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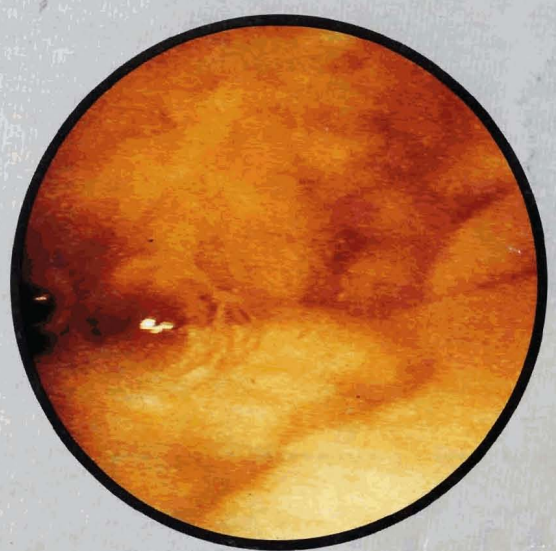

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EQUINE PRACTICE – MEDICINE

Liver biopsy in llamas is a useful diagnostic aid in the assessment of nutrient adequacy or toxicity. If properly collected, the procedure is simple and safe. The procedure for liver biopsy is described and illustrated. Thirteen llamas of different sexes, age range 4 to 18 years, and weight range 90 to 204 kg had liver biopsy and systemic responses were monitored by physical examinations and laboratory assessment of complete blood counts and multiple serum chemistry analytes. Results are provided.

Liver Biopsy in Llamas

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Introduction

The number of South American camelids in North America is expanding, and so is the likelihood that a clinician will be presented with a llama in need of extensive medical examination. Hepatic diseases reported in llamas include primary and metastatic neoplasia,[1] ketosis-fatty liver syndrome,[1] and fluke infections.[1,2] A liver biopsy may aid the clinician in making a diagnosis of one of these conditions. Liver biopsy can be of diagnostic value when suspecting iron deficiency anemia.[3] Biopsy and subsequent liver tissue analysis also can be useful when assessing dietary adequacy in the diagnosis of toxic or deficient mineral intake (i.e., copper, selenium, or zinc).[4]

Even with the advantages in diagnostic capability gained by performing a liver biopsy, there is much resistance from veterinarians and llama owners to use of this technique. Some complications of liver biopsy are: 1) hemorrhage from the biopsy site, 2) initiation of systemic inflammatory response, and 3) initiation of hepatic inflammation and cholestasis. The present study was undertaken to help determine the safety of standing liver biopsy technique, which is applicable to field conditions.

Llamas

Thirteen llamas with a mean age of 7.5 years (range 4 to 18 years), of different sexes (eight castrated males, four females, and one intact male), with an average weight of 127.3 kg (range 90 to 204.5 kg) were used for liver biopsy. Physical examinations were performed (including rectal temperature, heart rate, respiratory rate, gut sounds in all four abdominal quadrants, mucous membrane color, and capillary refill time), general attitude was assessed, and blood was collected via jugular venipuncture for a complete blood count (CBC) and serum biochemistry analyses (SB).

Liver Biopsy Technique

The fiber was clipped on the right side of the body between an area 8 to 12 inches from the top of the back and from the eighth to 10th intercostal space (Fig. 1). The remaining fiber around this region was taped out of the biopsy site and the area was surgically prepared with povidone iodine scrub, rinsed with distilled water and dried. The skin, 9 to 10 inches from the top of the back and over the ninth intercostal space, was infil-

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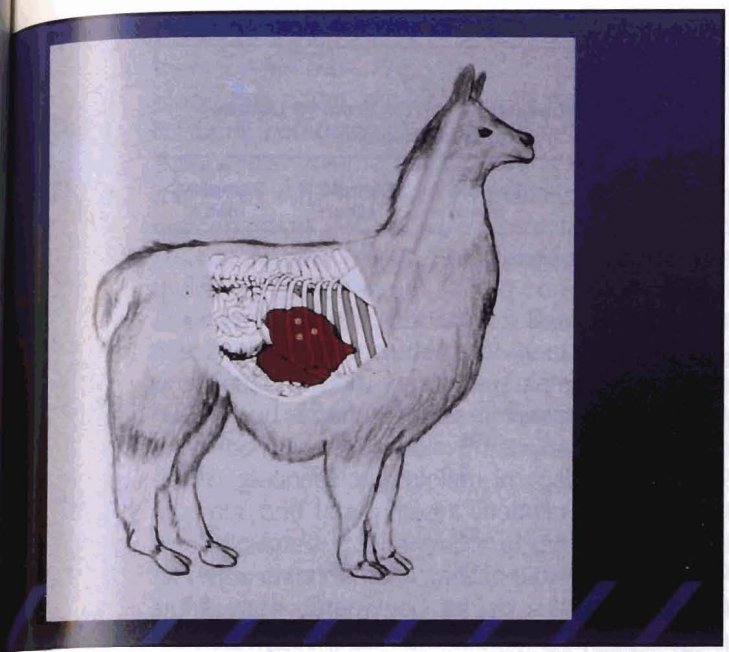

FIG. 1 — Anatomic location of the liver. Biopsy specimens can be harvested from areas denoted by asterisks (*).

FIG. 2 — After the biopsy site has been surgically prepared (clipped, cleaned, and locally anesthetized), an incision is made through the skin.

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TABLE 1
Vital Signs of 13 Llamas With Liver Biopsy

	Sample A Mean	Sample B Mean	Sample C Mean
Heart rate	61	70	62
Respiratory rate	41	40	35
Temperature (F)	102.2	101.8	102.1

Sample A = at the time of liver biopsy, Sample B = 24 hours post liver biopsy, Sample C = 72 hours post-biopsy

TABLE 2
Complete Blood Count Data From 13 Llamas With Liver Biopsy

	Sample A Mean	Sample B Mean	Sample C Mean
Fibrinogen (mg/dl)	377	308	177[a]
PP/fib ratio	20	23	41[a]
WBC (cells/μl)	15,708	18,400	17,692
Segs (cells/μl)	11,310	14,386	13,038
Bands (cells/μl)	205	124	45
Lymphs (cells/μl)	2,863	1,974[a]	2,888
Monos (cells/μl)	419	447	648
Eos (cells/μl)	911	959	1,072
Hematocrit (%)	34	33	33
MCV (fl)	26.6	26.7	26.7
MCHC (%)	41.1	41.1	41.8
Platelets (cells/μl)	231,692	260,800	239,385
MPV (fl)	2.95	3.05	2.87

[a] = Sample mean is significantly different from mean of Sample A at $P \leq 0.01$
Sample A, B, and C: see Table 1

TABLE 3
Serum Biochemistry Analytes From 13 Llamas With Liver Biopsy

	Sample A Mean	Sample B Mean	Sample C Mean
Sorbitol dehydrogenase (IU/L)	2.9	4.5	3.9
Total bilirubin (mg/dl)	0.2	0.2	0.2
g-glutamyl transferase (IU/L)	25	25	27
Aspartate transaminase (IU/L)	195	198	203
Creatinine (mg/dl)	3.0	2.9[b]	2.6[b]
Urea nitrogen (mg/dl)	23	21	23
Glucose (mg/dl)	119	123	62[a]
Calcium (mg/dl)	8.5	8.5	8.3
Inorganic phosphate (mg/dl)	5.6	6.1	6.1
Total protein (g/dl)	6.1	6.0	6.2
Albumin (g/dl)	3.5	3.5	3.5
Globulin (g/dl)	2.6	2.5	2.7[b]
Sodium (mmol/L)	143	148[b]	149[b]
Potassium (mmol/L)	4.4	4.8[b]	4.8[b]
Chloride (mmol/L)	115	118	118
Total CO_2 (mmol/L)	20	19[b]	23[b]
Anion gap	9	11[b]	9
Osmolality (mosmol/kg)	297	299	301

[a] = Sample mean is significantly different from mean of Sample A at $P \leq 0.01$, [b] = Sample mean is significantly different from Sample A at $P \leq 0.05$ but there is no physiologic difference
Sample A, B, C: see Table 1

trated with 1 ml of a 2.0% lidocaine solution. The area was scrubbed again with Betadine® (The Purdue Frederick Company, Norwalk, CT) rinsed twice with distilled water, and dried. A small skin incision was made through the anesthetized site (Fig. 2), and a 14-gauge biopsy needle (14-gauge × 6-inch, 20-mm specimen notch, Tru-Cut Biopsy Needle:Baxter Healthcare Corporation, Deerfield, IL) was inserted through the skin incision (Fig. 3). The needle was directed medially and caudally through the edge of the diaphragm (Fig. 4).[5] The liver is in direct contact with the diaphragm, therefore a biopsy can be harvested after the needle has been inserted only 2 to 3 mm through the diaphragm (Fig. 5).[5] After the biopsy was obtained, the skin incision was closed with non-absorbable suture material.

Laboratory Assessment

Physical examinations were performed, blood for CBC and SB was collected, and the cutaneous biopsy sites were inspected for swelling, redness, and drainage at 24 and 72 hours after obtaining the biopsy (PB). The animals then were monitored daily for 3 weeks for signs of inappetence and systemic disease. All animals were examined monthly for the following year for signs of subcutaneous abscess, systemic disease, or weight loss.

Plasma protein was measured by use of a refractometer. Fibrinogen was determined as the difference between refractometric measurement of plasma protein and serum protein. A complete blood count (CBC) was performed by use of an automated blood analyzer (Baker 9000:Serono-Baker Instru-

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ments, Allentown, PA), which included total white blood cell count, differential leukocyte count, hematocrit, hemoglobin, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and mean platelet volume (MPV). Serum chemistries, determined by use of an automated computer-assisted chemistry analyzer (Cobas Mira S:Roche Diagnostics, Nutley, NJ), included activity of sorbitol dehydrogenase (SDH), gamma glutamyl transferase (GGT), and aspartate transaminase (AST); and concentration of total bilirubin, creatinine, urea nitrogen (BUN), glucose, total calcium, inorganic phosphate, albumin, and total protein. Globulin concentration was calculated. Concentration of serum electrolytes (sodium, chloride, total carbon dioxide, and potassium) were determined by ion specific electrode method (Beckman E4A:Beckman Instruments, Brea, CA). Anion gap was calculated. Serum osmolality was determined by use of a vapor pressure osmometer (Model 5500 Vapor Pressure Osmometer:Westcor, Inc., Logan, UT). Data from each physical, hematologic, and biochemical parameter were analyzed using a general linear model (Proc GLM, Statistical Analysis System:SAS Institute, Cary, NC) for repeated measures analysis of variance. Data from samples at 24 hours (Sample B) and 72 hours (Sample C) after biopsy collection were thus contrasted with those from 0 hours (Sample A, at the time of biopsy collection). Contrasts were considered significant if the F value exceeded that corresponding to $P = 0.01$.

Results and Discussion

All llamas were alert and active at each sample time. Handling the llamas in order to obtain the biopsy and/or blood was stressful as indicated by increased heart rate, respiratory rate, and body temperature. Respiratory rate and body temperature decreased at times of collection for samples 24 hours PB and 72 hours PB as compared with Sample A taken at the time of biopsy (Table 1). Systemic inflammatory process caused by biopsy of the liver was not evident. The concentration of fibrinogen, an acute phase reactant produced in the liver, decreased over the times of sample collection (Table 2). Total protein concentration remained stable over time, while plasma protein to fibrinogen ratio increased. These data indicate that inflammation was not occurring and that the liver, even after being biopsied, did not increase acute phase protein production.

The total white blood cell count remained unchanged (Table 2). Concentration of mature, segmented neutrophils predominated, band neutrophil concentration decreased over the sampling times, concentrations of lymphocytes decreased at 24 hours PB, eosinophils were unchanged, and monocyte concentration increased at the 72-hour PB sample. These alterations were within sample variability owing to handling stress and difficulty in sample obtainment, rather than being indicative of systemic or localized inflammation.

All llamas had consistent hematocrit over the sampling times (Table 2). One llama, a 4-year-old castrated male, was anemic at the time the biopsy was taken and his hematocrit remained unchanged during the trial. These data indicate that hemorrhage from the hepatic surgical site was insufficient to alter the hematocrit. Size and hemoglobin content of erythrocytes remained constant throughout the collection time.

Platelet concentration (Table 2) was variable owing to the degree of difficulty in sample attainment. Platelet concentration was decreased and mean platelet volume was increased in samples with evidence of platelet activation, such as small fibrin clots in the blood tube or large clumps of platelets on blood smears.

Mean activity of SDH (Table 3), a highly liver-specific leakage enzyme with a short half-life, increased slightly 24 hours PB and decreased by 72 hours PB but was still above the mean of sample taken at the time of biopsy. Individual llamas had increased SDH activity at 24 hours PB (nine of 13) or decreased activity (four of 13). At 72 hours PB activity of SDH had decreased in nine of 13 llamas and had continued to increase in four llamas. One llama, an 18-year-old female, had markedly increased SDH activity (13.3 IU/L) at the time of biopsy and maintained high SDH activity at 24 hours PB (12.9 IU/L) and 72 hours PB (10.2 IU/L), but the activity decreased over this time. The increased activity of SDH indicates that damaged hepatocytes leaked enzyme. The degree of enzyme activity increase was minimal and in most llamas the activity was returning toward baseline by 72 hours PB; therefore, this is indicative of transient, mild damage to hepatocytes. Total bilirubin concentration and GGT activity were unchanged over time, which indicated that liver biopsy caused insignificant cholestasis. The 18-year-old female llama with marked increase in SDH activity also had markedly increased GGT activity that remained virtually unchanged over sampling times (106 IU/L at biopsy, 103 IU/L at 24 hours PB, and 100.8 IU/L at 72 hours PB).

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FIG. 3 — The biopsy instrument is manually pushed through the skin incision and body wall.

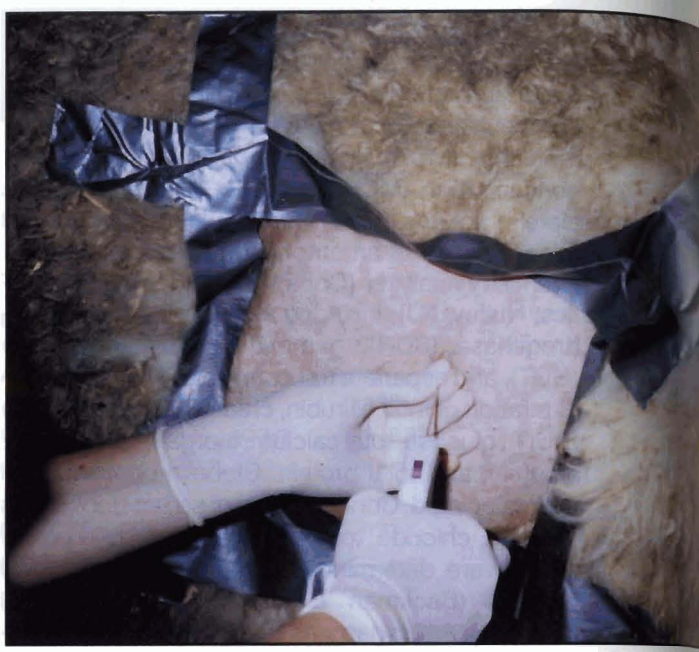

FIG. 4 — Medial and caudal advance of the biopsy instrument through the diaphragm is made slowly.

Other serum chemistry analyses (Table 3), including concentrations of BUN, calcium, inorganic phosphate, total protein, albumin, Cl, and osmolality remained consistent ling times. Concentrations of creatinine, globulin, Na, K, TCO_2, and anion gap showed differences over time when analyzed statistically. However, all values were physiologically normal and within the reference ranges determined for llamas in our laboratory. Glucose concentration decreased at 72 hours PB as compared with the other samples. This may indicate decreased hepatic hyperglycemic influence of catecholamines (excitement, fear) and corticosteroids (stress) during handling of the llamas at the time of sample collection.

Conclusion

The procedure for liver biopsy collection is easy and safe. Liver biopsy as a diagnostic aid in the assessment of nutrient adequacy or toxicity, if properly collected, will provide valuable information with minimal risk to the patient. ■

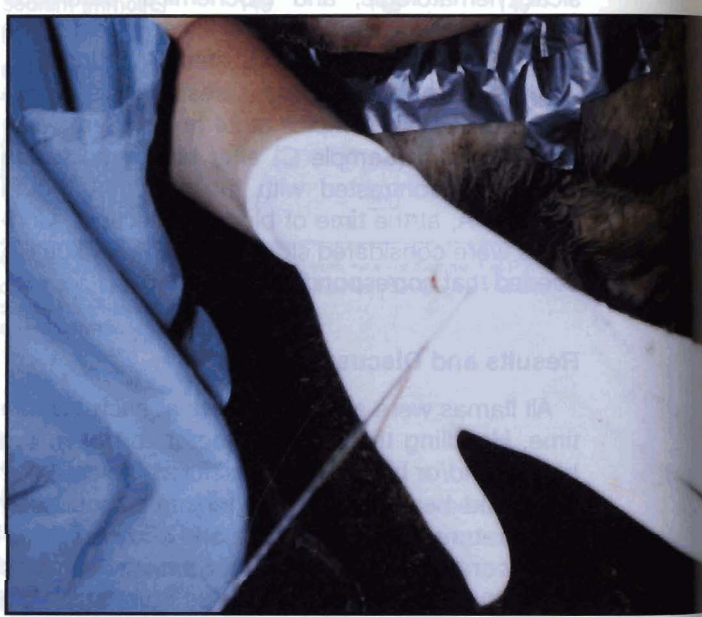

FIG. 5 — The harvested liver sample is examined for adequacy and then placed in an appropriate container of fixative.

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