Determination of reference intervals for fluid analysis and cytologic evaluation variables in synovial fluid samples obtained from carpal and tarsal joints in commercial nonlame growing swine

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OBJECTIVE
To determine reference intervals for total nucleated cell count, total protein concentration, pH, RBC count, and percentages of neutrophils, lymphocytes, and large mononuclear cells in synovial fluid samples (SFSs) obtained from the carpal and tarsal joints of healthy swine.

ANIMALS
54 healthy commercial finisher pigs that had no evidence of lameness or gross joint swelling.

PROCEDURES
Each pig was anesthetized, and SFSs were collected from 1 carpal and 1 tarsal joint for fluid analysis, cytologic evaluation, bacterial culture, and PCR analyses for common swine joint pathogens. Each pig was euthanized after SFS collection, and synovial tissue samples were collected for histologic assessment. If necessary, postmortem SFSs were collected.

RESULTS
Overall, 37 of 50 tarsal and 46 of 53 carpal SFSs met inclusion criteria of sufficient volume, no gross blood contamination, and negative results of bacterial culture and PCR analyses, and were from joints with histologically normal synovial tissues. For the carpal and tarsal joints, upper reference limits were as follows: total nucleated cell count, 3,281 cells/µL and 2,368 cells/µL, respectively; total protein concentration, 3.6 g/dL and 3.6 g/dL, respectively; pH, 7.2 and 7.0, respectively; RBC count, 0.8×10^6 cells/µL and 0.1×10^6 cells/µL, respectively; and percentage of neutrophils, 46.5% and 33.7%, respectively; percentage of lymphocytes, 40.6% and 56.3%, respectively; and percentage of large mononuclear cells, 92.0% and 95.3%, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
Results have provided reference intervals for selected variables in SFSs obtained from the carpal and the tarsal joints of healthy swine, which should be useful in diagnostic investigations of swine lameness and arthritis. (*Am J Vet Res* 2018;79:858–866)

Infectious and noninfectious lameness in growing swine are a welfare concern and can increase production costs. Lameness diagnosis has historically relied on detection of gross changes coupled with results of postmortem microbial culture and PCR testing of synovial fluid samples.[1] This strategy requires euthanasia of affected pigs, thereby limiting sample size selection and preventing follow-up investigation of the effect of postdiagnosis treatments. Thus, antemortem diagnostic assessments of synovial fluid samples that are applicable to a broad range of lameness agents would be useful. Diagnosis of the cause of lameness by use of an antemortem sample collection technique would afford practitioners the opportunity to monitor treatment success of interventions.

In equine and canine medicine, fluid analysis and cytologic evaluation of synovial fluid samples are core antemortem diagnostic tests for multiple types of arthritis.[2,3] Routine analysis of synovial fluid samples typically determines fluid pH, TPC, and differential nucleated cell percentages. Combined with PCR analysis and microbial culture, fluid analysis and cytologic evaluation could be key tools for diagnostic testing of swine synovial fluid samples. For swine, published values derived from synovial fluid

ABBREVIATIONS

%LMC	Percentage of large mononuclear cells
%LYMPH	Percentage of lymphocytes
%NEUT	Percentage of neutrophils
ISU VDL	Iowa State University Veterinary Diagnostic Laboratory
LMC	Large mononuclear cells
MHR	*Mycoplasma hyorhinis*
MHS	*Mycoplasma hyosynoviae*
RBCC	RBC count
TNCC	Total nucleated cell count
TPC	Total protein concentration

analysis and cytologic evaluation are currently limited. The objective of the study reported here was to determine reference intervals for TNCC, TPC, pH, RBCC, %NEUT, %LYMPH, and %LMC in synovial fluid samples obtained from the carpal and tarsal joints of healthy swine.

Materials and Methods

Sample size

Two experiments were conducted to create the reference intervals for the variables of interest. Both experiments were approved by the Iowa State University Institutional Animal Care and Use Committee. The American Society of Veterinary Clinical Pathologists has established a minimum sample size of > 20 animals to provide data for determination of reference intervals.[4] For these studies, it was anticipated that collection of synovial fluid samples would frequently yield an inadequate fluid volume or blood contamination in the sample; thus, a study sample size of 54 pigs was used, and the study was divided into 2 experiments that were undertaken at different times.

Animals and housing

Thirty healthy, nonlame pigs were used in experiment 1 and 24 healthy, nonlame pigs were used in experiment 2. The interval between experiments 1 and 2 was approximately 6 weeks. For each experiment, the pigs were weighed and then moved into individual pens for lameness scoring, joint swelling scoring, and anesthesia. Both experimental groups included commercial, crossbred finisher pigs (16 to 18 weeks of age) that were housed in the same continuous flow finisher barn before enrollment in the study. The pens were rectangular and partially slatted, with 15 to 20 pigs/pen; the stocking density was approximately 9 $0.84m^2$/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. Both groups were an equal mix of barrows and gilts. All pigs were fed commercial finisher feed without antimicrobials ad libitum for the duration of each experiment. The diet met all National Research Council nutritional requirements for swine.[5] At selection, pigs underwent physical examination by a veterinarian (PC), which included palpation of joints. Pigs had to be free of clinical signs to be entered into either experiment. Pigs were assessed while standing and during locomotion prior to selection.

Scoring of lameness and joint swelling

Once selected, each pig was assigned a lameness score and a joint swelling score on the basis of the physical examination findings. The scoring system used has been previously reported.[6] For inclusion in the study, pigs had to have a lameness score of zero and a gross joint swelling score of zero.[6] Briefly, for the lameness scoring, a score of zero indicated that the animal moved freely and used all 4 limbs with even weight distribution. The joint swelling score was modified slightly, and in this study, a score of zero for joint swelling indicated no swelling of any joint in the appendicular skeleton.

Anesthesia treatment groups

All pigs were administered anesthetic agents IM and were under general anesthesia during collection of synovial fluid samples. Pigs were not intubated during sample collection. In experiment 1, each pig received an IM injection of tiletamine hydrochloride and zolazepam hydrochloride[a] (4.4 mg/kg), ketamine hydrochloride[b] (2.2 mg/kg), and xylazine hydrochloride[c] (4.4 mg/kg) that had been combined in the same syringe. In experiment 2, pigs were randomly allocated to 1 of 4 anesthesia treatment groups. Group 1 received the same anesthetic protocol as pigs in experiment 1. Group 2 received acepromazine maleate[d] (0.03 mg/kg), ketamine[b] (2.2 mg/kg), and tiletamine-zolazepam[a] (4.4 mg/kg) combined in the same syringe IM. Group 3 received an IM injection of acepromazine[d] (0.3 mg/kg) and tiletamine-zolazepam[a] (4.4 mg/kg) that had been combined in the same syringe and an aseptically placed lumbosacral epidural injection. For the lumbosacral epidural injection, 2% lidocaine hydrochloride solution[e] (dose, 2.2 mg/kg up to a volume of 10 mL) was used. Group 4 received an IM injection of acepromazine[d] (0.5 mg/kg) and ketamine[b] (5 mg/kg) that had been combined in the same syringe and an aseptically placed lumbosacral epidural injection. For the lumbosacral epidural injection, 2% lidocaine solution[e] (dose, 2.2 mg/kg up to a volume of 10 mL) was used. Individual pig weights were used to calculate doses of the anesthetic agents. The anesthetic treatment groups in experiment 2 were part of a separate study to assess the quality of anesthetic procedures.

To be considered eligible for the synovial fluid collection procedure, each pig had to be fully anesthetized and recumbent and have a negative palpebral response and toe withdrawal response. Following the anesthetic injection, each pig was monitored closely during the collection procedure. Heart rate, respiratory rate, rectal temperature, and depth of sedation were monitored at least every 10 minutes while the pig was anesthetized and at 30-minute to 1-hour intervals during recovery from anesthesia.

Antemortem carpal and tarsal joint centesis

Once anesthetized, each pig was positioned in dorsal recumbency. One tarsus and carpus of each pig were randomly selected, shaved, and further prepared with a chlorhexidine soap scrub, an alcohol scrub, and final application of a tincture of chlorhexidine. The chlorhexidine soap and alcohol scrubs were repeated to ensure asepsis of the centesis sites. Once the joints were prepared for synovial fluid sample collection, each joint was dried with sterile gauze and a sterile adhesive drape was applied (experiment

1 only). In experiment 2, the joints were left to air dry for approximately 45 seconds.

To aspirate the synovial fluid, an 18-gauge, 1.5-inch needle attached to a 12-mL sterile syringe was inserted into the dorsolateral aspect of the carpus or tarsus, and negative pressure was used to aspirate synovial fluid into the syringe. Given the continuity between joint spaces within the tarsal and carpal joints, needle insertion at the same location in each joint was attempted; however, depending on the anatomy and position of the pig, it is possible that a sample could have been collected from a slightly differently location within the carpal or tarsal joint for each pig. Clean garments and clean gloves were worn by the veterinarian (PC) completing the sample collection from each pig. A gross description including color, clarity, fibrin, purulent material, or whole blood contamination was recorded for each synovial fluid sample. One veterinarian (PC) with previous experience with swine synovial fluid aspiration collected all the synovial fluid samples for the entire study.

Postmortem carpal and tarsal joint centesis

If an acceptable carpal or tarsal joint synovial fluid sample was not collected from a pig in either experiment because of insufficient anesthetic plane, insufficient sample volume, or grossly visible whole blood contamination of the sample, a postmortem sample was collected from the contralateral joint by means of the methods used for antemortem sample collection.

Sample handling

The minimum volume of synovial fluid required to complete all the tests was 0.5 mL. If this volume was not attained, the sample was discarded. Half of the volume of synovial fluid collected was immediately placed into a 2-mL tube containing EDTA,[f] agitated, and submitted for fluid analysis and cytologic evaluation within 8 hours after collection. For each pig, the remaining aliquots of fluid from each joint were pooled at the laboratory and submitted for aerobic and anaerobic bacterial culture, MHS PCR assay, and MHR PCR assay at the ISU VDL. These pooled samples were stored on ice within 2 minutes after collection and until analysis.

Euthanasia and experiment end point

Following antemortem synovial fluid sample collection in experiment 1, pigs were euthanized by use of a penetrating captive bolt and exsanguination. Insensibility was determined by absent corneal reflex, and death was confirmed by absence of respiration and a detectable heartbeat. In experiment 2, pigs were allowed to recover from anesthesia and were monitored for 7 days before they were euthanized by use of the procedure described for pigs in experiment 1.

Sample collection at necropsy

A systematic postmortem examination was completed for each pig, which included collection of tonsils for testing for MHS DNA and MHR DNA by PCR assays (experiment 1 only) and clinical examination of joints. Tonsils were not collected from pigs in experiment 2 because of the very low prevalence of MHS or MHR in pigs in experiment 1. Identification and recording of gross abnormalities of synovial fluid, synovium, and cartilage were completed for the left and right scapulohumeral, humeroradial, carpal, hip, femorotibial, and tarsal joints. The joints that were opened included the carpal and tarsal joints that were aspirated under anesthesia as described above. When gross abnormalities were identified in a joint, samples of synovial fluid and synovial tissue from that joint were submitted for diagnostic testing including histologic examination, aerobic and anaerobic bacterial culture, and MHS and MHR PCR assays. Internal organ evaluation was performed; when gross abnormalities were identified, fresh and fixed tissue samples were submitted for diagnostic testing including histologic examination, molecular testing, and aerobic and anaerobic bacterial culture as directed by the pathologist (DM).

Oral fluid samples

Oral fluid samples were collected on a pen-based basis from pigs at 2 weeks before the commencement of either experiment and at the end of either experiment. Samples were collected by use of clean cotton rope as described previously.[7] The oral fluid samples were submitted for MHR and MHS PCR assays.

Sample processing, DNA extraction, and PCR assay for MHR or MHS

For samples of oral fluid, synovial fluid, and tonsil tissue, DNA extraction was performed with a nucleic acid isolation kit[g] and a magnetic particle processor,[h] according to manufacturers' instructions and as described previously.[8] Each synovial fluid sample was processed in a homogenizer[i] for 5 minutes at maximum speed as an additional step during the extraction process. For each pig, the MHS and MHR PCR assays were performed on pooled samples of synovial fluid from the carpal and tarsal joints. Tonsil tissues were processed following the standard operating protocol at the ISU VDL. By use of forceps and scissors, tonsil tissue was weighed and subsequently minced or ground prior to placement in a blender bag and processed as a 10% homogenate. Oral fluid samples were processed by means of a high-volume modified lysis procedure.[9]

The procedures used for MHS and MHR DNA amplification and detection were as previously reported.[8] In brief, samples (joint fluid and tonsil) were processed routinely for the detection of MHR and MHS nucleic acid by use of a real-time PCR assay with SYBR green. Specifically, 1 g of tonsil tissue and 10 mL of balanced salt solution[j] were processed in a disposable

tissue grinder system[k] to make an approximately 10% homogenate. Samples were centrifuged at 4,200 X *g* for 10 minutes at 4°C to remove particulate debris. Joint fluid samples did not require processing.

The DNA extraction was performed with a viral isolation kit[l] and a purification system[m] according to manufacturer's instructions. Tonsil homogenates were extracted by means of a standard lysis procedure with 50 µL of the sample added to 130 µL of lysis-binding solution with carrier RNA (that was prepared according to the kit instructions), 20 µL of magnetic bead mix, and 90 µL of elution buffer. The standard lysis protocol used 150 µL of wash solution I and II provided with the kit. The standard lysis extractions were conducted with the purification system program[n] with a 5-minute pause added at the end of the program to allow the eluate to attain room temperature (approx 21°C).

Joint fluid was extracted with the same kit and a bead-beating standard lysis procedure. Briefly, 150 µL of PBS solution and 60 µL of joint fluid were added to a microcentrifuge tube that was placed into a bead beater[o] at maximum speed for 3 minutes followed by centrifugation at 1,000 X *g* for 1 minute. Bead tubes were used to combine 235 µL of lysis-binding solution and 175 µL of sample lysate, which were placed in the bead beater at maximum speed for 5 minutes. The sample was pelleted by centrifugation at 16,000 X *g* for 3 minutes. Next, 65 µL of isopropanol, 20 µL of bead mix, and 115 µL of lysate were added to a lysis-binding plate. Extractions were conducted by use of the same number and volume of wash solutions, elution buffer, and purification system program[n] as used for the tonsil homogenates, with a 5-minute pause added at the end of the program to allow the eluate to attain room temperature.

The real-time PCR procedure was performed on nucleic acid extracts with a SYBR green PCR mastermix[o] and appropriate primer and probe sequences **(Appendix)**. Reactions included 12.5 µL of 2X SYBR green PCR mastermix,[o] 9.8 µL of nuclease-free water, 0.1 µL of each forward and reverse primer (400nM), and 2.5 µL of the extract. One positive extraction control, 1 positive amplification control, 1 negative extraction control, and 1 negative amplification control were also included with each extraction or PCR run.

The real-time PCR assay was performed with a fast thermocycler[p] in standard mode with the following cycling conditions: 1 cycle at 95°C for 15 minutes, 45 cycles at 94°C for 15 seconds, 61°C for 30 seconds, and 72°C for 30 seconds. One cycle was used for dissociation at 95°C for 15 seconds, 60°C for 1 minute, 95°C for 15 seconds, and 60°C for 15 seconds. Assays conducted on the fast thermocycler used the auto-baseline setting to determine fluorescence baselines and cycle thresholds set at 0.04. Samples were considered positive if cycle threshold values were < 44 with a melting temperature of 75.9°C (tolerance, ± 1°C) for MHR and 81.5°C (tolerance, ± 1°C) for MHS.

Aerobic and anaerobic bacterial cultures

Synovial fluid samples were submitted for routine culture processing at the ISU VDL. Carpal and tarsal synovial samples from each pig were pooled, and a standard culture protocol was used. The culture protocol included aerobic and anaerobic conditions, the inclusion of a *Staphylococcus aureus* nurse for organisms requiring nicotinamide adenine dinucleotide, and a selective medium (brilliant green agar) for isolation *Salmonella* spp other than *Salmonella enterica* serovars Typhi and Paratyphi. No additional selective or nonselective enrichment was performed on the samples, and plates were incubated for a minimum of 48 hours prior to reporting results. The samples were considered to have no clinically relevant growth if nonpathogenic organisms were isolated. The clinical relevance of bacterial growth was determined by the veterinary pathologist for each pig.

Histologic examination of synovia

After collecting the synovial fluid, the joints sampled under anesthesia were then opened to obtain a sample of synovium, which was immediately placed in neutral-buffered 10% formalin solution. Tissue samples were routinely processed for histologic examination of tissue sections. Histologic findings were scored by a board-certified veterinary pathologist (DM) who was unaware of the experimental group assignment of each pig. A scoring rubric was modified from that used in a study[10] of experimentally induced infectious arthritis in pigs and included evaluation of synovial proliferation, synovial alteration, inflammation, edema, neutrophil infiltration, and presence or absence of fibrin or hemorrhage within the synovial tissue. Abnormal synovial histologic features were defined as proliferation, alteration, inflammation, or edema that were assigned a score of ≥ 2; for each category, the scale was 0 (no change), 1 (mild change), 2 (moderate change), or 3 (severe change).[10] Neutrophil infiltration, presence of fibrin or hemorrhage were recorded as present or absent for each sample.[10]

Fluid analysis and cytologic evaluation

The fluid analysis and cytologic evaluation was performed individually on each tarsal and carpal synovial sample of each pig. Each synovial fluid sample (collected in an EDTA-containing[f] tube) was checked visually for blood clots, which was recorded as a dichotomous outcome (presence of blood clots, yes or no). After measurement of pH (by use of pH paper[q]) and TPC (by use of a digital refractometer[r]), hyaluronidase (approx 0.01 mg of lyophilized hyaluronidase powder) was added to the synovial fluid sample.[11] A TNCC and RBCC were performed with a hematology analyzer.[s] Color and clarity of the synovial fluid sample (after the addition of hyaluronidase) were reported. A sample was discarded and not included in the reference interval assessments if it was red, which was considered to be a result of gross diffuse blood

contamination. Colorless, light-yellow, light-orange, or red-tinged samples were considered acceptable. Clarity was considered acceptable if the sample was clear, slightly hazy, or cloudy. Any samples with fibrin, blood clots, or purulent material were rejected. If a sample was too turbid to read print through it and the tube, some of the fluid was put in an Hct tube and centrifuged; the percentage of total solids was determined from the supernatant. If the original fluid volume was < 0.5 mL, the TPC measurement was not valid and it was recorded as an insufficient volume.

For cytologic evaluation of each synovial fluid sample, a direct smear preparation was made with a camel hair brush. One hundred microliters of each synovial fluid sample was cytocentrifuged[t] at 72 X *g* for 10 minutes and a slide preparation made. The slides were prepared as per routine processes from the direct smear and cytocentrifuge sample, stained with a modified Wright-Giemsa stain and analyzed by a board-certified clinical pathologist (AV); the direct fluid preparation was used to assess overall sample nucleated cell and erythrocyte cellularity. The cytocentrifuged fluid preparation was used to perform a differential count of 300 nucleated cells.

Large mononuclear cells were identified on the basis of their morphologic appearance. In a normal joint, the LMC in synovial fluid are typically round cells with a moderate nuclear-to-cytoplasmic ratio. They often contain round, centrally placed nuclei with dense chromatin; their cytoplasm is basophilic and lacks vacuolization. Both quiescent macrophages and synoviocytes may have this appearance; thus, the encompassing term LMC was used to categorize them.

Overview of inclusion criteria

For inclusion in the study, each pig had to be healthy and nonlame with no grossly visible swelling of any joints of the appendicular skeleton. In addition, for experiment 1, results of the MHS and MHR PCR assays performed on tonsil tissue had to be negative. For a synovial fluid sample to be included in the study, the fluid had to have acceptable clarity and color, as stipulated earlier. Each synovial fluid sample had to yield no clinically relevant growth on aerobic or anaerobic bacterial culture and be negative for MHS and MHR as determined by PCR assay results. Histologic examination of the joints from which synovial fluid samples were collected must have been free from histopathologic changes indicative of active joint disease. A carpal or tarsal synovial fluid sample volume of least 0.5 mL of was required for complete fluid analysis.

Statistical analysis

Reference intervals for TNCC, TPC, pH, RBCC, %NEUT, %LYMPH, and %LMC of synovial fluid samples from the carpal and tarsal joints of the study pigs were established. Separate reference interval databases were created for carpal and tarsal synovial fluid samples. Reference intervals were established in accordance with the published guidelines for reference interval determination by the American Society of Veterinary Clinical Pathologists.[4] Data were evaluated with the D'Agostino and Pearson omnibus test to determine normality; data were assessed with the Tukey test for identification and removal of statistical outliers only. Suspect outliers were not removed. For normally distributed variables, a robust method was used on native data to calculate the upper and lower reference value limits (limits that encompass the central 95% of values determined in the reference population), and a bootstrap method was used to calculate the 90% confidence intervals around those limits.[4] For non-normally distributed variables, a Box-Cox transformation was first performed and then a robust method was used to calculate the upper and lower reference value limits, and a bootstrap method was used to calculate the 90% confidence intervals around those limits.[4,12] To assess the contribution of blood contamination to %NEUT, a correlation analysis was performed between RBCC and %NEUT for both carpal and tarsal synovial fluid samples. If the distributions of RBCC and %NEUT were normal, then a Pearson correlation coefficient was calculated. If the distributions were skewed, then a Spearman correlation coefficient was calculated.

In addition, the assessed variables for each joint were evaluated to determine whether differences existed between antemortem and postmortem samples. For normally distributed variables, a simple *t* test was used to compare the means of the antemortem and postmortem samples; for non-normally distributed variables, a Wilcoxon rank sum test was used to compare the medians of the antemortem and postmortem samples. Significance was set at a value of $P < 0.05$. Reference interval determination and statistical comparisons were performed by graphing and with reference interval analysis software packages.[u,v]

Results

Analysis of oral fluid samples

Test results for MHS and MHR in oral fluid samples from the pens holding pigs used in experiments 1 and 2 were negative 2 weeks prior to the start and at the end of both experiments.

Selection of pigs

Approximately 100 pigs housed in several pens within the study barn were eligible for the study. Pigs were randomly selected individually (1 pig/pen) and assessed to meet inclusion criteria. This process was repeated until the requisite number of eligible pigs was identified; therefore, not every eligible pig from the larger group was examined. A total of 66 pigs were examined, and 8 were rejected on the basis of physical examination findings. Reasons for rejection included umbilical hernia (n = 3), diarrhea (2), and conjunctivitis (3). No pigs were excluded from the study because of lameness. However, another 4 nonlame pigs were excluded because of joint swelling; palpation revealed very slight

swelling of a bursa on the lateral aspect of both tarsi in each pig. Six synovial fluid samples, representing carpal and tarsal samples from 3 pigs, were excluded because of positive PCR assay results for MHR in tonsil tissue. All tonsil tissue samples were negative for MHS on the basis of PCR assay results. None of the pigs used in the study were clinically lame, had abnormal gaits, or had grossly visible joint enlargements.

Inclusion and exclusion of synovial fluid samples

In total, 50 tarsal and 53 carpal synovial fluid samples from 54 finisher pigs were collected before or immediately after euthanasia. Fluid samples without major gross blood contamination could not be obtained before or immediately after euthanasia from 5 pigs (from the tarsus of 4 pigs and from the carpus of 1 pig). Mean ± SD weight of pigs was 130.3 ± 8.2 kg. The remaining synovial fluid samples were all negative for MHS and MHR as determined by PCR assays. Ten samples were excluded because of mild histologic abnormalities of the synovium. Of these 10 samples, 7 were tarsal joint samples and 3 were carpal joint samples. Four samples (3 tarsal and 1 carpal joint samples) were excluded because bacterial cultures yielded growth of *Staphylococcus epidermis* and *Acinetobacter johnsonii*, which were considered skin contaminants.[13]

Samples from 7 tarsi and 3 carpi were discarded because of blood clots in the collection tubes. In 1 carpal synovial fluid sample, TPC and pH could not be determined because the volume of fluid was insufficient. Among the tarsal synovial fluid samples, insufficient sample volume prevented the determination of TPC in 2 synovial fluid samples and determination of pH in 1 sample.

No visible osteochondrosis lesions were identified in the carpal and tarsal joints used for determination of the reference intervals for synovial fluid. In the humeroradial joint of 11 of the 54 (20%) pigs, there were small (< 1-cm-wide and < 3-mm-deep), linear, irregular depressions or folds in the cartilage (suspicion of osteochondrosis manifesta). Synovial fluid and synovial tissue collected from those joints revealed no bacterial pathogens and mild or no inflammatory changes to the synovium.

Overall, 37 of 50 tarsal and 46 of 53 carpal synovial fluid samples met inclusion criteria for reference interval determination of sufficient volume, no gross blood contamination, and negative results of bacterial culture and PCR analyses and were from joints with histologically normal synovial tissues **(Tables 1 and 2)**. Data that were considered outliers were not used in the reference interval determinations.

Antemortem and postmortem synovial fluid samples

Among the 37 tarsal synovial fluid samples that were considered acceptable for analysis, 23 were collected before euthanasia and 14 were collected after euthanasia of the pigs. Among the 46 carpal synovial fluid samples that were considered acceptable for analysis, 23 were collected before euthanasia and 23 were collected after euthanasia of the pigs. For pigs that underwent the group 3 anesthetic treatment in experiment 2, samples were obtained after euthanasia exclusively because of the ineffectiveness of ketamine and acepromazine to induce an anesthetic plane sufficient for synovial joint centesis. There were no differences in any of the fluid analysis and cytologic evaluation variables between antemortem and postmortem carpal synovial fluid samples; thus, these 2 datasets were combined for determination of reference intervals. For the tarsal synovial fluid samples, the antemortem samples had a higher RBCC relative to that of the postmor-

Table 1—Data regarding reference intervals for variables in synovial fluid samples collected from carpal joints of healthy, nonlame commercial finishing hogs.

Variable	TNCC (cells/μL)	TPC (g/dL)	pH	RBCC (X 10^6 RBCs/μL)	%NEUT (%)	%LYMPH (%)	%LMC (%)
Lower limit of reference interval	199.4	2.0	6.1	0.002	0.3	2.6	27.5
Upper limit of reference interval	3,280.7	3.6	7.2	0.3	46.5	40.6	92.0
90% CI for lower limit	186–228.4	1.9–2.2	6.0–6.2	0.002–0.003	0.1–0.6	0–5.9	0.5–43.0
90% CI for upper limit	1,977.3–5,164.8	3.4–3.8	7.1–7.2	0.03–0.50	32.0–66.0	35.9–44.8	88.5–95.0
Gaussian distribution of data	No	Yes	No	No	No	Yes	No
No. of samples	43	42	42	43	46	46	46
No. of samples used for reference interval calculation	43	42	42	43	46	46	46
Outliers removed	0	0	0	0	0	0	0
Type of reference interval determination	Box-Cox, robust	Robust	Box-Cox, robust	Box-Cox, robust	Box-Cox, robust	Robust	Box-Cox, robust

Fifty-four healthy commercial finisher pigs with no evidence of lameness or gross joint swelling were used. Each pig was anesthetized, and 1 carpal and 1 tarsal synovial fluid sample were collected for fluid analysis and cytologic evaluation, aerobic and anaerobic bacterial culture, and MHR and MHS PCR analyses. After synovial fluid sample collection, each pig was euthanized; synovial fluid and synovial tissue samples were collected for histologic assessment. Overall, 46 of 53 carpal synovial fluid samples met the inclusion criteria of sufficient volume, no gross blood contamination, and negative results of bacterial culture and PCR analyses, and were from joints with histologically normal synovial tissues. Among the 46 carpal synovial fluid samples that were considered acceptable for analysis, 23 were collected before and 23 were collected after pigs were euthanized.

Table 2—Data regarding reference intervals for variables in synovial fluid samples collected from tarsal joints of healthy, nonlame commercial finishing hogs.

Variable	TNCC (cells/μL)	TPC (g/dL)	pH	RBCC (X10^6 RBCs/μL)	%NEUT (%)	%LYMPH (%)	%LMC (%)
Lower limit of reference interval	189.2	1.3	6.2	0	0.7	0.5	24.4
Upper limit of reference interval	2,367.6	3.6	7.0	0.1	33.7	56.3	95.3
90% CI for lower limit	154.8–235.8	1.1–1.5	6.1–6.2	0–0.001	0.2–1.9	0–7.4	15.7–33.7
90% CI for upper limit	1,488.7–4,094.0	3.1–4.0	6.9–7.0	0.05–0.2	26.2–41.8	48.4–63.9	87.5–104.8
Gaussian distribution of data	No	No	No	No	No	Yes	Yes
No. of samples	30	28	29	30	37	37	37
No. of samples used for reference interval calculation	28	28	28	27	36	37	37
Outliers removed	2	0	1	3	1	0	0
Type of reference interval determination	Box-Cox, robust	Box-Cox, robust	Box-Cox, robust	Box-Cox, robust	Box-Cox, robust	Robust	Robust

Overall, 37 of 50 tarsal synovial fluid samples met the inclusion criteria. Among the 37 tarsal synovial fluid samples that were considered acceptable for analysis, 23 were collected before and 14 were collected after pigs were euthanized.

See Table 1 for key.

tem samples. The main determinant of RBCC was 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, data from the antemortem and postmortem tarsal synovial fluid samples were combined for determination of reference intervals.

Synovial fluid analysis and cytologic evaluation

Analysis and cytologic evaluation of carpal and tarsal synovial fluid samples from healthy nonlame pigs provided data from which reference intervals for variables of interest were determined (Tables 1 and 2). The number of samples used to establish reference intervals for each fluid analysis and cytologic evaluation variable differed slightly. All but 1 tarsal synovial fluid sample was slightly hazy or cloudy. With regard to color, the tarsal synovial fluid samples were red tinged (n = 27), light yellow (5), colorless (4), or light orange (1). The carpal synovial fluid samples were all slightly hazy or cloudy; the colors of the samples were red tinged (n = 22), light yellow (3), colorless (9), or light orange (2).

Among carpal or tarsal synovial fluid samples, the distributions of RBCC and %NEUT were skewed to the right, and correlation of blood contamination and %NEUT was assessed by calculation of the Spearman correlation coefficient. For carpal synovial fluid samples, the Spearman correlation coefficient was 0.6346 ($P < 0.001$), indicating moderate correlation. For tarsal synovial fluid samples, the Spearman correlation coefficient was 0.2954 ($P = 0.113$), indicating weak or no correlation.

Necropsy results

Prior to euthanasia, none of the pigs had clinical signs such as coughing, nasal discharge, or diarrhea. At necropsy, minor gross lesions in internal organs were identified in most of the pigs (45/54 [83%]) in each experiment. 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 as the cause. The lung consolation was attributed to previous infectious insult, postmortem blood pooling, or the method of euthanasia (penetrating captive bolt).

Discussion

The data obtained in the present study were used to establish cytologic reference intervals for carpal and tarsal synovial fluid from nonlame commercial finisher pigs. To the authors' knowledge, there is only 1 other reported study[14] of synovial fluid analysis and cytologic evaluation variables for swine. In that study,[14] synovial fluid variables reported for 5 healthy pigs included pH, specific gravity, glucose concentration (per 100 mL), TPC, RBCC, TNCC, and percentages of various WBCs. The joints from which the synovial fluid was obtained and the fluid samples themselves were grossly normal and sterile (as determined by culture results), although it is unclear which joints were used. Upper reference limits were calculated from variables from the 5 pigs and defined as the mean plus 2 SDs. The upper reference limits for TPC, TNCC, and RBCC were 6.1 g/dL, 352 cells/μL, and 0.019 X 10^6 RBCs/μL.[14] With the exception of TPC, those upper limits fall within the reference intervals determined in the present study for carpal and tarsal synovial fluid samples; TPC was greater than the 90% confidence interval for the carpal or tarsal joint upper limit in the present study. With regard to the differential WBC percentages determined in the study of the 5 pigs,[14] %LYMPH was higher (mean, 49%) than the value determined for the carpal joint but not higher than the value determined for the tarsal joint in the present study. Other WBC percentages[14] appeared to be within the reference intervals established in the present study.

Compared with data for horses, the upper reference limits for synovial fluid TNCC and TPC were higher for swine, as determined in the present study. For horses, normal values of TNCC and TPC are reported to be < 1,000 cells/µL and < 2.0 g/dL, respectively.[2,15] On the basis of the present study data, normal values of < 5,000 cells/µL and < 3.8 g/dL seem more appropriate for swine. In dogs and cats, the TNCC is generally < 1,000 to 3,000 cells/µL and < 1,100 cells/µL, respectively.[3,16] For canine synovial fluid, normal values of TPC, as with all other variables, vary among laboratories but are generally between 1.8 and 4.8 g/dL, with < 2.5 g/dL considered normal.[3]

In the present study, the lower and upper reference limits for synovial fluid pH were 6.1 to 7.2 for the carpus and 6.2 to 7.0 for the tarsus, which were lower than pH ranges published for synovial fluid from horses and companion animals. It should be noted that the lower limit of detection for pH in the present study was 6.0. For horses, a synovial fluid pH of 7.30 (SD, 0.06) is considered normal.[1,2] For dogs and cats, a synovial fluid pH of 7.2 to 7.4 is considered normal.[3]

Normal synovial fluid should have low cellularity, and data from other species suggest that %LMC, %NEUT, and %LYMPH should be 80% to 90%, < 10%, and < 20%, repspectively.[2,3,17] In horses and dogs, neutrophils account for approximately < 10% to 12% of WBCs in synovial fluid.[3,17–19] In cats, the %NEUT in synovial fluid samples that is considered normal is < 39%, which is closer to values identified for pigs in the present study (ie, < 46% for carpal samples and < 33% for tarsal samples).[17] As with other species, LMC are the predominant cell type in swine synovial fluid. In the present study, there were large ranges for all the differential WBC percentages, particularly for LMC.

In the present study in pigs, there was some correlation between RBCC and %NEUTS for the carpal synovial fluid samples, which may indicate that a portion of neutrophils in each sample was a result of blood contamination. However, for the tarsal synovial fluid samples, it did not appear that %NEUT was affected by sample collection–related hemorrhage, given the weak correlation between RBCC and %NEUT.

During infectious and degenerative pathological processes, changes to the morphologies and percentages of LMC, neutrophils, and lymphocytes can be observed. In horses and small animals, fluid analysis and cytologic evaluation of synovial fluid samples can be used to identify joints that are normal and those that have degenerative disease or acute injury, inflammatory disease, or diseases that can have an infectious or immune-mediated cause.[2,3,18,20] To the authors' knowledge, there are no reports of experimental studies describing the changes in synovial fluid associated with various swine-specific joint pathogens, and extrapolation from other species would be necessary to interpret changes in swine synovial fluid.

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

An additional consideration is that mild osteochondrosis manifesta lesions were observed in the forelimbs of some pigs in the present study. However, calculation of the reference intervals did not include data from any joints with evidence of osteochondrosis manifesta. Osteochondrosis can cause changes to fluid analysis and cytologic evaluation results in circumstances where it causes degenerative joint disease and osteoarthritis.[20] Lesions consistent with osteoarthritis or degenerative joint disease were not identified in any joints in the pigs of the present study.

The creation of a reference interval dataset for synovial fluid from nonlame, healthy finisher pigs in the present study has provided a novel diagnostic tool for practitioners and production companies. In equine and canine medicine, synovial fluid analysis and cytologic evaluation are core diagnostic tests for lameness and arthritis. For horses, they are considered extremely useful tools for the diagnosis of septic arthritis.[2,21] Coupled with findings of microbial culture, molecular testing for *Mycoplasma* spp, and histologic examination of joint tissue, results of synovial fluid analysis and cytologic evaluation of samples obtained from pigs help complete the diagnostic picture.[2] Owing to the transient nature of many infectious arthritis agents, multiple pieces of evidence indicative of infectious agents are needed for accurate diagnosis. Such a complete diagnostic picture allows veterinarians to more confidently and accurately determine a diagnosis and create a treatment plan for a given patient. Improved diagnostic procedures and treatment plans have direct benefits to pigs and caretakers alike. Moreover, these antemortem reference intervals for synovial fluid variables allow veterinarians to document the effect of health interventions in populations of lame pigs without the need to euthanize individuals.

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Footnotes

a. Telazol, tiletamine HCl and zolazepam HCl injection, (100 mg/mL when reconstituted), Zoetis, Kalamazoo, Mich.

b. Ketamine HCL injection (100 mg/mL), Zoetis, Kalamazoo, Mich.
c. Xylazine injection (100 mg/mL), VetOne/Akorn Inc, Lake Forest, Ill.
d. Acepromazine Maleate injection (10 mg/mL), VetOne/Akorn, Inc, Lake Forest, Ill.
e. 2% lidocaine hydrochloride, MWI Veterinary Supply Co, Boise, Idaho.
f. BD Bioscience, San Jose, Calif.
g. MagMAX Total Nucleic Acid Isolation Kit, Thermo Fisher Scientific, Waltham, Mass.
h. KingFisher 96 magnetic particle processor, Thermo Fisher Scientific, Waltham, Mass.
i. BioSpec Bead Beater, BioSpec Products Inc, Bartlesville, Okla.
j. Earle's Balanced Salt Solution, Thermo Fisher Scientific, Waltham, Mass.
k. Disposable tissue grinder system, Thermo Fisher Scientific, Waltham, Mass.
l. MagMAX Viral RNA Isolation Kit, Thermo Fisher Scientific, Waltham, Mass.
m. Kingfisher 96 Flex purification system, Thermo Fisher Scientific, Waltham, Mass.
n. Kingfisher AM1836_DW_50_v3, Thermofisher Scientific, Waltham, Mass.
o. Qiagen QuantiTect SYBR Green I PCR mastermix, Qiagen, Germantown, Md.
p. AB 7500 fast thermocycler, Applied BioSystems, Foster City, Calif.
q. Hydrion pH 6.0 to 8.0, Micro Essential Laboratory Inc, Brooklyn, NY.
r. Clinic-Check refractometer, Reichert Technologies, Depew, NY.
s. ADVIA 2120, Siemens, Malvern, Pa.
t. Cytopsin 4, Thermo Scientific, Waltham, Mass.
u. GraphPad Software Inc, La Jolla, Calif.
v. Reference Value Advisor, Biostatistiques Ecoli Nationale Vétérinaires de Toulouse, Toulouse, France.

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Appendix

Primers used for real-time PCR reactions for *Mycoplasma hyorhinis* and *Mycoplasma hyosynoviae* at the ISU VDL.

Oligo name	Sequence
MHR-F	5′-GCA TGT TGA ACG GGA TGT AGC AAT-3′
MHR-R	5′-TGA AGC TGT GAA GCT CCT TTC TAT TAC TC-3′
MHS-F	5′-CAG TTG AGG AAA TGC AAC TGA AC-3′
MHS-R	5′-CGT CAG TGA TTG GCC ACC G-3′