Collection and analysis of peritoneal fluid from healthy llamas and alpacas

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Objective—To describe a technique for abdominocentesis in camelids and report peritoneal fluid biochemical and cytologic findings from healthy llamas and alpacas.

Design—Prospective study.

Animals—17 adult llamas and 5 adult alpacas.

Procedures—Right paracostal abdominocentesis was performed. Peritoneal fluid was collected by gravity flow into tubes containing potassium-EDTA for cell count and cytologic evaluation and lithium heparin for biochemical analysis. Blood samples were collected via jugular venipuncture into heparinized tubes at the same time. Cytologic components were quantified. Fluid pH and concentrations of total carbon dioxide, sodium, potassium, chloride, lactate, and glucose were compared between peritoneal fluid and venous blood.

Results—All but 3 camelids had peritoneal fluid cell counts of < 3,000 nucleated cells/μL, with < 2,000 neutrophils/μL and < 1,040 large mononuclear cells/μL. All but 1 had peritoneal fluid protein concentrations of ≤ 2.5 g/dL. Peritoneal fluid of camelids generally contained slightly less glucose, lactate, and sodium and roughly equal concentrations of potassium and chloride as venous blood.

Conclusions and Clinical Relevance—Peritoneal fluid was collected safely from healthy camelids. Compared with blood, peritoneal fluid usually had a low cell count and protein concentration, but some individuals had higher values. Electrolyte concentrations resembled those found in blood. High cell counts and protein concentrations found in peritoneal fluid of some healthy camelids may overlap with values found in diseased camelids, complicating interpretation of peritoneal fluid values. (*J Am Vet Med Assoc* 2008;232:1357–1361)

Peritoneal fluid analysis has been used in many species to diagnose abdominal disorders, including gastrointestinal diseases, uterine diseases, bacterial infections, splenic or hepatic torsion or rupture, urinary tract leakage, neoplasia, and pancreatitis. Although reported occasionally,[1–9] this tool historically has been underused in New World camelids because of lack of reference values and the perception that adequate volumes of uncontaminated sample were difficult to obtain, or that the collection technique could lead to health complications for the patient. Similar to cattle, abdominocentesis performed in the cranioventral region of the abdomen in camelids bore the risk of puncture into the gastrointestinal tract or interference from the omentum, and unlike cattle, camelids appeared to deal poorly with accidental abdominal contamination. As a result, prominent reference works on New World camelids contain no information on peritoneal fluid values or suggest that the values presented are not based on a comprehensive study.[3,4]

The purpose of the study reported here was to describe a technique for abdominocentesis in camelids and report peritoneal fluid biochemical and cytologic findings from healthy llamas and alpacas. Recently, we described a technique that yielded sufficient uncontaminated peritoneal fluid samples for analysis from a small number of healthy camelids, without identifiable danger to the patient.[5] Using an improved version of this technique, we collected fluid from a larger population of healthy llamas and alpacas to establish reference values. To further classify the nature of peritoneal fluid of camelids, we compared these results to blood values from the same camelids.

Materials and Methods

Animals—This study included 17 llamas and 5 alpacas from the Oregon State University teaching herd. Camelids were healthy adults and ranged in age from 3 to 17 years old. Good health was judged on the basis of physical examination findings and lack of adverse health events for the preceding 6 months. None of the llamas or alpacas was pregnant or had been pregnant within the last 2 years, and regular monitoring of this herd consistently revealed relatively low numbers of parasite eggs in fecal samples. This study was approved in advance by the institutional animal care and use committee.

Procedure—Peritoneal fluid was collected by use of a modified version of the paracostal approach.[5] In brief, camelids were placed in a restraint chute and received butorphanol tartrate (0.04 mg/kg [0.02 mg/lb],

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IM). The region caudal to the last rib and approximately a third to a half of the distance from the ventral to dorsal midline was clipped and surgically prepared. Two milliliters of lidocaine hydrochloride was infused SC and IM at a location approximately 1 cm dorsal and 3 cm caudal to the costochondral junction of the last rib in alpacas, and at a location approximately 2 cm dorsal and 5 cm caudal to the costochondral junction of the last rib in llamas; SC and IM infusion of lidocaine was within the ventral border of the muscular portion of the external abdominal oblique muscle in alpacas and llamas. This same site could be found slightly less than midway between the ventral midline and the ventral aspects of the lumbar vertebrae, when viewed from the side, at the spot where a line drawn caudad from the caudal-most aspect of the last rib intersected the diagonal formed by the muscular edge of the external abdominal oblique muscle.

A stab incision was made into the anesthetized muscle with a No. 15 scalpel blade. A sterile teat cannula was introduced perpendicular to the skin through the incision and pressed through the remaining muscle and peritoneal membrane into the abdomen, and fluid was collected by gravity flow into tubes containing potassium-EDTA for cell count and cytologic evaluation and lithium heparin for biochemical analysis. In some instances, the hub of the cannula was depressed slightly to facilitate flow. A minimum of 1 mL and a maximum of 10 mL of peritoneal fluid were collected into the tubes within 60 seconds. The cannula was removed and the stab incision left to heal by second intention. A blood sample was collected at the same time via jugular venipuncture into a tube containing lithium heparin. The rectal temperature of each llama or alpaca was determined to provide a baseline value and for input into the blood gas analyzer.

Camelids were monitored for 24 hours, including hourly observation of behavior and a second physical examination the day after the procedure, then returned to the herd at the conclusion of the trial. Camelids were subsequently monitored for abnormal behavior or loss of body condition for 1 year.

Peritoneal fluid and blood analyses—Volume, color, and clarity of peritoneal fluid samples were recorded. For cytologic analysis, peritoneal fluid samples were processed within 30 minutes of collection. Nucleated cell counts of peritoneal fluid samples were determined by use of a disposable diluting pipette system[a] and hemocytometer. For differential cell counts, a cytocentrifugation preparation was made from peritoneal fluid samples and stained with Wright-Giemsa stain; cell counts were determined by microscopic analysis of 200 nucleated cells in the sediment. Peritoneal fluid protein concentration was determined with a refractometer. The pH and the total carbon dioxide, glucose, lactate, sodium, potassium, and chloride concentrations of peritoneal fluid were determined within 10 minutes of sample collection by use of an automated analyzer.[b] The same analytes were determined in venous blood by use of the same analyzer.[b]

Statistical analysis—Data were checked for normality by the Kolmogorov-Smirnov test with a Lilliefors correction.[c] Data from llamas and alpacas pooled after comparison by use of a Student *t* test or the Mann-Whitney *U* test revealed no significant differences between species. Differences in concentration of specific biochemical constituents between blood and peritoneal fluid were calculated. Concentrations of the biochemical constituents in blood and peritoneal fluid were compared by use of the paired *t* test. Values of $P < 0.05$ were considered significant.

Results

The procedure yielded 1 to 10 mL of peritoneal fluid from each llama or alpaca. The peritoneal fluid was clear to hazy and colorless to light yellow or slightly pink. No adverse effects were observed following abdominocentesis, and all tested camelids remained in the herd for at least a year.

Nucleated cell counts in peritoneal fluid ranged from 110 to 20,500 cells/µL (median, 780 cells/µL; **Table 1**). Thirteen peritoneal fluid samples had < 1,000 cells/µL, and only 3 had > 3,000 cells/µL (**Figure 1**). Neutrophils accounted for 15% to 98% (median, 65%) of the nucleated cells, large mononuclear cells for 2% to 83% (median, 32%), and lymphocytes for 0% to 26% (median, 2%). Eosinophils accounted for up to 4% of nucleated cells in 3 peritoneal fluid samples. The 3 highest cell counts

Table 1—Selected cytologic and biochemical results for peritoneal fluid from 22 healthy New World camelids.

Variable	Median (range)	IQR
Nucleated cells (cells/µL)	780 (110–20,500)	250–1,890
Neutrophils (cells/µL)	426 (18–20,090)	67–16,300
Neutrophils (%)	65 (15–98)	41–85
Large mononuclear cells (cells/µL)	194 (23–4,163)	84–410
Large mononuclear cells (%)	32 (2–83)	14–55
Lymphocytes (cells/µL)	9 (0–203)	0–14
Lymphocytes (%)	2 (0–26)	0–4
Eosinophils (cells/µL)	0 (0–9)	0–0
Eosinophils (%)	0 (0–9)	0–0
Protein (g/dL)	< 1.0 (< 1.0–4.0)	< 1.0–1.4
Glucose (mg/dL)	135 (108–297)	119–146

IQR = Interquartile range (25th to 75th percentile).

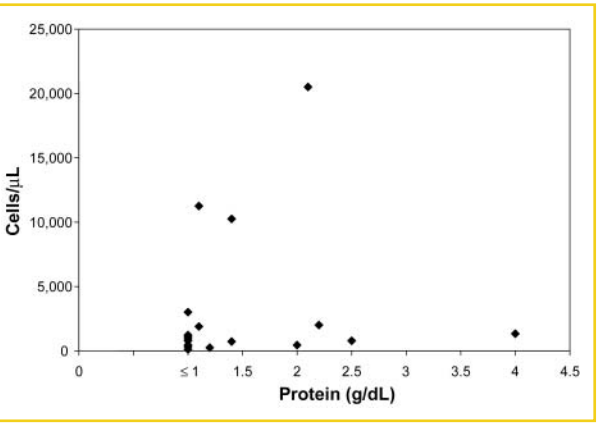

Figure 1—Association between peritoneal fluid protein concentration and nucleated cell count in 22 healthy New World camelids.

ranged from 10,000 to 20,500 cells/µL, with 63% to 98% neutrophils. The peritoneal fluid sample with the highest cell count had low volume. Except for the 3 camelids with the highest nucleated cell counts, all peritoneal fluid samples had < 2,000 neutrophils/µL and < 1,040 large mononuclear cells/µL.

Twelve camelids had peritoneal fluid protein concentrations of < 1.0 g/dL, and all but 1 of the camelids had peritoneal fluid protein concentrations of ≤ 2.5 g/dL (Table 1; Figure 1). The 3 camelids with high peritoneal fluid cell counts all had peritoneal fluid protein concentrations of ≤ 2.1 g/dL. Interestingly, the 1 animal with the highest protein concentration (4 g/dL) had a low cell count but also the lowest volume yield.

Biochemical analysis revealed that neither blood nor peritoneal fluid glucose concentrations were normally distributed. All but 2 peritoneal fluid samples had glucose concentrations between 100 and 150 mg/dL (median, 135 mg/dL; Table 1). Eighteen peritoneal fluid samples had glucose concentrations of 4 to 30 mg/dL lower than the corresponding blood samples. The remaining samples had equal to slightly higher peritoneal fluid glucose concentrations. The difference between mean ± SD glucose concentrations in peritoneal fluid versus venous blood (140 ± 39 mg/dL vs 152 ± 32 mg/dL, respectively) was significant ($P < 0.001$).

Sodium concentrations of peritoneal fluid samples from all camelids ranged from 141 to 153 mEq/L and were lower than that of the corresponding blood sample (**Table 2**). The difference between mean ± SD sodium concentrations in peritoneal fluid versus venous blood (147 ± 3 mEq/L vs 154 ± 4 mEq/L, respectively) was significant ($P < 0.001$). The difference in mean sodium concentrations between venous blood and peritoneal fluid was 6.7 ± 3.4 mEq/L.

Eighteen peritoneal fluid samples had potassium concentrations that were within 0.4 mEq/L of the corresponding blood sample; all but 1 peritoneal fluid sample had potassium concentrations that ranged from 3.7 to 5.1 mEq/L (Table 2). Roughly equal numbers of these peritoneal fluid samples had potassium concentrations that were higher or lower than the corresponding blood sample. The remaining 4 peritoneal fluid samples had potassium concentrations that were 0.59 to 1.32 mEq/L higher than that of the corresponding blood sample. The difference between mean ± SD potassium concentrations in peritoneal fluid versus venous blood (4.4 ± 0.6 mEq/L vs 4.3 ± 0.4 mEq/L, respectively) was not significant ($P = 0.119$).

Table 2—Mean ± SD selected cytologic and biochemical values for peritoneal fluid and venous blood from 22 healthy New World camelids.

Variable	Peritoneal fluid	Venous blood	Difference
pH	7.72 ± 0.14*	7.44 ± 0.07	–0.28 ± 0.13
Tco_2 (mEq/L)	36.3 ± 4.3*	31.3 ± 4.1	–5.9 ± 4.4
Lactate (mg/dL)	0.72 ± 0.61*	1.08 ± 0.69	0.36 ± 0.47
Sodium (mEq/L)	147 ± 3*	154 ± 4	7 ± 3
Potassium (mEq/L)	4.4 ± 0.6	4.3 ± 0.4	–0.1 ± 0.4
Chloride (mEq/L)	116 ± 3	116 ± 4	–0.3 ± 3

*Values significantly different between blood and peritoneal fluid.
Tco_2 = Total carbon dioxide.

Chloride concentrations of peritoneal fluid samples from all camelids ranged from 111 to 121 mEq/L and were within 5 mEq/L of that of the corresponding blood sample (Table 2). Roughly equal numbers of these peritoneal fluid samples had chloride concentrations that were higher or lower than the corresponding blood sample. The difference between mean ± SD chloride concentrations in peritoneal fluid versus venous blood (116 ± 3 mEq/L vs 116 ± 4 mEq/L, respectively) was not significant ($P = 0.665$).

The pH values of peritoneal fluid samples from all camelids ranged from 7.49 to 7.95; all peritoneal fluid samples had higher pH values than that of the corresponding blood sample (Table 2). The difference between mean ± SD pH values in peritoneal fluid versus venous blood (7.72 ± 0.14 vs 7.44 ± 0.07, respectively) was significant ($P < 0.001$).

Total carbon dioxide concentrations of peritoneal fluid samples from all camelids ranged from 27.6 to 42.4 mEq/L (Table 2); all but 1 peritoneal fluid sample had total carbon dioxide concentrations that were 1 to 14 mEq/L higher than that of the corresponding blood sample (Table 2). The difference between mean ± SD total carbon dioxide concentrations in peritoneal fluid versus venous blood (36.3 ± 4.3 mEq/L vs 31.3 ± 4.1 mEq/L, respectively) was significant ($P < 0.001$).

Lactate concentrations ranged from 0.2 to 1.05 mg/dL in all but 2 peritoneal fluid samples. All but 3 peritoneal fluid samples had lactate concentrations within 0.53 mg/dL of the corresponding blood samples (Table 2). In samples from all but 3 camelids, peritoneal fluid lactate concentration was lower than that of the corresponding blood sample. The difference between mean ± SD lactate concentrations in peritoneal fluid versus venous blood (0.72 ± 0.61 mg/dL vs 1.08 ± 0.69 mg/dL, respectively) was significant ($P = 0.002$).

Discussion

The technique of abdominocentesis described in this study readily provided adequate volumes of relatively uncontaminated peritoneal fluid from camelids. The fact that none of the camelids had signs of discomfort or inappetence, weight loss, or any other clinical sign referable to the procedure over an extended period suggested that the procedure was generally safe. Given that sick camelids frequently have hypoproteinemia,[10,11] which frequently results in the development of ascites, and that camelids specifically with abdominal lesions may be more prone to accumulate peritoneal fluid, the technique described here would logically also be likely to yield samples of adequate volume from sick camelids.

Based on these results, we submit that most healthy New World camelids have peritoneal fluid containing < 2,000 neutrophils/µL and < 1,040 large mononuclear cells/µL, with up to a total of 3,000 nucleated cells/µL. Neutrophils commonly outnumbered large mononuclear cells. The protein concentration should be < 2.5 g/dL, with higher values permissible if the cell count is within reference values and only a small volume of fluid is present. Cell counts and protein concentrations were usually at the lower end of these ranges. However, findings in this report and another study[5] suggest that

higher cell counts and protein concentrations can be found in apparently healthy camelids. Even previously suggested reference limits for protein concentrations of < 2.5 g/dL and < 3.0 g/dL and for nucleated cell counts of < 5,000 cells/μL[3,4] fail to encompass all clinically healthy camelids. Some of the high cell counts and protein concentrations could be attributed to low fluid volume. Another possible explanation would be the mass resumption of extravasation of cells after a period of inhibited margination. Camelids are known to develop neutrophilia with excitement or stress.[12] The ultimate fate of these neutrophils is unknown. In the present study, the high peritoneal cell counts were also predominately composed of neutrophils. The occurrence of noninflammatory peritoneal neutrophilic pleocytosis has not been described for other species, but stress neutrophilia of the magnitude commonly seen in camelids is also rare in other domestic mammals. It is also possible that some of the apparently healthy camelids had previous or ongoing bouts of abdominal disease, which were otherwise not detected.

The finding of high values for peritoneal fluid cell counts and protein concentration in some apparently healthy camelids may limit the specificity of these values in the diagnosis of abdominal disease. On the other hand, considering these high-end values as within the reference range may impact the sensitivity of peritoneal fluid analysis in the diagnosis of abdominal disease. In the 2 largest reports[1,6] of peritoneal fluid analysis from sick camelids, many camelids with clear abdominal abnormalities were reported to have peritoneal fluid cell counts and protein concentrations at the upper end of, yet within, the broader reference ranges for protein concentration (> 2.5 g/dL) and nucleated cell counts (> 3,000 cells/μL). In these studies,[1,6] volume of fluid collected and differential cell counts were not reported. On the basis of the current and previous findings,[1,6] results at the higher end of this range should be interpreted with caution. Factors that might indicate disease include copious amounts of cellular or proteinaceous fluid, or a combination of high cell count (> 3,000 nucleated cells/μL) and high protein concentration (> 2.5 g/dL). The effects of disease on differential cell counts have yet to be evaluated.

Peritoneal fluid of camelids was found to be more alkaline and contain slightly less glucose, lactate, and sodium and roughly equal concentrations of potassium and chloride as venous blood. Given the large surface area for exchange between peritoneal fluid and blood, relative equality of glucose and lactate might be expected, but concentrations may be affected on either side of the peritoneal membrane by local production or consumption. Concerning electrolytes, the overall calculated near-electroneutral character of peritoneal fluid samples met the theoretic criteria of a low-protein body fluid.[13] The lower peritoneal sodium concentrations fit with the principles of the Gibbs-Donnan equilibrium, whereby the presence of anionic proteins in plasma does not prevent the influx of interstitial chloride down concentration gradients, and some interstitial sodium accompanies the chloride to preserve an electroneutral environment.[14] The lack of the corresponding theoretic higher chloride concentration in peritoneal fluid may reflect an inaccuracy in the measurement of this anion. Previous research has highlighted the possibility of cross-reaction on diagnostic assays for anions,[15] and the diversity of anions potentially present in body fluids makes the possibility of cross-reaction a greater consideration for chloride than for sodium assays.

Peritoneal fluid sodium, potassium, and chloride concentrations were higher in healthy New World camelids than in horses with colic when analyzed by the same method.[16] These differences likely relate to the fact that camelids also often have higher blood concentrations of these electrolytes than horses.

Compared with venous blood values, the higher peritoneal fluid pH and total carbon dioxide concentration were similar to peritoneal fluid values found in horses[16,17] and humans[18] and appear to be attributable to the low concentration of protein in peritoneal fluid. In plasma, most proteins are dissociated at a physiologic pH, contributing to the acidity of plasma over that of peritoneal fluid.[19] In humans, each 1 g/dL decrease in albumin is estimated to increase plasma bicarbonate concentration by 3.7 mEq/L.[20]

Despite the small numbers of alpacas tested in our study, it is likely that our findings are applicable to llamas and alpacas as well as potentially to some of the wild species of New World camelids. To date, no differences in basic clinicopathologic tests have been identified between llamas and alpacas, and even though they have recently been classified as belonging to different genera,[21] mitochondrial DNA analysis suggests that llamas and most alpacas share a common ancestry or have undergone extensive hybridization at some point.[22]

Clinical reports[7,8] containing information on the biochemical analysis of peritoneal fluid of camelids are rare and mainly confined to the diagnosis of uroperitoneum. Anecdotally, this analysis has shown similar benefits to that seen in horses for the diagnosis of strangulating lesions or peritonitis. The use of blood gas analyzers to analyze equine peritoneal fluid has been reported previously and appears to offer useful results in that species.[16,17] Because peritoneal fluid often has a biochemical composition that is similar to plasma, most values fall well within the optimal range for such analyzers. Also, the likelihood of aberrant biochemical values in healthy camelids appears to be less likely than the likelihood of high cell counts or protein concentrations. Hence, biochemical analysis of peritoneal fluid of camelids by use of a blood gas analyzer appears to offer a rapid, convenient diagnostic option.

a. Unopette reservoirs and 20 μL capillary pipettes, Becton Dickinson, Franklin Lakes, NJ.
b. Rapidlab 865, Chiron Diagnostics, Norwood, Mass.
c. SigmaStat 2.0, SPSS Inc, Chicago, Ill.

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