Clinical Case Conference

Chronic weight loss in an immunodeficient adult llama

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A 3.5-year-old 118-kg (260-lb) female llama was examined at the Washington State University Veterinary Teaching Hospital to determine the cause of weight loss that had become evident throughout the preceding 2 months. The llama was kept on pasture with 31 other llamas and fed a diet that included a pelleted supplement. The llama had given birth to 2 healthy crias at 2 and 3 years of age and was currently in the seventh month of gestation. The llama had recently been vaccinated against clostridial organisms and had been dewormed with ivermectin. Six months prior to admission, the llama had been examined for weight loss and intermittent lameness. Although an affected organ system or site of infection had not been identified, the llama responded favorably to use of antimicrobials (procaine penicillin G, 22,000 U/kg [10,000 U/lb] of body weight), fenbendazole, and supportive care and was discharged.

Physical examination (day 0) revealed that the llama was tachycardic (96 beats/min; reference range, 60 to 80 beats/min), emaciated, and diarrheic. Mucous membranes were pale. A fetus, palpable per rectum in the left uterine horn, was consistent with a gestational age of 7 months. All other physical findings were considered normal. Initial examination included CBC, serum biochemical analysis, urinalysis, fecal examination for parasite ova, and examination of fluid obtained from the C1 compartment of the stomach for protozoal viability. Abnormal results included leukopenia (7,000 cells/μl; reference range, 8,000 to 21,000 cells/μl), neutropenia (3,360 cells/μl; reference range, 4,700 to 14,800 cells/μl), with a degenerative left shift (2,240 bands/μl; reference range, 0 to 128 bands/μl), toxic neutrophils, Döhle's inclusion bodies, hypoproteinemia (3.7 g/dl; reference range, 5.7 to 7.0 g/dl), hypoalbuminemia (1.8 g/dl; reference range, 3.5 to 4.8 g/dl), hyperfibrinogenemia (700 mg/dl; reference range, 100 to 400 mg/dl), macrocytic normochromic anemia (PCV, 13%; reference range, 25 to 45%; mean corpuscular volume, 41 fL; reference range, 23 to 30 fL), and anisocytosis. All other results of CBC, serum biochemical analysis, and urinalysis were within reference ranges. Protozoal activity was not detected in the sample obtained from the C1 compartment of the stomach. *Trichuris* sp, Trichostrongylidae ova, and *Eimeria* sp oocysts were found on fecal examination. *Eperythrozoon* sp were observed on examination of a blood film. Because clinical eperythrozoonosis has been associated with llamas that are immunosuppressed or debilitated,[1,2] serum IgG concentration was measured and was found to be low (310 mg/dl; reference range > 1,200 mg/dl). Low serum IgG concentration was suggestive of an immunodeficiency and is a consistent finding in llamas with **juvenile llama immunodeficiency syndrome (JLIDS)**.[1,2]

Anemia, anisocytosis, and pale mucous membranes were attributed to overwhelming eperythrozoonosis. Leukopenia, neutropenia, degenerative left shift, toxic neutrophils, Döhle's inclusion bodies, and hyperfibrinogenemia were suggestive of an infectious process. Infections, often caused by opportunistic pathogens, are common in immunodeficient animals. Other than eperythrozoonosis, a specific infection was not detected in this llama.

Initial treatment consisted of administration of oxytetracycline hydrochloride (11 mg/kg [5 mg/lb], IV, followed by 11 mg/kg, IM, of long-acting oxytetracycline, q 72 h, for 15 days) for treatment of eperythrozoonosis, deworming with fenbendazole (15 mg/kg [6.8 mg/lb], PO), administration of amprolium (10 mg/kg [4.5 mg/lb], PO, q 24 h, for 21 days), dental prophylaxis by filing down sharp points on the teeth, and administration via an orogastric tube of 1 L of fresh rumen fluid obtained from a donor cow.

On day 2, feces were submitted for bacterial culture, and serum was collected for *Mycobacterium paratuberculosis* testing via an agar gel immunodiffusion serologic examination. To evaluate humoral immune function, serum was obtained for determination of prevaccination titers for *Clostridium perfringens* type C and D, and the llama was then vaccinated with *C perfringens* type-C and -D toxoid. After collecting samples for determination of prevaccination titers, plasma (620 ml) and blood (1,125 ml) were administered IV to provide immunoglobulins, serum proteins, and erythrocytes. Crossmatching was not performed, because the llama had not been previously transfused, and only 1 transfusion was anticipated. Rumen fluid obtained from a donor cow (2 L) was mixed with softened alfalfa pellets and was administered via an orogastric tube. Serum was submitted for trace mineral analysis, and feed samples were analyzed. Serum iron,

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selenium, zinc, and copper concentrations were within reference ranges. Total digestible nutrients and percent digestible protein contents in feed samples were adequate.

Throughout the next week, the llama's appetite and attitude improved and diarrhea resolved. On day 9, CBC and serum biochemical analysis revealed continued leukopenia (3,100 cells/µl), neutropenia (589 cells/µl), macrocytic normochromic anemia (PCV, 14%; MCV, 40 fL), anisocytosis, hypoproteinemia (3.5 g/dl), and hypoalbuminemia (1.7 g/dl). Bacterial pathogens were not cultured from feces, and results of serologic examination for *M paratuberculosis* were negative. On day 12, an increase in titers to *C perfringens* type C and D was not detected (prevaccination titer, 1:100; postvaccination titer < 1:100; reference range ≥ twofold increase), suggesting a deficient humoral immune response. Measurement of serum IgG concentration was repeated, a lymph node biopsy specimen and bone marrow aspirate were obtained, and blood was collected for lymphocyte blastogenesis assay. Serum IgG concentrations remained low (400 mg/dl). Histologic examination of the lymph node specimen indicated hypoplasia or atrophy, and examination of the bone marrow aspirate revealed erythroid hyperplasia and possible granulocytic hypoplasia. Although we were unable to differentiate llama lymphocyte populations, which limited our interpretation of results, a decreased response to poke weed mitogen and *Streptococcus* protein A (insoluble form) were similar to results for llamas with JLIDS. Findings of persistent low serum IgG concentrations, lymph node hypoplasia or atrophy, failure to respond to clostridial vaccination, and eperythrozoonosis were consistent with our primary diagnosis of an immunodeficiency syndrome. After the owners were informed of the grave prognosis, they requested that we continue supportive care until gestation was complete.

The llama gained weight during the next month. By day 41, body weight was 126 kg (277 lb). On day 44, CBC and measurement of serum IgG concentrations were repeated. Abnormalities included leukopenia (4,100 cells/µl), neutropenia (2,501 cells/µl), thrombocytopenia (9,000 platelets/µl; reference range > 100,000 platelets/µl), macrocytic normochromic anemia (PCV, 21%; MCV, 38 fL), and eperythrozoonosis. Hypoproteinemia had resolved, and WBC and PCV values had improved, but serum IgG concentrations remained low (210 mg/dl). Recurrent eperythrozoonosis was treated with oxytetracycline, as described previously.

On day 63, CBC was repeated. Abnormalities included leukopenia (3,600 cells/µl) and neutropenia (3,300 cells/µl). On day 86, *Eperythrozoon* organisms were observed on examination of a blood film, and oxytetracycline was again administered. By day 89, *Eperythrozoon* organisms were not observed. On day 93, a 10-month fetus was aborted. Fetal or placental abnormalities were not observed during gross and histologic examinations. Recovery from abortion was uneventful, and the llama continued to eat well and gain weight. On day 107, eperythrozoonosis was diagnosed, and tetracycline was initiated (600 mg/d, PO). For the next month, the llama's attitude and appetite improved and it gained weight. By day 128, the llama had negative results when tested for eperythrozoonosis and weighed 130 kg (286 lb). Despite the llama's improved condition, the grave prognosis was reiterated. Recurrent eperythrozoonosis or systemic infections were anticipated. For emotional reasons, the owners wanted to transfer the llama to their farm, and the llama was discharged to them on day 133.

Three months later (228 days from initial admission), the llama was readmitted because of weight loss. Physical examination revealed severe emaciation and weakness. Body weight was 97 kg (213 lb). Because of the physical condition of the llama, abnormalities on CBC and serum biochemical analysis, and the recurrent nature of the llama's condition, the owners elected to euthanatize the llama. Gross necropsy was unremarkable. Histologic examination revealed diffuse lymph node atrophy, evidenced by a lack of germinal center activity, immature blast cells, and a decrease in the number of plasma cells and macrophages in sinusoids. Histologic examination of bone marrow indicated erythroid and megakaryocytic hypoplasia. The granulocytic profile was consistent with marrow exhaustion resulting from chronic inflammation.

The llama reported here had characteristics consistent with llamas that have JLIDS; however, the age at which clinical signs were recognized (34 months) was substantially greater than the median age previously reported (11.6 months).[2] When evaluating older llamas that have signs consistent with JLIDS, it must be remembered that prior signs of JLIDS may not be recognized. Furthermore, if JLIDS is an acquired illness, the llama may not have been exposed to the causative factor (infectious, environmental) until it was older than the juvenile phase; however, if JLIDS is genetic in origin, it may not be expressed until later in the llama's life. Conversely, older llamas may be affected by a comparable immunodeficiency syndrome other than JLIDS. The fact that the llama reported here successfully gave birth to and nursed 2 crias and was not reportedly ill until approximately 3.5 years of age implied that this llama was clinically normal until shortly before being admitted to our hospital. This would suggest that the causative factor of the immunodeficiency syndrome in this llama was acquired, genetic, and variably expressed or was a specific immunodeficiency syndrome that exists in older llamas and is distinct from JLIDS.

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The llama described here forces all clinicians to be cognizant of immunoincompetence as a possible differential diagnosis for chronic weight loss in adult llamas. The overwhelming causes of chronic weight loss in llamas that I have examined are malnutrition (inadequate or imbalanced nutrient intake) or parasitism (internal or external). Rarely do we examine an adult llama that has other causes of chronic weight loss (eg, neoplasia, dental disease, immunoincompetence).

In llamas with signs similar to the one described, a complete history of feeding management would be of great value. Similar to other herbivores, llamas establish a feeding hierarchy, and many times, timid, smaller animals may be deprived of feed. This may have been particularly true with this llama, because it was 1 of 32 llamas grazing on the same pasture. Client education on the proper use and implementation of a **body condition scoring (BCS)** system enhances early detection of weight loss and promotes appropriate revision of feeding practices.[1] Llamas should have their BCS assessed and recorded every time they are handled or at least bimonthly. Owners who use a BCS system may be alerted early in the course of the weight loss. Llamas of this age and stage of gestation should be consuming 1.2 to 1.8% of their body weight in dry matter; the diet should be approximately 10% crude protein and 60% total digestible nutrients. The energy portion of the diet should be modified with changing climatic conditions, body condition, and stages of gestation. The authors mentioned that the diet was adequate on the basis of a feed analysis, but that would only be the case if adequate quantities of feed were being consumed.

The llama had strongyle and *Trichuris* sp infections, despite a history of deworming with ivermectin and fenbendazole. Llamas infected with *Trichuris* sp (most commonly *T tenuis*) at our hospital have had diarrhea or bloody diarrhea. Although some llamas infected with *Trichuris* sp are successfully treated with a single administration of benzimidazole or ivermectin, many infections appear refractory. Furthermore, when fenbendazole is selected as the choice for treatment, a proper amount should be administered (15 mg/kg [6.8 mg/lb] of body weight, PO) for 3 consecutive days. We routinely perform a parasitologic examination, using a concentrated salt solution for flotation of ova on feces collected prior to administration of the anthelmintic, and we repeat the examination in 10 to 14 days. We expect to see a minimum of a 90% reduction in the number of parasite eggs per gram of feces, or other forms of treatment will be instituted.

Our experience with eperythrozoonosis appears to be quite similar to that reported for the llama described here. That is, *Eperythrozoon* sp appear to be opportunistic organisms that are controlled only by vigilant administration of oxytetracycline in an animal with a functioning immune system. We have thus far successfully managed to control eperythrozoonosis in llamas by use of a protocol that advocates administration of oxytetracycline (11 mg/kg [5 mg/lb], IV) and long-acting oxytetracycline (20 mg/kg [9.1 mg/lb], SC) on day 1, followed on days 3, 6, 9, and 12 by administration of long-acting oxytetracycline (20 mg/kg, SC). An oral form of chlortetracycline (22 g/kg [10 g/lb]) is administered on days 15 to 50.[2] Owners, handlers, and clinicians should ensure adequate daily intake of the oral form of the antibiotic. Unfortunately, when we have varied from this protocol, we have had recrudescence of infections similar to that of the llama described here.

The authors did an excellent job of performing a thorough diagnostic evaluation, but usefulness of serum selenium concentrations has been questioned.[3] In animals undergoing any dietary change (as this llama did when hospitalized), serum selenium values do not accurately reflect long-term dietary intake or body selenium status. Selenium concentration in whole blood would be a superior indicator to body selenium status, because it reflects selenium intake during the past 90 days. With the exception of dietary analysis and evaluation of hepatic biopsy specimens, serum or plasma analysis is the only tool currently available for ascertaining dietary copper intake or body copper status; however, both are seriously flawed. Plasma copper concentrations may be slightly more meaningful than serum concentrations, because much of the copper is bound in the clot. Still, blood copper concentrations appear unpredictable as to reflecting dietary adequacy and may rarely be of value in the diagnosis of deficiency when concentrations are overtly low.[3] Selenium and copper deficiencies may cause a compromised immune system, but concentrations within reference ranges have been reported for llamas with confirmed cases of juvenile llama immunodeficiency syndrome.[4]

In llamas with chronic weight loss that have concurrent immune deficiency, determination of titers to, or viral isolation of, **bovine viral diarrhea virus (BVDV)**

should be considered. The BVDV has been isolated from llamas admitted to the veterinary teaching hospital at Oregon State University.[5] Llamas infected with BVDV may have signs consistent with an immunodeficiency or may be diarrheic and abort.

The clinicians made a valiant effort to prolong the llama's life and maintain pregnancy. Although probably not applicable in this llama, the fetus may be carried to term if body weight and condition are maintained via use of parasite control and dietary manipulation. If other causes of weight loss are ruled out and immunodeficiency syndrome is suspected, a diagnosis can be attained by detecting a low resting γ-globulin concentration (particularly in the face of chronic infectious disease) and low initial serum titers to *Clostridium perfringens* type C and D and lack of a twofold increase in titers 14 days after administration of toxoid.[4] In our experience, use of immunostimulants, immunomodulators, and dietary modification have been unrewarding for long-term survival of adult llamas with immunodeficiency diagnosed by methods described for this llama. Currently, in llamas with this form of immunodeficiency, treatment to ensure adequate intake of energy, protein, and macro- and micronutrients and for control of internal, external, and bloodborne parasites may prolong life, but only for a short period.

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The authors described the history and clinical signs of an adult female llama with chronic weight loss and diarrhea and guided us through their diagnostic approach, treatment, and clinical progress. Ultimately, immunodeficiency syndrome was diagnosed, and the llama aborted and failed to thrive (despite an initial partial recovery) until it was euthanatized. Chronic weight loss and failure to thrive are relatively common clinical problems in llamas and alpacas. Determination of the cause of this condition is frustrating, and a thorough physical examination and diagnostic investigation is of paramount importance to the ultimate success or failure of clinicians. The authors have outlined a systematic and thorough progression, from diagnosis through treatment, of a llama affected with immunodeficiency syndrome that had chronic weight loss.

The clinical approach to chronic weight loss in camelids may vary with geographic location and historical information. High stocking density of llamas on pasture is a problem on many farms. The authors did not indicate the number of acres the 32 llamas were maintained on, but they indicated that the deworming regimen included ivermectin. Resistance to ivermectin by *Nematodirus* parasites has been observed. The authors performed a fecal examination and administered fenbendazole to this llama. Although overfeeding and obesity appear to be more common problems in camelids in North America, malnutrition also has been diagnosed. The authors did not elaborate as to the body condition of the herdmates, but this would be prudent for a veterinarian examining any llama with weight loss.

Changes in hematologic and serum biochemical variables are complex in llamas with immunodeficiency syndrome, and changes associated with secondary infections may disguise an immunodeficiency problem early on in the process.[1,2] As the authors have described, diagnostic testing for any llama suffering chronic weight loss that does not appear to be associated with parasitism or malnutrition (including poor feed intake because of malocclusion) should be intensive. Chronic infection, subclinical pneumonia, hepatic and renal disease, gastrointestinal diseases including paratuberculosis and salmonellosis, and neoplasia should be ruled out. Hypoproteinemia, characterized by hypogammaglobulinemia and hypoalbuminemia, is consistently found in immunodeficient llamas. Hypogammaglobulinemia appears to be associated with B-lymphocyte abnormalities, but a plausible explanation has not been established for hypoalbuminemia. As stated by the authors, anemia observed in the llama of this report did not appear to be associated with enteric parasitism, ulceration of the third compartment of the stomach, hemolysis, or decreased RBC production. Anemia appeared to be associated with eperythrozoonosis; however, anemia has been seen in immunodeficient

llamas that are apparently free of *Eperythrozoon* parasites. Although clinical signs of eperythrozoonosis are most commonly seen in immunodeficient llamas, I have observed severe anemia caused by *Eperythrozoon* infection in llamas with normal results on tests of the immune system.

To date, response to vaccination with *Clostridium perfringens* type-C and -D toxoid, examination of a biopsy specimen of a lymph node, and results of lymphocyte flow cytometry and blastogenesis assays are the best tools available for diagnosis of immunodeficiency syndrome. However, the clinical state of the llama may make interpretation of vaccination titer results more difficult. The results reported by the authors appeared to be clear, but severe debilitation of the llama and eperythrozoonosis also may suppress immune response.

Retrovirus infection was detected in a yearling llama with immunosuppression.[3] That yearling llama may be unique, may represent opportunistic infection by a retrovirus, or may shed light into the cause of immunodeficiency in llamas. The demographics of immunodeficiency syndrome, however, do not tend to support retrovirus infection as the cause. The llama reported here was a 42-month-old sexually mature adult female. Immunodeficiency syndrome has been described in llamas ranging from 2.3 to 30.1 months old.[1-3] Therefore, it is conceivable that the llama described here had not been challenged sufficiently to cause manifestation of clinical signs, that the clinical signs had not been observed, that the disease was not expressed until later in life, or that the disease was acquired. Although specific information was not provided, the amount of immunoglobulin transferred from the dam to the 2 apparently healthy crias would be interesting. If both crias had received plasma or other immunoglobulin supplements after birth, this would be evidence that the llama was immunosuppressed, but that it had not manifested clinical signs yet.

This was an interesting report, and it provided a logical method for evaluation of chronic weight loss in llamas after the most common causes have been ruled out (parasitism, malnutrition, inadequate feed intake because of being the lowest llama on the social hierarchy). Immunodeficiency syndrome should not be discarded as a diagnostic possibility simply on the basis of the age of the llama.

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