Evaluation of serum trace mineral, vitamin D, and sex steroid hormone concentration, and survey data in llamas and alpacas with metacarpophalangeal and metatarsophalangeal hyperextension

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Objective—To characterize serum trace mineral, sex steroid hormone, and vitamin D concentrations and identify factors associated with metacarpophalangeal and metatarsophalangeal hyperextension in llamas and alpacas.

Samples—Serum samples from 79 llamas and 15 alpacas and owner survey data for 573 llamas and 399 alpacas.

Procedures—Serum samples were stored at –20°C until analysis and were evaluated for trace mineral, vitamin D, estradiol, progesterone, and testosterone concentrations. Information regarding age of onset, number of affected animals in herd, feed and supplements given, type of housing, and management practices was obtained in an owner survey.

Results—Higher serum zinc and iron concentrations were associated with metacarpophalangeal and metatarsophalangeal hyperextension in camelids, compared with controls. In summer and fall months, vitamin D concentrations were significantly higher in affected camelids than controls. Overall prevalence was 13.3% in llamas, compared with 0.7% in alpacas. No management factors were found to be predictive of this condition. No other factors examined were associated with metacarpophalangeal and metatarsophalangeal hyperextension.

Conclusions and Clinical Relevance—Despite similar supplementation practices and environmental conditions between affected and unaffected animals, an association of high serum zinc, iron, and vitamin D concentrations in affected camelids, compared with controls, may indicate differences of intake or absorption of dietary supplements. (*Am J Vet Res* 2013;74:48–52)

Hyperextension of the metacarpophalangeal and metatarsophalangeal joints can affect camelids of any age and may ultimately lead to degenerative joint disease, soft tissue calcification, and decreased ambulation.[1] Previous studies[2–4] have characterized metacarpophalangeal and metatarsophalangeal hyperextension in llamas by ultrasonographic, radiographic, biochemical, and molecular techniques. In juvenile llamas, there is evidence of collagen and proteoglycan changes associated with trauma and healing of the suspensory ligament.[4]

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Trace mineral alterations have also been found in llamas with metacarpophalangeal and metatarsophalangeal hyperextension. A previous study[2] of mainly adult llamas identified low liver copper and high serum zinc concentrations in affected llamas. However, when solely adult llamas were compared, differences were not significant, although high serum zinc concentration was found in affected llamas ($P = 0.08$), likely because of the low number of animals (6 affected animals and 6 control animals). A follow-up study in juvenile llamas[4] revealed high serum molybdenum and low liver cobalt concentrations in llamas with this condition. Although liver samples provide superior results for trace mineral analysis, there is no minimally invasive means of obtaining sufficient liver tissue in a field setting. Serum samples offer the next best alternative, as they are easy to obtain and yield adequate results to help differentiate affected from unaffected animals.

Concentrations of estradiol, progesterone, and testosterone have not been determined in camelids with metacarpophalangeal and metatarsophalangeal hyperextension. Data on these sex steroid hormones will help to determine whether there is an association with this disease because estrogen is thought to play a role in ligament injury in other species.[5] In addition, low vitamin

D concentrations have been found in juvenile camelids with developmental orthopedic disease, including angular limb deformities and physeal abnormalities,[6] but it is not known whether vitamin D plays a role in metacarpophalangeal and metatarsophalangeal hyperextension of camelids.

Speculation by owners and veterinarians regarding environmental and hereditary factors associated with metacarpophalangeal and metatarsophalangeal hyperextension is common. However, demographic data and herd management information have not been reported in camelids with this disease.

Therefore, the objective of the study reported here was to evaluate serum trace mineral, vitamin D, and sex steroid hormone concentrations and identify factors associated with metacarpophalangeal and metatarsophalangeal hyperextension in llamas and alpacas. The hypothesis was that serum trace mineral imbalances, including low copper and high zinc concentrations, and high estradiol and low vitamin D concentrations would be found in affected animals, compared with controls.

Materials and Methods

Serum trace mineral, vitamin D, estradiol, progesterone, and testosterone concentrations—Serum samples from 79 llamas (46 affected and 33 control) and 15 alpacas (4 affected and 11 control) were shipped on ice by referring veterinarians and stored at −20°C until analysis. Animals were located in the Pacific Northwest (primarily western Oregon and Washington). Animals were selected by referring veterinarians, with an attempt to collect blood for serum samples from a portion of affected (if any) and unaffected animals from each herd. Owner consent was obtained for use of serum samples. This protocol was performed in compliance with institutional guidelines for research on animals. Serum samples were analyzed in 1 batch for concentrations of trace minerals[a] (copper, zinc, manganese, molybdenum, selenium, iron, and cobalt), vitamin D[a] (25-hydroxyvitamin D_3), and sex steroid hormones[b] (estradiol, progesterone, and testosterone). Serum samples were collected year-round for this study, with 44 samples obtained from January to June (31 affected and 11 control) and 52 samples obtained from July to December (19 affected and 33 control). Serum vitamin D concentrations were evaluated by use of these seasonal groupings on the basis of previous research by Smith and Van Saun.[7]

Survey data—Surveys were sent to all llama and alpaca owners in the 2009 database of the Northwest Camelid Foundation (n = 170); 59 owners (35% response) returned completed surveys for evaluation from February 2010 to May 2011 with data from 972 animals, including 573 llamas and 399 alpacas (79 camelids affected with metacarpophalangeal and metatarsophalangeal hyperextension). The geographic coverage of the survey sample was the Pacific Northwest (primarily western Oregon and Washington). Information regarding all camelids in each selected herd, including species, sex, age of onset, number of affected and unaffected animals, feed and supplements given, type of housing and fencing, and management practices, was included. Additionally, a cover letter was included with the survey, which included photographs of camelids with mild and severe metacarpophalangeal and metatarsophalangeal hyperextension and unaffected P1-to-ground angles for reference (**Figure 1**). Owner surveys were completed for 57 of 94 animals that had serum submitted concurrently for analysis.

Statistical analysis—A Mann-Whitney rank sum test was used to analyze serum concentration comparisons between affected and control animals because normality was violated between groups. Juvenile (< 4 years) and adult (≥ 4 years) age groups were initially evaluated separately but were combined together in the final statistical analysis because of the low number of serum samples from affected juveniles (n = 5). A χ^2 test was used for prevalence comparisons between llamas and alpacas. Multiple logistic regression was used to analyze survey data, with affected-unaffected as the outcome variable and herd, species, sex, access to pasture, access to trace minerals, access to galvanized materials, and type of hay fed as the predictor variables. Species, access to pasture, and type of hay were subsequently omitted from the analysis because of colinearity with other predictor variables. All analyses were calculated via statistical software.[c,d] Descriptive statistics of affected animals included frequency of signalment findings, disease progression, and management and hereditary factors. A value of $P < 0.05$ was considered significant.

Results

Serum trace mineral, vitamin D, estradiol, progesterone, and testosterone analysis—Median serum concentrations of trace minerals were determined (**Table 1**), comparing camelids affected with metacar-

Figure 1—Photographs of the forefeet of llamas illustrating the angle between P1 and the ground in a study of metacarpophalangeal and metatarsophalangeal hyperextension in camelids.

Table 1—Median (interquartile range) serum concentrations of trace minerals from llamas and alpacas with metacarpophalangeal and metatarsophalangeal hyperextension (n = 50) and unaffected controls (44).

Variable	Laboratory reference range	Affected	Control	*P* value
Copper (µg/mL)	0.2–0.6	0.53 (0.46–0.58)	0.49 (0.43–0.53)	0.052
Zinc (µg/mL)	0.2–3.0	0.48 (0.39–0.66)	0.36 (0.30–0.43)	< 0.001
Manganese (ng/mL)	1.5–2.5	1.1 (0.90–1.90)	1.1 (0.9–1.75)	0.54
Molybdenum (ng/mL)	NA	2.05 (0.88–3.83)	1.35 (0.82–4.1)	0.72
Selenium (ng/mL)	120–160	186 (83–229)	188 (91–219)	0.73
Iron (µg/dL)	75–170	139 (112–160)	126 (98–144)	0.03
Cobalt (ng/mL)	> 0.25	1.22 (0.60–2.27)	1.86 (0.71–2.96)	0.18

Table 2—Median (interquartile range) serum concentrations of estradiol, progesterone, and testosterone (laboratory reference ranges not available) in llamas and alpacas of various sexes with metacarpophalangeal and metatarsophalangeal hyperextension (11 females, 30 castrated males, and 8 sexually intact males) and unaffected controls (15 females, 11 castrated males, and 18 sexually intact males).

Variable	Affected	Control	*P* value
Estradiol (pg/mL)			
Female	22.9 (19.7–31.5)	27.6 (18.0–31.3)	0.64
Castrated male	17.7 (15.7–21.3)	17.9 (15.3–22.8)	0.57
Sexually intact male	28.5 (24.0–37.4)	28.5 (23.6–40.6)	0.85
Progesterone (ng/mL)			
Female	0.34 (0.05–2.23)	0.07 (0.04–0.82)	0.84
Castrated male	0.03 (0.01–0.04)	0.04 (0.0–0.05)	0.93
Sexually intact male	0.27 (0.22–0.33)	0.38 (0.18–0.76)	0.22
Testosterone (ng/mL)			
Female	0.0 (0.0–0.01)	0.0 (0.0–0.01)	0.98
Castrated male	0.0 (0.0–0.02)	0.0 (0.0–0.08)	0.99
Sexually intact male	1.58 (0.69–5.47)	1.62 (0.55–3.22)	0.82

pophalangeal and metatarsophalangeal hyperextension with unaffected controls. Metacarpophalangeal and metatarsophalangeal hyperextension in llamas and alpacas was significantly associated with higher serum zinc concentrations ($P < 0.001$) and higher serum iron concentrations ($P = 0.03$). No significant differences were found in serum concentrations of copper, manganese, molybdenum, selenium, or cobalt between affected and control animals. When juveniles and adults were evaluated separately, the results for the adults were similar to the combined results, whereas no significant differences were found for any of the juvenile variables. Vitamin D concentrations were significantly higher in affected camelids than unaffected controls in serum samples obtained from July to December (median for affected camelids, 160 nmol/L; for control camelids, 96 nmol/L; $P = 0.006$), but not in samples taken from January to June (for affected camelids, 65 nmol/L; for control camelids, 40 nmol/L; $P = 0.30$). When segregated by sex, no significant differences were found in testosterone, estradiol, or progesterone concentrations between affected and unaffected camelids (**Table 2**). No significant differences were found between llamas and alpacas for any of the variables evaluated.

Survey data analysis—On the basis of owner survey information of all affected and control camelids, llamas were significantly ($P < 0.001$) more likely to be affected by metacarpophalangeal and metatarsophalangeal hyperextension than alpacas: overall prevalence was 13.3% in llamas, compared with 0.7% in alpacas. On the basis of the multiple logistic regression analysis, none of the predictors (herd, sex, access to trace minerals, and access to galvanized materials) were significant. Thus, no differences were detected in management factors between affected and unaffected animals. Most owners (93%) supplemented all animals with trace minerals.

From survey data collected on 76 affected llamas and 3 affected alpacas, median age of initial onset of the condition was 10 years (interquartile range, 6 to 11 years). Only 10% of affected animals initially developed the condition as juveniles (< 4 years of age). Disease onset was described as acute in 35% of animals and chronic in 65%. Affected camelids were mostly castrated males (63%), followed by females (30%) and sexually intact males (6%). Forelimbs were mainly affected (80%), followed by all 4 limbs (19%) and hind limbs only (1%). Severity of hyperextension was reported as mild (40%), moderate (23%), or severe (37%), and worsened over time in 60% of animals, remained the same in 37%, and improved in 3%. Body weight of affected llamas was estimated to be < 136.4 kg in 39%, 136.4 to 181.8 kg in 29%, and > 181.8 kg in 32%. Most affected camelids were used as pets (55%); others were used for breeding (15%), packing (15%), or showing (5%) or as fiber animals (5%) or guard animals (5%). Twenty-nine percent of affected animals had related animals (siblings, offspring, sire, or dam) that were known to be affected with metacarpophalangeal and metatarsophalangeal hyperextension, whereas 20% did not (for 51%, a relationship was unknown). The majority of owners did not attempt to treat the condition (81%), whereas the remaining 19% of owners attempted to treat affected animals by use of homeopathy[e] (8%), weight loss (5%), phenylbutazone (3%), glucosamine (2%), or vitamin A, D, and E (1%). Most owners (80%) did not see any improvement with treatment, but 3 owners believed they saw improvement, including 2 whose animals lost weight and 1 whose animal received homeopathy.

Discussion

Despite similar supplementation practices between affected and control animals, an association of higher serum zinc and iron concentrations was found in affected animals. In addition, vitamin D concentrations were higher in affected animals when samples were drawn in the summer and fall months. However, none of the management factors examined were predictive of metacarpophalangeal and metatarsophalangeal hyperextension, which may indicate that individual intake or nutrient absorption differences or other factors unrelated to general herd management were associated with the condition.

In a previous study[2] in llamas, which included mostly adults and a few juveniles, investigators also identified higher serum zinc concentrations in affected animals, compared with unaffected controls. However, when juveniles were evaluated separately in another study,[4] no significant difference was found in zinc concentrations of affected animals, which concurs with results of the present study. Therefore, increased zinc concentrations may be more important in adult camelids than juveniles with the condition. Potential sources of zinc include mineral supplements and galvanized fencing or feed and water tubs. Zinc is added to camelid mineral supplements to help prevent zinc-responsive dermatosis,[8] but may be present in high enough concentrations to inhibit copper absorption. It is theoretically possible that affected camelids may have consumed more mineral supplements (if given free choice) to attain higher zinc concentrations. Alternatively, affected animals may preferentially seek greater oral contact with galvanized fencing or feed and water tanks than unaffected herdmates despite having similar access. The clinical importance of increased zinc concentrations remains to be determined because both affected and unaffected animals had median zinc concentrations within established laboratory reference ranges (Table 1). However, the effects of subclinical differences of trace mineral concentrations are largely unknown, and physiologic interactions between these and other vitamin or trace mineral alterations may combine to exert a clinical effect.[9,10]

Copper deficiency has been associated with increased incidence of developmental orthopedic disease in foals,[11] and molybdenum-induced copper deficiency in yaks and camels[12–14] may result in metacarpophalangeal and metatarsophalangeal hyperextension similar to that seen in llamas. However, contrary to our hypothesis, serum copper concentrations were not significantly different between affected and unaffected camelids in the present study. These findings are in contrast to pooled juvenile and adult llama data from previous studies,[2,4] which revealed significantly lower copper concentrations in serum and liver of llamas with metacarpophalangeal and metatarsophalangeal hyperextension. Liver copper concentrations are considered to be a better indication of cumulative copper concentrations than serum concentrations.[15] Because the present study only evaluated serum concentrations of copper, it is possible that differences in cumulative copper concentrations between affected and unaffected camelids were missed. Other trace mineral differences found in previous studies,[2,4] including increased serum molybdenum concentrations, were not confirmed in the present study.

The finding of higher serum iron concentrations in affected llamas and alpacas in this study was unexpected, especially because previous studies[2,4] had not identified this alteration. Iron is an essential part of hemoglobin, and increased iron concentrations may reduce cobalt, copper, selenium, or zinc absorption.[16] Dietary iron intake can also affect Hct and serum concentrations of hemoglobin, ferritin, and iron. Interactions between dietary iron, zinc, and copper and the resultant serum concentrations of these trace minerals have been found.[17] In addition, age-related changes in serum iron concentrations of llamas in a previous study[18] decreased during the first year after birth. In the study reported here, only 2 animals (1 affected and 1 control) were < 1 year of age, so the effect on the results was expected to be minimal. The clinical importance of high serum iron concentrations of affected camelids in the present study, compared with controls, must be further evaluated because the concentrations remained within reported reference ranges.[18,a]

On the basis of previous research by Smith and Van Saun,[7] vitamin D concentrations in llamas and alpacas are expected to be lowest from January to June and highest from July to December in the Pacific Northwest, lagging behind the period of the highest solar radiation concentrations. The present study confirmed clear differences in vitamin D concentrations between winter-spring months and summer-fall months. Contrary to our hypothesis of lower vitamin D concentrations in affected camelids, higher vitamin D concentrations were found in affected animals than in controls, at least in the summer months. However, vitamin supplementation practices were reported to be similar between affected animals and unaffected herdmates. Skin pigmentation may also play a role in vitamin D production,[7] but coat color was not recorded in this study. Similar to zinc and iron concentrations, affected animals had median vitamin D concentrations within established laboratory reference ranges, and the clinical importance of this alteration remains to be determined.

We hypothesized that higher concentrations of estradiol may be contributing to metacarpophalangeal and metatarsophalangeal hyperextension in affected animals. However, there was no evidence in the present study to support this hypothesis. Affected camelids were predominantly castrated males (63%), suggesting a possible link to lower sex hormone concentrations. However, when affected and unaffected camelids were segregated by sex and neutering status, no differences were found in sex hormone concentrations.

Previous studies[2–4] evaluated metacarpophalangeal and metatarsophalangeal hyperextension in adult and juvenile populations. It is not known whether the etiologies of juvenile and adult forms of this condition are the same because previous studies have provided conflicting conclusions. Affected juvenile animals had evidence of injury and repair of the suspensory ligaments, unlike their adult counterparts, and different trace mineral alterations were identified in juveniles versus adults.[2–4] In the present study, the low number of affected juvenile animals precluded a complete analysis of serum concentration differences, and initial evaluation of trace mineral concentrations revealed no significant differences between affected juvenile animals and controls. The later age of onset of this condition (median, 10 years) likely contributed to the low number of available affected juveniles. In the future, a larger sample size may be necessary to obtain a sufficient number of juvenile samples. Nonetheless, this study provided important information on a mainly adult population of camelids with metacarpophalangeal and metatarsophalangeal hyperextension, corresponding well with the distribution of affected camelids within the general population.

Another limitation to the present study included the inherent subjectivity of owner surveys. Inclusion of camelids in the affected group potentially relied on use of photographs illustrating unaffected and affected animals, but veterinarians and owners were ultimately responsible for deciding whether an animal belonged in the affected or unaffected group. Ideally, all of the animals would have been evaluated by one of the authors, but this was not possible because of the extended geographic location of sampled camelids and reliance on referring veterinarians for sample collection. Additionally, data sets were often incomplete: surveys were variably filled out, and not all animals that had serum samples submitted had surveys returned. Therefore, statistical analysis was limited to those categories with enough data to make meaningful comparisons between affected and unaffected animals. Despite these limitations, we believe the present study provided important information concerning disease prevalence and etiologic factors associated with metacarpophalangeal and metatarsophalangeal hyperextension because no previous studies have addressed overall prevalence and environmental factors, to our knowledge.

The effect of genetic influence on the prevalence of metacarpophalangeal and metatarsophalangeal hyperextension in camelids has yet to be determined. Almost a third of affected animals in this study had dams, sires, offspring, or siblings that were also affected with this condition. Several llama owners have claimed that specific lineages produce offspring with greater prevalence of metacarpophalangeal and metatarsophalangeal hyperextension. Further evaluation is necessary to determine the heritability and familial influence on the development of this condition in camelids.

Llamas had a higher prevalence of metacarpophalangeal and metatarsophalangeal hyperextension than did alpacas. Higher concentrations of zinc, iron, and vitamin D in affected animals provided evidence for possible nutritional or absorptive differences in these camelids. On the basis of on these findings, it may be recommended that veterinarians evaluate trace mineral and vitamin D concentrations of affected and unaffected animals in herds with this condition. The clinical importance of the findings remains to be determined, mainly because observed serum concentrations were within previously established laboratory reference ranges. However, physiologic interactions between these vitamin and trace minerals may combine to exert a clinical effect. Because none of the management factors examined in the survey were predictive of metacarpophalangeal and metatarsophalangeal hyperextension, future studies may need to focus on other factors, including monitoring individual animal intake and nutrient absorption differences within herds, evaluating coat color, or performing heritability studies.

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a. Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, Mich.
b. Endocrinology Laboratory, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY.
c. Stata/IC, version 11.2, StataCorp, College Station, Tex.
d. Statistix, version 1, Analytical Software Inc, Tallahassee, Fla.
e. Pollard's Camelid Tendon Repair Herbal Blend, Starwest Botanicals Inc, Rancho Cordova, Calif.

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