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CASE REPORT

Haemonchus-induced Anemia in Llamas (*Lama glama*) from Argentina

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ABSTRACT

This report documents clinical manifestation of anemia associated with *Haemonchus* spp. in naturally infected llamas from Argentina. Infected animals presented conjunctival pallor, discolored coat, edema in the submandibular area, weakness, lack of exercise tolerance, sternal recumbence and emaciation. The range of hematocrit values was between 4 to 16% with marked erythrocyte pleomorphism. *Haemonchus* spp.were recovered from the C3 compartment during a necropsy performed on a weak and recumbent pregnant female llama that was euthanized. Stressful conditions derived from farming management, production stress, long-term treatment with suboptimal doses of anthelmintics drugs and climatic conditions were concomitant causes that favored the herd infestation.

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INTRODUCTION

Three of the four species of South American Camelids (SACs) such as llamas (*Lama glama*), guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*), inhabit Argentina. The first one is a domestic species, while the other two are wild species. The llama is the largest (weighing ~130 kg) of the SACs and has been used since pre-Hispanic times as a multipurpose animal providing fiber, meat and leather, and as a beast of burden. Nowadays, these animals have been revalued for their use as fine fibers producers, companion animals and trekking on tourism trails. These situations have forced llamas to colonize new geographic areas different from their natural environment (Andean Puna of South America) which may negatively affect their health status.

Severe acute diarrhea, respiratory and reproductive problems caused by viral, bacterial or parasitic agents, were previously described in domestic and wild SACs bred under intensive conditions (Mattson 1994; Fowler 1998; Parreño *et al*. 2001; Marcoppido *et al*. 2011). The susceptibility of SACs to infectious agents that cause disease in domestic animals was also reported (Marcoppido *et al*. 2011). Etiological agents of parasitic gastroenteritis in cattle (*Ostertagia* spp., *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp and *Oesophagostomum venulosum*) also cause clinical disease in SACs (Cafrune *et al*. 2001). Likewise, host-specific nematodes (*Graphinema aucheniae*, *Camelostrogylus mantulatus*, *Nematodirus lamae*, *Lamanema chavezi*, *Trichuris tenis*) (Cafrune *et al*. 2001; Baldomenico *et al*. 2003), protozoa (*Eimeria* spp.) and hemoparasites (*Mycoplama haemolamae*) (Correa *et al*. 2011) have been described in SACs in their original habitats in South America.

Haemonchus spp. is the most common parasitic infestation in grazing ruminants in tropical and subtropical areas (Gatongi *et. al*. 1998). In warm areas of Argentina, it is the main anemia-causing agent in sheep and young cattle (Guzman *et al.* 2010). *Haemonchus* spp. is a blood-sucker which removes proteins and red blood cells from the blood stream. The chronic phase of the disease is characterized by weakness, anemia and progressive emaciation. Massive infestation affects fleece, milk, ova and sperm production, metabolism, development and maintenance of immunity. The presence of adult *Haemonchus contortus* (Barber Pole Worm) has been described in the 3rd compartment (C3) of llamas and alpacas in USA (Fowler, 1998), but haemonchosis in SACs has never been reported. This is the first report that documents clinical manifestations of anemia associated with *Haemonchus* spp. present in naturally infected llamas from Argentina.

MATERIALS AND METHODS

Farm Management

In a rainy spring of 2010 (October), a llama breeding farm, located in Entre Rios province (central-east of Argentina), reported 4% mortality (5 animals) among pregnant females and mothers with 3 to 5 -month-old crias which were born in perfect conditions. Female llamas grazed a natural pasture paddock, crossed by a creek. They did not share the paddock with male llamas or any other species. Preventive veterinary treatment of the entire llama herd included: deworming 3 times a year with 1% doramectin (Dectomax, Pfizer) and 10% fenbendazole (Axilur, Intervet, Argentina) at double body weight dose. A prevention protocol was completed with an anti-clostridial vaccination 4 times a year (T5 Plus, Sanidad Ganadera, Argentina) that includes *Clostridium* (*C*) *chauvoei*, *C. welchii* C and D, *C. septicum*, *C. novyi* and *C. sordellii*. The area is subject to a rainfall pattern with long periods of rain during October to March, with an annual rainfall average of about 100 mm.

Clinical syndrome and sampling

More than 30% of the herd showed clinical symptoms. Affected animals presented with conjunctival pallor, discolored coat, edema in the submandibular area, weakness, lack of exercise tolerance, sternal recumbency and emaciation. Sick animals had been treated with long-acting oxytetracycline (Oxitetraciclina LA, Bayer Laboratories, Argentina), but did not show any noticeable improvement. A quantitative copro-parasitological analysis conducted previously to our visit showed negative results. A slightly similar picture was observed 6 months ago during the rain fall, but affected animals recovered during the dry winter. A weak and recumbent pregnant female llama was euthanized with sodium pentobarbital (Euthanyle, Brouwer Laboratories, Argentina) and a complete necropsy was performed. Liver, kidney, intestine, lung, gastric compartments 1, 2 and 3 (C1, C2 and C3, respectively), spleen, lymph nodes and brain samples were collected and stored in buffered 10% formalin for further histopathology studies. C3 was removed and the contents were saved in a 10-L plastic bucket. For qualitative and quantitative parasitic analysis, an individual fecal sample was collected prior to euthanizing the animal. Also, fresh fecal samples were collected directly from the pasture and pooled. All samples were refrigerated until arrival at the laboratory. Additionally, blood samples were collected by jugular venipuncture (Fowler 1998) with 5-ml EDTA-coated Vacutainer tubes ^{TM} (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) from 3 affected llamas. One drop of blood was smeared on a slide, fixed with methanol and dried at room temperature (22°C).

Laboratory studies

Samples for histopathology studies were processed according to the standard procedures for paraffin embedding, sectioned at 5-µm thickness, stained with hematoxylin and eosin, and examined under light microscopy. The fecal egg output was determined according to the modified McMaster technique for cattle and sheep (Robert and O'Sullivan 1949). Briefly, five grams of the feces were placed in a mortar and suspended in 100 ml of saturated solution of sodium chloride (specific gravity 1.2). Samples were disintegrated using a pylon and contents were poured through a tea strainer to a beaker. A magnet was placed on the magnetic stirrer. The supernatant was removed by aspiration and transferred to a counting chamber (modified McMaster chamber INTA) that has 4 cells of 0.5 cc capacity each. Eggs per gram were observed under light microscopy (100X).

The parasites recovered from C3 were processed following previously described techniques (Ueno and Goncalves 1988). The parasites were observed under a microscope (100X) and identified according to the recommendations of Lukovich (1968) and Niec (1968). For hematocrit determination, collected blood was centrifuged at 1,500 rpm for 10 minutes at room temperature. The blood smear was stained with Giemsa and observed at 40X magnification using a light microscope.

RESULTS

Post-mortem studies

The euthanized llama showed hydrothorax, hydropericardium and ascites with digestive and respiratory mucosa of pearly white color. The content in the three gastric compartments was fluid, and C3 content showed self-directed movement when transferred to a plastic bucket due to the abundant worms present. C3 rugae were edematous and *Haemonchus* parasites were observed on the mucosal surface (Fig. 1). Moreover, the liver was yellow and slightly firm, and the blood was pale red and watery. Histological studies of the C3 mucosal layer identified helminthes in dilated mucosal glands (Fig. 2). Other histological changes were observed in the liver and spleen. The liver sample showed centrilobular necrosis with leukocytic infiltration while the spleen had red pulp atrophy.

Laboratory analysis

The range of hematocrit values was between 4 and 16%. Hematology studies showed marked erythrocyte pleomorphism, reflecting an expanded erythropoiesis to compensate for the loss of red blood cells. No hemoparasites or intra- or extra- erythrocytic microorganisms were found. The fecal egg counts were 300 eggs per gram (epg) and 360 epg in the euthanatized llama feces and the pooled fecal samples, respectively. One sample collected from a diarrheic llama contained 18,360 epg. Oocysts were also present in fecal samples. The oocysts per gram (opg) counts ranged from negative in the euthanatized llama to 620 opg on pasture. Analysis of the C3 content detected 6000 adults and 160 pre-adults of *Haemonchus* spp., and 160 adults of *Ostertagia* spp. Tapeworms were concomitantly identified in fecal samples collected from the pasture.

DISCUSSION

The recovery of adult's worms from the C3 of one llama with clinical signs of anemia constitutes to our knowledge, the first report of *Haemonchus*-induced anemia in llamas in Argentina. The presence of nematodes

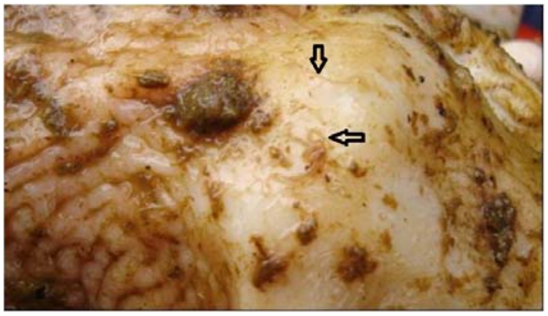

Fig. 1: *Haemonchus* spp. parasites on the mucosal surface of C3 (arrows).

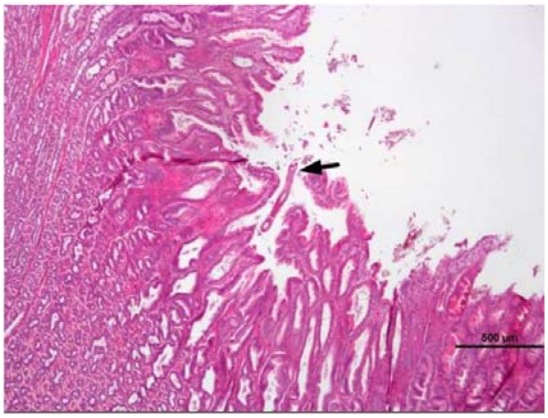

Fig. 2: Histological section of C3 layer, showing helminthes in the glands (arrow). H/E, 400X.

from genera *Haemonchus* was previously reported in SACs (Fowler 1998; Cafrune et al. 2001; Baldomenico et al. 2003), but those publications indicated unexpected findings without clinical manifestations of disease. In this study, hematological and fecal analysis indicated a large-scale herd infestation. *Haemonchus* spp. was found to be the most prevalent gastrointestinal nematode in this study. This result agrees with previous reports in cattle from tropical and subtropical regions worldwide (Gatongi *et. al*. 1998). Llamas bred under farming conditions have to adapt to many different situations such as housing, management, restricted access to pasture, environmental conditions, overcrowding and forced proximity to humans. Some of these are stressful situations that immunocompromise the animals and decrease their inherent resistance to external agents. This conclusion is consistent with the situation presented in this study, showing severe clinical manifestations in pregnant females with young crias, corresponding to the last stage of lactation for yearling llamas and late pregnancy, a period currently associated with stress for the females (van Rijswijk and Vorster 1995), subjecting the animals more susceptible to infection (Cichón *et al*. 2002). The overcrowding and overgrazing of the pasture, plus the watercourse that crossed the paddock allowed heavy parasite development. The annual rainfall recorded in 2010 tended to be lower than the one registered in 2009 (109.41 mm and 88.3 mm, respectively; Dirección de Hidráulica, Pcia de Entre Rios). However, this could influence the vegetation growth over the studied period, critical for nematode transmission (Gatongi *et al*. 1998). Altogether, these causes may have favored a high larvae density of parasites on the female´s grazing pastures, leading to the observed clinical manifestations of disease. In addition, some third-stage *Haemonchus* larvae ingested earlier may persist in stomach glands in a hypobiotic state due to climate adversity. Under favorable environment conditions (optimal temperature and moisture), parasites reactivate their development, mature synchronically and emerge in large numbers from the mucosa (Gatongi *et al*. 1998). This simultaneous maturation of large numbers of previously hypobiotic larvae could cause acute disease, such as a rapid onset of anemia and hypoproteinemia and even death in heavily infected animals, sometimes death may ensue even before the maturation of the worms. This fact may explain the negative epg counts found in previous copro-parasitological analyses, in agreement with other literature which states that the fecal egg counts alone cannot be used as a gauge of severity of the disease (Fowler, 1998).

Treatment information for *Haemonchus* spp. in SACs is scarce. In this report, llamas were routinely dewormed with 0.02 to 0.04 mg/kg of doramectin. Sarre *et al*. (2012), reported doramectin resistance in a 2-year-old alpaca stud infested with *H. contortus* in Belgium. In that case, the authors reported 1000 epg even after two treatments with 0.3 to 0.6 mg/kg of doramectin (Sarre *et al*. 2012). In other reports, gastrointestinal nematodes, including *Haemonchus contortus*, showed resistance to avermectins, benzimidazoles and moxidectin on farms from USA (Gillespie *et al.* 2010). Some publications (Caracostantogolo *et al*. 2005) and personal reports from local veterinarians and technicians mentioned a rising doramectin and ivermectin resistance in ruminants from neighboring areas. Studies regarding the possible presence of doramectin – resistant *Haemonchus* recovered from C3 of the euthanized llama are currently being performed.

In conclusion, knowledge about parasite agents affecting SACs is crucial to achieve appropriate management for successful llama production. Stressful conditions derived from farming management, production stress and long-term treatment with suboptimal doses of antiparasitic drugs were concomitant causes that favored the herd infestation. Our report highlights the importance of *Haemonchus* spp. as an anemia-inducing etiological agent in llamas.

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