J Vet Intern Med 2013;27:970–976

Evaluation of Methods to Improve the Diagnosis of Systemic Inflammation in Alpacas

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Background: The stoic nature of alpacas and limitations of current diagnostic tests make early recognition of inflammatory diseases in this species challenging.

Objectives: In a model of mild systemic inflammation, this study evaluated the utility of different clinical and clinicopathologic variables as accurate predictors of inflammation in alpacas.

Animals: Twelve clinically healthy alpacas were randomly assigned to equal-sized treatment (TG) and control (CG) groups. After collection of initial blood samples (0 hour), lipopolysaccharide (LPS; 20 μg/kg/24 h) or saline was administered by SC osmotic mini-pumps (OMP) for 96 hours. Additional blood samples were collected at 12, 18, 24, 36, 48, 72, 96, 120, 144, and 240 hours and differential leukocyte counts and concentrations of globulin, albumin, iron, haptoglobin, and serum amyloid A were measured.

Results: Mild swelling was observed at OMP implantation sites in both groups. Other clinical signs of systemic inflammation were not observed. Total leukocytes, neutrophils, albumin, and globulin concentrations were not significantly different between groups. Compared with CG-alpacas, TG-alpacas had fewer lymphocytes ($P = .0322$), more band neutrophils ($P = .0087$), and higher neutrophil/lymphocyte ratios ($P = .0295$) during the first 96 hours of the study. During LPS administration, serum iron concentrations were significantly decreased in TG-alpacas ($P < .0001$). Haptoglobin concentrations of TG-animals exceeded those of CG-animals after removal of OMP ($P = .0056$). Serum amyloid A was not detectable in alpacas in this study.

Conclusion and Clinical Importance: These results indicated that neutrophil/lymphocyte ratios and serum iron concentrations are early indicators of inflammation in alpacas. Additional research is needed to evaluate the acute phase protein responses of alpacas.

Key words: Acute phase proteins; Haptoglobin; New World camelid; Osmotic mini-pump; Serum amyloid A; Serum iron concentration.

Abbreviations:

APP	acute phase proteins
CG	control group
LPS	lipopolysaccharide
N/L	neutrophil-to-lymphocyte ratio
OMP	osmotic mini-pump
SAA	serum amyloid A
TG	treatment group

Many disorders induce inflammatory responses in alpacas, and severe infections can result in life-threatening systemic inflammation. Untreated systemic inflammation may lead to septic shock and multiple organ failure. Prompt intervention with early goal-directed treatment is critical for management of severe inflammatory diseases.[1] However, early recognition of inflammation in alpacas is difficult using conventional diagnostic techniques such as clinical signs, CBC, and plasma fibrinogen concentrations.

The stoic nature of alpacas makes early recognition of inflammatory diseases challenging. Clinical signs signaling an inflammatory process often are nonspecific and vary considerably among stages of disease.[2,3] Septic animals can be hypothermic, normothermic, or hyperthermic as a result of changes to cardiovascular function during endotoxemia.[3] Respiratory rate, pulse rate, and body temperature are influenced by exogenous factors and noninflammatory processes, including ambient temperature or excitement.

Similarly, changes in leukocyte counts vary with duration and severity of inflammatory processes. Despite its common clinical use, diagnostic ambiguity restricts the reliability of plasma fibrinogen concentration as a marker of inflammation. Plasma fibrinogen concentration increases relatively late and may not peak until day 2 or 3 of the inflammatory process.[4] In llamas experimentally infected with *Streptococcus zooepidemicus* by intratracheal inoculation, fibrinogen concentrations exceeded the reference interval on day 4, but not on day 1, after infection.[2] Furthermore, wide reference intervals of plasma fibrinogen concentration in healthy alpacas decrease diagnostic validity.[5]

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Submitted October 23, 2012; Revised March 15, 2013; Accepted April 2, 2013.

10.1111/jvim.12102

In various species, including horses and ruminants, acute phase proteins (APP) are widely applied as biomarkers of inflammation, but these markers have not been evaluated in alpcas.[6] APP are liver-derived blood proteins that change in concentration in response to infection, inflammation, or injury.[6,7] APP aid in restoration of homeostasis, restraint of microbial growth, and enhanced tissue repair, and play major roles in aspects of the systemic reaction to inflammation, including opsonization of pathogens, scavenging of toxic substances, and regulation of different stages of inflammation.[6,8] Because blood concentrations of APP are related to disease severity, their quantification provides valuable diagnostic and prognostic information.[7] In cattle, haptoglobin and SAA were demonstrated to be useful in detection of mastitis, reproductive conditions, gastrointestinal disorders, respiratory diseases, and viral infections.[8] A third APP, hepcidin, restrains growth of bacterial pathogens by decreasing serum iron concentrations upon host infection.[9] Decreased serum iron concentration is a more sensitive marker of acute inflammation than increased plasma fibrinogen concentration in horses.[10] In llamas experimentally infected with *S. zooepidemicus*, low serum iron concentration also was the most consistent biochemical abnormality.[2]

Prompted by limitations of current diagnostic tests of inflammatory disease in alpacas, this study evaluated the utility of different clinical and clinicopathologic variables as accurate predictors of inflammation in alpacas.

Materials and Methods

Animals

This research was performed under approval of the Institutional Animal Care and Use Committee of Auburn University (2011–1866). Twelve adult, castrated, clinically healthy alpacas from the teaching herd of the College of Veterinary Medicine, Auburn University were included in the prospective cohort study. Animals were transported for 200 meters to a 2-acre research pasture on the same premises and acclimated for a period of 7 days. Animals were fed free-choice grass hay and water, supplemented with 1 kg of concentrate per animal. Alpacas were randomly assigned to equal-sized treatment (TG) or control (CG) groups, comprised of 6 animals each.

Model of Systemic Inflammation and Sample Collection

At 0 hour, animals were sedated by SC injection of butorphanol tartrate[a] (0.04 mg/kg), ketamine HCl[b] (4.5 mg/kg), and xylazine[c] (0.4 mg/kg). In recumbent alpacas, long-term IV jugular catheters[d] were placed after aseptic preparation and collection of initial blood samples. Catheters were maintained until 144 hours and irrigated with 10 mL of 1% heparinized saline after each sample collection. In TG-animals, bacterial LPS[e] was administered in adaptation of a model of systemic inflammation in sheep,[11] delivering continuous low-dose LPS from osmotic mini-pumps (OMP)[f] placed SC in the left thoracic area. For TG-animals, OMP were filled according to manufacturer instructions to deliver a solution of LPS in physiologic saline at a dosage of 20 μg/kg/24 h. OMP for controls were filled with physiologic saline. In sedated alpacas, the left thoracic area was prepared aseptically and 2% lidocaine hydrochloride[g] was administered at the implantation site. A 3-cm incision was made in the thoracic skin at a point caudal to the elbow when the elbow and shoulder joints were maximally flexed. Blunt dissection was used to create a 5 × 2.5 cm SC pocket in which an OMP was placed before skin closure. This model was chosen to prevent severe clinical signs associated with the commonly used single-bolus IV LPS injection, in which adverse respiratory and cardiovascular effects are common.[12] Continuous low-dose LPS administration was used to emulate natural disease and evaluate which biomarkers are sensitive indicators of inflammation. Osmotic mini-pumps were removed under sedation and local analgesia (as described above) at 96 hours. Wound closure was achieved using skin staples.[h]

Physical Examination and Sample Collection

Initial physical examinations and blood sample collection were performed before OMP placement (0 hour). Clinical, hematologic, and biochemical data were collected while OMP were in situ (12, 18, 24, 36, 48, 72, and 96 hours) and after removal of OMP (120, 144, and 240 hours). This schedule allowed characterization of inflammatory marker responses during a continual period of inflammation and the postinflammatory period. Catheters were removed at 144 hours to prevent catheter infection, and blood samples at 240 hours were collected by jugular venipuncture. During examinations, objective and subjective clinical variables were recorded. Objective variables included body temperature, heart rate, and respiratory rate. Subjective variables (activity level, nasal discharge, mucosal congestion, presence of diarrhea, and appetite) were scored individually from 0 to 3 and summarized as a clinical score with a maximum of 15. After physical examination, blood samples were collected into anticoagulant-free and EDTA-containing blood tubes. Samples were processed for CBC and serum biochemistry immediately after collection, and serum aliquots were stored at −80°C until APP measurement.

CBC and Serum Biochemistry

Hematologic and biochemical analyses were performed at the clinical pathology laboratory of the College of Veterinary Medicine, Auburn University. Total cell counts were performed by automated cell counter,[i] followed by manual differential cell counts. Serum biochemistry analysis was performed by chemical analyzer[j] to detect total protein, albumin, globulin, and iron concentrations.

Acute Phase Protein Analysis

Haptoglobin concentrations were determined in sera using a commercially available colorimetric assay as per the manufacturer's instructions.[k] The analytical sensitivity of this test is 0.005 mg/mL of haptoglobin. A volume of 7.5 μL of samples or calibrator standards were added in duplicate to wells of 96-well microtiter plates. Five minutes after addition of reagents, the optical density of each well was determined by microplate reader at 615 nm wavelength.

Serum amyloid A concentrations were analyzed by a commercially available ELISA assay.[l] Standards and samples were analyzed in duplicate as per the manufacturer's instructions. Briefly, the solubilized standard was diluted 1:5 with diluent buffer to obtain the top working standard. Five subsequent standards then were prepared by 2-fold serial dilutions. Sera were diluted 1:500 with diluent buffer before analysis. The top standard and subsequent dilutions were the basis for calculation of species-specific

SAA concentrations. Because these results are unknown for camelids, calculations for SAA concentrations were based on bovine standards, as previously performed for goats.[13] In addition to provided standards, serum from a calf with septic arthritis and serum from an adult alpaca with parelaphostrongylosis were included as positive controls with a presumed detectable SAA response. The calf's serum sample was diluted at 1:500. Serum from the hospitalized alpaca was included without dilution and at dilutions of 1:50 and 1:500. Negative controls were included for each assay.

Statistical Analysis

Response data were analyzed using repeated measures methodology using PROC GLIMMIX of SAS 9.2.l.[m] All but 2 response variables were analyzed using a normal distribution function. The exceptions were (1) band neutrophil counts, which were arcsine square root transformed before analysis using a normal distribution function and (2) neutrophil-to-lymphocyte (N/L) ratios, which were analyzed using a lognormal distribution function. The random effect used to test main effects for treatments was animals (treatment). The repeated nature of the experiment resulted in a correlated residual error structure. Among all possible error structures, we chose a power covariance structure based on a lower AICC fit statistic compared with competing models.

The analysis proceeded along 2 paths. First, we analyzed all time and treatment points jointly using the SLICEDIFF option to (1) compare time > 0 to time = 0 in either group (control, treatment) by means of a Dunnett's test, and (2) compare groups at each time point. Second, differences between groups were analyzed within 2 time periods: period of inflammation (0–96 hours) and postinfusion period (120–240 hours) to account for the 2 unique phases of the experiment. The stated type I error rate was $\alpha < 0.05$.

Results

Physical Examinations and White Blood Cell Counts

At physical examination, objective metrics including temperature, pulse, and respiratory rate were within reference ranges. With the exception of soft feces (diarrhea score 1/3) in a control animal at 0, 12, and 24 hours, physical examinations did not identify abnormalities, and all remaining clinical scores were 0/15 at each time point. In both groups, mild local swelling containing a small amount of serous fluid was present at the OMP implantation site at the time of OMP removal.

Hematological and biochemical variables were compared within groups to samples collected at time 0 hour (Table 1a,b). For all variables, except band neutrophils, statistical differences were detected over time. Among hematologic variables in TG-animals, total leukocyte counts, neutrophil counts, and N/L ratios varied significantly from time 0 hour. Significant variation from 0 hour also occurred in albumin, globulin, haptoglobin, and serum iron of TG-alpacas. In CG-alpacas, significantly increased lymphocyte counts and haptoglobin concentrations were observed.

Total leukocyte counts of both groups remained within reference intervals (7,100–18,600/μL)[14] and did not differ significantly between groups (Table 1a). Statistically different leukocyte counts were not detected between groups while OMP were in situ (period 1) or after their removal (period 2). Segmented neutrophil counts in CG-animals remained within the reference interval (3,500–12,100/μL).[14] Although segmented neutrophil counts in TG-alpacas exceeded reported reference ranges at 5 time points (Table 1a), segmented neutrophil counts did not vary significantly between groups. During the administration of LPS or saline (period 1), TG-alpacas had significantly higher band neutrophil counts ($P = .0087$), exceeding published reference intervals (0–100/μL)[15] at 12 and 18 hours (Table 1a). After removal of OMP (period 2), band neutrophil counts did not differ significantly between groups.

In the first 24 hours, average lymphocyte counts decreased in TG-alpacas and were below reported reference intervals (1,300–4,800 μL)[14] at 24 hours (Table 1a). During the period of LPS administration, TG-alpacas had significantly lower lymphocyte counts than controls ($P = .0322$), but differences were not evident after removal of OMP. The N/L ratios were above the reported normal value of 1.54[16] in both groups during the study (Table 1a). A significantly higher N/L ratio was detected in TG-alpacas during LPS administration ($P = .0295$), but not after removal of OMP ($P = .4119$).

Serum Proteins and Serum Iron

Serum albumin and globulin concentrations remained within reference intervals (2.05–4.78 and 1.76–5.47 g/dL, respectively)[16] in both groups (Table 1b). Although albumin and globulin concentrations varied significantly over time ($P = .0090$ and .0007, respectively), no significant treatment effects were detected. A significant treatment by time interaction ($P = .0173$) was identified for globulin concentrations that were higher in CG-animals at 12, 18, and 24 hours. In contrast, serum globulin concentrations were higher in TG-animals at all other sampling times. Albumin and globulin concentrations did not differ significantly during LPS administration ($P = .6858$ and .3918, respectively) or in the period after OMP removal ($P = .9354$ and .3729, respectively).

At 96 hours, serum haptoglobin concentrations of TG-animals were increased 2-fold over baseline and remained increased until the end of the study. At 96, 120, and 144 hours, haptoglobin concentrations of TG-animals significantly exceeded those of CG-animals ($P = .0366$, .0039, and .0012, respectively). Haptoglobin concentrations did not differ significantly during LPS administration ($P = .3540$), but significant treatment effects ($P = .0056$) occurred after removal of OMP.

The 6 serially diluted standards and negative control of the commercially available SAA ELISA provided expected optical densities, and back-calculated SAA concentrations corresponded to actual concentrations of standard solutions. Although SAA was detected in

Table 1. Hematologic and biochemical markers of inflammation in treatment and control groups.

(a)

	WBC (*1,000/μL)		Neutrophils (*1,000/μL)		Bands (*1,000/μL)		Lymphocytes (*1,000/μL)		N/L Ratio	
Time	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0	13.66	13.65	9.70	9.83	0.00	0.05	1.99	1.99	4.62[c]	4.58[c]
12	15.65	16.83[a]	10.43	13.38[a,c]	0.00	0.23[c]	2.78	1.71	3.82[c]	9.61[b,c]
18	16.11	16.64[a]	10.82	13.73[a,c]	0.03	0.27[c]	2.77	1.45[b]	4.05[c]	9.89[b,c]
24	15.25	16.41	10.07	13.98[a,c]	0.00	0.00	2.39	1.00[b,c]	4.11[c]	13.67[a,b,c]
36	16.54	16.89	9.58	11.10	0.01	0.03	4.07[a]	2.78	2.33[c]	3.79[c]
48	14.18	16.46	9.63	12.73[c]	0.00	0.00	2.49	2.67	3.93[c]	4.89[c]
72	14.03	14.89	8.80	11.40	0.00	0.00	2.84	1.37[b]	3.40[c]	8.38[b,c]
96	13.68	14.23	8.41	10.45	0.00	0.03	3.00	1.68[b]	3.14[c]	6.12[c]
120	14.62	14.52	9.40	10.65	0.01	0.01	2.52	2.00	3.88[c]	5.12[c]
144	13.90	14.47	8.28	10.00	0.00	0.00	3.05	2.79	3.06[c]	3.64[c]
240	14.84	16.94	9.38	12.55[c]	0.00	0.00	3.07	2.85	3.18[c]	4.27[c]
SE_{max}	1.76	1.69	1.63	1.57	0.01	0.01	0.47	0.44	1.33	1.31

(b)

	Albumin (g/dL)		Globulin (g/dL)		HPT (mg/dL)		Iron (mg/dL)	
Time	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0	3.62	3.70	2.33	2.43	1.46	1.38	96.0	85.2
12	3.70	3.57	2.40	2.30	1.43	1.40	88.2	38.5[a,b,c]
18	3.68	3.65	2.47	2.35	1.50	1.64	100.3	31.0[a,b,c]
24	3.62	3.55	2.47	2.35	1.75	1.71	108.7	29.8[a,b,c]
36	3.63	3.62	2.39	2.47	1.72	2.11[a]	115.2	30.0[a,b,c]
48	3.57	3.50[a]	2.40	2.50	1.74	1.92	122.1	45.7[b,c]
72	3.54	3.47[a]	2.41	2.52	1.68	2.19[a]	122.1	75.5[b]
96	3.56	3.48[a]	2.56	2.60	2.29[a]	3.06[a,b]	130.3	83.3[b]
120	3.60	3.48[a]	2.62[a]	2.65	2.11	3.16[a,b]	99.3	91.0
144	3.56	3.47[a]	2.48	2.77[a]	2.27[a]	3.20[a,b]	123.4	97.5
240	3.60	3.48[a]	2.55	2.67	2.20[a]	2.67[a]	127.0	120.8
SE_{max}	0.10	0.09	0.12	0.12	0.26	0.24	14.4	13.3

[a]Denotes a significant difference from time 0.
[b]Denotes statistically different treatment effect.
[c]Denotes values outside published reference intervals.[14–16]

serum from a calf with septic arthritis (284.45 ng/mL), optical densities in sera from alpacas in this study and a hospitalized alpaca did not differ from those of negative controls. To verify these results, randomly selected serum samples from both groups were submitted to the laboratory of 1 of the co-authors (CC). A turbidimetric immunoassay[n] developed for measuring human serum SAA concentrations was utilized by chemical analyzer[o] to substantiate our results. Although this immunoassay provides reliable detection of SAA in various species, including horses and wildlife,[17,18] no significant reactivity was detected in samples from alpacas in this study using this alternate assay (data not shown).

In TG-animals, the average serum iron concentrations were below the reference interval (70–148 mg/dL)[16] from 12 to 48 hours (Table 1b), but remained within the reference interval throughout the study in controls. Compared with CG-alpacas, serum iron concentrations were significantly lower in TG-alpacas from 12 to 96 hours, corresponding to an overall statistical difference for the period of LPS administration ($P < .0001$). After OMP removal, serum iron concentrations of TG-alpacas increased rapidly and were not significantly different from controls ($P = .2702$).

Discussion

The inflammatory challenge used in this study was adapted from a study evaluating neuropeptide Y in endotoxemic sheep.[11] In that study, LPS delivery from OMP was favored over IV administration to conduct experiments in the absence of pyrexia. Prevention of severe clinical disease also was a goal of this study, and continuous LPS delivery was chosen to emulate natural disease in which gram-negative bacteria persistently produce LPS. The OMP were removed at 96 hours to enable characterization of inflammatory marker responses during a continual period of inflammation and the postinflammatory period. During the period of continual inflammatory challenge, significant differences were identified between groups in band neutrophil and lymphocyte counts, N/L ratios, and serum iron concentrations suggesting utility as early markers of inflammation. During the postinflammatory period, haptoglobin concentrations varied significantly between

groups, and other inflammatory markers approached prechallenge concentrations. A disadvantage of using subcutaneous OMP could be mild, local inflammation as detected in all alpacas in this study, which could prompt alterations of inflammatory markers in control animals. In this study, haptoglobin concentrations of control animals were significantly higher at 96, 144, and 240 hours as compared with time 0, likely reflecting inflammation at the OMP implantation site.

Large variability exists among LPS doses used to experimentally induce endotoxemia in different species.[19] To the authors' knowledge, LPS has not been utilized to experimentally induce endotoxemia in New World camelids. Dromedary camels given 0.1 μg/kg of LPS IV as a single dose developed markedly increased hematocrit and biochemical alterations in addition to various clinical signs, prompting the conclusion that dromedary camels are highly sensitive to endotoxin.[20] In cattle challenged IV with increasing doses of LPS at 3-week intervals, depression, anorexia, hyperthermia, tachypnea, ruminal stasis, and various hematological and biochemical alterations were observed with challenge doses of 10, 100, and 1000 ng LPS/kg.[12] Direct comparison of clinical effects after single IV infusion of LPS and continuous infusion by SC OMP is difficult. Similar to a study in sheep, the dosage of 0.83 μg/kg per hour used in this study resulted in the desired absence of severe disease, but resulted in detectable changes in markers of inflammation.[11] Limiting the severity of clinical signs and extent of inflammatory marker responses made it possible to evaluate which markers of inflammation would be most sensitive in camelids.

The observed increases in total leukocyte and neutrophil counts in TG-alpacas were relatively mild and cell counts did not differ significantly from CG-alpacas. Such subtle changes associated with mild or early inflammation may be difficult to assess in clinical practice because of the wide range of normal reference intervals[16,21] and a lack of baseline values from the period preceding the onset of illness. Similarly, band neutrophils of TG-alpacas exceeded some published reference intervals for adult alpacas,[14,15] but not others.[16] In a retrospective study of culture-positive, septic neonatal camelids, total leukocyte and neutrophil counts were diagnostically ambiguous, and leukocyte counts, including band neutrophils, were within reference intervals.[22] In TG-alpacas, lymphopenia was observed during LPS challenge, and lymphopenia is a common sequel of endotoxemia.[23] However, a significantly increased N/L ratio in TG-alpacas was the most diagnostically useful hematological metric, observed as early as 12 hours after LPS administration and decreasing after challenge cessation. N/L ratios in animals without LPS administration (time 0 and control group) were considerably higher than stated in a reference text in which the normal N/L ratio was reported as 1.5.[16] Because all alpacas in this study were clinically normal before inclusion, the cause for higher N/L ratios is uncertain. A review of the medical records at the Large Animal Teaching Hospital, Auburn University indicated that in alpacas presented for noninflammatory conditions similarly large N/L ratios (>3) were present (data not shown). Although stress responses may cause neutrophilia and lymphopenia in hospitalized animals, in this study, transport of animals to the research pasture was very brief. Alpacas remained as 1 group and were acclimated for 7 days before the experiment, minimizing external stressors. Despite higher than expected normal N/L ratios, this study demonstrated their clinical usefulness, but also highlighted the necessity for establishment of locally appropriate reference intervals. A previous study demonstrated that hematologic reference intervals should be regularly re-established to account for geographical and genetic variations.[24]

Previous reports on the use of haptoglobin assays in alpacas are sparse, but increased haptoglobin concentrations were observed in alpacas vaccinated with a *Mycobacterium bovis* vaccine.[25] In that study, intradermal tuberculin skin testing resulted in increased serum haptoglobin concentrations of up to 0.44 mg/mL.[25] The earliest significant increase in haptoglobin concentration was detected in TG-alpacas at 36 hours, which is similar to cattle in which large increases are detected 2 days after inflammatory challenge.[26,27] In this study, increases in haptoglobin concentrations were observed in both groups, likely arising from an inflammatory response induced by OMP implantation. Peak increases, however, were higher in TG-alpacas as compared with CG-alpacas, suggesting that measurement of haptoglobin concentrations may be diagnostically useful in alpacas with suspected inflammatory conditions. In cattle, haptoglobin is considered a major APP as immune-stimulation can raise concentrations from undetectable levels to concentrations increased by up to 100-fold.[27] This appears to be in contrast to alpacas in this and another study in which concentrations of approximately 1 mg/mL occurred before experiments.[25] Measureable increases in haptoglobin concentrations after low-dose LPS challenge in the present study suggest that more pronounced haptoglobin changes can be expected in clinical practice, where bacterial infections are common.

Serum amyloid A has been utilized as a marker of infection and inflammation in large animal species including ruminants, horses, pigs, and wildlife,[6] but no reports of its use in New World camelids exist. In healthy dromedary camels, higher SAA concentrations were detected when compared to healthy sheep and cattle.[28] Using the same multispecific ELISA assay used in this study, a previous study evaluated the influence of transportation on plasma concentrations of APP including SAA in dromedary camels.[29] Although the acute phase response and APP are common to all species, differential sensitivity exists according to the type of disease and species under investigation.[6] Serum amyloid A may not be a major APP of alpacas or the challenge could have been too mild to prompt increased SAA concentrations. However, measurable concentrations were expected in the hospitalized animal from which undiluted samples also were

included. Although no previous reports on the successful application of the utilized ELISA assay in New World camelids exist, SAA is highly conserved among vertebrates, and the ELISA was effective in detecting SAA in diverse domestic and wildlife species.[30,31] Additional studies are needed to evaluate SAA and its detection in alpacas.

At 12 hours of the study, serum iron concentrations in TG-alpacas were significantly lower compared with 0 hour, indicating inflammation. Serum iron concentrations were significantly different between groups only during LPS administration and rapidly returned to normal concentrations after removal of OMP. Decreasing serum iron concentrations during inflammation result from increased cellular uptake of iron under the regulation of hepcidin, making iron less available for invading micro-organisms.[9,32] Rapid decrease in serum iron concentration could be diagnostically useful in clinical patients and facilitate early goal-directed treatment. Rapid reduction in serum iron concentrations also occurs in other species including rats and ponies after experimental turpentine injection.[33,34] In horses inoculated IV with *S. zooepidemicus,* serum iron concentrations were beneficial in predicting severity of clinical signs and often were decreased before the onset of clinical signs and pyrexia.[35] The serum iron concentration was a sensitive and specific marker of inflammation, and concentrations were significantly lower in horses with systemic inflammation compared with horses with localized inflammation.[10]

In conclusion, a model of continuous, low-dose LPS challenge was successfully utilized to evaluate clinical benefits and limitations of markers of inflammation in alpacas. Ambiguity existed when evaluating differential white blood cell counts that fell within published reference intervals, but changes of individual leukocyte populations were observed, and the response may be best evaluated diagnostically by N/L ratios. Among biochemical markers of inflammation, serum iron concentration appeared to be a sensitive and specific marker of inflammation. Additional research is needed to evaluate the APP responses of alpacas, especially SAA.

Footnotes

[a] Butorphic; Lloyd Laboratories, Shenandoah, IA
[b] Ketaset; Fort Dodge Animal Health, Fort Dodge, IA
[c] AnaSed; Lloyd Laboratories
[d] Abbocath-T; Hospira, Inc, Lake Forest, IL
[e] Escherichia coli 055:B5, L6529; Sigma-Aldrich, St. Louis, MO
[f] ALZET 2ML1 Osmotic Pump; Durect Corporation, Cupertino, CA
[g] Med-Pharmex Inc, Pomona, CA
[h] Weck, Visistat 35W; Teleflex Medical, Research Triangle Park, NC
[i] ADVIA 120 Hematology System; Siemens Healthcare Diagnostics, Deerfield, IL
[j] Cobas C 311; Roche Diagnostics, Indianapolis, IN
[k] Phase Haptoglobin Assay; Tridelta Development Ltd, Maynooth, CO Kildare, Ireland
[l] Phase Serum Amyloid A Assay; Tridelta Development Ltd
[m] SAS Institute Inc, Cary, NC
[n] LZ test 'Eiken' SAA, Eiken Chemical Co, Ltd, Bunkyo-ku, Tokyo, Japan
[o] RX Daytona, Randox Laboratories Ltd, Kearneysville, WV

Acknowledgments

The authors thank Drs Robert Judd and Desiree Wanders and Mrs Yijing Zhang for assisting with the analysis of acute phase protein concentrations and providing expertise in their interpretation.

This study was funded by Auburn University Animal Health Research.

Conflict of Interest Declaration: Authors disclose no conflict of interest.

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