Analysis of synovial fluid from clinically normal alpacas and llamas

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Objective—To establish reference range values for synovial fluid from clinically normal New World camelids.

Animals—15 llamas and 15 alpacas.

Procedure—Llamas and alpacas were anesthetized with an IM injection of a xylazine hydrochloride, butorphanol tartrate, and ketamine hydrochloride combination. Synovial fluid (1 to 2 ml) was obtained by aseptic arthrocentesis from the radiocarpal and tarsocrural joints. Synovial fluid evaluation included determination of total nucleated cell count (NCC), absolute number and percentage of polymorphonuclear (PMN) and mononuclear leukocytes, total protein, and specific gravity.

Results—Synovial fluid evaluation revealed a total NCC of 100 to 1,400 cells/µl (mean ± SD, 394.8 ± 356.2 cells/µl; 95% confidence interval [CI], 295.2 to 494.6 cells/µl). Mononuclear leukocytes were the predominant cell type with lymphocytes, composing 50 to 90% (mean, 75.6 ± 17.2%; 95% CI, 70.8 to 80.4%) of the mononuclear leukocytes. Approximately 0 to 12% (mean, 1.3 ± 2.9%; 95% CI, 0.49 to 2.11%) of the cells were PMN leukocytes. Total protein concentrations ranged from 2.0 to 3.8 g/dl (mean, 2.54 ± 0.29 g/dl; 95% CI, 2.46 to 2.62 g/dl); the specific gravity ranged between 1.010 and 1.026 (mean, 1.017 ± 0.003; 95% CI, 1.016 to 1.018).

Conclusion and Clinical Relevance—In llamas and alpacas, significant differences do not exist between species or between limbs (left vs right) or joints (radiocarpal vs tarsocrural) for synovial fluid values. Total NCC and absolute number and percentage of PMN and mononuclear leukocyte are similar to those of other ruminants and horses. However, synovial fluid total protein concentrations in New World camelids are high, compared with other domestic species. (*Am J Vet Res* 2002;63:576–578)

Joint disease, including inflammatory and infectious synovitis and arthritis, is found in New World camelids of all ages.[1] Infectious arthritis in New World camelids may develop by direct inoculation of microorganisms, extension from periarticular injuries, or hematogenous spread. Other causes of arthritis involving camelids include traumatic arthritis and degenerative osteoarthritis as a sequela to an angular limb deformity.[1] Because microbiologic culture of synovial fluid has been reported to yield bacterial growth in only approximately 50% of clinically infected joints, synovial fluid analysis is vital for differentiation between the various types of synovitis.[2] Regardless of the cause, early identification and treatment of synovitis are essential for successful management and return to function. Currently, reference range values for synovial fluid from New World camelids are extrapolated from values of small ruminants or cattle.

The specific data routinely included in synovial fluid analysis are color, turbidity, total **nucleated cell count (NCC)**, absolute number and percentage of **polymorphonuclear (PMN)** and mononuclear leukocytes, total protein concentration, and specific gravity.[3] Synovial fluid from clinically normal horses and other ruminants is reported as being clear and having a total NCC of < 500 cells/µl[4,5] (< 25% PMN leukocytes on cytologic examination) and a protein concentration of 1.8 ± 0.26 g/dl[4] and 1.5 g/dl,[6,7] respectively. Characteristic signs of degenerative joint disease or nonseptic inflammation in the horse and other ruminants include yellow and translucent synovial fluid with a total NCC of 2,000 to 10,000 cells/µl and > 75% PMN leukocytes.[8] Recommendations for values of synovial fluid total NCC for which joints should be considered to be septic range from 30,000 to 100,000 cells/µl.[8] In other large animals, including horses, sheep, goats, and cattle, total protein concentrations between 2.0 and 4.0 g/dl indicate inflammation. Total protein concentrations ≥ 4.0 g/dl (small ruminants, ≥ 3.5 g/dl) and identification of degenerative neutrophils with activated macrophages are indicative of sepsis.[4,6-8] The purpose of the study presented here was to establish reference range values for synovial fluid from clinically normal New World camelids (alpacas and llamas). These values will be helpful for accurate diagnoses of acute synovitis.

Materials and Methods

Camelids and anesthesia—Fifteen llamas and 15 alpacas (20 females, 8 geldings, and 2 intact males; 1 to 12 years of age) were anesthetized with an IM injection of a xylazine hydrochloride (0.83 mg/ml), butorphanol tartrate (0.083 mg/ml), and ketamine hydrochloride (83 mg/ml) combination at a dosage of 0.05 ml/kg. Signs of clinical joint disease were not identified by physical and lameness examinations.

Arthrocentesis and cytologic examination—Aseptic arthrocentesis of the radiocarpal and tarsocrural joints was performed in all llamas and alpacas. The particular limb (left vs right) on which the arthrocentesis was performed was determined in a random fashion. The area over the particular joint was clipped and surgically prepared with a 10% solution of povidone iodine. Synovial fluid (0.5 to 2 ml) was obtained

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by arthrocentesis from the radiocarpal and tarsocrural joints. The radiocarpal joint was aspirated with the carpus in flexion. A 21-gauge 1.5-in-long needle was inserted into the dorsolateral joint capsule adjacent to the extensor tendon.[1] To obtain fluid from the tarsocrural joint, a 20-gauge 1.5-in-long needle was inserted ventral to the lateral malleolus of the tibia and directed medially and ventrally.[1] Forty-nine synovial fluid samples were evaluated. Total NCC were performed, using a hematology analyzer.[a] Cytologic evaluation was performed after the samples were collected in tubes containing EDTA to preserve cytologic detail.[3] For cytologic examination, synovial fluid smears were stained with Wright stain. With this stain, specific cell types were differentiated, including PMN and mononuclear leukocytes as well as RBC. A refractometer was used to measure total protein and specific gravity. The same individual (JSS) performed all measurements.

Data and statistical analysis—Data evaluated in our study included synovial fluid total NCC, absolute number and percentage of PMN and mononuclear leukocytes, total protein, and specific gravity of fluid from the radiocarpal and tarsocrural joints. Data were expressed as mean ± SD, 95% **confidence intervals (CI)**, and ranges. Analysis of variance was used to simultaneously contrast the effects of species (llama vs alpaca), limb (left vs right), and joint (radiocarpal vs tarsocrural) on each variable. Forty-nine samples were used in the ANOVA. The differences were considered significant at values of $P \leq 0.05$

Results

The gross appearance of the synovial fluid collected was uniformly clear and free of flocculent material. An occasional sample became contaminated with fresh blood at the time of aspiration; hemorrhagic samples were discarded. The total volume of synovial fluid obtained in general varied in direct proportion to the size of the joint. Arthrocentesis of the tarsocrural joint was readily obtained from the dorsal pouch with minimal risk of contamination and resulted in a sufficient amount (1 to 2 ml) of synovial fluid for analysis. Arthrocentesis of the radiocarpal joint was difficult as a result of a narrow joint space, so redirection of the needle often resulted in hemorrhagic contamination of the sample. The radiocarpal joint was entered through the dorsolateral aspect of the joint capsule adjacent to the extensor tendons with the joint in flexion to obtain an adequate amount (500 µl) of fluid with minimal contamination. Eleven samples were not included in the study because of hemorrhagic contamination or because the aspirated volume was inadequate for analysis.

When evaluating the total NCC, absolute values for PMN and mononuclear leukocytes, total protein, and specific gravity of the llama and alpaca synovial fluid, no significant differences were found between camelid species, limbs, or joints. New World camelid synovial fluid total NCC ranged from 100 to 1,400 cells/µl with a mean ± SD of 394.8 ± 356.2 cells/µl and a 95% CI of 295.2 to 494.6 cells/µl (**Table 1**). Mononuclear leukocytes were the predominant cell type within the joint. Lymphocytes composed 50 to 90% of the mononuclear leukocytes with a mean of 75.6 ± 17.2% and a 95% CI of 70.8 to 80.4%. Approximately 0 to 12% (mean ± SD, 1.3 ± 2.9%; 95% CI, 0.49 to 2.11%) of the cells found in the synovial fluid were PMN leukocytes (**Table 2**). No eosinophils were observed on cytologic examination. The remaining 4 to 48% of the cells were monocytes (mean, 21 ± 14.9%; 95% CI, 17.2 to 25.6%). Total protein concentrations ranged from 2.0 to 3.8 g/dl (mean, 2.54 ± 0.29 g/dl; 95% CI, 2.46 to 2.62 g/dl); the specific gravity ranged between 1.010 and 1.026 (mean, 1.017 ± 0.003; 95% CI, 1.016 to 1.018).

Discussion

Currently, synovial fluid values for New World camelids are based on values from small ruminants or cattle. We determined reference range values for total NCC and absolute number and percentage of PMN and mononuclear leukocytes, because cytologic examination of synovial fluid can provide valuable information in addition to that gained by clinical and radiographic examination. Quantitative and qualitative changes in the leukocytes can also provide an indication of the

Table 1—Reference range values for synovial fluid from clinically normal New World camelids

	Llama		Alpaca		Combined	
Variables	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**
Total NCC (cells/µl)	100–1,200	369.6 (308.3)	100–1,400	428.5 (417.0)	100–1,400	394.8 (356.2)
PMN leukocytes (%)	0–4	0.731 (1.5)	0–12	2.0 (4.0)	0–12	1.3 (2.9)
Monocytes (%)	4–48	17.9 (9.5)	4–38	25.9 (19.2)	4–48	21.4 (14.9)
Lymphocytes (%)	52–92	80.8 (9.9)	50–96	68.5 (22.2)	50–96	75.6 (17.2)
Protein (g/dl)	2–3.8	2.5 (0.35)	2.1–2.5	2.3 (0.23)	2.0–3.8	2.54 (0.29)
Specific gravity	1.010–1.026	1.017 (0.003)	1.014–1.019	1.017 (0.001)	1.010–1.026	1.017 (0.003)

Total NCC = Total nucleated cell count. PMN = Polymorphonuclear.

Table 2—Absolute PMN leukocyte, monocyte, and lymphocyte count for synovial fluid of New World camelids

	Llama		Alpaca		Combined	
Variables	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**
PMN leukocytes (cells/µl)	0–16	1.8 (4.1)	0–168	11.2 (37.8)	0–168	5.8 (25.2)
Monocytes (cells/µl)	8–192	63.3 (50.8)	12–884	127 (197.0)	8–884	91.2 (137.0)
Lymphocytes (cells/µl)	68–1,032	319 (278.0)	40–1,064	304 (308.0)	40–1,064	312 (288.0)

See Table 1 for key.

magnitude of synovial membrane inflammation and presence of infection, as culture results are often unrewarding.

Synovial fluid from cattle is relatively acellular with a mean total NCC of 103.50 ± 14.23 cells/µl. The majority of the leukocytes are mononuclear (lymphocytes, 49.08 ± 2.77%; monocytes, 38.22 ± 2.47%).[5,9,10] Small ruminant values are grossly and cytologically equivalent.[7] Reference values for total NCC of synovial fluid from horses have been reported as 167 ± 21 cells/µl. The percentage of PMN leukocytes is generally < 10% in synovial fluid of clinically normal horses.[4] The results of our study indicate that the total NCC and absolute number and percentage of PMN and mononuclear leukocytes in the synovial fluid of New World camelids are similar among species, joints, and other large domestic animals, including horses, sheep, goats, and cattle.

Total protein concentrations are evaluated in synovial fluid analysis, because increases are observed with inflammatory and infectious synovitis.[3] Reference values for total protein concentrations of synovial fluid from New World camelids in our study were similar between llamas and alpacas but much higher (mean, 2.54 ± 0.29 g/dl; 95% CI, 2.46 to 2.62 g/dl) than other domestic species. The reference values for total protein concentrations in small ruminants and horses are documented at 1.5 mg/dl[7] and 1.8 ± 0.26 g/dl,[4] whereas the protein concentration in cattle synovial fluid is considered within reference range if it is < 1.8 g/dl.[9,10] The reason for the higher total protein concentration in synovial fluid from llamas and alpacas is unknown. Paper electrophoresis of synovial fluid from clinically normal horses after treatment with hyaluronidase has been performed to identify various protein fractions.[11] Further investigations using electrophoresis may be helpful in determining the reason for the high protein concentration in synovial fluid of New World camelids.

[a]Baker 9118 hematology analyzer, Biochem Immunosystems, Allentown, Pa.

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