Diseases of New World Camelids

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Dedication

To my mostly patient but always loving husband Patrick

And to my son Ian, who knows what alpacas say

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CHAPTER ONE

Literature Review

Introduction: Camelid Diseases

Camelids are a relatively recent addition to the domestic herbivores of North America. The first camelids were imported by multiple private owners in the 1970s (llamas) and 1980s (alpacas),[1] although the species already existed in select zoo populations. These unique animals have become more popular in the last several decades and their monetary value has been high.[2] Meanwhile, their owners have struggled to find veterinarians with sufficient knowledge of camelid medical problems. In recent years there has been increasing study of the medical aspects of their unique metabolism as well as an interest in identifying their most common diseases. Little is known, however, about the true prevalence of camelid conditions or about which diseases are responsible for the majority of camelid morbidity and mortality. One study surveyed the causes of death in camelids submitted to the University of Guelph Animal Health Laboratory and identified digestive disorders as the most frequent cause of death in camelids, with neurologic diseases as the next most common cause of death.[3]

Practitioners working with camelids report that they commonly encounter gastrointestinal parasitism, including strongyles,[4] small coccidia and the more pathogenic large coccidia such as *Eimeria macusaniensis*,[5-8] as well as cryptosporidiosis.[9] In sufficient numbers and in combination with other stresses such as temperature extremes and competition for food, these gastrointestinal parasites can cause morbidity and mortality.[10, 11] External parasites such as

Chorioptes are a common finding but are not expected to cause mortality.[12, 13] There is also concern about the potential for gastrointestinal conditions such as ulceration of the glandular portion of the third compartment[14-16] or foreign body ingestion.[17, 18] Congenital abnormalities such as choanal atresia[19-23] or congenital heart defects[24-26] can account for mortality in young crias. Meningeal worm is considered a major differential for a neurologic camelid, depending on geographic location,[27-32] and polioencephalomalacia can occur under similar circumstances as in other ruminants.[33-35] Lymphoma and other round cell tumors are the most commonly reported form of malignant neoplasia in camelids.[15, 36-38]

Metabolic disorders such as hyperglycemia,[39-41] hepatic lipidosis,[42] hyperosmolar syndrome,[39, 43] and hyperlipidemia[44] can also cause morbidity and mortality in camelids. These metabolic derangements often occur as a secondary condition in sick and hospitalized camelids but can frequently be the medical issue that requires the most intensive treatment.[45] The glucose and fat metabolism derangements seen in camelids are thought to be related to their unique properties of glucose and fat mobilization regulation,[41, 46-51] in comparison to other species. Although camelids are forestomach fermenters, with the majority of their energy needs coming from short-chain fatty acids produced by microbial fermentation, their resting glucose levels are high compared to domestic ruminant species.[46] The increased glucose levels observed in camelids are likely due to decreased insulin dependent uptake by muscle and adipose tissue in

comparison to other species.[40, 41] This is supported by the fact that camelids have low resting levels of insulin, with fasting values ranging from approximately 3-6 μU/ml,[46, 52] compared to cattle with a range of 5-25 μU/ml. They have also been shown to clear glucose slowly from the bloodstream even after treatment with exogenous insulin.[46-48] The distinctive glucose metabolism shown by New World camelids is proposed to be an adaptation to living in areas with poorly or intermittently available feed.[41, 53]

Normal Pancreatic Function

The endocrine pancreas is responsible for regulation of energy metabolism, including blood levels of glucose and lipids. Insulin is released from the β cells of the pancreas in response to an increased blood glucose level. Insulin is the major energy storage hormone and has many functions, including the suppression of fat and glucose mobilization and enhancement of tissue uptake of energy substrates including glucose, non-esterified fatty acids (NEFAs), and amino acids.[54] Glucagon is the catabolic hormone secreted by pancreatic α cells that acts to promote glycogenolysis and gluconeogenesis.[55] Under normal circumstances, the opposing actions of insulin and glucagon maintain blood glucose in a narrow physiologic range.

Glucose is the primary stimulus for insulin secretion, though other sources of energy (lipids, amino acids), hormones, and nitric oxide can also directly

stimulate insulin release.[56] Glucose enters the β cells via glucose transporter-2 (GLUT-2) and is phosphorylated by glucokinase.[57] The phosphorylated glucose is metabolized into pyruvate, ATP, and NADH by standard glycolytic pathways. Oxidation of NADH by the mitochondrial transport chain generates additional ATP and the high ratio of ATP to ADP in the β cell inhibits ATP-sensitive potassium channels to cause depolarization of the cell membrane. This depolarization opens voltage-gated calcium channels and the calcium influx stimulates exocytosis of insulin-containing granules.[56, 57] Insulin secretion follows a biphasic pattern with a large but short-lived rise in insulin levels following the initial stimulus and then a smaller but prolonged secondary phase of secretion.[58] In humans with type 2 diabetes mellitus, the first phase of insulin secretion in response to a glucose load is no longer present;[59] however, other insulin secretogogues are still able to elicit a first phase response.[60]

Once released, insulin binds to its receptor which is found on the cell surface of insulin-sensitive tissues such as muscle, adipose, and liver. In response to insulin binding at the α subunit, the receptor forms a dimer and is autophosphorylated, setting off a signaling cascade that results in many downstream effects, one of which is the translocation of the glucose transporter-4 (GLUT4) molecule to the cell surface, primarily in muscle cells.[61] This insulin-dependent uptake of glucose by muscle cells is responsible for a large proportion of glucose disposal after a meal.[54]

Glucagon secretion is triggered by the generation of electrical signals by sodium and calcium channels that are active when blood glucose levels are low.[62] Glucagon binds to a G-protein coupled receptor on the surface of cells throughout the body but particularly in the liver, where it acts to stimulate gluconeogenesis by upregulating key enzymes in that pathway.[63] Thus, defects in glucose homeostasis can be caused by decreased insulin secretion, lack of normal β cell responsiveness to blood glucose levels, or peripheral insulin resistance, as well as abnormalities in glucagon secretion and α cell function.

Endocrine Pancreatic Function Testing

There are many methods by which to assess the function of the endocrine pancreas and the ability of the peripheral tissues to process glucose. Until recently, in humans, diabetes mellitus was diagnosed based on elevated levels of blood glucose, either after fasting or after an oral glucose challenge.[64] An individual's response to oral glucose tolerance testing depends on many factors, including intestinal absorption of glucose, pancreatic endocrine function, and peripheral insulin sensitivity. Due to the many possible sources of variation in response as well as greater convenience of testing, measurements of glycated hemoglobin (also known as A1C) have supplanted oral glucose testing as the diagnostic test of choice in humans.[64] Although oral glucose tolerance testing has

been used in monogastric species including horses,[65] it is not suitable for forestomach digesters.[66]

Intravenous glucose tolerance testing (IVGTT) removes the variability of intestinal absorption from the analysis and assesses a combination of pancreatic endocrine function and peripheral insulin sensitivity. This method has been used in adult and juvenile camelids.[46, 67] After an overnight fast, 0.5 g/kg of glucose is rapidly infused intravenously and blood glucose values are measured at 0, 1, 2, 3, 4, 5, 15, 30, 60, 90, 120, 180, and 240 minutes after glucose infusion. In healthy animals of other species, the blood glucose value returns to normal less than 90 minutes of glucose infusion.[68] However, in adult camelids blood glucose levels were still elevated 4 hours after glucose infusion, indicating slow glucose clearance attributable to a decreased insulin response to glucose load compared to other species.[46] Llama crias demonstrated a stronger insulin response to IVGTT than adult camelids, though the fractional turnover rate of glucose was still lower than values observed in healthy cows.[67]

Administration of exogenous insulin in the form of an insulin tolerance test (ITT) provides additional information about tissue uptake of glucose in response to insulin. After a 0.1 U/kg dose of regular insulin, blood glucose levels are expected to decrease by 50% within 30 minutes and return to baseline within 2 hours.[66] Insulin administration can also be coupled with glucose administration to

perform a combined glucose-insulin tolerance test (CGIT).[69] A study in alpacas used a CGIT wherein 0.2 U/kg regular insulin was administered at T = 15 minutes after a 0.5 g/kg glucose infusion. In that study, even with exogenous insulin, blood glucose levels remained elevated for 3 hours, demonstrating decreased peripheral insulin sensitivity.[47] The half life of plasma glucose was 69 minutes in camelids that received insulin compared to 111 minutes in those that did not. In comparison, healthy cows have a plasma glucose half life of 35 minutes without the assistance of exogenous insulin.[66]

All of the previous methods have the drawback that treatment with glucose and insulin can result in multiple competing processes, including glucose uptake into the tissues, release of endogenous insulin, and gluconeogenesis depending on the fluctuations in blood glucose. The hyperglycemic clamp (HGC) and the hyperinsulinemic euglycemic clamp (HEC) are techniques that have been developed in other species[70] and performed in camelids[51] that help to separate the components of systemic glucose production and disposal. Using the HGC, blood glucose levels are raised to supraphysiologic levels and maintained there for 2 hours to suppress endogenous glucose production.[70] The rate of glucose infusion needed to maintain the predetermined blood glucose level depends on insulin secretion by the pancreas and thus is a measurement of β cell sensitivity. During the HEC, a supraphysiologic level of insulin is infused at a constant rate to remove the contribution of the pancreas and the rate of glucose infusion is

adjusted to maintain euglycemia. Under these conditions, the rate of glucose needed is a measure of tissue insulin sensitivity. This method is considered the gold standard method for measurement of insulin sensitivity.[70-72] In camelids, the rate of glucose required to maintain hyperglycemia during the HGC was lower than in horses[73] or humans,[70] indicating a comparatively decreased responsiveness of pancreatic β cells to hyperglycemia.[51] Similarly, the glucose infusion rate required to maintain euglycemic conditions during the HEC in camelids was roughly half of that needed in humans[70] and cats,[74] indicating relative peripheral insulin insensitivity.[51]

Arginine Stimulation Testing

The intravenous arginine stimulation test (IVAST) is an additional method to assess pancreatic endocrine α and β cell function. Arginine is one of several pancreatic secretogogues that stimulate release of insulin and glucagon.[60, 75, 76] The transport of a positively-charged amino acid into the cell is thought to change the electrical gradient of the membrane and stimulate calcium influx, thereby promoting exocytosis of insulin-containing granules.[77, 78] To perform the IVAST, a series of baseline measurements of glucose, insulin, and glucagon are taken, followed by intravenous infusion of arginine over 30 seconds. Blood samples are taken at T = 2, 3, 4, 5, 10, 15, and 20 minutes after infusion and glucose, insulin, and glucagon levels are measured at each time point.[76] The hormone levels peak within 2-5 minutes,[76, 79] providing an assessment of the first phase of insulin and

glucagon release.[80] The IVAST can also be combined with a hyperglycemic clamp technique to assess pancreatic response at different blood glucose levels.[81] It has been used extensively to evaluate both type 1 and type 2 diabetes mellitus in humans[60, 75, 81-83] as well as veterinary species including foals,[84-86] cattle,[87-91] and both normal and diabetic cats.[92-94] In comparison to the more time-consuming clamp procedures, the IVAST is simple to perform and does not require maintenance of supraphysiologic glucose or insulin levels, and thus can be used to assess pancreatic function in clinical cases of disease. The IVAST has been successfully performed in healthy alpacas and llamas using the method described above with a dose of 67 mg/kg arginine.[95]

Disorders of Camelid Lipid Metabolism

New World camelids are also prone to developing disorders of fat mobilization such as hyperlipemia and hepatic lipidosis, even in the absence of risk factors such as pregnancy, lactation, or obesity.[40, 42, 44, 96-98] A negative energy balance (due to feed restriction, anorexia, or increased demand) leads to fat mobilization, primarily in the form of non-esterified fatty acids (NEFAs) released from adipose tissue by the action of hormone sensitive lipase (HSL). NEFAs not used by peripheral tissues are processed in the liver to form ketone bodies such as β-hydroxybutyrate (BHBA) as well as triglycerides (TGs). TGs are packaged as very-low-density lipoproteins (VLDLs) and are exported to the peripheral circulation, where lipoprotein lipase (LPL) releases the TGs to be used by

peripheral cells. Insulin inhibits the action of HSL and stimulates the action of LPL in order to decrease circulating fat fractions.[99] In the face of low insulin levels or insulin resistance, as occurs in camelids, fat mobilization can spiral out of control, particularly because TGs themselves interfere with the normal function of insulin. High levels of TGs, NEFAs, and ketones cause anorexia and depression, and can cause hepatic lipidosis if the liver's ability to process the excess fat becomes overwhelmed.[99]

In camelids, fat mobilization can be induced under experimental conditions by treatment with epinephrine[49] but not corticosteroids.[52, 100] Treatment with corticosteroids was studied as a possible mechanism to induce fat mobilization because in other species corticosteroids have complex effects on lipid metabolism including increased expression of HSL, thereby releasing more NEFAs into circulation.[101] Additionally, corticosteroids increase de novo lipogenesis within the liver and increase the exportation of VLDL.[102] At the same time, corticosteroids cause peripheral and hepatic insulin resistance by inactivating insulin receptor proteins in skeletal muscle and by inducing expression of gluconeogenic enzymes in the liver.[103] On the other hand, catecholamines stimulate HSL activity to promote fat breakdown and inhibit LPL, and at higher doses also suppress insulin release. One hour after a single treatment with epinephrine, levels of glucose, NEFAs, TGs, and BHBA increased,[49] suggesting that epinephrine might play a role in the development of

hypertriglyceridemia and hyperlipemia in camelids. Treatment of camelids with short-acting insulin at the same time as epinephrine prevented the rise in NEFAs and delayed the increase in TGs and BHBA, indicating that insulin could be an effective treatment for disorders of fat mobilization, despite systemic insulin resistance.[49]

HYPOTHESES AND SPECIFIC AIMS

OBJECTIVES:

1. To describe the various causes of death in alpacas and llamas submitted to the University of Minnesota Veterinary Diagnostic Laboratory

2. To investigate the effect of fat mobilization on camelid pancreatic function

STUDY 1

Justification

The camelid patient can present a diagnostic challenge to practitioners. A thorough description of the causes of mortality among alpacas and llamas in the Upper Midwest will provide a list of common differential diagnoses and will help identify specific aspects of husbandry and routine veterinary care that can be modified to reduce morbidity and mortality.

Hypotheses

Animals will present with a wide range of fatal disorders; however, the systems most commonly affected will be the digestive and nervous systems.

Specific Aims

a) To categorize diagnostic laboratory submissions by body system and pathophysiologic process

b) To describe the range of diagnoses of alpacas and llamas submitted to the Veterinary Diagnostic Laboratory

c) To identify the most common causes of death in camelids submitted to the University of Minnesota Veterinary Diagnostic Laboratory for necropsy evaluation.

STUDY 2

Justification:

Understanding how pancreatic function changes in camelids in a state of fat mobilization will help guide treatment protocols to improve the ability of camelid practitioners to successfully treat sick animals. Pilot studies have shown that the arginine stimulation test can be performed on clinical cases without adverse effects. However, it is impractical to enroll a sufficient number of clinical cases and control for their individual variability. Epinephrine can be used to induce a transient state of fat mobilization in healthy camelids in order to study pancreatic function using the arginine stimulation test.

Hypothesis

Camelids in a state of epinephrine-induced fat mobilization will display a reduced pancreatic β cell response and an exaggerated pancreatic α cell response to arginine.

Specific Aims

To use the arginine stimulation test to determine whether pancreatic β and α cell responses to arginine differ between healthy camelids and those with epinephrine-induced fat mobilization.

CHAPTER TWO

Necropsy findings in alpacas and llamas from the Upper Midwest of the United States of America: a retrospective study of 359 necropsies (2001-2011)

Necropsy findings in alpacas and llamas from the Upper Midwest of the United States of America: a retrospective study of 359 necropsies (2001-2011)

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Summary

Knowledge of medical conditions that affect camelids has increased greatly in recent years. However, sometimes the first signs of illness in camelids can be subtle and nonspecific or animals may be found dead with no abnormalities observed. The purpose of this study was to catalog the fatal conditions seen in camelids in the Upper Midwest in order to recognize trends and ultimately to prevent disease. The reports of 234 alpacas and 125 llamas submitted to the University of Minnesota Veterinary Diagnostic Laboratory (UM-VDL) for post-mortem examination from 2001 to 2011 were reviewed. The cause of death was identified in 73% of the cases. The digestive system was the most common site of the major disease condition, accounting for 29% of alpacas and 31% of llamas. In adults, gastrointestinal parasitism associated with emaciation, neoplasia, and hepatic lipidosis were among the most common diagnoses identified. Meningeal worm (*P. tenuis)* was the most commonly identified cause of death associated with the nervous system in adults, since 13% of llamas and 2.4% of alpacas had central nervous system lesions consistent with verminous migration. The most common cause of death or euthanasia among alpaca crias was choanal atresia, which accounted for mortality in 19% of alpacas under 6 months of age. Other congenital abnormalities and bacterial infections were also common in neonates. In older crias (age 2 weeks to 6 months), enteritis and septicemia were most common. In conclusion, necropsy evaluation was successful in identifying the cause of death in the majority of camelids submitted to the UM-VDL. The risk of morbidity or mortality related to many of the most common diseases identified in this study can be reduced by taking steps to ensure good husbandry practices, including close monitoring of body condition score, regular fecal testing to identify parasite burdens, basic biosecurity measures to limit spread of pathogens, and IgG measurement to ensure adequate passive transfer of immunity.

Introduction

New World camelids are a relatively recent addition to the herbivore fauna of North America. There are currently more than 200,000 registered alpacas (Alpaca Registry, Inc.) and more than 162,000 registered llamas (International Lama Registry) in the United States. As these animals have become more common in the last several decades, additional information has become available describing the medical conditions affecting alpacas and llamas. Diseased New World camelids may show only nonspecific signs of illness such as weakness or recumbency,[104, 105] and antemortem diagnosis of many diseases can be difficult due to their stoic nature.[17, 106] Therefore, postmortem examination is often necessary to attain an accurate diagnosis in these animals.

Disease conditions commonly encountered in camelid practice include parasitism caused by endoparasites such as strongyles,[4] small coccidia and large coccidia such as *Eimeria macusaniensis*,[5-8, 107] cryptosporidia,[9] and ectoparasites such as mange mites (*Chorioptes* sp.).[12, 13] Camelids are also subject to non-infectious gastrointestinal conditions such as ulceration of the third compartment[14-16, 108] or foreign body ingestion.[17, 18] Congenital abnormalities that include conditions such as choanal atresia[19-23] or labial fusion[25, 109] are an important aspect of camelid disease. The most common neurologic diseases include meningeal worm infection (where the habitat of white-tailed deer and camelids overlap[27, 29-32]) and polioencephalomalacia.[33-35] Lymphoma and other round cell tumors represent

the most common neoplastic diseases.[15, 36-38] Important metabolic conditions include hepatic lipidosis,[42, 97, 98] hyperosmolar syndrome,[39, 43] and hyperlipidemia.[44] However, little is known about which diseases are responsible for the majority of camelid mortality.

Retrospective studies of necropsy examinations have been performed in other species and have provided a more complete understanding of their common disease processes.[110-119] One report summarizing data from camelid necropsy submissions to Guelph's Animal Health Laboratory has been published[3] as has been a review of the gross pathology of camelids.[15] Awareness of the most common fatal disorders in camelids may help identify specific aspects of husbandry or routine veterinary care that can be altered to prevent mortality and can also aid clinicians by narrowing the range of differential diagnoses for animals in recumbency. Additionally, regional differences in camelid diseases are known to occur and thus the aim of this retrospective study was to determine the causes of death observed in New World camelids submitted to a veterinary diagnostic laboratory for postmortem evaluation in the Upper Midwest region of the United States of America.

Materials and Methods

Necropsy records were reviewed from all alpaca and llama submissions to the University of Minnesota Veterinary Diagnostic Laboratory (UM-VDL). Only full

body necropsies that were performed between January 2001 and December 2011 were included in this study. Three hundred and ninety-two carcasses were submitted by animal owners and private practice veterinarians as well as cases first presenting for referral treatment at the University of Minnesota Veterinary Medical Center (VMC). Submissions that were in a state of advanced autolysis were eliminated from the study (4 alpacas and 3 llamas). Furthermore, animals submitted as part of research studies (21 alpacas and one llama) and those donated for teaching followed by euthanasia were also excluded (3 alpacas and one llama).

All postmortem examinations were performed at the UM-VDL under the supervision of veterinary pathologists. The necropsy included a gross examination and in most cases histopathologic evaluation. In general, the gross examination consisted of an assessment of the body condition score (BCS) using a 1 to 5 scoring system. Scoring was based on a system adapted for camelids[120], and serous atrophy of bone marrow fat and/or coronary fat was used to identify animals with a score of 1. General findings were recorded. Body cavities and each body system (e.g. integument, respiratory, cardiovascular, alimentary, nervous) were dissected and evaluated. Samples of each system and lesions noted were fixed in 10% buffered formalin. Representative tissue sections were paraffin-embedded and examined histologically. Additional testing such as

bacterial culture, molecular analysis, and fecal flotation was performed if deemed necessary by the pathologist.

The necropsy reports were examined retrospectively for this study. A variety of variables were recorded whenever available. These included age, sex (male, gelding, female), weight (kg), method of death (euthanized, died, or unknown), BCS (1 to 5), body system affected by disease (nervous, urogenital, musculoskeletal, respiratory, gastrointestinal, cardiovascular, hemic/lymphatic, systemic), gross and microscopic necropsy findings, presumptive cause of death, and results of fecal analysis, bacterial cultures, and molecular or serologic diagnostics, when performed. Gross or histologic diagnoses described by the pathologists were recorded using a predefined list of six-letter codes. The codes were defined by body system (e.g. nervous, cardiovascular, digestive). Diagnoses interpreted as incidental or agonal changes (such as lung congestion) were not recorded. The presumptive cause of death in each case was based on the magnitude and nature of the abnormalities noted as interpreted by the pathologist. If no specific cause of death was identified, the major abnormalities were recorded.

Data Analysis

Animals were separated into the following age groups: fetus, neonate (<14 days), cria (2 weeks to 6 months), youngster (> 6 months to 18 months), adult (>18

months). Animals without specific ages listed on the report were included in the appropriate age group (e.g. adult) if age category could be determined from the necropsy report or by contacting the owner for additional information. Statistical analyses were conducted in *R*.[a] Categorical variables were presented as percentages and continuous variables were described as mean ± standard deviation or median.

Results: Table of Contents

I. Alpacas

I.A: Alpaca Summary

Two hundred and thirty-four necropsies were performed on alpacas over the 11 years of the retrospective study. The mean age was 3.4 +/- 4.2 years old (range

0-22 years) and the median was 1.5 years. One hundred and thirty-five were female (58%), 69 were intact males (29%), 17 were neutered males (7%), 6 were unspecified males (2.5%), and 7 (3%) had no sex recorded. The median BCS was 2 out of 5. Sixty-six cases had no BCS recorded. Ninety-nine animals were submitted via the VMC (42%). One hundred nineteen cases died (51%), 60 were euthanized (26%), 28 cases were abortions (12%), and the method of death was unrecorded in 27 cases (12%). Forty-five alpacas (19%) were found dead without previous signs of illness reported by the owners and 26 were found recumbent (11%). The specific cause of death was determined in 166 cases (71%). Table 1 and Figure 1 display the frequency of body system affected and Table 3 and Figure 3 show the distribution of pathophysiologic processes.

I.B: Fetuses

Thirty alpaca fetuses were submitted. Fifteen were female (50%), 12 were male (40%), and 3 (10%) had no sex recorded.

The cause of abortion was considered idiopathic in 22 cases (73%). One submission was a pair of twins; death was thought to be caused by the unsustainability of a twin pregnancy. One fetus had an undifferentiated round cell tumor found throughout the body, including the heart, lung, liver, spleen, lymph nodes, and bone marrow. One was positive for bovine viral diarrhea virus (BVDV) by polymerase chain reaction. One submission had intranuclear

inclusion bodies identified in the placenta, but testing for bovine and equine herpesviruses by fluorescent antibody techniques performed on the lung, liver, and kidney and immunohistochemistry of the placenta and kidney yielded a negative result. One fetus had bilateral renal aplasia. One fetus was aborted after the dam underwent colic surgery and no specific lesions were identified. One fetus was aborted after surgical correction of a uterine torsion.

I.C: Neonates

Twenty-eight alpaca neonates (age 0 to 14 days) were submitted for postmortem examination. The mean age was 3.8 +/- 3.7 days and the median was 1.8 days. There was no numerical age recorded for 4 animals. Sixteen (57%) were female, 11 (39%) were male, and 1 had no sex recorded. The mean weight was 7.1 +/- 1.6 kg with a median of 6.8 kg (range 5.4-10.8 kg). Thirteen animals (46%) died, 6 (21%) were euthanized, and no method of death was recorded for 9 animals (32%). Three neonatal alpacas were found dead without previous signs of illness reported by the owners (10%) and 1 was found recumbent (3%). The cause of death was identified in 25 cases (89%).

Twelve animals in this age group (39%) had choanal atresia. Many had concurrent abnormalities in the olfactory bulb, cribiform plate, and eyes, and one also had a genital anomaly (fused labia). Two other animals had congenital malformations; one had multiple cardiac abnormalities and the other had abnormal lateral facets in the caudal cervical spine that were compressing the

spinal cord. There were 4 animals in which the digestive system was primarily affected. One had an intestinal perforation and peritonitis likely due to a penetrating foreign body. This animal also had hydronephrosis of the left kidney. One neonate had an enteritis due to infection with zygomycetes, suspected secondary to failure of passive transfer. One 10 day old neonate had cryptosporidiosis. Another animal had hemorrhagic and necrotizing inflammation of compartments 2 and 3 but the cause of the inflammation was not identified despite aerobic, anaerobic, and *Salmonella* cultures and electron microscopy of intestinal contents. There were 4 animals in which the respiratory system was primarily affected. Two had aspiration pneumonia; one of these was also septicemic and had hepatic lipidosis. One case of aspiration was related to feeding via nasoesophageal tube and the cause of aspiration could not be determined for the other. One neonate had pneumonia with *Enterococcus* sp. and *Escherichia coli* isolated from the lungs. Another animal was born approximately 30 days premature and had evidence of atelectasis consistent with prematurity and insufficient surfactant. One animal had evidence of myocardial degeneration possibly suggestive of ionophore toxicosis because liver vitamin E and selenium levels were considered adequate. One female neonate had a ruptured urinary bladder. One neonate had an *E. coli* septicemia with no obvious portal of entry. One neonate died due to exsanguination caused by umbilical artery hemorrhage.

I.D: Crias

Thirty-eight alpaca crias (age 2 weeks to 6 months) were submitted for postmortem examination. The mean age was 2.5 +/- 1.5 months and the median was 2 months. Three animals (8%) had no numerical age recorded. Twenty-one cases were female (55%), 14 were intact male (37%), and 3 (8%) had no sex recorded. BCS ranged from 1 to 3 out of 5, though 9 had no BCS recorded. The median BCS was 1.5. The mean weight was 15.7 +/- 7.9 kg with a range of 6.1 to 39 kg. Thirteen animals had no weight recorded. Twenty-four animals died (63%), 8 were euthanized (21%), and no method of death was recorded for 6 animals (16%). Ten crias (26%) were found dead without previous signs of illness reported by the owners and 3 (8%) were found recumbent. The cause of death was determined in 30 cases (79%).

A total of 8 animals (21%) had culture-confirmed septicemia as the cause of death and an additional 2 crias (5%) had histological changes consistent with septicemia but did not have positive cultures. Bacteria cultured included *E. coli*, *Pasteurella* sp., *Klebsiella pneumoniae, Actinobacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Listeria monocytogenes*. In three animals emaciation was considered the cause of death; underlying concurrent conditions included ulceration of the first compartment, giardiasis, and multiple intestinal parasitoses (coccidia, *Nematodirus* spp., and *Trichuris* spp.).

Five crias had primary musculoskeletal diagnoses. Two crias had congenital malformations of the cervical spine with spinal cord compression. One animal had a congenital absence of the intervertebral disc between lumbar vertebrae 1 and 2. One animal had a *Staphylococcus* osteomyelitis and arthritis of the coxofemoral joint secondary to internal surgical fixation of a mid-diaphyseal femur fracture. One cria had skeletal muscle degeneration and necrosis with low liver selenium concentration (1 mcg/kg dry weight, normal range 1.2-2.5 mcg/kg) suggestive of white muscle disease. The respiratory system was primarily affected in three alpaca crias. One four-week old cria had choanal atresia with secondary aspiration pneumonia. One case had fibrinosuppurative and necrotizing pneumonia caused by *Actinobacillus sp.* and one case had a fibrinohemorrhagic bronchopneumonia with no specific cause recognized despite aerobic culture of tissues and immunohistochemistry of the lung to identify bovine respiratory syncytial virus as well as viral isolation from a tissue homogenate. One cria was euthanized for poor growth and depression and was diagnosed with a ventricular septal defect. One animal with severe pulmonary edema was euthanized for lack of response to treatment for *Mycoplasma haemolamae*. There were thirteen crias with the digestive system primarily affected. Five had parasitic enteritis; specific causes included a variety of pathogens such as *Cryptosporidium* sp., *Eimeria macusaniensis*, small coccidia, *Moniezia* sp., undifferentiated strongyles, and *Nematodirus* spp. Two animals had evidence of

hepatic lipidosis without an identified underlying cause. One animal with a recorded age of 4 weeks had atresia coli. One cria had an esophageal obstruction with aspiration pneumonia. One animal died of *Salmonella typhimurium* enteritis with a concurrent coronavirus infection. One cria had necrosis of the second compartment with *Clostridium perfringens* type A isolated from the intestines and third compartment.

There were three crias with primary neurologic diagnoses. One had *Actinobacillus suis*-associated encephalitis secondary to necrotizing stomatitis and sinusitis. One had a small brain abscess in the thalamus (approximately 1 cm in diameter). Cultures were not performed and the route of infection was unclear. One case had encephalitis caused by *Listeria monocytogenes*.

I.E: Youngsters

Thirty-six alpacas (age 6 months to 18 months) were submitted for postmortem examination. The mean age was 11.9 +/- 4 months and the median was 12 months. Two animals (6%) had no numerical age recorded. Twenty-two cases were female (63%) and 13 were intact males (37%). BCS ranged from 1 to 4 out of 5, though 11 had no numerical BCS recorded. The median BCS was 2. The mean weight was 40.6 +/- 12.9 kg with a range of 20 to 65 kg. Ten animals had no weight recorded. Twenty-two animals died (61%), 9 were euthanized (25%), and no method of death was recorded for 5 animals (14%). Eight cases (22%)

were found dead without previous signs of illness reported by the owners and 3 (8%) were found recumbent. The cause of death was determined in 25 cases (69%).

Emaciation (BCS = 1) was considered the cause of death of five of the youngsters. One of those had concurrent hepatic lipidosis, two had gastrointestinal parasites (coccidia and strongyles in one case and coccidia, *Nematodirus* spp., *Trichuris* sp., *Capillaria* sp., and strongyles in the other) that may have contributed to death. One of these had pericardial effusion, possibly related to hypoproteinemia. One of the youngsters died due to jugular vein trauma presumably caused by an animal attack. One had evidence of *Actinobacillus* sp. septicemia secondary to pneumonia. Two animals died after exploratory abdominal surgery with pulmonary edema and acute aspiration.

Four animals had significant lesions of the cardiovascular system. Three cases had mural or valvular endocarditis; two of these cases had evidence of previous liver fluke infection. One animal died of pulmonary hemorrhage of an unknown cause. The hemolymphatic system was primarily affected in three alpaca youngsters. Two animals had multicentric round cell tumors; one of these was positive by immunohistochemistry for CD79, consistent with a B cell lymphoma, and the other was predominantly positive for CD3, consistent with a T cell

lymphoma. One animal was found dead in late August with multifocal subcutaneous hemorrhages; heat stroke was suspected.

Thirteen animals had lesions primarily affecting the digestive system. Four had hepatic lipidosis; all four also had concurrent multifocal hemorrhage suggestive of a coagulation disorder. Two animals (aged 7 months and 12 months) had active liver fluke infection. Two animals had intussusceptions (one that was ileo-ileal and one jejuno-jejunal) and one of those was positive for *Salmonella enterica* serotype Senftenberg. Two animals had hemorrhagic enteritis and *Clostridium perfringens* type A was isolated from intestinal contents of both cases; one isolate was positive for the β2 toxin gene on PCR. Another animal had enteric salmonellosis (*Salmonella enterica* serotype Newport). One case was found dead with peritonitis due to a foreign body induced intestinal perforation (fibrous plant matter).

Three animals had primary urinary system abnormalities. One seven-month-old intact male was found dead with evidence of urethritis with luminal obstruction but no stone or plug was identified and bacterial culture from the urethra was negative. One ten-month-old intact male had an urachal abscess with secondary cystitis and pyelonephritis; *Trueperella pyogenes* was cultured from the abscess. A one-year-old male was euthanized for straining to urinate and suspected obstruction; however, the only abnormality identified was dilatation of the urinary

bladder and hypertrophy of the muscular layer of the bladder with no obvious cause.

Four cases had primary nervous system abnormalities. Two cases had polioencephalomalacia. One animal had a five centimeter abscess compressing the cerebrum and another animal had suppurative meningitis. Bacterial culture yielded a negative result in both animals and a possible portal of entry was not identified.

I.F: Adults

One hundred and two adult alpacas (age > 18 months) were submitted for postmortem examination. The mean age was 6.5 +/- 4.1 years and the median was 5 years. Seven animals (7%) had no numerical age recorded. Sixty-one cases were female (60%), 19 were intact males (19%), 16 were geldings (16%), and 6 were unspecified males (6%). BCS ranged from 1 to 4.5 out of 5, though 18 had no numerical BCS recorded. The median BCS was 2.25. Forty-two animals (41%) were underweight with a BCS of 2 or less. The mean weight was 61.0 +/- 13.6 kg with a range of 31 to 97 kg. Twenty-five animals had no weight recorded. Fifty-eight animals died (57%), 37 were euthanized (36%), and no method of death was recorded for 7 animals (7%). Twenty-four cases (24%) were found dead without previous signs of illness reported by the owners and 19

(19%) were found recumbent. The cause of death was determined in 82 cases (80%).

Emaciation was considered the cause of death in eleven of the adult alpacas (11%). Three of those had concurrent gastrointestinal parasitism, two (aged 10 and 13 years) had dental abnormalities (uneven wear of the molar arcades in one case and severe incisor malalignment in the other), and one had hepatic lipidosis. Septicemia was considered the cause of death in six adults; *Streptococcus* spp. were isolated from four animals, while one animal each had *E. coli* and *Listeria monocytogenes* septicemia. One animal had peritonitis after exploratory abdominal surgery. One animal was found dead lodged in a fence with no other gross abnormalities on necropsy; suffocation was the presumed cause of death.

Five animals had the musculoskeletal system primarily affected. Four had fractured bones. There was an animal with a fractured third cervical vertebra with spinal cord transection that was found dead, a case that was euthanized with a fractured third metacarpal, and a case that was euthanized for neurologic signs caused by a fractured tympanic bulla. One animal died due to fractures of the occipital condyles and the atlas that were possibly the result of an atlanto-occipital malformation. One animal was euthanized with chronic lameness issues and had chronic proliferative bursitis of the right hock.

Five animals had primary respiratory diagnoses. Two animals had aspiration pneumonia; one of these was found dead several days after castration under general anesthesia. One animal had a food bolus lodged in its trachea. One animal had a granulomatous pneumonia that was negative for acid-fast organisms and one had an *E. coli* pneumonia with evidence of septicemia.

The cardiovascular system was primarily affected in four cases. Two animals had endocarditis; one of those had concurrent liver fluke infection and bacterial culture was negative, while the other grew *Acinetobacter* sp. from the right ventricle. One animal was found dead with a ventricular septal defect and pericardial effusion. One animal had fibrosis of the endocardium and myocardium of the right ventricle without a clear underlying cause.

The hemolymphatic system was primarily affected in 14 animals. Eight adult alpacas had round cell tumors affecting multiple organs and lymph nodes; one of these had a concurrent *Listeria monocytogenes* septicemia. Three of the tumors were positive for CD20 on immunohistochemistry, which is consistent with B cell lymphoma. The other five tumors did not have consistent immunoreactivity to either B cell or T cell markers. Three adults had caseous lymphadenitis (CLA) abscesses and were culture positive for *Corynebacterium pseudotuberculosis*; two had both internal and external abscesses while one had only internal

abscesses. All three animals with CLA had abscessation of the liver; the lungs, heart, aorta, and portal vein each were affected in a single animal. Three animals had widespread petechiae of unknown pathogenesis and one of these also had hepatic lipidosis.

The digestive system was primarily affected in 38 adult alpacas. Eight animals had a primary diagnosis of hepatic lipidosis. Seven had parasitic gastroenteritis; four had *Eimeria macusaniensis*, one had severe *Haemonchus contortus* infection, and two had a variety of parasites including undifferentiated strongyles, *Nematodirus* spp*., Capillaria* sp., *Trichuris* spp., and *Moniezia* sp. Six animals had intestinal obstructions; one had a spiral colon intussusception, one had a trichophytobezoar in the spiral colon, one had an adhesion of the jejunum to the urinary bladder, one had a foreign body in compartment 1, one had a stricture at the duodenal-pyloric junction after two foreign body surgeries, and one had a perforation of the spiral colon. There were four cases of intestinal neoplasia consisting of two small intestinal adenocarcinomas (one with metastasis to the lungs), a rectal carcinoma, and a leiomyoma of the first compartment. Two animals had severe third compartment ulcers; one of which was perforated in one animal. Two animals had ulcerative lesions in the first compartment and another had diphtheritic inflammation of the esophagus as well as compartments 1 and 3; *Fusobacterium* sp. and *Bacteroides* sp. were cultured from the lesions in one of these animals. *Pseudomonas aeruginosa* was isolated from C1 in two of

the cases of first compartment ulceration. Two cases died shortly after abdominal surgery (one cesarean section, one abdominal exploratory) and lymphoplasmacytic enteritis was the most significant necropsy finding. One animal had portal hepatitis with fibrosis and one had a focal area of bile duct proliferation with no other significant lesions identified. One case had a liver fluke infection as the major cause of death. One animal had necrohemorrhagic enteritis with *C. perfringens* type A isolated (positive for the gene for β2 toxin) with concurrent coccidiosis. One animal had an impaction of the first compartment. One case had centrilobular liver necrosis; *E. coli* was isolated but the cause and significance of the liver lesion was undetermined.

There were six animals with the urogenital system primarily affected. Two cases died or were euthanized with severe postpartum metritis 3-4 days after delivery. One animal died two days after a dystocia with uterine and vaginal perforation. One animal had a 17 kg periovarian abscess from which *E. coli* was isolated. Two cases died with chronic glomerulonephritis without specific underlying cause.

The nervous system was primarily affected in ten cases. Five animals ranging in age from 4 to 12 years had eosinophilic inflammation and linear tracts in the central nervous system consistent with *Parelaphostrongylus tenuis* (meningeal worm) infection; 4 of the 5 cases involved the brain. There were two cases of

West Nile virus encephalitis and one of Eastern equine encephalitis. There was one animal with fungal osteomyelitis of the temporal bone resulting in encephalitis and compression of the cerebrum and cerebellum. The fungus was not cultured but the histologic appearance was consistent with *Aspergillus* sp. One animal had cerebral hemorrhage but the underlying cause was uncertain.

I.G: Found Dead

Forty-five alpacas were found dead without previous illness reported by their owners, comprising 19% of alpaca submissions. The mean age of alpacas suffering unexpected death was 4.7 +/- 5.0 years and the median was 4 years with a range of 2 days to 18 years. Six animals (13%) had no numerical age recorded. Eighteen cases were female (40%), 19 were intact males (42%), 5 were geldings (11%), 2 were unspecified males (4%), and one had no sex recorded (2%). BCS ranged from 1 to 4.5 out of 5, though 8 had no numerical BCS recorded. The median BCS was 2. The cause of death was determined in 34 cases (76%).

The most common cause of unexpected death in alpacas was emaciation, accounting for 8 cases (18%). Parasitic infections were the next most frequent cause of sudden death, affecting 7 cases; three animals had massive liver fluke infections, one had a meningeal worm infection, one had *Cryptosporidium* sp. infection, one had *Eimeria macusaniensis*, and one was infected with a variety of

gastrointestinal parasites. Six died from bacterial infections, including three cases of pneumonia, two cases of neonatal sepsis, and one case of *Actinobacillus suis* encephalitis. There were five cases of neoplasia that resulted in death without previously noted clinical signs (three round cell tumors and two small intestinal carcinomas), and five traumatic causes of death (including suffocation, spinal fracture, and animal attack). There were three cases of hepatic lipidosis.

I.H: Found Recumbent

Twenty-six alpacas were found recumbent (11%). The mean age of down alpacas was 5.0 +/- 4.3 years and the median was 4 years with a range of 9 days to 18 years. There was one animal with no numerical age recorded. Fourteen cases were female (54%), 6 were intact males (23%), 5 were geldings (19%), and one was an unspecified male (4%). BCS ranged from 1 to 4 out of 5 with a median of 2.25, though 8 had no numerical BCS recorded. The cause of death was determined in 21 cases (81%).

There were a wide variety of ultimate causes of death in animals that were found down; however, gastrointestinal parasites accounted for 4 cases, including infection with *Eimeria macusaniensis*, *Haemonchus contortus*, and small coccidia. There were two cases each of emaciation, round cell tumors, West Nile virus, widespread hemorrhage consistent with a coagulation disorder, and meningeal worm. The nervous system was affected in only 5 of the 26 animals

found down (19%), while the digestive system was primarily affected in 11 cases (42%).

I.I: Ancillary Testing

One hundred and ninety-five animals had aerobic cultures performed on swabs of organs and/or serosal surfaces (83%). Sixty-seven cultures were positive and a wide range of bacteria were isolated, including *Enterococcus* sp., *Staphylococcus* spp., *Streptococcus* spp., *Listeria monocytogenes, Trueperella pyogenes*, *Actinobacillus* spp., *Actinomyces* spp., *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bordetella bronchiseptica*, and *Acinetobacter* sp. Sixty-three anaerobic cultures were performed (27%). Forty-three cultures were positive, including 36 cultures of intestinal contents which isolated *Clostridium perfringens*, two endocarditis lesions and one aortic abscess that were positive for *Peptostreptococcus anaerobius*, two esophageal swabs that grew *Fusobacterium necrophorum*, and one liver swab that was positive for *Clostridium sordelli*. One hundred and twenty-six *Salmonella* cultures were performed on pooled tissue samples (54%) and five samples were positive (one cria, 2 youngsters, and 2 adults; 2% of alpacas). The isolation of *Salmonella* may have been incidental in the two adult alpacas (who died of Eastern equine encephalitis and emaciation). However, *Salmonella* appeared to cause disease in the younger animals, where it was considered the cause of fatal enteritis in two cases and was isolated from an animal with an intussusception. Eighteen

animals were tested for *Mycobacterium avium* subsp. *paratuberculosis* by fecal PCR (8%) and all were negative.

One hundred and thirty-nine animals were tested for bovine viral diarrhea virus (BVDV) by PCR (59%) and the one positive specimen was an aborted fetus. Fifteen animals (6%) were tested for rabies by the direct fluorescent antibody test and all were negative. Nine cases (4%) were tested for viral encephalitides and three (all adults) were positive for Eastern equine encephalitis virus and West Nile virus. Forty-six cases (20%) were tested for viruses by electron microscopy of intestinal contents and four were positive; there was one diagnosis each for coronavirus, adenovirus, enterovirus, and one small unidentified virus. The significance of these observed viruses is unclear. Eight abortion cases underwent viral isolation by passage on bovine turbinate cells or other tests for viral pathogens other than bovine diarrhea virus; all were negative.

Fecal flotations were performed on one hundred and forty-nine cases (64%) and 82 were positive (55% of floats performed). A variety of gastrointestinal parasites were identified, including small coccidia, *Eimeria macusaniensis*, *Cryptosporidium* spp.*, Giardia* sp., undifferentiated strongyles, *Nematodirus* spp., *Trichuris* spp., *Capillaria* sp., and *Moniezia* sp.. Twenty-four immunofluorescence assays for *Cryptosporidium* sp. and *Giardia* sp. were performed (10%) and a single 4-month-old alpaca was positive.

II. Llamas

II.A: Llama Summary

One hundred and twenty-five necropsies were performed on llamas over the 11 years of the retrospective study. The mean age was 6.7 +/- 5.2 years old (range 0-20 years) and the median was 5.5 years. Sixty-seven of the llamas were female (54%), 8 were intact males (6%), 29 were neutered males (23%), 11 were unspecified males (1%), one was a hermaphrodite (1%), and 9 (7%) had no sex recorded. The median BCS was 2 of out 5. Fifty cases had no BCS recorded. Fifty-two cases died (42%), 44 were euthanized (35%), 2 cases were abortions (2%), and the method of death was unrecorded in 27 cases (22%). Thirty llamas were found dead (24%) without previous signs of illness reported by the owners and 29 were found recumbent (23%). The specific cause of death was determined in 104 cases (83%). Table 2 and Figure 2 display the frequency of body system affected and Table 4 and Figure 4 show the distribution of pathophysiologic processes.

II.B: Fetuses

There were 3 llama fetuses submitted. If there was any evidence that the animal had breathed (aerated lung), it was classified as a neonate and not included in this group (one case). Two were female (67%) and one was male (33%).

The cause of abortion was considered idiopathic in 2 cases (67%). One of these cases had hemoperitoneum with no underlying cause identified and the other had no gross abnormalities. The third fetus was found dead and partially delivered and was considered to have died from asphyxia.

II.C: Neonates

Five llama neonates (age 0 to 14 days) were submitted for postmortem examination. The mean age was 4.5 +/- 4.0 days and the median was 5.1 days. Three (60%) were female, one (20%) was male, and one had no sex recorded. The range of weight was 9 to 10 kg and three animals had no weight recorded. Three animals (60%) died, one (20%) was euthanized, and no method of death was recorded for one animal (20%). The cause of death was identified in all 5 cases.

One llama neonate died of *Actinobacillus* sp. septicemia with pneumonia, meningitis, and laryngitis. One case died at one day of age with a ventricular septal defect with an overriding aorta and a patent ductus arteriosus. One animal was euthanized at 5 days of age with a meconium impaction. One case died during dystocia but had no other abnormalities identified; it was classified as a neonate rather than a fetus by the pathologist. The remaining case had suppurative meningitis and encephalitis; *Enterobacter* sp. and *Haemophilus* sp. were isolated.

II.D: Crias

Five llama crias (age 2 weeks to 6 months) were submitted for postmortem examination. The mean age was 4.0 +/- 1.9 months and the median age was 5 months. Three cases were female (60%), one was an intact male (20%), and one (20%) had no sex recorded. BCS ranged from 1 to 3 out of 5, though two had no BCS recorded. The median BCS was 1.5. The weight ranged from 14 to 43 kg but three animals had no weight recorded. Two animals died (40%), two were euthanized (40%), and no method of death was recorded for one animal (20%). Two cases (40%) were found dead without previous signs of illness reported by the owners and one (20%) was found recumbent. The cause of death was determined in 4 cases (80%).

Emaciation was considered the cause of death in one case that had concurrent infection with *Trichuris* spp. and undifferentiated strongyles. One case had a subluxation of the fifth cervical vertebra that was thought to be congenital. One case had marked *Haemonchus contortus* infection of the third compartment and was anemic and emaciated. One llama cria had *Listeria monocytogenes* septicemia. The last case was anemic and had acidosis of the first compartment but no specific cause of death was identified.

II.E: Youngsters

Eleven llamas aged 6 months to 18 months were submitted for postmortem examination. The mean age was 13 +/- 3 months and the median was 12 months. One animal (9%) had no numerical age recorded. Six cases were female (55%), three (27%) were intact males, one (9%) was a gelding, and one (9%) had no sex recorded. BCS ranged from 1 to 3 out of 5 and one animal had no BCS recorded. The median BCS was 1.75. The mean weight was 55.4 +/- 20.7 kg with a range of 28 to 91 kg. Two animals had no weight recorded. Six animals died (55%) and five were euthanized (45%). Five cases (45%) were found dead and one (9%) was found recumbent. The cause of death was determined in 10 cases (91%).

One case had degeneration of the heart muscle but no specific cause was identified; histology of skeletal muscle was within normal limits and feed samples were negative for ionophores. One case had an *Actinobacillus* sp. arteritis that eroded into the lung and caused fatal pulmonary hemorrhage. Liver flukes (*Fascioloides magna*) were the cause of death in four llama youngsters. Two other cases had marked gastrointestinal parasitoses; *Haemonchus contortus* accounted for one case and a large *Moniezia* burden along with *Trichuris* sp. and strongyles was observed grossly in the other. One llama youngster had ulceration of the first compartment. One case was euthanized for poor response after a tube cystotomy for obstructive urolithiasis and had a ruptured bladder due

to necrosis of the surgical site. One llama aged 14 months had spinal cord lesions consistent with *Parelaphostrongylus tenuis* migration.

II.F: Adults

One-hundred and one adult llamas (age > 18 months) were submitted for postmortem examination. The mean age was 8.2 +/- 4.7 years and the median age was 7.5 years. Fifteen animals (15%) had no numerical age recorded. Fifty-three cases were female (52%), 3 were intact males (3%), 28 were geldings (28%), 10 were unspecified males (10%), one was a hermaphrodite (1%), and the sex was not recorded for six cases (6%). BCS ranged from 1 to 4.5 out of 5, though 41 had no numerical BCS recorded. The median BCS was 2.5. Twenty-nine animals (29%) were underweight with a BCS of 2 or less. The mean weight was 133 +/- 43 kg with a range of 56 to 260 kg. Forty-seven animals had no weight recorded. Forty animals died (40%), 36 were euthanized (36%), and no method of death was recorded for 25 animals (25%). Twenty-two cases (22%) were found dead and 27 (27%) were found recumbent. The cause of death was determined in 84 cases (83%).

Emaciation was considered the cause of death in twelve adult llamas. Seven of these had gastrointestinal parasites (namely *Trichuris* sp. but also strongyles; one also had *Fascioloides magna*), two had dental abnormalities, two had fungal rhinitis, stomatitis, and/or pharyngitis, and one had ulceration of the first

compartment. A multisystemic primary cause of death was identified in another seven animals. The cause of death in one case was *Streptococcus equi* subsp. *zooepidemicus* septicemia with concurrent hepatic lipidosis. The cause of death for one animal was polyserositis and cystitis without a bacterial cause identified. There were two cases of suspected toxicity, one of copper overload (299.4 ppm wet weight, normal range 30-100 ppm wet weight)[121] and one with lead exposure (liver lead value 2.9 ppm wet weight). Other causes of death included one case of *Actinomyces* spp. and *Clostridium sordelli* and *perfringens* peritonitis secondary to liver fluke infection, and one case of a traumatic body wall herniation with hemoabdomen. One case was found dead with no significant abnormalities; heat stroke was suspected.

The integumentary system was primarily affected in two adult llamas. One llama had squamous cell carcinoma of the mammary gland area with metastasis to the sublumbar lymph nodes and lungs as well as secondary abscessation of the skin tumors. One animal was found dead with marked *Chorioptes* infestation; however, no cause of death could be identified and tests for strychnine were negative.

The musculoskeletal system was primarily affected in five adult llamas. There were two spinal fractures: one at the sixth cervical vertebra with concurrent suppurative osteomyelitis and one at T12-L1. The latter case also had evidence

of linear tracts in the spinal cord consistent with meningeal worm infection, which may have caused ataxia that predisposed the animal to trauma. Three animals were euthanized for chronic lameness issues; one had bilateral rupture of the lateral collateral ligaments of the stifle, one had chronic arthritis and osteomyelitis of the carpal joint, and one had ankylosis of the tibiotarsal joint.

The respiratory system was primarily affected in eight adult llamas. There were three cases with changes suggestive of chronic obstructive pulmonary disease. These findings included hyperinflated and resilient lungs on gross examination with lymphocytic peribronchiolitis, hyperplasia of bronchiolar smooth muscle, and interstitial and alveolar fibrosis seen histologically. No bacteria, fungi, *Mycoplasma* species, or viruses were isolated from these three cases. There were two cases of aspiration pneumonia, one pneumonia caused by a *Bacillus* sp., one case with pulmonary edema of unknown cause, and one chronic interstitial pneumonia with no identified underlying cause.

Five adult llamas had a primary lesion in the cardiovascular system. Two animals had a ruptured pulmonary artery that resulted in cardiac tamponade. There were two cases from one farm with degeneration of the heart muscle; ionophore toxicosis was suspected but feed samples tested negative. One case had a focally extensive area of fibrosis in the left ventricular free wall that was considered to be the cause of death.

The hemolymphatic system was primarily affected in three adult llamas. There were two cases of multicentric round cell tumors. One was described as a well differentiated lymphosarcoma but no immunohistochemistry was performed. One was immunonegative when using standard markers for leukocytes including lysozyme, CD79, and Mac387, was negative for cytokeratin, and was variably positive for vimentin and CD3; the pathologist's conclusion was that the tumor origin could not be identified based on the tests performed. There was one case of caseous lymphadenitis with internal abscessation that was culture positive for *Corynebacterium pseudotuberculosis*; abscesses were found in the lungs, heart, kidneys, and brain.

The digestive system was primarily affected in 30 cases. There were seven cases of liver fluke infection (*Fascioloides magna*). Seven animals had marked compartment 3 ulcers, two of which were perforated. Three cases had dilation and impaction of compartment 1 and one of these also had megaesophagus. There were two cases of intestinal volvulus, both affecting the spiral colon. Two cases had ulcerative inflammation of the esophagus and the first compartment of the stomach but no underlying cause was identified. Two cases died of intestinal perforation and peritonitis and one case had concurrent third compartment ulcers. One animal had an enteritis with concurrent hepatic lipidosis; *Clostridium perfringens* type A with the gene for enterotoxin was isolated from intestinal

contents. There was one case of anaplastic liver carcinoma with metastasis to internal lymph nodes. One animal was euthanized with neurologic signs and had severe hepatic cirrhosis of unknown etiology. Hepatic encephalopathy was suspected.

Six animals had primary lesions in the urogenital system. There were three cases of urinary tract obstruction; one had a ruptured bladder due to urolithiasis (though stone composition was not determined), one had a urethritis with rupture but no stone detected, and the third case had silica uroliths and an *Enterobacter* sp. cystitis. There was one case of chronic glomerulonephritis with no underlying cause identified. One animal was diagnosed as intersex with bilateral ovotestes, female internal tubular structures, and masculinization of the external genitalia. One animal was diagnosed with a spindle cell sarcoma of the kidney that had metastasized to the lung and pleural surface.

Twenty-three adult llamas had primary lesions of the nervous system. Eighteen animals had lesions consistent with meningeal worm infection; however, parasites were directly observed in only two cases. The brain was affected in eight of the eighteen cases (44%). There were two cases of polioencephalomalacia. There was one case with evidence of chronic cervical spinal cord compression, one case of listeriosis, and one case with cerebral edema of unknown cause.

II.G: Found Dead

Thirty llamas were found dead without previous illness reported by their owners, which comprised 24% of llama submissions. The mean age of llamas suffering unexpected death was 6.0 +/- 5.6 years and the median was 4 years with a range of 0-20 years. Six animals (20%) had no numerical age recorded. Twenty-two cases were female (79%), one was an intact male (4%), 4 were geldings (14%), and three had no sex recorded (11%). BCS ranged from 1 to 4 out of 5, though 7 had no numerical BCS recorded. The median BCS was 1.5. The cause of death was determined in 24 cases (80%).

The most common cause of unexpected death in llamas was emaciation, with 9 cases (32%). Liver fluke infection was the next most frequent cause of sudden death, affecting 6 cases (20%). Three cases had degenerative changes in the heart resulting in sudden death. There were two cases of suspected toxicity: one each involving copper and lead. There were two animals found dead with rupture of the pulmonary artery.

II.H: Found Recumbent

Twenty-nine llamas were found recumbent, comprising 23% of total llama submissions. The mean age of down llamas was 6.6 +/- 4.6 years with a median of 4 years. Two animals had no numerical age recorded. Fourteen cases were

female (48%), one was an intact male (3%), 10 were geldings (34%), three were unspecified males (10%), and one had no sex recorded (3%). A cause of death was determined in 27 cases (93%).

The most common cause of death in llamas initially found down was meningeal worm, affecting 12 cases or 41% of recumbent llamas. Other causes of death in this subset included 2 cases of parasitic enteritis involving strongyles and coccidia, 2 cases of spinal fractures, and 2 cases of intestinal perforation. The other 12 cases found down died from a variety of causes including *Streptococcus* septicemia, caseous lymphadenitis, chronic interstitial pneumonia, spiral colon volvulus, third compartment ulceration, first compartment impaction, ulcerative stomatitis and esophagitis, urethral rupture, *Listeria* encephalitis, and bacterial meningitis.

II.I: Ancillary Testing

Ninety-four aerobic cultures (75%) were performed on swabs of organs and/or serosal surfaces; 38 were positive and the bacteria isolated included *Enterococcus* sp., *Staphylococcus* spp., *Streptococcus equi* subsp. *zooepidemicus*., *Streptococcus bovis*, *Listeria monocytogenes, Trueperella pyogenes*, *Actinobacillus* spp., *Bacillus* sp., *E. coli*, *Pseudomonas aeruginosa*, *Bordetella bronchiseptica*, *Haemophilus* sp., *Acinetobacter* sp., and *Enterobacter* sp.. Nine anaerobic cultures were performed (7%); three were positive for

Clostridium perfringens from intestinal contents and liver swabs from two cases with liver flukes were positive for a variety of anaerobes: *Peptostreptococcus anaerobius, Bacteroides* sp., *Clostridium perfringens* and *Clostridium sordelli*. Fifty-five *Salmonella* cultures were performed on pooled tissues (44% of cases) and one adult llama was positive for *Salmonella enterica* serotype Agona. Thirteen animals were tested (10%) for *Mycobacterium avium* subsp. *paratuberculosis* (5 by culture, 4 by PCR, and 4 by both methods) and all were negative.

Forty-four llamas (35%) were tested using PCR for bovine viral diarrhea virus and all were negative. Twenty animals (16%) were tested for rabies and all were negative. Five cases (4%) were tested for viral encephalitides and all were negative. One fetus underwent viral isolation but no viruses were identified.

Fecal flotations were performed on eighty-six cases (69%) and 53 were positive (62% of floats performed). A variety of gastrointestinal parasites were identified, including small coccidia, *Eimeria macusaniensis*, *Cryptosporidium* spp.*, Giardia* sp., undifferentiated strongyles, *Nematodirus* spp., *Trichuris* spp., *Capillaria* sp., *Cooperia* sp., *Strongyloides* sp., *Dictyocaulus* sp., and *Moniezia* sp.. Three immunofluorescence assays for *Cryptosporidium* and *Giardia* were performed (2%) and all were negative.

Discussion

There was a distinct difference in the demographics of the alpacas and llamas submitted for necropsy during this time period. Although the range of ages was similar for both groups, the median age was 5 years for llamas and 1.5 years for alpacas. There were 96 alpaca crias, neonates, and fetuses compared to only 13 llama crias, neonates, and fetuses, whereas the numbers of adult llamas and alpacas were similar (101 and 102 respectively). This may be due to a more vigorous breeding program among alpaca owners in the sampled region and different intended uses of the animals. It may be that llamas were more likely to be kept as pets or guard animals in small herds whereas alpacas in the area were more likely to come from larger farms and be used for breeding and showing. The distribution of male and female animals was similar between alpacas (58% female) and llamas (54% female); however, intact males were more common in the alpaca group (29% compared to 6% among llamas). This difference may be due in part to the ages of the animals submitted, since camelids often are not castrated before they are several years old. However, the difference was also apparent when comparing only adult llamas and alpacas; only 3% of adult male llamas were intact compared to 19% of adult male alpacas. This may also be due to differences in intended use.

Although a specific cause of death could be identified in the majority of cases submitted (75% of all cases), the cause of abortion was determined in relatively

few fetuses, both alpaca and llama (only 5 out of 33 total). This can be frustrating for camelid owners and veterinarians, but this finding is similar to a previous report[122] where 67% of camelid abortions were considered idiopathic. In addition, abortions may occur due to maternal stress without pathologic findings.[15] Interestingly, bovine viral diarrhea virus infection appears to be uncommon in camelids submitted to this veterinary diagnostic laboratory, with only one positive animal identified, an alpaca fetus examined in 2001.[123] The virus was detected by viral culture of tissue homogenate as well as polymerase chain reaction. Concern over bovine viral diarrhea virus infection among alpacas arose in the mid-2000s after the identification of persistently infected crias.[124, 125] Although a survey of alpaca owners in the United States identified that 25% of herds had at least one animal that was seropositive for BVDV and 6% had a persisently infected cria,[126] there was only a single positive test in this study. There may also be a regional variation because no herds from Minnesota agreed to participate in the survey.[126] However, in the present study, only 183 animals (51%) were tested by PCR, so there may have been additional positive animals and it is also possible that serologic testing (which was not performed) would have identified evidence of exposure to BVDV.

The most common cause of death in alpaca neonates (age 0-14 days) was choanal atresia, a failure of normal craniofacial development that results in obstruction of the nasal passages.[22, 23] This is considered a relatively common

congenital abnormality in camelids, though specific data on the prevalence of the condition is lacking. An informal survey of camelid practitioners estimated a prevalence of 0.75% of llama births and 0.48% of alpaca births.[21] In this study, there were 13 cases of choanal atresia in alpacas (comprising 19% of alpacas <6 months of age) and none in llamas. Most animals died or were euthanized within the first day of life; however, one animal lived to two months of age with bilateral obstruction of her choanae before dying from secondary suppurative bronchopneumonia. Concurrent congenital abnormalities were common; eight of the thirteen cases had additional craniofacial abnormalities such as brain, eye, and optic nerve malformations as previously described,[23] one had spina bifida, and one had a genital anomaly (fused labia).[25, 109, 127] During part of the study period, researchers at the University of Minnesota were actively studying camelid choanal atresia cases in order to attempt to identify a genetic component to the disorder, so there likely is a bias in the number of choanal atresia cases submitted. However, any cases received from outside the standard area (e.g. from California or Michigan) were excluded from this retrospective study.

Congenital malformations accounted for a total of 21 deaths among alpacas when the data from liveborn animals under 6 months of age was combined (31% of this age group and 9% of all alpaca submissions). A list of the congenital defects reported in camelids has been previously published.[26, 128, 129] After choanal atresia, the most common abnormalities in this study were related to

malformation of the spinal column. Vaughan *et al.* described a male alpaca with deformities in his coccygeal spine which passed the abnormalities to eight of his offspring, and the report included one unrelated case of cervical vertebral malformation in an alpaca cria.[130] The much larger number of congenital defects noted in alpacas versus llamas in our study is likely due to the much lower numbers of llama neonates and crias submitted for evaluation, as previously discussed, rather than a true difference in the incidence of such defects. In general, alpacas and llamas appear to have higher incidences of congenital malformations than other species.[129, 131] This may be a consequence of a reduced genetic pool caused by large scale decimation of camelid herds during the Spanish conquest of the Andes[132, 133] as well as selective breeding for color and fiber quality.[134, 135] Llamas may have been under less selection pressure for fiber characteristics, since that is not their primary use.

Septicemia was considered the cause of death in 11 alpaca crias under six months of age (16% of that age group). However, in contrast to other species such as calves or foals that are primarily affected in the neonatal period (<4 weeks of age),[136-139] the median age at death was 6 weeks. Previous reports on the clinical progression of septicemia in crias have found other differences between camelids and other species. For instance, septic crias often do not display the clinical signs such as fever, recumbency, or lack of suckle reflex which are considered classic for sepsis in other species. Additionally,

hematologic and biochemical measurements can remain within reference intervals even in cases of culture-confirmed sepsis.[104] Similar to the results in the present study, Dolente *et al.* identified *E. coli*, *Enterococcus* sp.*,* and *Listeria monocytogenes* as the most frequent causes of septicemia.[104] Additionally, in this study there was often no obvious portal of entry for the bacteria, nor was there evidence of an umbilical infection in any case, while Dolente *et al.* identified only a single cria with an abnormal umbilicus,[104] suggesting that this is not a common source of neonatal septicemia in camelids.

As a whole, bacterial infections accounted for 47 of 234 cases of alpaca mortality (20%), including 19 of 38 crias age 2 weeks to 6 months (50%) and 18 of 102 adult alpacas (18%). In addition to the septicemia described above, there were also cases of bacterial pneumonia, caseous lymphadenitis, other abscesses, peritonitis and pleuritis, encephalitis and meningitis, systemic and neurologic listeriosis, and enteritis. Bacterial infections have been previously described as the most common cause of death or referral of camelids to tertiary hospitals.[140] This was true of alpacas in this study, but in llamas bacterial infections comprised only 11 of 125 causes of mortality (8.8%). Both parasite infections and inflammatory conditions were more frequent than bacterial infection as a cause of death in llamas (Table 4, Figure 4).

Caseous lymphadenitis (CLA), caused by *Corynebacterium pseudotuberculosis,* has been occasionally described in camelids[141-144] and was an infrequent cause of death in this study, with four total cases (1%) among adult alpacas and llamas. Two of the alpacas were submitted from a single herd in a two-month period. The llama lived in a pasture with sheep, but it was not possible to determine the specific source of infection from the histories provided. However, spread may occur when camelids are in contact with sheep or goats or when shearing equipment is not thoroughly disinfected.[131] Anderson *et al.* monitored an outbreak of CLA in a North American alpaca herd and only external abscesses were identified,[141] in contrast to the findings in the present study where all four cases had internal abscesses. Data from South American alpaca herds showed that internal abscessation may have a predilection for renal lymph nodes but in contrast to sheep, alpacas did not appear to develop frequent lung lesions.[142] Experimental inoculation of alpacas with *Corynebacterium pseudotuberculosis* resulted in a similar predilection for abscessation of renal lymph nodes.[143] To the contrary, none of these cases in the present study had involvement of the renal lymph nodes and two animals had pulmonary abscesses.

"Alpaca fever," as bacteremia and polyserositis caused by *Streptococcus equi* subsp. *zooepidemicus* is sometimes known,[10, 140, 145] was an infrequent cause of death in this study, affecting one adult llama (0.8%) and two adult alpacas (0.8%), though there were an additional two adult alpacas diagnosed with

Streptococcus spp. septicemia without concurrent evidence of polyserositis. Contrary to previous reports that most affected animals were under 3 years of age,[10] the affected animals were 3.5, 4.5, and 10 years old. Alpaca fever can occur as a herd outbreak;[10, 26] although all of the cases in this report resulted in mortality in single animals from a given location. However the histories included with the submissions of these cases did not state whether there were other sick animals on the property at the same time.

Enteritis with isolation of *Clostridium perfringens* was an infrequent diagnosis in this study, accounting for four cases (2 alpaca youngsters, one adult alpaca, and one adult llama). *Clostridium perfringens* type A was isolated from each of these cases, though only three cases were positive for the genes for toxin production (two for the β2 toxin, one for enterotoxin). In Peru, clostridial enteritis is considered the leading cause of death among neonatal alpacas[8] and often occurs in conjunction with coccidial infection. Damage to the intestines from coccidia may allow proliferation of *C. perfringens,* as has been demonstrated in chickens.[146] However, *C. perfringens* type A and even the gene for the β2 toxin can be isolated from normal intestinal contents and the contribution to disease is not always clear.[147, 148]

Hepatic lipidosis and derangements of glucose metabolism are a common concern in sick and hospitalized camelids due to inherent differences in their

metabolism, including decreased insulin production and peripheral insulin resistance.[40, 46, 47, 51, 67, 149] Under experimental conditions, animals with additional metabolic stressors such as pregnancy and particularly lactation were more likely to develop hepatic lipidosis during feed restriction.[52] However, lipidosis has also been identified in very young crias and was present in 12% of cria necropsies at Oregon State University.[40] In our study, hepatic lipidosis was considered the primary cause of death in 8 adult alpacas, 7 of which were female. An additional 7 adult female alpacas had a secondary diagnosis of lipidosis. There was not enough information in the record to accurately determine how many of these animals were currently lactating. Among the 4 cases of hepatic lipidosis in alpaca youngsters (aged 6-18 months), all 4 were female, though at this age the metabolic drains of pregnancy and lactation are likely not playing a role. In contrast, the two cases of hepatic lipidosis in crias (under 6 months of age) occurred in male animals. Whether or not hepatic lipidosis was considered the primary cause of death, there were 23 alpacas with hepatic lipidosis (10% of alpacas) but only two llamas (1.6%). The reason for the species difference is unclear; however, if the population of llamas was less likely to be reproductively active, those llamas may have been at lower risk of developing lipidosis.

Emaciation was considered the cause of death in 19 alpacas (8%) and 12 llamas (10%). Some of these animals had underlying conditions such as gastrointestinal parasitism or dental abnormalities that may have reduced their ability to digest

and absorb nutrients; however, protein-energy malnutrition likely also played a role.[150] Animals with increased energy expenditures such as growth, lactation, and pregnancy are at higher risk of developing protein-energy malnutrition, and cold weather also increases the energy needed to maintain a normal temperature.[150] Twenty-seven of the 32 animals that died with evidence of emaciation were submitted between October and March, suggesting that the cold winters in the Upper Midwest contributed to mortality; however, there was no nutritional information available in the necropsy reports. Routine observation of body condition score by camelid owners may be hampered by dense fiber[10, 150] and hands-on evaluations should be performed on a monthly basis to identify animals in need of supplemental nutrition.

Dental disease may contribute to decreased feed intake and subsequent emaciation. Bony swellings of the mandible are commonly identified in camelids[16] and are frequently caused by tooth root abscesses.[151-153] However, dentition was remarked upon in only 8 cases (2% of total submissions; 4 adult llamas and 4 adult alpacas). There were two cases with fractured incisors, two cases of wave mouth, two cases of overly worn molars, one case of severe incisor malalignment, and one case with chronic abscessation of the root of the third molar. Because the small number of animals diagnosed with dental abnormalities in this study does not match the frequency of antemortem diagnosis of dental

disease, it is possible that closer attention to the oral cavity during necropsy may be warranted, particularly in cases where animals are emaciated.

Gastrointestinal parasites, particularly nematodes[11, 154, 155] and coccidia[5, 8, 107, 156-163] are commonly identified in camelids and can be a cause of serious economic loss.[11] In this study, the primary cause of death was gastrointestinal parasitism in thirteen alpacas (6%) and three llamas (2%). Furthermore, semi-quantitative fecal flotation was performed on 158 alpacas and 86 llamas and was positive in 81 cases (51%) and 53 cases (62%), respectively. Some parasite infections are likely to be incidental; however, as discussed above, parasite burdens may have played a role in the death of a subset of the animals that died due to emaciation. Despite frequent identification of parasite burdens, only a small proportion of cases had gross evidence of infection with the *Haemonchus contortus*, *Ostertagia*, *Trichostrongylus* (HOT) complex. Eight animals (2%) showed evidence of thickened glandular mucosa in compartment 3 similar to ostertagiosis of cattle, while there were only two llamas diagnosed with severe haemonchosis with anemia. The frequent use of anthelmintics for suppressive deworming and meningeal worm prevention has led to recent evidence of anthelmintic resistance in camelid nematodes;[164-167] however, it is not possible to determine whether resistance played a role in the deaths of animals in this study. Although gastrointestinal parasitism is clearly an important cause of morbidity and mortality, it may no longer be possible to rely solely on chemical treatment

for prevention. *Eimeria macusaniensis* (*E. mac*) is a species of large coccidia that can cause serious disease and even sudden death in adults as well as crias.[6, 159] *E. mac* was the primary cause of death in five alpacas (one cria and four adults), but it was not identified in any llamas. The reason for the species difference is unclear; however, the flotation technique used in our study and histopathology are not the most sensitive method to identify the heavier *E. mac* oocysts[4, 168] and it is possible that low levels of *E. mac* were missed.

Aberrant larval migrans caused by the white-tailed deer parasite *Parelaphostrongylus tenuis*, also known as meningeal worm, is considered a common cause of neurological disease of camelids living in endemic areas.[27-32, 169-172] At the teaching hospital at the Ohio State University, one third of cases presenting with neurologic signs were diagnosed with meningeal worm.[30] Similarly, when alpacas and llamas are considered together in this study, there were 24 cases of meningeal worm out of 43 total fatal diseases involving the nervous system (56%). However, there were only five cases of meningeal worm in alpacas (2% of total submissions) compared to 18 in llamas (14% of total submissions). The reason for this difference in unclear, though it may be related to the frequency of prophylactic deworming. Routine meningeal worm prevention may be more common in larger breeding herds of alpacas compared to smaller farms with a few animals. Eight of the llama cases (44%) and four of the alpaca cases (80%) had involvement of the brain as well as the spinal cord, although

cases presenting for treatment at Ohio State less commonly had neurologic signs referable to the brain (such as seizures, head tilt, or cranial nerve deficits).[30] It may be that animals undergoing necropsy represent a more severely affected group.

Liver flukes, specifically *Fascioloides magna* (the large American liver fluke), caused the death of 4 of 234 alpacas (1.7%) and 11 of 125 llamas (8.8%). Additionally, there were 3 adult llamas with evidence of fluke infection but a separate cause of death was identified. All of the cases of liver flukes came from central and northeastern Minnesota, consistent with the known range of *F. magna* in the state,[173] and seven of the llamas were from a single location over a one year period. There are limited previous reports of *F. magna* in camelids,[105, 174, 175] but the clinical course of the disease appears to be more similar to that of cattle than to small ruminants, since the flukes are able to develop to maturity.[174] Two of 18 animals in this study with evidence of *F. magna* had concurrent mural endocarditis.[105]

Neoplasia accounted for the cause of death in 15 of 234 alpacas (6.4%) and 5 of 125 llamas (4%), for an overall prevalence of 5.5% (5.8% if excluding abortions). This is similar to a prevalence of 7% in camelids reported by the Purdue Veterinary Medical Diagnostic Laboratory[176] and 6.9% by the Oregon State University Veterinary Diagnostic Laboratory.[177] The mean age of alpacas with

neoplasia was 7.8 +/- 5.6 years (range fetus – 22 years) and median was 6.5 years. In contrast, the mean age of llamas with neoplasia was 12.8 +/- 2.9 years (range 9-15 years).

Round cell tumors (RCTs), which include lymphosarcoma and primitive malignant round cell tumors,[36, 178] are the most frequently reported form of neoplasia in camelids.[3, 15, 36-38, 176-184] In a retrospective evaluation of camelid neoplasia, RCTs accounted for only 5 of 40 tumors identified (12.5%); however, biopsy samples were also included.[177] In our study, RCTs made up 73% of all neoplasia as cause of death in alpacas. The mean age of alpacas with RCTs was 6.0 +/- 4.1 years of age with a median of 5.5 years. Three alpaca cases were under 2 years of age and all three were diagnosed with RCTs. This is in contrast with other forms of neoplasia and although the total number of alpacas with other neoplastic diseases was low, the animals affected had a mean age of 12.4 +/- 6.8 years and a range of 6-22 years with a median of 10.75 years, which is consistent with past studies.[177] There were only two llamas with round cell tumors (accounting for 40% of neoplastic disease among llamas) with a mean age of 12 years. The age difference in alpacas and llamas with RCTs is consistent with previous reports;[37, 177] however, the reason for the different signalment between species is unclear. Using immunohistochemistry, 4 tumors were identified as B cell lymphoma (based on CD20 or CD79 staining) and one was categorized as T cell lymphoma (based on CD3 staining). This ratio of B cell

to T cell malignancy is similar to other reports.[37] The remaining eight tumors either did not undergo immunohistochemistry or were negative to most markers and may represent primitive malignant round cell tumors (RCTs).

A variety of neoplasms other than RCTs have been described in llamas and alpacas.[185-195] However, in this population of alpacas, all fatal neoplasms other than RCTs involved the gastrointestinal tract. This is in contrast to the population from Purdue, where the second most common site of neoplasia was the pulmonary parenchyma, accounting for 23% of camelid tumors.[176]

Ulceration of the third compartment (C3) is considered a common disease of camelids,[3, 14, 15, 108] and although this condition can lead to perforation of the stomach and peritonitis, it may also be found incidentally in some cases.[3] In our population of alpacas, C3 ulcers were less common than previously reported and were an infrequent cause of death. Previous reports have determined the prevalence of C3 ulcers as a cause of death to be 5%, with ulcers additionally contributing to mortality in more than 20%.[14] In a series of 45 animals at Oregon State University from whom gastric contents were sampled, 14 had ulcers (31%).[108] Our data showed that there were two out of 234 alpacas (0.9%) with C3 ulcers as the primary cause of death, whereas there were seven of 125 llamas (5.6%) that were considered to have died due to C3 ulceration. An additional 13 alpacas ranging in age from 5 months to 12 years had evidence of

C3 ulceration for an overall prevalence of 6% in alpacas. Among llamas there were another 8 cases with evidence of C3 ulcers, giving an overall prevalence of 12% in New World camelids in this study.

Interestingly, although C3 ulceration was less common than expected, there were 6 alpacas (2.5%) and 6 llamas (4.8%) with ulceration of the esophagus and/or first compartment (C1). The C1 ulcers were considered of secondary importance in 4 of the llamas but no specific cause was identified. One llama youngster had concurrent evidence of disseminated intravascular coagulation and may have been septicemic, and one adult llama had widespread ulceration of the esophagus and C1. Among the alpacas, 4 had evidence of septicemia or other concurrent disease processes such as generalized hemorrhage, and one of these had evidence of fungal invasion of the compartment wall. The other two alpacas with C1 ulceration had evidence of renal compromise; one was a cria with hydronephrosis caused by an ectopic ureter and one was markedly dehydrated with elevated creatinine prior to death. The specific cause of the ulceration in these two cases is not known but may be related to uremia as is seen in other species, although dogs with uremic gastropathy develop edema and mineralization of the oral and glandular mucosa rather than ulceration of the esophagus.[196]

There were three cases of spontaneous (non-traumatic) pulmonary artery rupture in llamas (two adults and one youngster). This is rarely reported in the veterinary literature[197-199] though it occurs in humans, most often secondary to pulmonary hypertension.[200] Other known causes in humans include disorders of connective tissue such as Ehlers-Danlos syndrome or Marfan syndrome, infection (including bacteria, fungal, and mycobacterial), congenital malformation, or iatrogenic rupture during cardiac procedures.[200] The llama youngster had evidence of a bacterial arteritis from which *Actinobacillus sp.* were isolated; however, there was no underlying disease identified in the other two cases.

Of note was the fact that the urinary tract and respiratory tract were uncommon causes of death in the alpacas and llamas in our study. Urogenital system disorders accounted for 11 (5%) deaths among alpacas and 8 (6%) llamas, if abortions are excluded. There were two cases of glomerulonephritis without an underlying cause identified. Data from necropsies at Oregon State University suggests that this type of kidney disease may occur secondary to systemic inflammation.[201] Urolithiasis was not a frequent finding, in contrast to sheep and goats, in which obstructive urolithiasis is a common presenting complaint and can result in death if not treated promptly.[202, 203] Urolithiasis and urinary tract obstruction are reported in camelids[204, 205] but the reason for a lower prevalence of disease compared to sheep and goats is unknown. There may be a regional variation in the prevalence of urolithiasis in camelids, with the Pacific Northwest

more heavily affected by silicate stones.[201] Pulmonary disease was also an uncommon cause of death in this study. This is in contrast to similar necropsy retrospectives among other ruminants such as farmed deer and elk, where pneumonia was second only to trauma as a primary cause of death.[111-113]

In both llamas and alpacas found dead, the two most commonly identified causes of death were emaciation and parasitism. However, bacterial infections, neoplasia, and hepatic lipidosis were also identified in animals that died unexpectedly, suggesting that camelids can develop severe systemic compromise without displaying clinical signs obvious enough to be recognized by owners. As previously mentioned, dense fiber may make observation of declining body condition score less obvious, which means that regular hands-on evaluation is important to identify animals in need of a workup for weight loss.

Acute recumbency (e.g. a down camelid) is a frequent presenting complaint for veterinary evaluation. In endemic areas, many camelid owners consider meningeal worm to be the primary differential. In this study, the single most common diagnosis in llamas that were found down was meningeal worm, accounting for 38% of down llamas submitted for necropsy. In contrast, only 8% of down alpacas were diagnosed with meningeal worm and in fact abnormalities with the digestive system (42%) were more commonly the cause of eventual death in an alpaca found recumbent. This confirms the need for a thorough clinical workup for a down camelid.

Previous investigation into the causes of mortality in camelids is limited. A survey of alpaca and llama owners in the United Kingdom showed that the most common causes of death were believed to be trauma, intestinal disorders, perinatal disorders including congenital defects and failure of passive transfer, and vitamin E/selenium deficiency.[206] Because this was a survey of owners, many of the causes of death were unconfirmed by necropsy. In our study, intestinal disorders and perinatal causes of death were also common. In contrast, in our study traumatic death was not a frequent diagnosis (9 of 234 alpacas and 6 of 125 llamas), although it is possible that trauma cases were not submitted for necropsy. While selenium toxicosis was diagnosed in two alpaca crias, there was only one suspected case of white muscle disease.

A brief report on the causes of camelid mortality in Canada[3] described digestive disorders followed by neurologic disorders as the most common causes of death in camelids over 1 year of age. In our study, although digestive disorders (including hepatic disorders) were the most common cause of mortality for both alpacas and llamas, the second most common cause of death in alpacas involved multisystemic disorders, primarily consisting of severe emaciation and septicemia.

Limitations to this retrospective analysis include variations in the method of necropsy, the supervising pathologist, and the specific data recorded. Although 42% of the animals submitted came through the Veterinary Medical Center (VMC), the overall numbers may still reflect an accurate representation of fatal conditions that would be encountered in the field because many local practices encourage the use of the VMC for after-hours emergencies. There is likely a degree of location bias, as owners closer to the UM-VDL may have been more willing to submit animals for necropsy; however, animals were submitted from throughout the state of Minnesota and from nearby areas of Wisconsin and Iowa (Figures 5-7).

Conclusions

In conclusion, although there are many different causes of death among alpacas and llamas, many of the disorders resulting in mortality can be at least partially prevented with attention to husbandry. Close attention to the provision of adequate nutrition and frequent monitoring of body condition score may reduce the likelihood of death due to emaciation. Rational parasite control programs could also help lower mortality rates. Bacterial infections are a common problem in young alpacas (under 6 months of age) and ensuring adequate passive transfer of antibodies may help prevent life-threatening infections.

Footnotes

a. R Development Core Team. (2010) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Table 1: Results of 234 alpacas submitted for necropsy evaluation to the UM-VDL grouped by age and by primary body system involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

	Age Group					
Primary Body System	Fetus	Neonate	Cria	Youngster	Adult	**Total**
Multisystemic	1	2	12	7	20	42
Musculoskeletal		1	5		5	11
Respiratory		16	3	2	5	26
Cardiovascular		3	1	4	4	12
Hemolymphatic	1		1	3	14	19
Digestive		4	13	13	38	68
Urogenital	28	2		3	6	39
Nervous			3	4	10	17
Total	30	28	38	36	102	234

Table 2: Results of 125 llamas submitted for necropsy evaluation to the UM-VDL grouped by age and by primary body system involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

	Age Group					
Primary Body System	Fetus	Neonate	Cria	Youngster	Adult	**Total**
Multisystemic	1	1	1		19	22
Integumentary					2	2
Musculoskeletal			1		5	6
Respiratory					8	8
Cardiovascular		1		2	5	8
Hemolymphatic			1		3	4
Digestive		1	1	7	30	39
Urogenital	2	1		1	6	10
Nervous		1	1	1	23	101
Total	3	5	5	11	101	125

Table 3: Results of 234 alpacas submitted for necropsy evaluation to the UM-VDL grouped by age and by primary pathophysiologic process involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

Pathophysiologic Process	**Fetus**	**Neonate**	**Cria**	**Youngster**	**Adult**	Total
Developmental	3	15	6		2	**26**
Degenerative			1		1	**2**
Metabolic			2	4	8	**14**
Neoplastic	1			2	12	**15**
Nutritional			3	7	12	**22**
Bacterial		4	19	6	18	**47**
Viral	1				3	**4**
Parasitic		1	5	2	13	**21**
Fungal		1			1	**2**
Inflammatory		1		5	15	**21**
Idiopathic	24					**24**
Toxic		1	1			**2**
Traumatic		2		1	6	**9**
Vascular	1			2	4	**7**
Miscellaneous		1	1	7	6	**15**
Unknown		2			1	**3**
Total	**30**	**28**	**38**	**36**	**102**	**234**

Table 4: Results of 125 llamas submitted for necropsy evaluation to the UM-VDL grouped by age and by primary pathophysiologic process involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

Pathophysiologic Process	**Fetus**	**Neonate**	**Cria**	**Youngster**	**Adult**	Total
Developmental		1	1		1	**3**
Degenerative				1	3	**4**
Metabolic						**0**
Neoplastic					5	**5**
Nutritional			1		16	**17**
Bacterial		2	1	1	7	**11**
Viral						**0**
Parasitic			1	7	28	**36**
Fungal						**0**
Inflammatory				2	20	**22**
Idiopathic	2					**2**
Toxic					2	**2**
Traumatic					6	**6**
Vascular	1	1			2	**4**
Miscellaneous		1			10	**11**
Unknown			1		1	**2**
Total	**3**	**5**	**5**	**11**	**101**	**125**

Figure 1: Graph showing result of 204 alpacas (excluding abortions) submitted for necropsy evaluation to the UM-VDL grouped by age and by primary body system involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

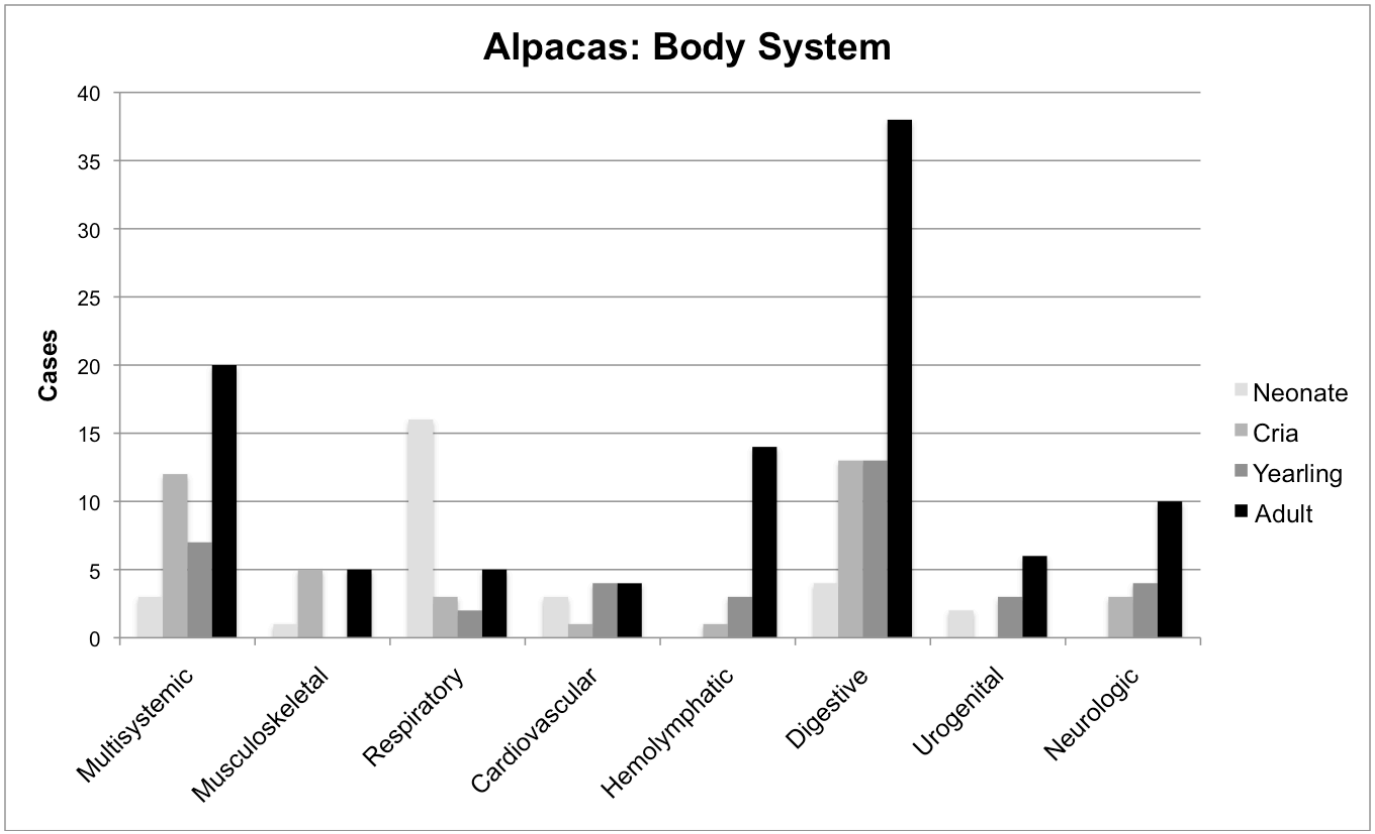


Figure 2: Graph showing result of 122 llamas (excluding abortions) submitted for necropsy evaluation to the UM-VDL grouped by age and by primary body system involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

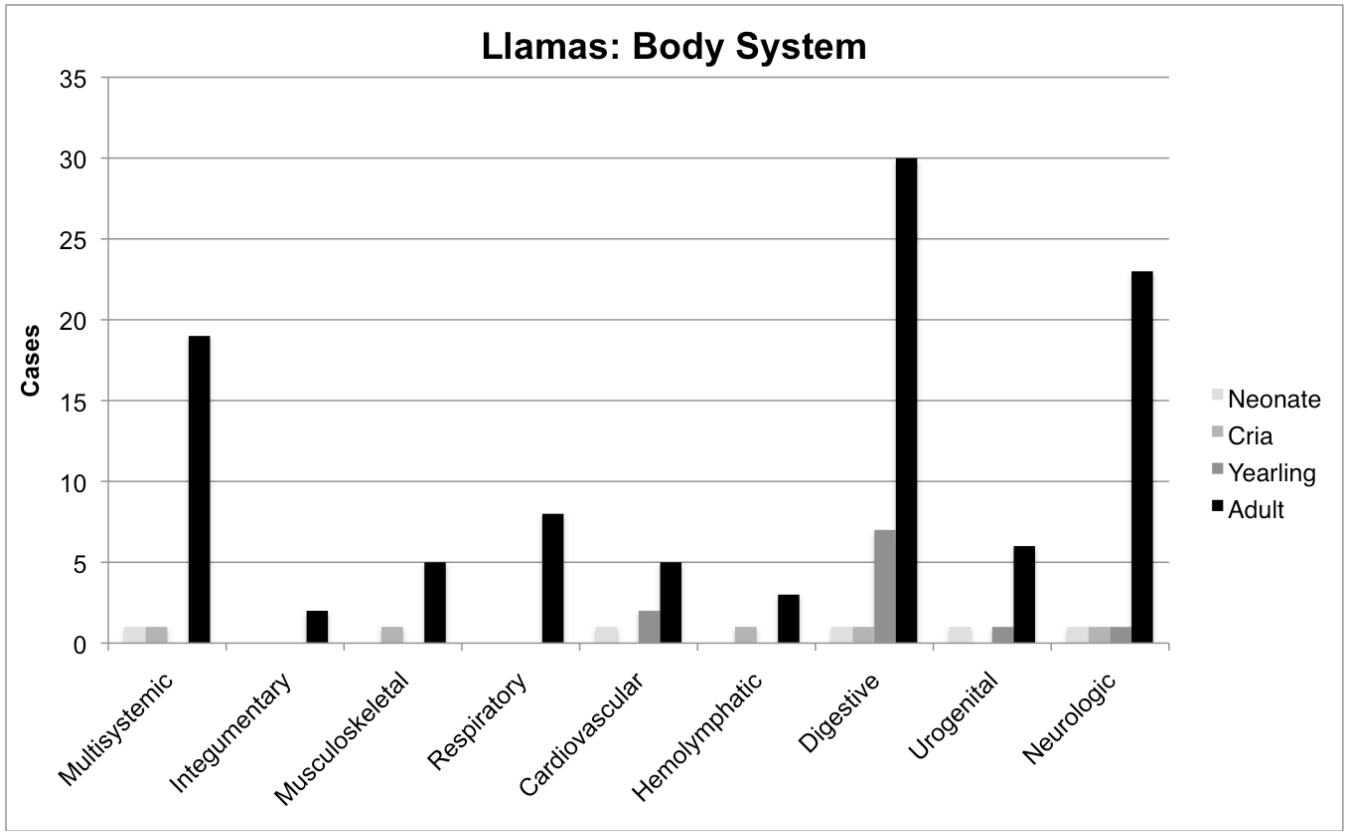


Figure 3: Graph showing result of 204 alpacas (excluding abortions) submitted for necropsy evaluation to the UM-VDL grouped by age and by primary pathophysiologic process involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

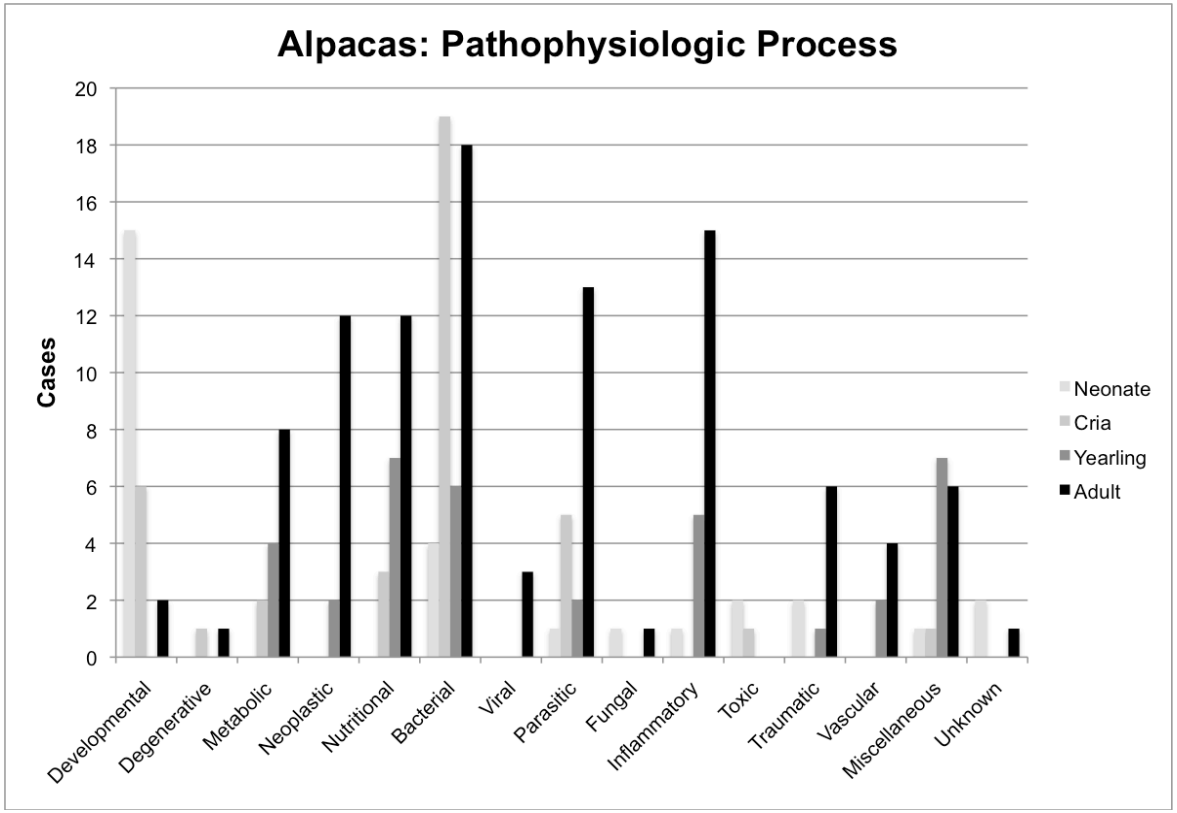


Figure 4: Graph showing result of 122 llamas (excluding abortions) submitted for necropsy evaluation to the UM-VDL grouped by age and by primary pathophysiologic process involved in cause of death. Age groups are neonate (<2 weeks), cria (2 weeks to 6 months), youngster (6 months to 18 months), and adult (>18 months).

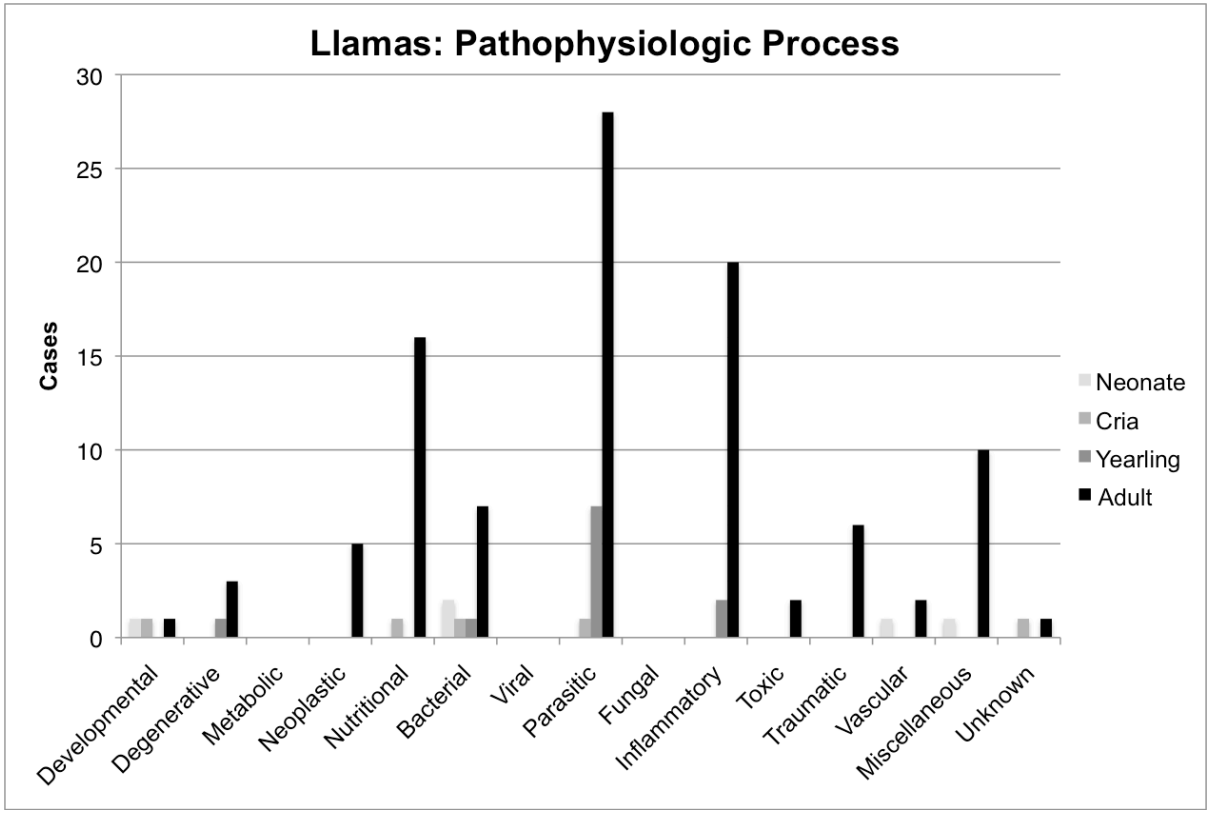


Figure 5: Map showing the distribution of location of alpacas and llamas submitted to the UM-VDL for necropsy from 2001-2011. Darker colors represent higher numbers of cases per county (range 1-41).

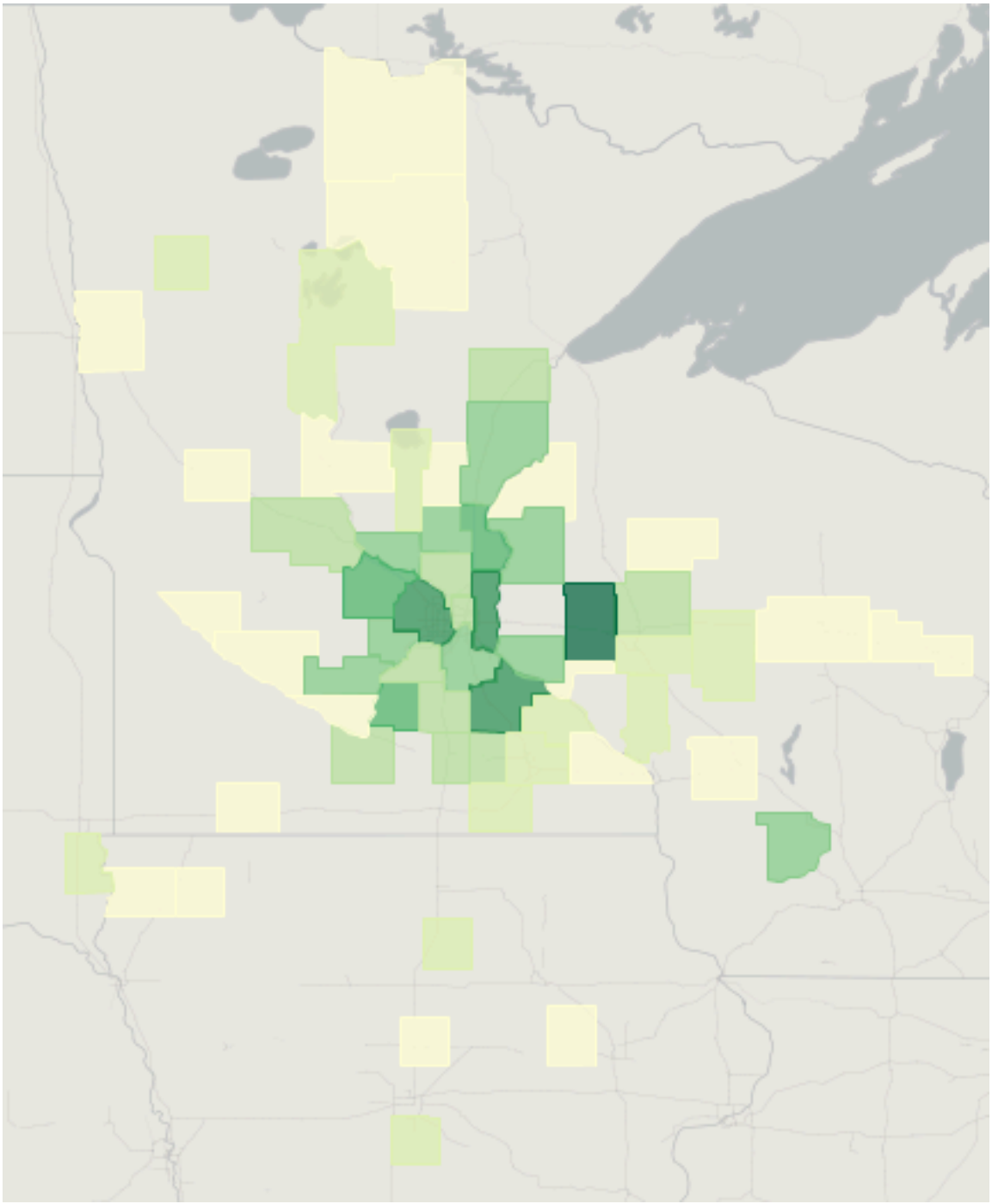


Figure 6: Map showing the distribution of location of alpacas submitted to the UM-VDL for necropsy from 2001-2011. Darker colors represent higher numbers of cases per county (range 1-41).

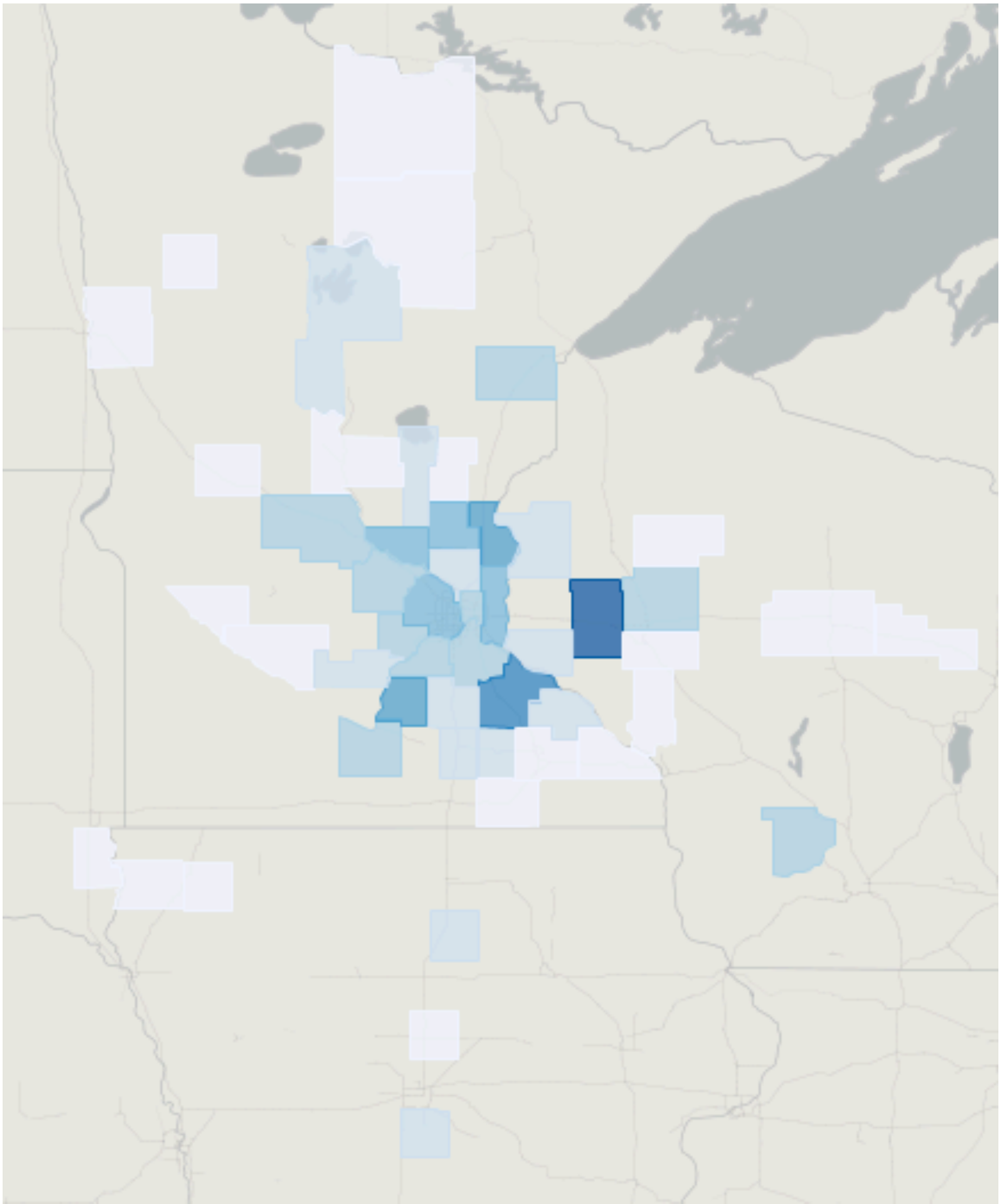
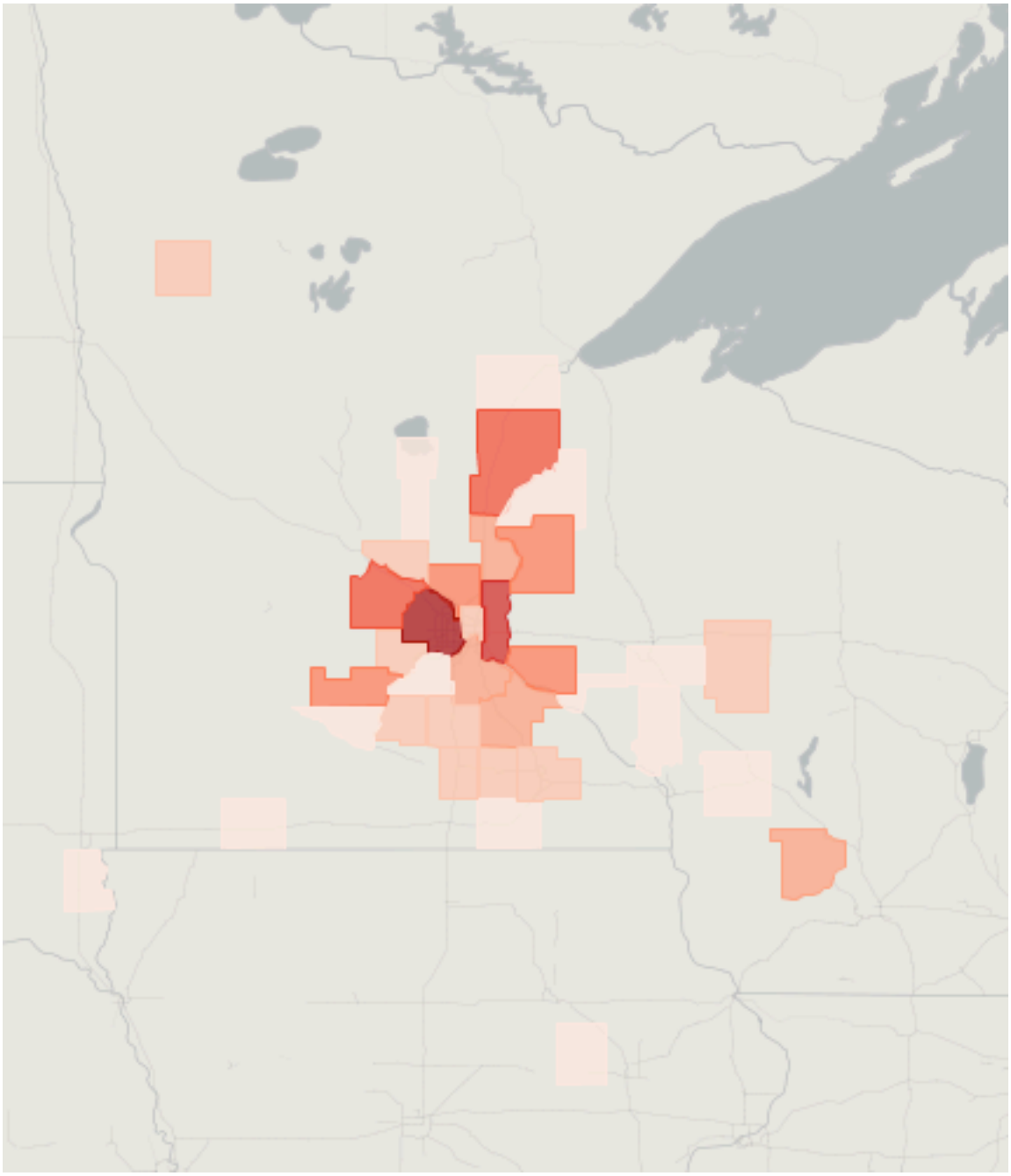


Figure 7: Map showing the distribution of location of llamas submitted to the UM-VDL for necropsy from 2001-2011. Darker colors represent higher numbers of cases per county (range 1-18).



CHAPTER THREE

Arginine Stimulation Testing in New World Camelids with Epinephrine-Induced Elevations in NEFAs, Triglycerides, and Ketones

Arginine Stimulation Testing in New World Camelids with Epinephrine-Induced Elevations in NEFAs, Triglycerides and Ketones

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Summary

New World camelids (NWCs) have reduced insulin production and peripheral insulin sensitivity compared to other species. Sick NWCs commonly develop hyperglycemia, hyperketonemia, hypertriglyceridemia, and hepatic lipidosis, but little is known about pancreatic function in this state. Our aim was to use epinephrine to induce transient fat mobilization in healthy NWCs and then intravenous arginine stimulation testing (IVAST) to assess pancreatic α and β cell function. The hypothesis was that NWCs in a state of epinephrine-induced fat mobilization would have reduced insulin secretion and increased glucagon secretion as measured by IVAST. Fourteen healthy llamas were used in a control design and intravenous catheters were placed. IVAST involved intravenous administration of 67 mg/kg 10% arginine HCl. Blood samples were collected at 0,2,3,4,5,7,10,15 and 20 minutes post-arginine injection for plasma glucose, insulin and glucagon measurement. All 14 animals underwent a baseline IVAST. 36 hrs later feed was withheld for 12 hours and then each animal received 1 μg/kg epinephrine IM (t=-60). One hour after epinephrine administration IVAST was repeated (t=0). Serum NEFAs, ketones, and triglycerides were measured at t=-60 and at t=0. Epinephrine induced mild elevations in NEFAs, ketones, and triglycerides ($p<0.0001$). Baseline insulin (but not glucagon) was lower after treatment with epinephrine ($p<0.01$). During IVAST the AUC at 10 minutes was lower for [insulin] ($p<0.05$) and higher for [glucagon] ($p<0.0001$) after epinephrine treatment. The results of this study may reflect a direct effect of α-adrenergic stimulation on the pancreas. Our findings may provide insights into changes that occur in pancreatic function in sick NWCs.

Introduction

New World camelids are prone to developing disorders of fat mobilization such as ketoacidosis, elevations in non-esterified fatty acids, hypertriglyceridemia, and hepatic lipidosis, even in the absence of risk factors such as pregnancy, lactation, or obesity.[40, 42, 44, 96-98] These metabolic derangements often occur as a secondary condition in sick and hospitalized camelids, but can frequently be the medical issue that requires the most intensive treatment.[9, 39, 43, 44, 98] Interestingly, transient fat mobilization can be induced in camelids by treatment with epinephrine[49] and it is possible that this model of induced fat mobilization could be utilized in order to study the metabolic responses of New World camelids in a state of abnormal metabolic function.

Camelids are known have low resting levels of insulin,[42, 46, 51] a decreased release of insulin in response to hyperglycemia,[46, 67, 149] and decreased glucose clearance with the administration of exogenous insulin[47, 48, 51, 67] when compared to other species. It is not known whether these responses are further reduced in sick camelids with fat mobilization, but the widespread occurrence of fat mobilization disorders[44, 45] along with hyperglycemic disorders such as marked stress hyperglycemia and hyperosmolar syndrome[39, 43, 46] suggests that there may be an association. Further evaluation of camelid energy metabolism, particularly insulin sensitivity and pancreatic function, will enable better understanding of the development of disorders of glucose and lipid metabolism

as well as help target treatment of these conditions to prevent mortality.

Techniques such as intravenous glucose tolerance testing[46, 67] and hyperinsulinemic euglycemic and hyperglycemic clamping[51] have been performed to investigate insulin and glucose dynamics in healthy camelids. However, no studies have been performed investigating how glucose tolerance, insulin production, and insulin sensitivity are affected in sick camelids in a state of fat mobilization. This may in part be due to the long procedure time of currently available testing methods and their potential to adversely affect recovery from the animal's original illness, due to the need to induce supraphysiologic levels of glucose and insulin in the bloodstream during these testing procedures.

Insulin secretagogues such as arginine have been used to study diabetes mellitus in humans[60, 76, 81, 83] and have also been used in veterinary species including cats,[92-94] pigs,[207, 208] foals,[84-86] sheep,[207, 209-211] and cattle.[87-91, 207] Intravenous infusion of arginine stimulates the release of insulin and glucagon from the pancreas and this response can be used to assess α and β cell function. The arginine stimulation test is a short procedure that allows blood insulin and glucose concentrations to remain at physiological levels and consequently can be used to study changes in pancreatic function in clinical cases. The purpose of this study was to use the intravenous arginine stimulation test (IVAST) to characterize and compare the pancreatic α and β cell responses in healthy

camelids with those in a state of epinephrine-induced fat mobilization. The hypothesis to be tested was that camelids in a state of fat mobilization would have decreased insulin production and increased glucagon production by the pancreas.

Materials and Methods

Animals

The study included 15 healthy llamas, 10 from the research herd at Oregon State University and 5 client-owned animals at the University of Minnesota. The study used a non-randomized crossover design in which each animal acted as its own control. Experiments were conducted in October and November 2011. There were 12 females and 3 neutered males with a mean age of 10.2 +/- 6.2 years (range 3-22 years). Median body condition score on a five point scale was 4 with a range of 2 to 5. Prior to the study all animals were screened by physical examination and found to be healthy. The llamas were maintained on a diet of grass hay. All procedures were approved by the Institutional Animal Care and Use Committees at Oregon State University and the University of Minnesota.

Arginine Stimulation Test Method

Procedures used for the IVAST were based on methods previously reported in humans[60, 76] and piloted in New World camelids.[95] A 14 gauge catheter[a] was placed in the right jugular vein of each llama (for medication administration and

blood sample collection). The next day, the baseline intravenous arginine stimulation test (IVAST) was performed. Llamas were tied in pairs in their stalls to maintain a calm state. Baseline blood samples were taken into heparinized and EDTA tubes immediately prior to the IVAST. Arginine HCl[b] at 67 mg/kg was administered intravenously over 30 seconds, with t = 0 set at the time at which half the solution had been given. Venous blood samples were taken into EDTA tubes at t = 2, 3, 4, 5, 7, 10, 15, and 20 minutes. Blood glucose was immediately measured at each time point using a handheld glucometer[c] previously validated for use with camelid whole blood samples.[51] The heparinized and EDTA tubes were immediately placed on ice and centrifuged promptly. The resulting plasma was then stored at -80 C until processing.

Fat Mobilization

After a 36-hour rest period from the first IVAST, the llamas were held off feed for 12 hours. Access to water was not restricted. Sixty minutes prior to the start of the IVAST (t = -60) a pre-epinephrine blood sample was taken into heparinized and EDTA tubes. The llamas then received 1 μg/kg epinephrine[d] by intramuscular injection into the semimembranosus muscle in order to induce transient fat mobilization. The dose of epinephrine was chosen based on the results of a study that evaluated the responses of serum fat fractions in llamas to treatment with intramuscular epinephrine.[49] Sixty minutes after treatment with epinephrine, the IVAST procedure was repeated (t = 0). A randomized crossover

design was not utilized in case the epinephrine treatment caused a delayed effect of fat mobilization on pancreatic response.

Analytes

The heparinized plasma from t = 0 and t = -60 was analyzed for triglycerides, β-hydroxybutyrate, and non-esterified fatty acids (NEFAs). EDTA plasma from each time point was kept frozen at -80 °C until analyzed for insulin[e] and glucagon[f] by radioimmunoassay, within 3 months of collection.

Analysis

For each time point insulin (I), glucagon (Gl), and glucose (G) were measured. For each animal baseline, (I_0, Gl_0, G_0), peak response (I_{max}, Gl_{max}, G_{max}), and time to peak response (IPT, GlPT, GPT) were recorded. The incremental area under the curve (AUC) for insulin and glucagon were calculated during the first 10 (AUC10) and the entire 20 minutes (AUC20) using the trapezoid method[g].

Statistics

Results were reported as median and range or mean +/- standard deviation depending on the distribution of the data. For all parameters, the normality of the data was evaluated using the Shapiro-Wilk test of normality. If the data were not normally distributed, the data was transformed with the natural log for calculation of the paired t-test to evaluate the difference between control and epinephrine-

treated values and the results were reported as geometric mean +/- geometric standard deviation. If the data were normally distributed, results were reported as mean +/- standard deviation and the paired t-test was performed on un-transformed data. Unless otherwise stated, paired t-tests were one-sided with the hypotheses that the control values of insulin were greater than the values after treatment with epinephrine and that the control values of glucagon and glucose were lower than the values after treatment with epinephrine. The changes in the fat fractions during the control experiment, after an overnight fast, and after treatment with epinephrine were analyzed with the Friedman rank sum test followed by the Wilcoxon signed rank test using the Bonferroni correction. A repeated measures ANOVA was used to compare insulin, glucagon, and glucose at each time point (t=0, 2, 3, 4, 5, 7, 10, 15, 20) before and after epinephrine treatment. For all values $p < 0.05$ was considered significant.

Results

Epinephrine treatment, but not overnight fasting, successfully induced mild elevations in β-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFAs), and triglycerides (TGs) (Table 1, Figure 1). Results of the intravenous arginine stimulation test are presented in Table 2. After epinephrine treatment, both I_0 and peak insulin were significantly lower ($p = 0.012$ and $p = 0.012$ respectively) and peak glucagon was significantly higher ($p = 0.0024$) when compared to the IVAST performed without epinephrine treatment (Figure 2, Figure 3). There was

no significant difference in GI_0 before and after epinephrine treatment. The insulin area under the curve (AUC) at 10 minutes was significantly lower after epinephrine treatment (p = 0.03) and the glucagon area under the curve (AUC) at both 10 and 20 minutes was significantly higher after epinephrine treatment (see Table 2).

Discussion

To the authors' knowledge, this is the first description of the use of the arginine stimulation test to examine pancreatic function in New World camelids. In other species such as humans, cats, sheep, and cattle, arginine administration stimulates release of both insulin and glucagon by the pancreas. Arginine alters the cell membrane potential in β cells as the positively-charged amino acid is transported across the membrane[77, 212, 213] and stimulates calcium influx,[77, 78, 214] resulting in insulin secretion. In humans, arginine stimulation results in a roughly three-fold increase (from baseline) in peripherally sampled insulin and glucagon[60, 76] with no significant differences between control and diabetic subjects when blood glucose levels are not controlled. However, when blood glucose is tightly regulated by insulin infusion or dextrose infusion, diabetic patients show reduced insulin secretion and exaggerated glucagon secretion in response to arginine stimulation in comparison to control subjects.[81] Cats have a more vigorous response to arginine stimulation than humans, with a six-fold increase in insulin and glucagon from baseline,[93] whereas cattle have shown a

range from 4-fold to 10-fold increases in insulin depending on the study and a roughly 5-fold increase in glucagon.[87, 88] When directly comparing change from baseline, this study indicates that healthy llamas show a similar response to arginine stimulation as seen in healthy humans. In contrast, previous studies of pancreatic function in camelids using intravenous glucose tolerance testing[46] and hyperglycemic clamping[51] have shown that healthy llamas and alpacas have lower insulin secretion in comparison to other species. It is important to note, however, that basal blood glucose values in llamas are higher than in healthy humans. In humans, hyperglycemia potentiates insulin secretion in response to non-glucose secretagogues (such as arginine) and similar patterns of response to arginine in healthy and diabetic patients are observed unless blood glucose values are uniform between the two study populations.[60, 76, 81] Thus it is possible that if the blood glucose level was lowered to that of humans in order to directly compare the IVAST response at the same level of glycemia, llamas may have an overall decreased insulin response to arginine.

New World camelids are prone to disorders of fat and glucose metabolism[39, 43-46, 97] and their poor pancreatic β cell responsiveness and glucose intolerance are thought to contribute. The study of New World camelids with naturally occurring fat mobilization is limited by the availability of appropriate numbers of clinical cases and the difficulty in performing research procedures that may have negative consequences in client-owned animals. In order to study the effect of

IVAST testing in animals with fat mobilization, this study aimed to induce fat mobilization in healthy animals through treatment with epinephrine. In addition to its cardiovascular effects, epinephrine plays an important role in the regulation of fat and glucose metabolism. In times of stress, epinephrine directly increases plasma glucose levels by promoting glycogenolysis in the liver. However, it also causes peripheral insulin resistance,[215, 216] thereby reducing tissue uptake of glucose by insulin-mediated mechanisms. At the same time, it increases the levels of plasma lipids by stimulating the activity of hormone-sensitive lipase to release NEFAs and decreasing clearance of triglycerides through inhibition of translation of lipoprotein lipase.[217] In addition, despite a resulting rise in blood glucose levels stimulated by catecholamine release, there is a direct inhibitory effect on insulin secretion by pancreatic β cells, mediated by α-adrenergic receptors.[218-220] In the state of hypoglycemia, the sympathetic nervous system (including epinephrine release) is partially responsible for increasing blood glucose values and epinephrine itself also stimulates the release of glucagon by pancreatic α cells.[221]

In previous models,[49, 52, 100] epinephrine but not cortisol was able to induce fat mobilization in treated animals, suggesting that endogenous catecholamine release may be important in the development of these disorders of metabolism. In the present study, epinephrine treatment resulted in mild elevations of fat fractions and the degree of fat mobilization was similar to a previous study in

llamas,[49] based on the mean values of fat fractions 60 minutes after epinephrine treatment. In that study, the mean basal insulin level was lower before treatment (2.17 μU/ml) than reported here and increased slightly but significantly to a mean of 2.88 μU/ml 60 minutes after administration of epinephrine, rather than decreasing as in the study reported here. Cebra *et al* attributed that increase in insulin to compensation for the increase in plasma glucose levels;[49] however, in the present study mean plasma glucose increased by 46% yet basal insulin decreased from 7.9 μU/ml to 5.7 μU/ml after treatment with epinephrine. A decrease in insulin in response to epinephrine is expected based on studies of continuous epinephrine infusion in other species,[218-220, 222] though the effects of a single dose rather than an infusion could be different. Interestingly, although the epinephrine treatment resulted in mild elevations of fat fractions, elevated blood lipids themselves are expected to enhance insulin secretion[56] rather than decrease it. It is possible that there may be less influence on insulin secretion due to the change in blood glucose after epinephrine administration because of the decreased responsiveness of the camelid pancreas to a glucose stimulus.[46, 51, 67]

During arginine stimulation testing, epinephrine-treated llamas with mild elevations in plasma NEFAs, ketones, and triglycerides showed a reduced acute insulin secretory response and an exaggerated acute glucagon secretory response, in comparison to their baseline measurement (see Figures 2 and 3). In

humans, the first phase of the insulin secretory response is highly correlated to the area under the curve of insulin release in response to arginine,[80] whereas measuring the area under the curve for time periods longer than 10 minutes is likely to include some degree of second phase insulin secretion.[76] In the current study the incremental area under the curve at 10 minutes for insulin was significantly lower after epinephrine treatment, while there was no significant difference at 20 minutes. A study in humans[223] determined that epinephrine is a dose-dependent inhibitor of insulin secretion (as measured by the acute insulin response to arginine injection) when plasma glucose levels are held constant, which would be consistent with the findings in the present study. Similarly, epinephrine is a dose-dependent stimulator of glucagon secretion when plasma glucose levels are held constant,[220] and in the data reported here the acute glucagon response to arginine was significantly higher after treatment with epinephrine. Plasma epinephrine levels were not quantified in this study, making it difficult to determine whether there is a direct correlation between epinephrine concentrations at the time of the IVAST and the attenuated insulin and exaggerated glucagon responses.

It is not possible to determine from this study whether the changes observed in IVAST response after treatment with epinephrine were due to a direct effect of adrenaline on pancreatic function, or whether the effect of causing fat mobilization in healthy animals contributed to the differences seen. In ruminant

species, the IVAST has been used to evaluate changes in pancreatic function in response to stressful environmental stimuli such as high or low temperatures. Similar to the epinephrine-treated llamas in this study, the environmental extremes resulted in decreased insulin secretion and increased glucagon secretion in response to arginine. The proposed mechanism for the stress-induced changes in IVAST response seen in ruminants was increased sympathetic tone in response to environmental stressors.[87, 88, 210, 211] Interestingly, a pilot trial examining IVAST response in clinical cases of camelids with deranged fat mobilization also follows a similar pattern, whereby insulin response is decreased and glucagon response is increased after IVAST in comparisons to normal animals.[95] This suggests that stress and the catecholamine response may be part of the mechanism by which pancreatic function is affected in sick camelids.

Conclusions

In conclusion, the IVAST was successfully used to evaluate pancreatic function in llamas. Healthy llamas showed a similar magnitude of insulin response to arginine as healthy humans. However, given previous evidence that camelid insulin secretion is reduced compared to other species,[46, 51] this response may be due to potentiation of pancreatic β cells due to a higher baseline glucose level in camelids. Further investigation using a combination of IVAST and hyperglycemic clamping to better characterize camelid insulin secretion at

multiple blood glucose levels is warranted. Additionally, although the effects of epinephrine may be directly responsible for the decreased insulin secretion and increased glucagon secretion observed, similar findings in stressed animals of other species suggest that epinephrine may be part of the mechanism by which sick camelids develop derangements of glucose and lipid metabolism. Further studies in clinically affected cases are needed to more thoroughly evaluate this possibility.

Footnotes

a. 14 gauge Abbo peripheral intravenous catheter, Abbott Laboratories, Abbott Park, Illinois.

b. R-Gene 10, 10% L-arginine hydrochloride, Pharmacia and Upjohn, New York, New York.

c. One touch Ultra blood glucose monitoring system, Lifescan, Inc, Milpitas, California.

d. Epinephrine 1:1000, MWI, Boise, Idaho.

e. Porcine Insulin RIA, Millipore Corp, Billerica, Massachusetts.

f. Glucagon Radioimmunoassay, American Laboratory Products, Windham, New Hampshire.

g. Excel 2008, Microsoft Corporation, Redmond, Washington.

h. R: A language and environment for statistical computing, Development Core Team, Vienna, Austria.

Table 1: Table showing median and range for B-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), and triglycerides (TG) immediately before the control intravenous arginine stimulation test (IVAST), 60 minutes prior to second IVAST after a 12 hour fast (fasted), and 60 minutes after epinephrine injection immediately prior to the second IVAST (epinephrine-treated), in 15 llamas. Different superscripts represent values that are significantly different according to post hoc testing. Values of $P \leq 0.05$ were considered significant.

	Control		**Fasted**		**Epinephrine-treated**		
Fat Fraction	Median	Range	Median	Range	Median	Range	*p* value
BHBA (mg/dl)	0.39^{a}	0.16-2.13	0.2^{a}	0.1-2.35	0.76^{b}	0.4-5.91	<0.0001
NEFA (mg/dl)	0.11^{a}	0.06-0.5	0.15^{a}	0.08-0.5	1.4^{b}	0.13-2.19	<0.0001
TG (mg/dl)	18^{a}	15-90	17^{a}	13-74	42^{b}	21-103	<0.0001

Table 2: Table showing insulin and glucagon parameters in 15 llamas during an intravenous arginine stimulation test (IVAST) with and without treatment with epinephrine 60 minutes prior to IVAST. I_0, baseline plasma insulin concentration; Peak I, insulin peak response; AUC10, incremental area under the curve at 10 minutes; AUC20, incremental area under the curve at 20 minutes; GI_0, baseline plasma glucagon concentration; Peak GI, glucagon peak response.

		Control		**Epinephrine-treated**		
Parameter	**Unit**	Geometric Mean	Geometric SD	Geometric Mean	Geometric SD	*p* value
I_0	μU/ml	7.90	1.59	5.70	1.95	0.012
Peak I	μU/ml	26.08	1.57	21.72	1.77	0.012
AUC10 insulin	μU/ml/10 min	116.65	1.79	103.58	1.73	0.03
AUC20 insulin	μU/ml/20 min	240.62	1.92	221.41	1.93	0.10
GI_0	pg/ml	47.82	1.32	53.06	1.42	0.13
Peak GI	pg/ml	99.75	1.39	123.55	1.50	0.0024
AUC10 glucagon	pg/ml/10 min	341.72	1.90	538.79	1.73	<0.0001
AUC20 glucagon	pg/ml/20 min	634.39	1.88	962.95	1.85	0.0002

Figure 1: Bar chart showing median fat fractions immediately before control intravenous arginine stimulation test (IVAST), at 60 minutes prior to second IVAST after a 12 hour fast, and at 60 minutes after epinephrine injection immediately prior to the second IVAST, in 15 llamas. Error bars represent interquartile range. Different superscripts represent values that are significantly different according to post hoc testing. Values of $P \leq 0.05$ were considered significant.

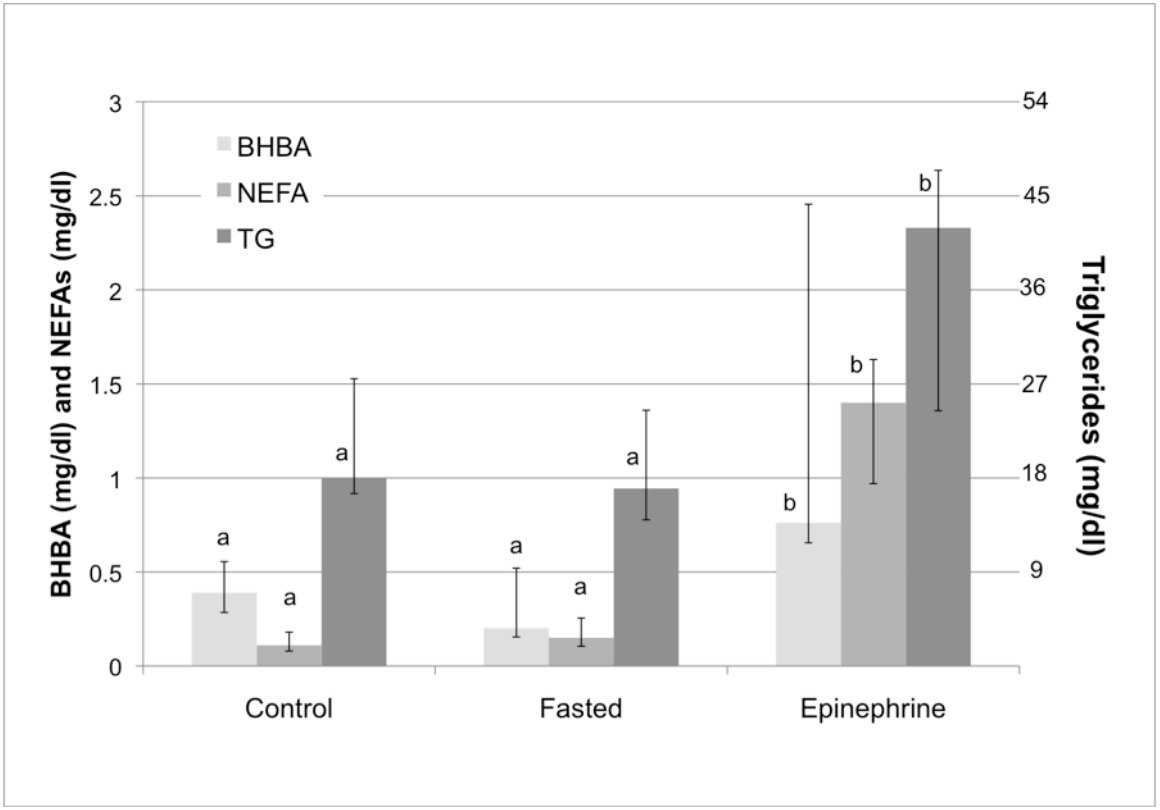


Figure 2: Figure showing geometric mean of plasma insulin concentrations during intravenous arginine stimulation test (IVAST) with and without treatment with epinephrine 60 minutes prior to IVAST, in 15 llamas. Error bars represent geometric standard deviation.

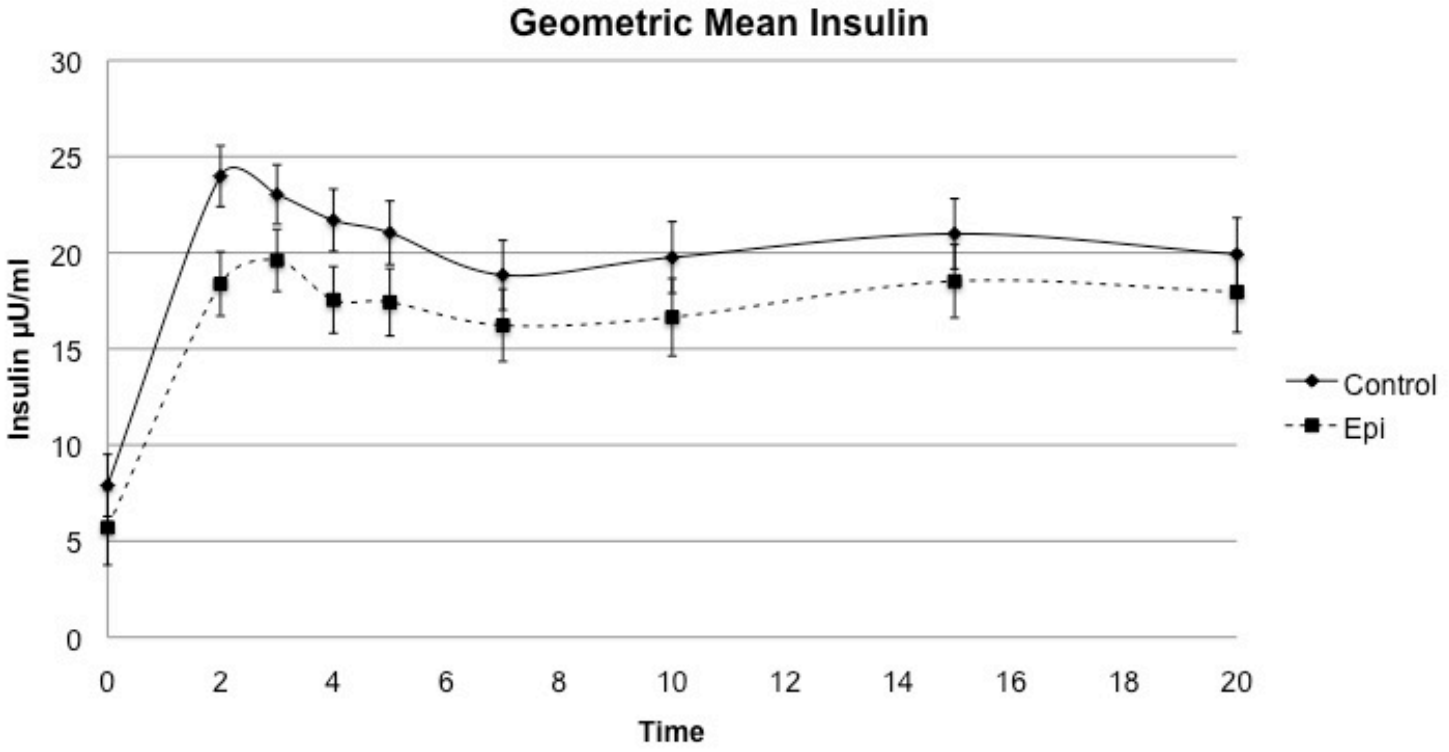
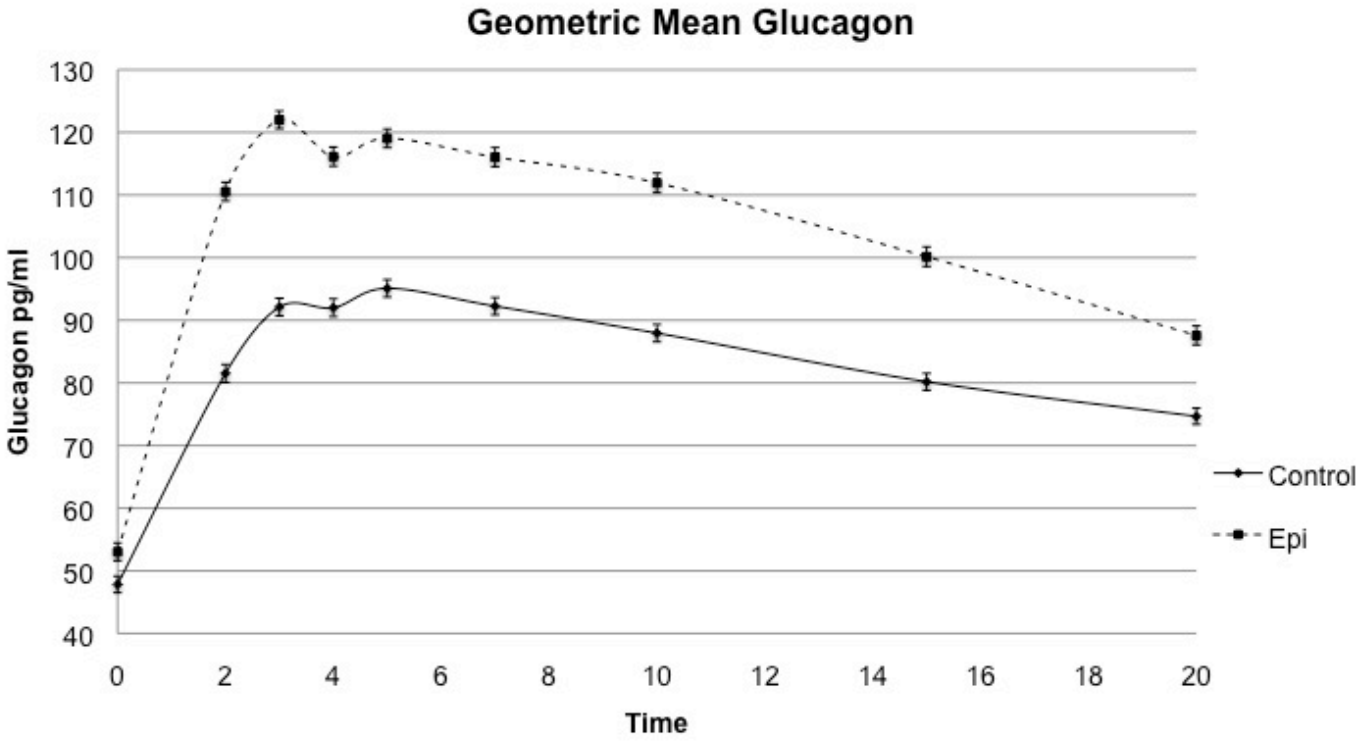


Figure 3: Figure showing geometric mean of plasma glucagon concentration during intravenous arginine stimulation test (IVAST) with and without treatment with epinephrine 60 minutes prior to IVAST, in 15 llamas. Error bars represent geometric standard deviation.



CHAPTER FOUR

Conclusions

In the present study, New World camelids were affected by a wide variety of fatal disorders. In both alpacas and llamas, abnormalities of the digestive system including gastrointestinal parasitism, hepatic lipidosis, liver flukes, intestinal neoplasia, and gastrointestinal ulcers were the most frequently identified cause of death. Other causes of death in alpacas included emaciation and systemic bacterial infections, whereas the nervous system (primarily meningeal worm infection) was the second most common site of abnormalities among llamas. Congenital abnormalities accounted for more than 30% of deaths among alpaca neonates and crias, suggesting the need for additional studies on the genetic diversity of alpaca populations and the heritability of particular malformations such as choanal atresia. Many of the causes of death may be preventable with routine husbandry measures such as nutritional evaluation, frequent body condition scoring, and routine diagnostics to assess gastrointestinal parasite burdens. Results from this study will provide veterinarians with more information to better assist camelid owners with routine health maintenance practices such as nutrition and parasite control that will help reduce mortality. The description of common causes of death in this population of animals will help camelid practitioners identify aspects of management that need closer attention as well as help in the determination of likely differential diagnoses when faced with a sick animal. Further studies such as similar descriptions of common fatal diseases in other regions of the United States are necessary to provide a complete picture of the causes of mortality in New World camelids.

The IVAST technique is quick and comparatively simple to perform, so it has the potential to be used to evaluate pancreatic function in camelids in a variety of metabolic states including growth, pregnancy, lactation, and illness. Under control conditions, llamas in this study had a similar magnitude of insulin and glucagon release as healthy humans. However, the combination of a hyperglycemic clamp and IVAST could be used to better characterize insulin and glucagon secretion at several blood glucose levels to determine the degree to which the camelid pancreas is potentiated by circulating glucose. Additionally, because arginine induced insulin secretion, it is possible that intravenous arginine could be used as therapy to augment or replace exogenous insulin for camelid cases with metabolic derangements. Further study in clinical cases is required.

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