study, the potential exists for physiological complications which necessitates active monitoring and veterinary management. Recovery time typically decreases in smaller/young pigs due to different body composition and metabolism rate. For example, in two studies on telazol and xylazine in 37 kg cross-bred pigs that assessed similar parameters to this study, the pigs reached sternal recumbency in 76-98 minutes and were standing at 100-130 minutes post initial injection. However, these papers did not use ketamine with telazol and xylazine, thus a direct comparison cannot be made to the recovery times published here.

Placement of the epidural was successful in the pigs in the TAL group but required additional equipment and technical skill beyond the joint aspiration. Compared to the TKX group, there was a longer delay between the initial IM injection, joint fluid collection and ultimately, recovery in the TAL group. Despite this, epidurals in swine may be useful for other procedures such as scrotal and inguinal hernia repair.

From the post mortem findings and elevated vital parameters while under anesthesia, it is believed that pig 185 experienced cardiac and/or respiratory complications that lead to death. In this study and in the field setting, it would be difficult to screen pigs for pre-existing conditions beyond a visual examination and thoracic auscultation. Since pigs have a relatively small lung capacity compared to horses and companion animals, knowledge of pre-existing conditions, such as previous bouts of
pneumonia, would be important for selecting good candidates for anesthesia and subsequent antemortem joint aspirations. Additionally, pig 185 was in the TKA group which received a larger dose of acepromazine than other groups. Acepromazine is known for its hypotensive effects that may have negatively affected cardiac output in this pig. For this reason, in addition to recovery time, it is not advised that practitioners use TKA in the field for market weight hogs.

The mean values for individual pig heart rate, respiration rate, and temperature were generally elevated compared to normal values for finishers reported in Anderson and St. Jean. Also, within each of the treatment groups, there was variability in vital parameters between individual pigs. For example, mean heart rate in the TKX group ranged between 82 and 116 beats per minute. Information about normal vital parameters for commercial pigs in field conditions is limited. Published values for vital parameter information under specific anesthetic regimes in the field are not available. Thus, Table 2 provides information for practitioners on the vital parameter values and variability they may encounter while performing field anesthesia in finisher pigs.

This study emphasizes the use of tools readily available to practitioners in the field to monitor vital parameters. This is unique from other anesthetic evaluation and comparison studies in which there is additional monitoring performed including blood pressure, arterial blood gases, and blood biochemistry. In those studies, expanded monitoring was critical to collect the data required to compare the physiological effects of different protocols which served as the primary objective of the research. The present study focused on the ability of anesthetic protocols to provide appropriate conditions for efficacious completion of a diagnostic task.
TKX performed well during this antemortem procedure and allowed for the successful collection of joint fluid and prompt recovery post procedure. An anesthetic protocol for antemortem joint taps that is applicable to commercial settings and cost effective is a valuable tool to practitioners for diagnostic investigations of lameness. The antemortem technique allows practitioners to increase their diagnostic sample size, monitor treatment success in sampled pigs, and can complement post mortem examinations in affected herds. Veterinary practitioners must carefully consider the local, state, and federal regulatory consequences and current rules or guidance before utilizing any anesthetic protocol in the field.

**Implications**

TKX was the best overall anesthetic protocol for antemortem joint fluid collection in this trial. Veterinary practitioners must carefully consider the local, state, and federal regulatory consequences and current rules or guidance before utilizing any anesthetic protocol in the field. Antemortem joint fluid collection was not associated with significant joint tissue damage; therefore, it has potential as a useful diagnostic tool for infectious arthritis.

**Acknowledgments**

Thank you to ISU Swine Nutrition Farm staff for their assistance, especially Trey Faaborg and Tim Hicks. Thank you to the SMEC summer interns, Megan Nickel and Rochelle Warner, for their help with the live animal work. SMEC veterinarians Justin Brown and Anna Forseth were instrumental in the monitoring of the animals and we sincerely appreciate their efforts.
Conflict of Interest

None. National Pork Board, Iowa Pork Producers Association and PIC provided funding for this project.

Disclaimer

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References


Table 1. Comparison of four anesthetic protocols with respect to cost, procedure success and recovery times.

<table>
<thead>
<tr>
<th>Group information for all pigs</th>
<th>TKX</th>
<th>KAL</th>
<th>TAL</th>
<th>TKA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pigs</td>
<td>6</td>
<td>3*</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mean body weight in kilograms (SD†)</td>
<td>83.1 (12.7)</td>
<td>92.1 (2.7)</td>
<td>82.2 (6.4)</td>
<td>90.1 (10.7)</td>
</tr>
<tr>
<td>Number of animals for which sufficient surgical plane was achieved to allow for joint aspiration (%)</td>
<td>6 (100%)</td>
<td>1 (33%)</td>
<td>6§ (100%)</td>
<td>2† (33%)</td>
</tr>
<tr>
<td>Number of pigs requiring at least one additional dose (%)</td>
<td>2 (33%)</td>
<td>3 (100%)</td>
<td>3 (50%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Number of pigs that reached maximum dosage</td>
<td>0 (0%)</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Mean cost of anesthesia protocol (SD)</td>
<td>$24.98 (4.16)</td>
<td>$22.37 (0.82)</td>
<td>$38.96 (4.65)</td>
<td>$50.99 (3.65)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Procedure and recovery time for pigs that reached sufficient surgical plane for joint aspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time to joint aspiration from first injection‡ in minutes (SD)</td>
</tr>
<tr>
<td>Mean time to sternal‡ in minutes (SD)</td>
</tr>
</tbody>
</table>
| Mean time to ambulatory‡ in minutes (SD) | 266 (73) | 317 (-5) | 378 (79) | 267 (-**)

*As the first three pigs anesthetized did not reach a sufficient plan to place an epidural or conduct joint aspiration, the remaining three pigs in the group were not dosed and were removed from the study.
†SD refers to standard deviation
‡for pigs that reached sufficient surgical plane for sampling
§in one pig, the joint was sampled but fluid was not collected
†n=1
**Pig died after achieving sternal recumbency
TKX: telazol, ketamine and xylazine; KAL: ketamine and acepromazine with lidocaine epidural; TAL: telazol and acepromazine with lidocaine epidural; TKA: telazol, ketamine and acepromazine.
Table 2. Mean, minimum, and maximum heart rate, respiratory rate, rectal temperature of pigs for four injectable anesthetic protocols. Each parameter was measured until pigs could stand.

<table>
<thead>
<tr>
<th>Pig</th>
<th>Heart rate (beats/min)</th>
<th>Respiratory rate (breaths/min)</th>
<th>Rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  Low  High</td>
<td>Mean  Low  High</td>
<td>Mean  Low  High</td>
</tr>
<tr>
<td>TKX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>178</td>
<td>116  104  140</td>
<td>34    28    44</td>
<td>38.9   38.0   39.9</td>
</tr>
<tr>
<td>179</td>
<td>98   88   128</td>
<td>43    24    60</td>
<td>38.7   37.8   39.5</td>
</tr>
<tr>
<td>180</td>
<td>111  90   140</td>
<td>39    28    52</td>
<td>39.1   38.5   39.7</td>
</tr>
<tr>
<td>181</td>
<td>101  80   120</td>
<td>38    24    52</td>
<td>38.1   37.3   38.9</td>
</tr>
<tr>
<td>182</td>
<td>96   84   112</td>
<td>41    20    76</td>
<td>38.2   37.5   39.6</td>
</tr>
<tr>
<td>183</td>
<td>82   64   100</td>
<td>50    32    80</td>
<td>37.7   36.7   38.6</td>
</tr>
<tr>
<td>KAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>190</td>
<td>115  68   148</td>
<td>35    24    48</td>
<td>38.2   37.6   39.0</td>
</tr>
<tr>
<td>191</td>
<td>117  72   148</td>
<td>45    44    48</td>
<td>37.3   36.6   38.3</td>
</tr>
<tr>
<td>192</td>
<td>89   52   128</td>
<td>34    28    44</td>
<td>37.4   36.4   38.4</td>
</tr>
<tr>
<td>TAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>193</td>
<td>121  96   160</td>
<td>40    28    52</td>
<td>36.7   35.9   38.9</td>
</tr>
<tr>
<td>194</td>
<td>109  64   140</td>
<td>41    28    64</td>
<td>37.9   36.7   39.6</td>
</tr>
<tr>
<td>195</td>
<td>119  104  132</td>
<td>44    24    76</td>
<td>37.3   36.6   39.2</td>
</tr>
<tr>
<td>196</td>
<td>108  72   120</td>
<td>34    20    48</td>
<td>36.6   36.0   37.7</td>
</tr>
<tr>
<td>197</td>
<td>122  72   160</td>
<td>57    40    80</td>
<td>37.8   36.2   39.4</td>
</tr>
<tr>
<td>198</td>
<td>90   72   108</td>
<td>37    28    60</td>
<td>36.7   35.2   38.8</td>
</tr>
<tr>
<td>TKA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>184</td>
<td>105  56   128</td>
<td>30    28    40</td>
<td>37.9   36.8   39.0</td>
</tr>
<tr>
<td>185*</td>
<td>160  120  200</td>
<td>55    36    84</td>
<td>38.2   37.0   39.3</td>
</tr>
<tr>
<td>186</td>
<td>122  60   160</td>
<td>39    24    60</td>
<td>38.2   37.5   39.1</td>
</tr>
<tr>
<td>187</td>
<td>96   78   116</td>
<td>53    36    78</td>
<td>38.0   36.6   39.6</td>
</tr>
<tr>
<td>188</td>
<td>100  80   140</td>
<td>29    18    44</td>
<td>38.2   37.4   39.5</td>
</tr>
<tr>
<td>189</td>
<td>100  80   120</td>
<td>42    36    44</td>
<td>37.3   36.5   38.5</td>
</tr>
</tbody>
</table>

*Pig number 185 died before it was observed to have stood

TKX: telazol, ketamine and xylazine; KAL: ketamine and acepromazine with lidocaine epidural; TAL: telazol and acepromazine with lidocaine epidural; TKA: telazol, ketamine, and acepromazine.
CHAPTER 4 FLUID ANALYSIS AND CYTOLOGIC EVALUATION REFERENCE
INTERVALS FOR SYNOVIAL FLUID FROM CARPAL AND TARSAL JOINTS IN
NON-LAME COMMERCIAL GROWING SWINE

Submitted to American Journal of Veterinary Research (AJVR) May 2017, revisions
submitted July 12th 2017 and August 15th 2017

Paisley Canning¹ DVM; Primary researcher, created protocol, completed live animal work,
data collection, data analysis, and preparation of the manuscript

Austin Viall² DVM, MS, Dip ACVP; co-advisor on protocol and data analysis, and reviewer
of manuscript

Katie O’Brien¹ BS; assisted with live animal work and data collection

Darin Madson³ DVM, PhD, Dip ACVP; co-advisor on protocol and data analysis, and reviewer
of manuscript

Kristin Skoland¹ BS; co-advisor on protocol and data analysis, assisted with live animal work
and reviewer of manuscript

Adam Krull³ DVM, PhD; co-advisor on protocol and reviewer of manuscript

Daniel Linhares³ DVM, MBA, PhD; co-advisor on protocol and data analysis, and reviewer
of manuscript

Phil Gauger³ DVM, MS, PhD; co-advisor on protocol and data analysis, assisted with live
animal work and reviewer of manuscript

Alex Ramirez²,³ DVM, MPH, PhD, Dip ACVPM; co-advisor on protocol and data analysis,
assisted with live animal work and reviewer of manuscript

Locke Karriker¹,³ DVM, MS, Dip ACVPM; co-advisor on protocol and data analysis,
assisted with live animal work and reviewer of manuscript
Acknowledgements

Thank you to ISU SNF farm staff (Trey Faaborg), Swine Medicine Education Center summer interns (Rochelle Warner, Megan Nickel, and Victoria Thompson), and post doctorates (Justin Brown and Anna Forseth) for their help for this project. Funding for this project was provided by means of an external grant funded by the National Pork Board, Iowa Pork Producers Council, and PIC. Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript. The authors declare that there were no conflicts of interest.

Abstract

Objectives

Cytological evaluation and fluid analysis of synovial fluid is a common diagnostic process used in equine and companion animal medicine to diagnosis joint disease. Diagnostic tests for lameness in swine are limited and there are no reference intervals available for swine synovial fluids.
The objective of this study was to create reference intervals for total nucleated cell count, total protein, pH, red blood cell count, percentage of neutrophils, lymphocytes, and large mononuclear cells for the carpus and the tarsus joints in healthy swine.

**Animals**

Fifty-four healthy finishing pigs that did not display lameness or gross joint swelling.

**Procedures**

Pigs were anesthetized and synovial fluid samples were collected from the carpus and tarsus for cytologic evaluation, fluid analysis, and culture. Animals were euthanized after collection and synovial tissue samples were collected for histologic assessment.

**Results**

Thirty-seven (37) tarsus and 46 carpus samples met inclusion criteria of sufficient volume, no gross blood contamination, negative by culture and polymerase chain reaction for common swine joint pathogens and normal synovial tissue histology. The upper reference limit for percentage neutrophils for the carpus was 46.5% and 33.7% for tarsal samples. The upper reference limit of total protein for carpal fluid samples was 3.6g/dL and 3.6g/dL for tarsal samples. Both anatomical locations had between 92% and 95% large mononuclear cells at their upper reference limit.

**Conclusions and clinical relevance**

The novel synovial fluid cytologic evaluation and fluid analysis reference intervals provided by this study aid diagnostic investigations of swine lameness and arthritis.
Abbreviations

IM intramuscular
ISU VDL Iowa State University Veterinary Diagnostic Laboratory
LMC large mononuclear cells
MHR *Mycoplasma hyorhinis*
MHS *Mycoplasma hyosynoviae*
PCR polymerase chain reaction
TNCC total nucleated cell count
TP Total protein
RBCC red blood cell count
%NEUTS neutrophils
%LYMPHS percentage of lymphocytes
%LMC percentage of large mononuclear cells
Introduction

Infectious and non-infectious lameness issues of growing swine are a welfare concern and can increase production costs. Lameness diagnosis has historically relied upon gross changes coupled with postmortem synovial fluid culture and PCR testing.\(^1\) This strategy requires the euthanasia of affected pigs, limiting sample size selection and preventing follow-up investigation of the effect of treatments post-diagnosis. Thus, antemortem diagnostic assessments of synovial fluid that are applicable to a broad range of lameness agents would be useful. Diagnosing the cause of lameness using an antemortem sample collection technique would afford practitioners the opportunity to monitor treatment success of interventions.

In equine and canine medicine, cytologic evaluation and fluid analysis of synovial fluid is a core antemortem diagnostic test for multiple types of arthritis.\(^2,3\) Routine synovial fluid analysis encompasses fluid analysis and cytologic evaluation, and reports fluid pH, total protein (TP), and differential nucleated cell percentages. Combined with PCR and culture, cytologic evaluation and fluid analysis could be key diagnostic tools for swine joint fluid. Published cytologic evaluation and fluid analysis values for swine joint fluid are currently very limited.

The objective of this study was to report cytologic evaluation and fluid analysis reference intervals from clinically healthy pigs for total nucleated cell count (TNCC), TP, pH, red blood cell count (RBCC), percentage of neutrophils (%NEUTS), percentage of lymphocytes (%LYMPHS), and percentage of large mononuclear cells (%LMC) for the carpus and the tarsus joint.
Materials and methods

Sample size

Two studies were conducted to create the reference intervals. Both trials were approved through the Iowa State University Institutional Animal Care and Use Committee. The American Society of Veterinary Clinical Pathologists establishes a minimum sample size of >20 animals to report reference intervals.\textsuperscript{4} It was anticipated that an inadequate fluid volume would be collected or blood contamination in the sample would occur frequently when collecting joint fluid samples, thus the sample size of 54 pigs was used and the study was divided and executed at two different time periods (trial 1 and 2).

Animals and Housing

Thirty healthy, non-lame pigs (trial 1) and 24 healthy, non-lame pigs (trial 2) were weighed and moved into individual pens during the studies. Both groups were commercial, crossbred finisher pigs (16 to 18 weeks of age) that were housed in the same continuous flow finisher barn before enrollment. The pens were rectangular, partially slatted, with 15 to 20 pigs per pen and the stocking density was approximately 9 ft\textsuperscript{2} per pig. The pigs were owned and supplied by an Iowa State University swine farm. Pigs were procured from a source with no reported clinical history of lameness. Trials 1 and 2 were separated by approximately six weeks. Both groups were an equal mix of barrows and gilts. All pigs were fed \textit{ad libitum} commercial finisher feed without antibiotics for the duration of the trial. The diets met all swine nutrition National Research Council requirements.\textsuperscript{5} At selection, pigs were given a physical exam by a veterinarian, which included palpation of joints, and pigs had to be free of clinical signs to enter the trial. Pigs were assessed while standing and during locomotion prior to selection.
Lameness and joint scoring

Upon selection, pigs were given a lameness score and joint swelling score. The scoring system used was previously reported. To be included in the study, pigs must have scored a zero on both lameness and gross swelling score. Briefly, for the lameness scoring, a score of zero indicated that the animal moved freely and used all four limbs evenly. The joint swelling score was modified slightly for this study, as a score of zero for the joint swelling scale indicated no swelling.

Anesthesia treatment groups

To achieve general anesthesia, all pigs were given intramuscular anesthetic agents and were under general anesthesia during collection of joint fluid. Pigs were not intubated. Trial 1 pigs received an IM injection of tiletamine hydrochloride and zolazepam hydrochloride (4.4mg/kg), ketamine (2.2mg/kg), and xylazine (4.4mg/kg) combined in the same syringe. Trial 2 pigs were randomly allocated to one of four treatment groups. Group one received the same anesthetic protocol as trial 1 pigs. Group two received 0.03 mg/kg of acepromazine, 2.2 mg/kg of ketamine, and 4.4 mg/kg of tiletamine hydrochloride and zolazepam hydrochloride given IM. Group three received an IM injection of 0.3 mg/kg of acepromazine and 4.4 mg/kg of tiletamine hydrochloride and zolazepam hydrochloride all from the same syringe and an aseptically placed epidural. The lumbosacral epidural consisted of 2% lidocaine dosed at 2.2 mg/kg up to 10 mL. Group four received an IM injection of 0.5 mg/kg of acepromazine and 5 mg/kg of ketamine all from the same syringe and an aseptically placed epidural. The lumbosacral epidural consisted of 2% lidocaine dosed at 2.2 mg/kg up to 10 mL. Individual pig weights were used to calculate anesthetic dosages. The anesthetic treatment groups in trial 2 were part of
a separate study to assess the quality of anesthetic procedures and results from this analysis are to be published elsewhere.

To be considered eligible for the joint fluid collection procedure, the pig must have been fully anesthetized, recumbent, and have a negative palpebral response and toe withdrawal response. From the anesthetic injection onwards, pigs were monitored closely. Heart rate, respiratory rate, temperature, and depth of sedation were monitored at least every 10 minutes while the pig was anesthetized and every 30 minutes to one hour during the recovery process.

**Antemortem centesis**

Once anesthetized, pigs were positioned in dorsal recumbency. One tarsus and carpus per pig was prepared by shaving followed by three steps: a chlorhexidine soap scrub, alcohol scrub, and final prep with tincture of chlorhexidine. Steps one and two were repeated to ensure asepsis of the centesis site. Once the joints were prepared for sampling, the joint was dried using sterile gauze and a sterile adhesive drape was applied (trial 1 only). In Trial 2, the joints were left to air dry for approximately 45 seconds.

To aspirate the joint fluid, an 18G 1.5 inch needle on a 12 cc sterile syringe was inserted into the dorso-lateral aspect of the joint and negative pressure was used to aspirate the joint fluid into the syringe. Clean garments and clean gloves were worn for sampling. A gross description including color, clarity, and blood contamination was recorded for each joint fluid sample. One veterinarian with previous experience with swine joint fluid aspiration collected all the joint fluid samples for the entire study.
Postmortem centesis

If two suitable joint fluid samples (one from the carpus and one from tarsus) were not collected antemortem for a pig in either trial due to insufficient anesthetic plane, insufficient volume, or gross blood contamination, the contralateral joint was sampled postmortem using the same methods as described above.

Sample handling

The minimum volume of joint fluid required to complete all the tests was 0.5 mL. If this was not attained, the sample was discarded. Half of the volume of joint fluid collected was immediately placed into a 2 mL EDTA tube, agitated and submitted for fluid analysis and cytologic evaluation within 8 hours of collection. The remaining aliquot for each joint was pooled by pig at the lab and submitted for aerobic bacterial culture, MHS PCR, and MHR PCR at the ISU VDL. Samples were stored on ice within two minutes of sample collection until submission to the diagnostic laboratory for analysis.

Euthanasia and trial end point

For trial 1 pigs, once antemortem sampling was completed, pigs were euthanized by penetrating captive bolt and exsanguinated. Insensibility was determined by absent corneal reflex and death confirmed by absence of respiration and heartbeat. For trial 2, pigs were recovered from anesthesia and monitored for seven days then euthanized using the same procedures described above.

Samples collected at necropsy

A systematic postmortem was completed for each pig including the collection of tonsils for testing of DNA MHS and DNA by MHR PCR assays (trial 1 only) and clinical examination of joints. Tonsils were not collected in trial 2 due to the very low prevalence
of MHS and MHR found in trial 1. Identification and recording of gross abnormalities to synovial fluid, synovium, and cartilage was completed for the left and right humeroscapular, radial-humeral, radial meta carpal, coxofemoral, tibiofemoral, metatarsal and tarsocurral joints. If gross abnormalities were identified in a joint, the corresponding joint fluid and synovial tissue were submitted for diagnostic testing including histology, culture and PCR as described above. Internal organ evaluation was performed and fresh and fixed tissue(s) were submitted for diagnostic testing including histology, molecular testing, and culture as per discretion of pathologist if gross abnormalities were identified.

**Oral fluids**

Oral fluids were collected from pens containing pigs enrolled in either trial two weeks before the commencement of the trial, and at the end of the trial using clean cotton rope as described in a previous study. The oral fluids were submitted for MHR and MHS PCR.

*Mycoplasma hyosynoviae and Mycoplasma hyorhinis sample processing, DNA extraction, and PCR*

For oral fluids, joint fluid, and tonsil tissue, DNA extraction was performed using a nucleic acid isolate kit and a magnetic particle processor as per the manufacturers’ instructions and as described previously. Joint fluid was processed using a homogenizer for five minutes at maximum speed as an additional step during the extraction process. The carpus and tarsus sample from each pig was pooled for the MHS and MHR PCR. Tonsils were processed as per standard operating protocol at the ISU VDL. By using forceps and scissors, tonsil was weighed and subsequently minced/ground prior to placing into a
stomacher bag and processed as a 10% homogenate. Oral fluids were processed using a high-volume modified lysis procedure. The procedures for DNA amplification and detection for MHS and MHR used in this study are provided in detail in another study.

**Culture**

Joint samples submitted for routine culture in the ISU VDL were setup following the standard protocol. The carpal and tarsal sample from each pig was pooled for the culture. This setup included aerobic and anaerobic conditions, the inclusion of *Staphylococcus aureus* nurse for organisms requiring *nicotinamide adenine dinucleotide*, and selective media consisting of brilliant green agar for *Salmonella* sp. isolation. No additional selective or non-selective enrichment was performed on the samples and plates were incubated for a minimum of 48 hour prior to reporting no growth. The samples were considered to have “no significant growth” if non-pathogenic organisms were isolated. The significance of the growth was determined by the veterinary pathologist on each case.

**Histopathology**

After collecting the joint fluid the joint was then opened to obtain a sample of synovium which was immediately placed in 10% buffered formalin solution. Histopathology scoring was performed by a board certified veterinary pathologist who was blinded to treatment groups. A scoring rubric was modified from another study on infectious arthritis and included evaluation of synovial proliferation, synovial alteration, inflammation, edema, neutrophil infiltration, presence or absence of fibrin, and hemorrhage. Abnormal synovial histology was defined as having a proliferation, alteration, inflammation, or edema
score of 2 or greater (moderate changes) or neutrophil infiltration or presence of fibrin or hemorrhage.\textsuperscript{10}

**Cytologic evaluation and fluid analysis**

The cytologic evaluation and fluid analysis procedures for joint fluids submitted in EDTA\textsuperscript{f} are as follows. The sample was checked for blood clots in the EDTA\textsuperscript{f} tube visually and this was a dichotomous outcome (yes/no to blood clot). A TNCC and RBCC was performed using a hematology analyzer.\textsuperscript{j} Hyaluronidase was added to the sample before the cell counts.\textsuperscript{11} Color and clarity was reported. Samples were discarded and were not included in the reference interval if they were red due to gross hemo-contamination. Clarity was considered acceptable if the sample was clear, slightly hazy, or cloudy. Samples with fibrin, blood clots, or purulent material were rejected. The pH was measured and recorded using pH paper\textsuperscript{k}. Total protein was measured with a digital refractometer\textsuperscript{l}. If a sample was too turbid to read, a hematocrit tube was spun down and the percent total solid was read from the supernatant. If volume was <0.5 mL, the TP measurement was not valid and it was recorded as insufficient volume.

For cytologic evaluation slide preparation a direct smear was made with a camel hair brush. A cytopsin sample was made using 100 µl of synovial fluid spun at 72 g for 10 minutes.\textsuperscript{m} The direct and cytopsin samples were analyzed by a board certified clinical pathologist and a 300-cell differential cell count were performed. The fluid analysis and cytologic evaluation was performed individually on each tarsus and carpal sample per pig. A description of the LMC morphology used for the cytologic evaluation was as follows: in a normal joint, the large mononuclear cells in fluid are typically round-shaped cells with a moderate nuclear:cytoplasmic ratio. They often contain round, centrally placed nuclei with
dense chromatin; their cytoplasm is basophilic and lacks vacuolization. Morphologically, both quiescent macrophages and synoviocytes may have this appearance and thus the encompassing term large mononuclear cells is used to categorize them.

**Summary of inclusion criteria**

For the individual animal to be included in the study, the pig must have been healthy, non-lame with no grossly visible swelling of joints. Additionally, for trial 1, the tonsil PCR for MHS and MHR must be negative. For an individual joint fluid sample to be included in the study, the fluid must have been within the acceptable clarity and color stipulations described above. The fluid sample must have no growth on aerobic culture and be PCR negative for MHS and MHR. Synovium histology from the joint must have been free from histopathologic changes indicative of active joint disease. At least 0.5 mL of volume was required for the complete fluid analysis.

**Statistical Analysis**

Reference intervals were established for TNCC, TP, pH, RBCC, %NEUTS, %LYMPHS, and %LMC for the carpus and tarsus joints. Reference intervals were established in accordance with the published guidelines for reference interval determination by the American Society of Veterinary Clinical Pathologists.\(^4\) Data was evaluated with the D’Agostino and Pearson omnibus test to determine normality; data was assessed with the Tukey test for identification and removal of statistical outliers only. Suspect outliers were not removed. For parametric variables, robust methodology on native data was used to calculate the upper and lower reference limits, and a bootstrap method was used to calculate the 90% confidence intervals around these limits.\(^4\) For nonparametric variables, a Box-Cox transformation was first performed and then robust methodology was used to calculate the upper and lower reference
limits, and a bootstrap method was used to calculate the 90% confidence intervals around these limits.\textsuperscript{4,12} To assess the contribution of blood contamination to \%NEUTS in the sample, a correlation analysis was performed between RBCC and \%NEUTS for both carpal and tarsal samples. If the distributions of RBCC and \%NEUTS were normal, then a Pearson correlation coefficient was calculated. If the distributions were skewed, then a Spearman correlation coefficient was calculated.

Additionally, for each joint, the assessed variables were evaluated to determine if differences existed between antemortem and postmortem collected samples. For parametric variables, a simple T-test was used to compare the means of the antemortem and postmortem samples; a Wilcoxon rank sum test was used for nonparametric variables to compare medians. Statistical significance was set at $p < 0.05$. Reference interval determination and statistical comparisons were performed using graphing and reference interval analysis software packages.\textsuperscript{\textit{n,o}}

\textbf{Results}

\textbf{Oral fluids}

Oral fluid testing for MHS and MHR from pens holding pigs used in trial 1 and 2 were negative two weeks prior to the start and at the end of both trials.

\textbf{Selection of individual pigs}

There were eight pigs that were rejected at physical exam for the following reasons: umbilical hernia (3), diarrhea (2), conjunctivitis (3). There were four pigs excluded due to joint swelling and no pigs were excluded for lameness. These four pigs were not lame and palpation revealed very slight swelling of a bursa on the lateral aspect of the tarsus and thus these pigs were excluded.
Inclusion and exclusion of joint samples

There was a total of 50 tarsus and 53 carpus joint fluid samples from 54 finisher hogs collected ante or postmortem from four hogs (tarsus) and one hog (carpus). The mean weight of pigs (standard deviation) was 130.3 kg (8.2 kg). A separate reference interval database was created for carpus and tarsus samples. Six samples, representing carpus and tarsus samples from three animals, were excluded due to tonsil positive PCR results for MHR. All tonsils were negative for MHS using PCR. The remaining samples were all negative using PCR for MHS and MHR. Ten samples were excluded due to mild abnormalities in synovium histology. Of these ten samples, seven were tarsus samples and three were carpus samples. Four samples, (three tarsus and one carpus) were excluded due to positive bacterial culture results. Culture results identified *Staphylococcus epidermis* and *Acinetobacter johnsonii*, and thereby were considered skin contaminants. Fluid analysis was unable to calculate TNCC, RBCC, TP, and pH data due to clotting in the EDTA tube for seven tarsus and three carpus samples. There was one carpus sample where TP and pH could not be determined due insufficient quantity of fluid. For the tarsus samples, insufficient sample volume prevented the determination of TP in two samples and pH in one. No visible osteochondrosis lesions were identified in carpal and tarsus joints used for the reference intervals. In the elbow of some pigs (11 total; 20%) there were small (<1 cm wide by <3 mm deep) linear, irregular depressions or folds in the cartilage that were suspect of osteochondrosis manifesta. Synovial fluid and synovial tissue collected from these joints revealed no bacterial pathogens and mild or no inflammatory changes to the synovium. None of
the pigs sampled were clinically lame, had abnormal gaits, or had grossly visible joint enlargements.

**Antemortem and post mortem samples**

Out of the samples that met eligibility criteria, for the tarsus, there were 23 antemortem and 14 postmortem samples. For the carpus, 23 samples were collected antemortem and 23 were collected postmortem. Group three in trial 2 was sampled exclusively postmortem due to ineffectiveness of the ketamine and acepromazine to achieve sufficient anesthetic plane for synovial centesis. There were no differences in antemortem and postmortem samples for the carpal samples for any of the cytologic evaluation and fluid analysis parameters and these two datasets were combined. For the tarsus samples, the antemortem samples had higher RBCC relative to the postmortem samples. The main determinant of RBCC is the sample collection process itself. Red blood cell count generally reflects blood contamination as opposed to pathological changes related to joint inflammation. For this reason, antemortem and postmortem samples were combined for the tarsal samples and used to create the reference intervals.

**Fluid analysis and cytologic evaluation**

Carpal and tarsal fluid analysis and cytologic evaluation reference variables of healthy pigs are listed in Tables 1 and 2. The final number of samples used to establish reference intervals for each cytologic evaluation and fluid analysis variable is available in Tables 1 and 2. For the tarsal samples, all but one sample was slightly hazy/cloudy. In terms of color, the tarsal samples were light pink or red (n=27), light yellow (n=5), colorless (n=4), or light orange (n=1). The carpus samples were all slightly hazy/cloudy and the colors reported were as follows: light pink or red (n=22), light yellow (n=13), colorless (n=9), or light orange (n=2).
For both carpal and tarsal samples, the distribution of RBCC and %NEUTS was skewed to the right and the Spearman correlation coefficient was used to assess correlation. For carpus samples, the Spearman correlation coefficient was 0.6346 (p-value <0.0001) indicating moderate correlation. For tarsal samples, the Spearman correlation coefficient was 0.2954 (p-value 0.1130) indicating there was weak or no correlation.

**Necropsy results**

At necropsy, minor gross lesions in internal organs were identified in most of the pigs (83%) from both trials. The most common lesion was minor lung edema, with atelectasis or consolidation (<15% of each lung affected). For all gross lesions, diagnostic investigation did not reveal an active infectious process contributing to gross pathology and none of the pigs showed clinical signs such as coughing, nasal discharge, or diarrhea. The lung consolidation was attributed to previous infectious insult, related to blood pooling postmortem or due to the cause of death (penetrating captive bolt).

**Discussion**

This study produced reference intervals from non-lame commercial finisher pigs for carpal and tarsal synovial fluid cytologic evaluation. There is only one other study to the author’s knowledge that has published cytologic evaluation and fluid analysis variables for swine synovial fluid. It reported pH, specify gravity, glucose per 100mL, TP, RBCC, TNCC, and differential white cell percentages for five normal adult pigs. The sampled joint and joint fluid was grossly normal and sterile based on culture although it is unclear which joints were sampled. The average TP reported was 3.9g/dL, TNCC was 220 cells/µL, and RBCC was 0.005 x 10^6 cells/µL. These averages fall within the reference intervals reported in this study for carpus and tarsus, except for TP which was slightly above the determined upper 90% confidence
interval for the carpus. For the differential cell percentages, reported %LYMPHS were higher (mean: 49%) than reported in this study for the carpus but not for the tarsus. Other differential cells percentages appeared to be within the intervals reported here.14

Compared to horses, it appears that the upper reference limit for TNCC and TP is higher for swine. Reference intervals for horses reported TNCC <1000cells/µL and TP <2.0g/dL as normal; however, for swine, <4000cells/µL and <3.90g/dL seems more appropriate based on the values we present here.2,15 In dogs and cats, TNCC is generally <1000-3000cells/µl (dogs) and <1100cell/µl (cats).3,16 For canine synovial fluid, normal TP levels, as with all other variables vary by laboratory but are generally between 1.8 to 4.8g/dl, with <2.5g/dl considered normal.3 In this study, the pH lower and upper reference limits were 6.1-7.2 for carpus and 6.2-7.0 for tarsus which was lower than pH ranges published for horses and companion animals. It should be noted that the lower limit of detection for pH in this study was 6.0. For horses, pH of 7.30±/0.06 is considered normal.2,1 For dogs and cats, a pH of 7.2 to 7.4 is considered normal.3 Normal joint fluid should have low cellularity and guidelines from other species report <80-90% LMC, <10% neutrophils, and <20% lymphocytes.2,3,17 In horses and dogs, neutrophils make up approximately <10-12% of the differential cell percentages.3,17-19 In cats, the percent neutrophils that is considered normal is less than 39%, which is closer to values identified for hogs in this study (<46% for carpus and <33% for tarsal samples).17 As with other species, LMC are also the predominant cell type in swine synovial fluid. There is a large range between the lower and upper reference limit for all the differential cell percentages in Tables 1 and 2, particularly for LMC.

There was some correlation between RBCC and %NEUTS for the carpus sample set which may indicate that a portion of neutrophils in each sample were due to non-grossly visible
blood contamination. For the tarsal samples, however, it does not appear that %NEUTS were contributed by sampling hemorrhage due to the weak correlation between RBCC and %NEUTS.

During infectious and degenerative pathological processes, morphological and percentage changes to the LMCs, neutrophils and lymphocytes can be observed. Cytologic evaluation and fluid analysis on synovial fluid can be used to identify normal joints, degenerative joint disease, acute injury and inflammatory joint disease, which can be infectious or immune mediated.\textsuperscript{2,3,18,20} Currently, there are no other experimental studies describing the changes in synovial cytologic evaluation and fluid analysis associated with various swine-specific joint pathogens and extrapolation from other species would be necessary to interpret changes in swine synovial fluid.

There are several variables which were not controlled for in this study and there is limited information on how these variables may impact cytologic evaluation and fluid analysis outcomes on normal swine synovial fluid. The pigs in these studies were crossbred, raised on partially slatted floors, and were close to market weight. Differences between genetic lines, age groups, stocking density, structural conformation, housing, and nutrition may impact cytologic evaluation and fluid analysis variables for synovial fluid.

An additional consideration is that mild \textit{osteochondrosis manifesta} lesions were observed in the forelimbs of some pigs. None of the joints included in the reference intervals had \textit{osteochondrosis manifesta}. \textit{Osteochondrosis} can cause changes to cytologic evaluation and fluid analysis results in circumstances where it causes degenerative joint disease and osteoarthritis.\textsuperscript{20} Lesions consistent with \textit{osteoarthritis} or degenerative joint disease were not identified in any joints.
The creation of a reference interval dataset for synovial fluid from non-lame, healthy finisher pigs provides a novel diagnostic tool to practitioners and production companies. In equine and canine medicine, synovial fluid cytologic evaluation and fluid analysis is a core diagnostic test for lameness and arthritis. For horses, it is considered an extremely useful tool for the diagnosis of septic arthritis.\textsuperscript{2,21} Coupled with culture, molecular testing for mycoplasma species, and histology on the joint tissue, swine cytologic evaluation and fluid analysis helps complete the diagnostic picture.\textsuperscript{2} Due to the transient nature of many infectious arthritis agents, multiple pieces of evidence indicative of infectious agents are critical for an accurate diagnosis. This complete diagnostic picture allows veterinarians to more confidently and accurately create a diagnostic and treatment plan for a given patient. Improved diagnostic and treatment plans are a direct benefit to pigs and caretakers alike. Moreover, these novel ante mortem reference intervals for synovial fluids allows veterinarians to document the effect of health interventions in lame pig populations without the need to euthanize pigs.
Footnotes

\(^a\) Telazol, tiletamine HCl and zolazepam HCl injection, (100mg/mL when reconstituted) Zoetis, Kalamazoo, MI

\(^b\) Ketamine HCL injection (100mg/mL) Zoetis, Kalamazoo, MI

\(^c\) Xylazine injection (100mg/mL) VetOne/Akorn, Inc. Lake Forest, IL

\(^d\) Acepromazine Maleate injection (10mg/mL), VetOne/Akorn, Inc. Lake Forest, IL

\(^e\) 2% Lidocaine, MWI, Boise, ID.

\(^f\) BD Bioscience, San Jose, CA

\(^g\) MagMAX™ Total Nucleic Acid Isolation Kit, Thermo Fisher Scientific; Waltham, MA

\(^h\) KingFisher® 96 magnetic particle processor, Thermo Fisher Scientific; Waltham, MA

\(^i\) BioSpec Bead Beater, Bartlesville, OK

\(^j\) ADVIA 2120, Siemens, Malvern, PA.

\(^k\) Hydrion pH 6.0 – 8.0, MicroEssentialLab, Brooklyn, NY

\(^l\) Clinic-Check refractometer, Reichert Technologies, Depew, NY

\(^m\) Cytopsin 4, Thermo Scientific, Waltham, MA

\(^n\) GraphPad Software, Inc., La Jolla, CA

\(^o\) Reference Value Advisor, Biostatistiques Ecole Nationale Vétérinaires de Toulouse, Toulouse, France
References


Table 1. Reference intervals for clinical pathology variables from carpal joint fluid samples from normal, non-lame commercial finishing hogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TNCC* (cells/µL)</th>
<th>Protein (g/dL)</th>
<th>pH</th>
<th>RBCC† (1x10^6 RBC/µL)</th>
<th>Neutrophil %</th>
<th>Lymphocyte %</th>
<th>LMC‡ %</th>
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<td>Lower limit of reference interval</td>
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*Total nucleated cell count
†Red blood cell count
‡Large mononuclear cell
Table 2. Reference intervals for clinical pathology variables from tarsal joint fluid samples from normal, non-lame commercial finishing hogs.

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<tr>
<th>Parameter</th>
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<th>Protein (g/dL)</th>
<th>pH</th>
<th>RBCC† (1x10^6 RBC/µL)</th>
<th>Neutrophil %</th>
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*Total nucleated cell count
†Red blood cell count
‡Large mononuclear cell
CHAPTER 5. CONCENTRATIONS OF TYLVALOSIN AND 3-O-ACETYLTYLOSIN ATTAINED IN THE SYNOVIAL FLUID OF SWINE AFTER ADMINISTRATION BY ORAL GAVAGE AT 50MG/KG AND 5MG/KG

Published in the Journal of Veterinary Pharmacology and Therapeutics (JVPT) in 2016, DOI 10.1111/jvp.12309

P. Canning,¹ primary researcher, contributed to protocol, completed live animal work, data collection, data analysis and preparation of the manuscript

J. Bates,¹ co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

K. Skoland,¹ co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

J. Coetzee,² co-advisor on protocol and data analysis and reviewer of manuscript

L. Wulf,² conducted analytical chemistry assays and reviewed manuscript

S. Rajewski,² conducted analytical chemistry assays and reviewed manuscript

C. Wang,³ assisted with data analysis and reviewed manuscript

L. Karriker¹ co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

¹Swine Medicine Education Center, College of Veterinary Medicine, Iowa State University, Ames, IA USA.

²Pharmacology Analytical Support Team, Veterinary Clinical Sciences, Iowa State University, Ames, IA USA.

³Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA USA.
Abstract

The objectives of this study were to determine the concentration of tylvalosin (TVN) and its metabolite, 3-O-acetyltylosin (3AT) in the synovial fluid of growing pigs when administered as a single bolus by oral gavage at target doses of 50 mg/kg (Trial 1) and 5mg/kg (Trial 2). TVN is a water-soluble macrolide antimicrobial used in swine production. The stability of the drug in synovial fluid samples stored at -70°C up to 28 days was also evaluated in Trial 2. In Trial 1, eight pigs were randomly assigned to one of eight time points for euthanasia and synovial fluid collection: 0, 1, 2, 3, 4, 6, 9, 12 hours post-gavage. For Trial 2, twenty-four pigs were randomly allocated to one terminal collection time point at 0, 2, 4, 6, 8, or 10 hours post-gavage. Synovial fluid was analyzed to determine TVN and 3AT concentrations. TVN and 3AT were detected in Trial 1 at all time points, except 0 hour. At 2 hours post-gavage for Trial 2, the mean concentrations peaked at 31.17 ng/mL (95% CI: 18.62 – 52.16) for TVN and at 58.82 ng/mL (95% CI: 35.14 – 98.46) for 3AT. Storage duration did not impact TVN or 3AT concentrations (p-value=0.9732).
Brief communication

Tylvalosin (TVN) has been approved for oral administration through drinking water (50 ppm equivalent to approximately 5 mg/kg/day for five consecutive days) to control of porcine proliferative enteropathy (PPE) associated with Lawsonia intracellularis infection in groups of swine in buildings experiencing an outbreak of PPE. Although extra-label use of tylvalosin to treat infectious arthritis caused by Mycoplasma spp. has been reported, detection and quantification of TVN in porcine synovial fluid has not been published to date. The objectives of this study were to determine if tylvalosin and its metabolite, 3-O-acetyltlylosin (3AT) distribute to the synovial fluid of pigs at a target dose of 50 mg/kg (Trial 1) and at 5mg/kg (Trial 2) after a single bolus via oral gavage. The label dose specifies administering the product at 50 ppm through the drinking water for five days. Based on estimated water consumption of 10% body weight, it is estimated that pigs would orally ingest 5 mg/kg of TVN throughout the course of one day. The objective of Trial 1 was to establish if TVN and 3AT distribute to the synovial fluid. As Trial 1 was a proof of concept trial, ten times label dose (50 mg/kg) was selected based on the hypothesis that a larger dose would result in larger TVN and 3AT concentrations in the joint which would increase the likelihood of detection of these compounds. As TVN and 3AT were detected in synovial fluid in Trial 1, Trial 2 then aimed to quantify joint concentrations under dosing conditions more similar to field administration. The dose chosen in Trial 2 reflected dosing rates used in the field (5 mg/kg/day) while serving as a continuation of Trial 1 before proceeding to larger scale oral medication field trial. The stability of TVN and 3AT in synovial fluid samples stored at -70°C for 7, 14, and 28 days was also evaluated in Trial 2.
In Trial 1, eight healthy pigs weighing approximately 22 kg were purchased from a high health commercial operation and housed in one indoor pen. In Trial 2, twenty-four clinically healthy pigs weighing approximately 45 kg each were housed in individual pens. A larger weight class was used in Trial 2 to facilitate the collection of sufficient quantities of joint fluid for the HPLC-MS analysis. Both studies were approved by the Iowa State University Institutional Animal Care and Use Committee. Pigs were fed an antibiotic-free, age-appropriate commercial diet of corn, soybean meal, dry distillers grains, and vitamin mineral premix ad libitum. Feed was withheld from 12 hours prior to oral gavage until the end of the study.

In Trial 1, one pig was randomly allocated to one of eight synovial fluid collection time points: 0, 1, 2, 3, 4, 6, 9, 12 hours post-gavage. In Trial 2, two pigs of each sex were randomly allocated to the following time points for synovial fluid collection: 0, 2, 4, 6, 8, 10 hours post-gavage. Animals received tylvalosin at a target dose of 50 mg/kg and 5 mg/kg for Trial 1 and 2, respectively. Aivlosin Water Soluble Granules™ (62.5% w/w tylvalosin as tylvalosin tartrate, ECO Animal Health) was dissolved in water and administered in a single bolus by oral gavage. Pigs were humanely euthanized at specified time points post-gavage and synovial fluid was collected aseptically via fine needle aspirate from all pigs. Synovial fluid samples with blood contamination were discarded. Synovial fluid samples collected from different leg joints were pooled by the individual pig and split into two aliquots (Trial 1) or four aliquots (Trial 2) per pig. Aliquot one was tested immediately for the presence of TVN and 3AT. Aliquot two, three (Trial 2 only), and four (Trial 2 only) were frozen at -70°C for 7, 14, and 28 days respectively and then analyzed for TVN and 3AT.
Concentrations of TVN and 3AT in porcine synovial fluid were measured with high-pressure liquid chromatography (1260 Infinity Pump and Autosampler, Agilent) with mass spectrometry detection (ABSciex QTRAP 4500). Calibration standards were prepared by spiking 100 µL blank porcine synovial fluid to obtain final concentrations of 1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 1000 ng/mL. Quality control final concentrations were 3, 300, and 750 ng/mL. Internal standard, roxithromycin, was added to each standard, unknown sample, and quality control sample at a concentration of 5 ng/mL. The samples were diluted with 900 µL of 0.1 molar ammonium acetate (pH 4.7), vortexed, and then centrifuged at 2000 rpm for 20 minutes. The drug was extracted using Phenomenex Strata-X Polymeric Reverse Phase (33 µm, 60 mg, 3 mL) solid phase extraction (SPE) cartridges. The SPE cartridge was conditioned with 2 mL of methanol, equilibrated with 2 mL of water, loaded with the sample, washed with 1 mL of 25% methanol in water and dried for 10 minutes. After elution with 2 volumes of 0.75 mL acetonitrile, the sample was dried under a stream of nitrogen, reconstituted in 25% (v/v) acetonitrile in water, and transferred to an injection vial. The vials were spun down at 2000 rpm for 10 minutes. Injection volume was set to 10 µL. The mobile phase consisted of A: 5 mM ammonium formate in water and B: 5mM ammonium formate in methanol at a flow rate of 0.3 mL/min. The mobile phase began at 35% B with a linear gradient to 85% B at 4 minutes which was maintained for 2 minutes, followed by a linear gradient to 98% B at 6.3 minutes, followed by re-equilibration to 35% B. Separation was achieved using a Phenomenex Gemini C18 column (50 x 2.0 mm, 3 µm particles, 110 Å) at room temperature. The SRM transition used for quantitation of TVN was 108.8 and the transition for quantitation of 3AT was 814.5.
The standard curves of TVN and 3AT concentration determined using porcine synovial fluid were linear from 1 to 1000 ng/mL and were accepted when the correlation coefficient exceeded 0.99 and measured values were within 20% of the actual values. The lower limit of quantification (LOQ), was 1.0 ng/mL for all samples in Trial 1 and for samples in the 0, 7, and 14-day storage duration groups in Trial 2. The LOQ for the 28-day storage duration samples in Trial 2 was 2.5 ng/mL.

No statistical analysis was performed on TVN and 3AT concentrations in Trial 1 due to the limited sample size per time point. For Trial 2, a linear mixed model (SAS 9.4) was generated to determine least squares means and 95% confidence intervals of the log concentration of TVN and 3AT at each sampling time and storage duration. Concentration of TVN was log transformed to reduce skewed distribution of raw data and satisfy assumptions of normal distribution. Drug (TVN or 3AT), storage duration time (0, 7, 14, and 28 days) and sampling time (0, 2, 4, 6, 8, and 10 hours post-gavage) were fixed variables. The dose received by each pig was added into the model as a co-variable. Pig (one to 24) was entered as a random variable. Interactions between drug and storage time, drug and group, storage time and group, and drug/storage time/group were included in the model. Partial F tests for fixed effects were conducted on the variables and interaction terms.

Results from Trial 1 are shown in Table 1 and demonstrate that TVN and 3AT were detected in the synovial fluid of the study pigs. Figures 1 and 2 demonstrate the mean concentration and its 95% confidence intervals for TVN and 3AT respectively at 2, 4, 6, 8 and 10 hours post gavage for all four storage durations evaluated. For the 0-hour time group, the synovial concentrations of both compounds were below the LOQ and are not included in the figures.
Sampling time point and drug (TVN or 3AT) significantly affected log concentration (both p-values <0.0001). Dose was weakly significant and thus remained in the model (p-value 0.0413). Storage duration did not impact the concentration of TVN and 3AT in the synovial fluid samples (p-value=0.9732).

There is limited minimum inhibitory concentration (MIC) data available for *Mycoplasma* spp. associated with infectious arthritis in swine and a lack of randomized controlled trials evaluating tylvalosin in an experimentally induced or naturally occurring *Mycoplasma* spp. arthritis models. Ninety percent of tylvalosin MICs for *M. hyosynoviae* were reported to be <60 ng/mL (Rosener et al., 2013). MIC data for *Mycoplasma* spp for 3AT are not published to date and the antimicrobial activity of 3AT is unknown. Clinical breakpoints have not been established for tylvalosin for any clinical scenario or pathogen. Tissue concentrations alone can be poor indicators of therapeutic efficacy for macrolides due to their propensity for intracellular accumulation. As a result, comparisons of synovial fluid concentrations to MIC values may be unrewarding in terms of predicting response to treatment. Additional studies to correlate TVN and 3AT MIC information to clinical improvements are crucial for the judicious use of TVN for the treatment of infectious arthritis. In the field, tylvalosin is administered in solution through the water lines and daily dose is determined by the daily water intake of each animal. This dosing mechanism is more variable than administration by oral gavage and may affect joint concentrations of TVN and 3AT attained in each pig.

Trial 1 was a proof of concept study designed to establish if TVN and 3AT can be detected in the synovial fluid of pigs orally gavaged at ten times label dose. Based on the identification of TVN and 3AT in joint fluid in Trial 1, Trial 2 furthered this work by
quantifying synovial fluid concentrations of these compounds with a dosing rate more similar to the label dose. Results from Trial 2 also demonstrated that TVN and 3AT concentrations remain stable for 28 days at -70°C in the joint fluid matrix. The authors are not aware of comparable studies or reports of the distribution and detection of TVN and 3AT in the synovial fluid of pigs for comparison.

Acknowledgements

The authors would like to acknowledge the Swine Medicine Education Center, ISU CVM Field Services, ISU Swine Nutrition Farm staff, and PhAST lab staff and interns that contributed to the studies. ECO Animal Health/Pharmgate provided financial support to both projects.

Reference


Table 1: Concentration of TVN and 3AT in the synovial fluid of eight pigs after oral gavage at 50mg/kg

<table>
<thead>
<tr>
<th>Time post-gavage (hours)</th>
<th>TVN (ng/mL)</th>
<th>3AT (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>264.8</td>
<td>602.2</td>
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<tr>
<td>2</td>
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<td>6</td>
<td>84.9</td>
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<tr>
<td>12</td>
<td>27.7</td>
<td>81.8</td>
</tr>
</tbody>
</table>
Figure 1. The mean and upper and lower 95% confidence intervals for the concentration of tylofosin (TVN) in the synovial fluid of pigs dosed at 5mg/kg at 2, 4, 6, 8 and 10 hours post gavage. Values not reported for the 14 day storage group at 8 hours and the 28 day storage group at 6, 8 and 10 hours as the TVN concentration for at least one pig per group of four at those time points was below the LOQ.
Figure 2. The mean and upper and lower 95% confidence intervals for the concentration of 3AT in the synovial fluid of pigs dosed at 5mg/kg at 2, 4, 6, 8 and 10 hours post gavage. Values are not reported for the 28 day storage group at 10 hours post-gavage as the 3AT concentration for at least one pig at that time point was below the LOQ.
CHAPTER 6 DETERMINATION OF THE CONCENTRATION OF TYLVALOSIN AND 3-O-ACETYLTYLOSIN IN THE SYNOVIAL FLUID AND PLASMA OF PIGS WHEN ADMINISTERED ORALLY IN THE DRINKING WATER FOR FIVE CONSECUTIVE DAYS

This manuscript was reduced to 1500 words and submitted to JVPT in July 2017 as a short communication as per the request of the JVPT editors. The full length paper is included below.

P. Canning, Primary researcher, created protocol, completed live animal work, data collection, data analysis and preparation of the manuscript

J. Bates, assisted with live animal work and data collection

K. Skoland, assisted with live animal work and data collection

J. Coetzee, co-advisor on protocol and data analysis and reviewer of manuscript

L. Wulf, completed analytical chemistry assays

S. Rajewski, completed analytical chemistry assays

C. Wang, advisor for statistical analysis

P. Gauger, co-advisor on protocol and data analysis and reviewer of manuscript

A. Ramirez, co-advisor on protocol and data analysis and reviewer of manuscript

L. Karriker, co-advisor on protocol and data analysis, assisted with live animal work and reviewer of manuscript

1Swine Medicine Education Center, College of Veterinary Medicine, Iowa State University, Ames, IA USA.

2RTI, LLC. Brookings, SD, USA
Abstract

Tylvalosin (TVN) is a water-soluble macrolide antimicrobial used in swine production. The objective of this study was to determine the concentration of tylvalosin and its metabolite, 3-O-acetyltlycosin (3AT), in the synovial fluid and plasma of growing pigs during five days of continuous water medication.

Sixty healthy finisher pigs were housed individually with individual waterers. Three pigs of each sex were randomly allocated to the following time points for sample collection: 0, 48, 60, 72, 84, 96, 102, 108, 114, and 120 hours on medication. TVN was administered daily in the water at a target concentration of 50 ppm. Water disappearance and medicated water concentration were measured daily. At each time point, six pigs were humanely euthanized and plasma and synovial fluid were collected for analysis.

Mean TVN synovial fluid concentrations ranged between <1 ng/ml (hour 0) to 4.5 ng/mL (hour 102). Synovial fluid and plasma concentration were moderately correlated (Pearson correlation coefficient of 0.9291). There was substantial variation between individual pigs in their daily water disappearance. Mean TVN water concentration ranged between 51 to 70 ppm.
Understanding variation between individual pigs in water intake, and subsequently ingested daily dose, is critical to ensuring animals are receiving an appropriate antimicrobial dose.

**Introduction**

Aivlosin® (65.5% w/w tylvalosin [TVN] tartrate, ECO Animal Health, London, UK) Water Soluble Granules (WSG) is a macrolide antibiotic with a zero-day meat withdrawal when administered in the drinking water (50 ppm tylvalosin) to pigs for five consecutive days in the United States. Anecdotally, tylvalosin has been reported to be effective in field conditions for treating *Mycoplasma spp.*-associated lameness. One previous study demonstrated that TVN and its metabolite (3-O-acetyltylosin, 3AT) can be found in the joints of healthy grower pigs after a single oral gavage at label dose (Canning et al., 2016). There is a need to expand on this study as gavage studies are not representative of field administration where pigs ingest portions of their daily dose at irregular intervals determined by their drinking behavior. The primary objective of this study was to determine the concentration of TVN and 3AT in the joints and plasma of healthy grower pigs during oral administration of Aivlosin® WSG through the drinking water for five consecutive days at label dose (50 ppm). The secondary objective was to quantify the correlation between joint and plasma in terms of TVN concentration.

**Materials and methods**

**Pigs and husbandry**

This study was approved by the Iowa State University Institutional Animal Care and Use Committee. Sixty healthy cross-bred pigs weighing approximately 45 kg were housed in individual pens. Pigs were acclimated to the individual pens for three days before the onset of
the study. Each pen was equipped with an individual pig waterer made from PVC pipe, which was the sole water source for each pig (Figure 1). The waterers were checked twice daily for proper function and troubleshooting leaking or plugged nipples, as needed. Each waterer was designed to hold 2.5 times the anticipated daily water intake of a 60 kg pig. At the same time daily, water disappearance was recorded for each pig and waterers were emptied and refilled with fresh, clean water. Water disappearance was recorded for each pig for two days (day -1 and 0) prior to the five-day medicated water administration (day 1 to 5).

Pigs were fed an antibiotic-free, age-appropriate, commercial diet of corn, soybean meal, dry distillers grains, and vitamin mineral premix *ad libitum*. Pigs enrolled in the trial did not consume antibiotics in the water or feed nor were they given injectable antibiotics for at least 21 days prior to the commencement of the trial.

**Administration of drug**

On day one (hour 0) to day 5 (hour 120) animals received a target concentration of 50 ppm TVN. To accomplish this, TVN (Aivlosin® WSG, ECO Animal Health, London, UK) was dissolved in water as per label directions into clean bulk tanks and then dispersed manually to pigs in each of their individual waterers. A new medication solution was provided to each pig every 24 hours. A digital scale (Ozeri, San Diego, CA, USA) was calibrated daily and used to weigh the TVN granules for dissolution into the bulk mixing tanks. Water samples from each bulk mixing tank were collected daily for the duration of the five-day water medication and submitted for 3AT and TVN analysis.
Experimental Design

Euthanasia and sample collection

Three pigs of each sex were randomly allocated (www.random.org) to the following time points for sample collection: 0 (day one), 48, 60, 72, 84, 96, 102, 108, 114, and 120 (day five) hours on medication. Hour 0 corresponds to 0700 (military time). There were ten sample collection time points in total over the five-day (120 hour) medication time period with six pigs per sample time point. At each time point, the six pigs were humanly euthanized using a penetrating captive bolt followed by exsanguination. A free catch, whole blood sample was collected in a heparin tube at exsanguination. At least one milliliter of synovial fluid was aseptically collected via fine needle aspirate from each pig. Synovial fluid samples with blood contamination were discarded. To attain sufficient volume, synovial fluid samples were collected from the tarsocrural and carpal joints were pooled by the individual pig. Whole blood samples were centrifuged for 5 minutes to reach 1000 g. The plasma was collected into a two milliliter cryotube (BD, Franklin Lakes, NJ, USA) and stored with the synovial fluid at -70°C prior to sample analysis.

A systematic gross assessment of the internal organs of the pigs was completed at necropsy. Any abnormalities were recorded and affected organs were submitted for further diagnostic investigation to the Iowa State University Veterinary Diagnostic Laboratory.
**Analytical Procedures**

**Water samples**

Concentration of TVN and 3AT in water samples were measured with high-pressure liquid chromatography (1260 Infinity Pump and Autosampler, Agilent, Santa Clara, CA, USA) with mass spectrometry detection ABSciex QTRAP 4500 (SCIEX, Framingham, MA, USA).

Calibration standards were prepared by spiking 100 µL 25% acetonitrile in 10% formic acid to obtain final concentrations of 1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 1000 ng/mL. Quality control samples were 15, 300, and 750 ng/mL. Water samples were diluted by a factor of 200 with 25% acetonitrile in 10% formic acid. Internal standard, roxithromycin, was added to each standard, sample, and quality control at a final concentration of 250 ng/mL, vortexed, and centrifuged at 2000 rpm for 10 minutes. This protocol is as per the standard operating procedures of the Pharmacology Analytical Laboratory at the Iowa State University Veterinary Diagnostic Laboratory.

Injection volume was 10 µL. The mobile phase consisted of A: 5 mM ammonium formate in water and B: 5 mM ammonium formate in methanol at a flow rate of 0.3 mL/min. The mobile phase began at 35% B with a linear gradient to 98% B at 6.3 minutes, followed by re-equilibration to 35% B. Separation was achieved using a Gemini C18 column (50 x 2.0 mm, 3 µm particles, 110 Å, Phenomenex, Torrance, CA, USA) at room temperature. The standard curve of TVN and 3AT concentration was linear from 1 to 1000 ng/mL and was accepted when the correlation coefficient exceeded 0.95 and measured values were within 20% of the actual values.
**Synovial fluid and plasma samples**

The extraction and detection methods used to quantify TVN and 3AT in the synovial samples is reported in Canning et al. (2016).

For plasma samples, the procedures reported in Canning et al. (2016) were performed using blank plasma in lieu of synovial fluid for the calibration curves. The plasma samples were diluted with 400 µL of acetonitrile containing 0.1% formic acid, vortexed, and centrifuged for 20 minutes at 630 g. The supernatant was poured off, dried down, and reconstituted with 200 uL of 25% (v/v) acetonitrile. The extraction and detection that followed was done as described in Canning et al. (2016).

The standard curves for TVN and 3AT concentrations for both blank porcine synovial fluid and plasma were linear from 1 to 1000 ng/mL and were accepted when the correlation coefficient exceeded 0.95 and measured values were within 15% of the actual values.

**Statistical Analysis**

Descriptive statistics using SAS (9.4 SAS Institute Inc, Cary, NC) were performed to determine the mean and standard deviation for the concentration of TVN and 3AT in the plasma, synovial fluid, and water samples. A linear regression between the mean plasma TVN concentration and mean synovial TVN concentration for each group was performed. A Pearson correlation coefficient with 95% confidence intervals was conducted to assess correlation between the two sample types. For each pig, daily water disappearance, individual weight, and medicated water concentration were used to calculate a daily dose of TVN in milligrams per kilogram. As some pigs were euthanized and sampled at twelve or six hour intervals, the calculated daily dose for these animals includes a partial daily dose.
Results

Concentration of TVN and 3AT in synovial fluid and plasma

Concentrations of TVN and 3AT for each sample time point for synovial fluid are summarized in Figure 2. For TVN, there are three time points (72, 96 and 108 hours), where there is at least one sample in which TVN was not detected in the joint fluid. For 3AT, the concentrations were less than TVN and at least one pig from every time point had a 3AT concentration of <1 ng/mL.

The concentrations of TVN and 3AT in the plasma are summarized in Figure 3. There appears to be a diurnal pattern for TVN with the largest concentrations detected at evening time periods (60, 84, and 102 hours) and the smallest concentrations at morning time points (48, 72, and 96 hours). Mean plasma 3AT concentrations were similar to that of synovial concentrations and fluctuated between 0 and 2 ng/mL (Figure 3).

Mean TVN concentrations were positively, linearly correlated between synovial and plasma samples (Figure 4). For the Pearson correlation coefficient, it was 0.9291 with a 95% confidence interval of 0.7217 to 0.9834 (p-value 0.0001).

Medicated water concentration, daily water disappearance and daily dose

The mean, standard deviation, and range of the medicated water concentration of the bulk bins are provided in Table 1.

For day -1, the mean (standard deviation) water disappearance for all pigs was 4.7 L (0.22). For day 0, the mean (standard deviation) water disappearance for all pigs was 4.4 L (0.22).

The mean and range of individual pig water disappearance and individual pig dose is provided in Table 2 for days 1 to 5 of medicated water administration.
On necropsy, there was lung consolidation affecting approximately 5% to 20% of the lung field identified in 22% (13/60) of the pigs. Diagnostic testing revealed chronic, non-active pneumonia indicative of a previous bacterial or viral pathogen. No viral pathogens were identified with molecular testing. None of the waterers were identified as having faulty equipment during the trial.

Discussion

This study documents the concentrations of TVN and 3AT in the joints and plasma of healthy growing pigs over ten time points during a five-day water administration of TVN. The targeted daily dose for pigs for TVN is approximately 5 mg/kg. As evidenced in Table 2, this concentration was met or exceeded for most groups and the dose range within groups was relatively large. This appears to be influenced by variation in individual pig water disappearance. In groups with a large range in water disappearance, the range in daily dose was also large. Additionally, there were pigs at several time points with TVN concentration of <1 ng/mL (Figures 2 and 3). It is challenging to make comparisons between groups, as each treatment group contained different individual pigs with different drinking behaviors. Additionally, an important consideration is the limitation of the water delivery system which only allows assessment of water disappearance and not water intake. It is generally recognized that pigs will play with water nipples and that there will be water wastage when pigs drink from a water nipple. Thus, the data reported here for daily drug dose are likely over estimates as they are calculated on the assumption that the pigs did not waste any water when drinking. The lack of observed leaky waterers may have reduced potential for difference between water intake and disappearance.
A key discussion point is that individual pig variation in water intake is a critical driver of daily ingested dose. More specifically, there is substantial variation between pigs on their water disappearance and therefore inter-pig variation in received drug dose. Some pigs may need injectable antimicrobial treatment to achieve and sustain therapeutic concentrations of antimicrobials, despite concurrent medication through the water. Unless pigs are demonstrating clinical signs while being observed by caretakers, it can be difficult to select pigs for individual treatment. Additionally, in groups of unhealthy pigs, individual variation in water intake is expected to increase (Pijpers et al., 1991).

There are several studies in pigs reporting on daily dose for water soluble antibiotics, but none of the studies included TVN and few of them provide individual animal water intakes or disappearances (Agerso, Friis, & Haugegaard, 1998; L. Gutiérrez, 2011; Mason, Baynes, Almond, Riviere, & Scheidt, 2009; Pijpers et al., 1991; Soraci, Amanto, Tapia, de la Torre, & Toutain, 2014). One study examined plasma concentrations of tetracycline at four different concentrations in healthy pigs in individual pens with individual water carboys. Water consumption data was not reported other than to detail that water consumption was not different between treatment groups. There was, however, substantial variation between individual pig plasma concentration within each treatment group, which is consistent with the results of this study (Mason et al., 2009).

As discussed in Canning et al. (2016), minimum inhibitory concentration (MIC) information for Mycoplasma spp. associated lameness in swine is limited. Clinical breakpoints and efficacy data from lameness challenge models or field studies for TVN have not been reported. Intracellular accumulation of macrolides poses an additional complexity to the interpretation and clinical application of MIC information (Giguère, 2013). MIC data for
3AT for *Mycoplasma* spp. are not available in peer reviewed literature to date but 3AT is believed to have antimicrobial activity (Giguère, 2013).

There was variation in the TVN concentration in the stock solution bins (Table 1) despite efforts to minimize this. Specifically, consistent procedures and the same people were used to mix the solution each day and weights and mixing calculations were verified by two people. Collecting multiple samples per bin may have helped better understand and quantify the variation in bin concentrations.

Plasma and synovial fluid concentrations were positively, linearly, and moderately correlated. Plasma could be an acceptable sample to collect in lieu of synovial fluid in future pharmacokinetic studies on TVN. Utilization of plasma would decrease the number of pigs required for studies because it would allow for repeated sampling of individual pigs.

This study reports on the concentration of TVN and 3AT in the synovial fluid and plasma of pigs medicated with TVN in the water for five days. Understanding and quantifying the dose variation amongst individual pigs in a mass medication scenario is important to ensuring that individual animals are receiving the intended and appropriate dose of antimicrobial.

**Acknowledgements**

The authors would like to acknowledge the Swine Medicine Education Center staff and interns, ISU CVM Field Services, ISU Swine Nutrition Farm (Trey Faaborg and Tim Hicks), and the PhAST lab for their contributions to this study.

**Conflict of Interest**

The Swine Medicine Education Center (affiliate for Dr. Locke Karriker and Dr. Paisley Canning) was compensated by ECO Animal Health/Pharmgate for the cost of supplies, live animals, and animal housing.
References


Figure 1. Depiction of the individual pig housing. The PVC pipe waterer the pig is drinking from was the only source of water for the pig. The bowl waterer in the picture was not functional.
Figure 2: Mean concentration of tylvalosin (TVN) and 3-O-acetyltlosin (3AT) in the synovial fluid of pigs across ten time periods during the study period. The bars above and below each marker represent the minimum and maximum concentration in each group.
Figure 3. Mean concentration of tylvalosin (TVN) and 3-O-acetyltlyosin (3AT) in the plasma of pigs across ten time periods during the study period. The bars above and below each marker represent the minimum and maximum concentration in each group.
Figure 4. Linear correlation between mean concentration of tylvalosin (TVN) in the plasma and mean concentration of TVN in the synovial fluid for each of the ten sample groups. R squared was 0.86. The slope on the linear regression equation was 1.290 and the intercept was 0.836.
Table 1. Mean concentration, standard deviation and range of tylvalosin (TVN) and 3-acetyltlylosin (3AT) in the bulk tank medicated water samples.

<table>
<thead>
<tr>
<th>Day of medicated water administration</th>
<th>Number of bulk tank samples*</th>
<th>Mean concentration (ppm)</th>
<th>Standard deviation</th>
<th>Range: Low (ppm)</th>
<th>Range: High (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TVN</td>
<td>3AT</td>
<td>TVN</td>
<td>3AT</td>
</tr>
<tr>
<td>Day 1</td>
<td>6</td>
<td>62</td>
<td>2</td>
<td>5.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>6</td>
<td>51</td>
<td>1</td>
<td>8.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Day 3</td>
<td>5</td>
<td>61</td>
<td>1</td>
<td>8.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Day 4</td>
<td>4</td>
<td>59</td>
<td>1</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Day 5</td>
<td>2</td>
<td>70</td>
<td>2</td>
<td>2.1</td>
<td>0.0</td>
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</table>

*The volume of each bulk tank was constant each day. A medicated water sample was taken from each bulk tank mixed and distributed to pigs each day. The number of bulk tanks required each day decreased as groups of pigs were euthanized.
Table 2. Concentration of tylvalosin (TVN) in the medicated water, individual pig water disappearance and individual pig dose of TVN for the five days of water medication (day one to day five).

<table>
<thead>
<tr>
<th>Day of trial</th>
<th>Sample Group (6 pigs per group)</th>
<th>Hours of water access per day†</th>
<th>TVN concentration in water (ppm)</th>
<th>Individual pig water disappearance (L)</th>
<th>Individual pig dose of TVN (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 to 10</td>
<td>24 hours</td>
<td>61.1</td>
<td>3.5</td>
<td>6.3</td>
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<tr>
<td>2</td>
<td>2 to 10†</td>
<td>24 hours</td>
<td>50.9</td>
<td>4.6</td>
<td>6.4</td>
</tr>
<tr>
<td>3*</td>
<td>3</td>
<td>12 hours</td>
<td>51.0</td>
<td>3.6</td>
<td>1.2</td>
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<td>3</td>
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<td>5</td>
<td>24 hours</td>
<td>75.0</td>
<td>4.1</td>
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<td>3</td>
<td>6</td>
<td>24 hours</td>
<td>61.0</td>
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<td>3</td>
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<td>64.0</td>
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<td>3.3</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>24 hours</td>
<td>64.0</td>
<td>4.7</td>
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</tr>
<tr>
<td>4</td>
<td>8</td>
<td>24 hours</td>
<td>55.0</td>
<td>4.8</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>24 hours</td>
<td>55.3</td>
<td>4.3</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>24 hours</td>
<td>57.0</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>5***</td>
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<td>68.0</td>
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</tr>
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<tr>
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<td>71.0</td>
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</tr>
<tr>
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<td>24 hours</td>
<td>71.0</td>
<td>4.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

†Pigs were sampled at 12 and 6 hour intervals during the study. As a result, water disappearance and dose (mg/kg) information reflects the number of hours the pig had access to the medicated water in each of the five 24 hour periods during the 5-day water medication. For each day, the number of hours each group of six pigs had access to medicated water is reflected in this column.

*Pigs from groups 1 and 2 were removed prior to day 3 as per the sampling schedule

**Pigs from groups 3 to 4 were removed prior to day 4 as per the sampling schedule

***Pigs from groups 5 to 6 were removed prior to day 5 as per the sampling schedule

†One pig in group 8 did not have water disappearance recorded for day 2 due to a data recording error and is not included in any subsequent results reported in this row.
CHAPTER 7. CONCLUSIONS

From the retrospective review of lameness cases involving joints and legs at the ISU VDL, it was demonstrated that there are four primary diagnosis that made up approximately 80% of the 464 cases assessed. These four diagnoses included Mycoplasma hyosynoviae, infectious arthritis (bacterial), metabolic bone disease, and lameness with inconclusive findings. This is consistent with practitioner reports that it is not uncommon for diagnostic investigations on lameness to generate non-specific findings as this category represented the largest number of cases in the review. Additionally, a secondary diagnosis was identified in about 25% of cases. This indicates that lameness etiologies are likely multifactorial. This review also highlighted the challenges of accounting for the numerous biases involved with sample collection, diagnostic submissions and retrospective review process itself. Based on these findings, it is likely that a singular diagnostic investigation is insufficiently comprehensive to adequately identify contributing etiologies in complex multifactorial cases. Despite this, the diagnostic investigation process is an important and essential tool for practitioners for the clinical management of infectious lameness. Additional diagnostic tools and systematic approaches to clinical assessment and sample collection would complement and augment most diagnostic submissions for lameness.

In Chapters 3 and 4, the development and refinement of fluid analysis and cytologic evaluation of swine joint fluid was explored from two perspectives. First, in Chapter 3, the question of feasibility of attaining antemortem joint fluid samples was assessed using several anesthetic protocols. Telazol, ketamine and xylazine were found to be the most effective combination for successful antemortem sample collection with the least recovery time and cost. It is essential that veterinarians review FDA guidances and statements about the use of
anesthetic agents in food animals and make an informed decision before using anesthetic agents for lameness work-ups. Secondly, in Chapter 4, joint fluid samples were analyzed to produce reference intervals for the carpus and tarsus for fluid analysis and cytology variables. Previously, these reference intervals were not available with the public body of literature. Practitioners can submit freshly collected joint fluid samples for clinical pathology and compare samples from lame pigs to the reference intervals published here. In addition to PCR and culture, clinical pathology results provide additional information about the pathological process occurring in the joints and are a critical tool especially for antemortem sampling when synovium is not accessible. As has been published for other species, it will be important to further define and characterize specific changes to clinical pathology variables with respect to different swine-specific pathogens and pathological processes to solidify clinical pathology as a useful tool for swine practitioners.

The last section of the dissertation addressed the lack of swine pharmacology data specific to joints. The focus of Chapters 5 and 6 were to determine if tylvalosin concentrations could be detected in synovial fluid and if so, to determine joint concentrations over the course of a five-day oral administration of the product. Tylvalosin and its metabolite were identified in joint fluid after oral gavage at label and ten times the label dose. This information gave rise to Chapter 6 where tylvalosin concentrations in synovial fluid were assessed under field-like conditions of administration. This project demonstrated that there was substantial individual pig variation in water disappearance and, consequently, joint concentration levels, between healthy, individually housed pigs. From a clinical management perspective, this information reinforces that it is imperative to ensure pigs are drinking medicated water during a treatment course. Lame and depressed pigs may need
injections in additional to site-wide water medication as their water intake will be decreased. Additionally, tylvalosin concentrations were below 1 ng/mL for some pigs indicating that steady state concentrations were not reached in individual pigs during the medication course. This is likely related to the half-life of the drug and individual pig drinking behavior. Lastly, this chapter provides data to support the use of plasma in lieu of synovial fluid for measuring tylvalosin concentrations as the concentrations in both of these samples are highly correlated. The use of plasma would allow for repeated, antemortem, sampling of individual pigs and would greatly facilitate sample collection for future projects.

As introduced in the literature review and statement of aims, the diagnosis and treatment of infectious arthritis in growing pigs is a broad topic. To address knowledge gaps in our understanding of infectious arthritis, the approach of this dissertation has been on applied projects to support the clinical management of IA in the field. Within that scope, there are many directions and opportunities for future projects based on this work. Amongst those, the top priority for future research would be the execution of challenge models and/or field trials to evaluate the efficacy of antimicrobials for infectious arthritis and to further elucidate clinical pathology changes associated with specific pathogens.