

Posterior paralysis in a Holstein cow with Enzootic Bovine Leukosis[□]

Parálisis posterior en una vaca Holstein con Leucosis Enzoótica Bovina

Paralise posterior numa vaca holandesa com Leucose Enzoótica Bovina

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Summary

A 6 year-old Holstein cow was euthanized after a 3 week course of progressive paraplegia. In spite of the increasing difficulties to rise and walk, the animal remained bright, alert, afebrile and with good appetite throughout most of the clinical course. Complete blood counts, biochemical profiles and analysis of the cerebrospinal fluid were reported within normal limits. Antibody was detected for bovine leukaemia virus using an enzyme-linked immunosorbent assay, supporting a tentative diagnosis of bovine leukosis. Post-mortem examination revealed a localized form of lymphosarcoma with few 2-5 cm nodular tumors confined to the walls of the gastrointestinal tract, particularly in the abomasum. In addition, soft grey tumors were found within the vertebral canal surrounding the lumbar spinal cord and associated nerve roots. Microscopic examination revealed the nodular masses were composed of neoplastic lymphocytes. Mass in the lumbar vertebral canal had extradural neoplastic lymphocytes infiltrating connective tissues around the spinal cord and spinal nerve roots. Unlike the more common chronic and wasting presentation of the disease with widespread lymphadenopathy, the rapid progression of the disease to total paraplegia in this animal could be explained by the localized presence of tumors in the spinal canal.

Key words: bovine leucosis, linfadenopathy, paraplegia, tumour.

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Resumen

Una vaca Holstein de 6 años de edad fue sacrificada después de un curso de paraplejía progresiva de 3 semanas de duración. Apesar de las crecientes dificultades para levantarse y caminar, el animal se mantuvo alerta, sin fiebre y con buen apetito casi todo el transcurso de la enfermedad. Los análisis del hemograma, perfil bioquímico y líquido cefalorraquídeo no mostraron alteraciones fuera del rango normal. El análisis de ELISA frente al virus de la Leucosis Bovina resultó positivo, apoyando el diagnóstico diferencial de leucemia. El examen post-mortem reveló una forma localizada de linfosarcoma, con escasas y pequeñas tumoraciones nodulares de 2-5 cm confinadas a las paredes del tracto gastrointestinal, sobre todo en el abomaso. Además, los tumores estaban presentes en el canal vertebral rodeando la médula lumbar espinal y raíces nerviosas adyacentes. El examen microscópico reveló que las masas nodulares estaban compuestas de linfocitos neoplásicos; igualmente, el tejido conectivo rodeando la medula lumbar presentaba gran infiltración de dichos linfocitos. A diferencia de la presentación más corriente y crónica de la enfermedad en que existe un desgaste progresivo del animal asociado a una linfadenopatía generalizada, en este caso la rápida progresión de la enfermedad hacia una paraplejía total se podría explicar por la presencia de tumores localizados en el canal espinal.

Palabras clave: leucosis bovina, linfadenopatía, paraplejía, tumor.

Resumo

Uma vaca holandesa de seis anos de idade foi abatida após um curso de paraplegia progressiva de três semanas de duração. Ainda que a vaca teve muitas e crescentes dificuldades para levantar-se e caminhar, o animal se manteve alerta, sem febre e com bom apetite quase todo o transcurso da doença. A análise do resultado de um hemograma, do perfil bioquímico e do líquido cefalorraquiano não mostraram alterações fora do padrão normal. A análise feita pelo teste de ELISA para o vírus da leucose bovina foi positivo, apoiando o diagnóstico diferencial de leucemia. O exame post-mortem revelou uma forma localizada de linfosarcoma, com escasas e pequenas tumoraciones nodulares de 2-5 cm confinadas às paredes do trato gastrointestinal, principalmente no abomaso. Além, os tumores estavam presentes no canal vertebral rodeando a medula espinal na região lombar e as raízes nervosas adjacentes. A avaliação microscópica revelou que as massas nodulares estavam compostas de linfócitos neoplásicos; igualmente, o tecido conjuntivo ao redor da medula espinal na região lombar apresentava uma grande infiltração desses linfócitos. A diferença deste caso com a forma mais comum e crônica da doença na qual existe um desgaste progressivo do animal associado a uma linfadenopatía generalizada, foi que neste caso houve uma rápida progressão da doença que evoluiu para uma paraplegia total que poderia explicar-se pela presença de tumores localizados na medula espinal.

Palavras chave: leucose bovina, linfadenopatía, paraplegia, tumor.

Introduction

The classification of bovine lymphoproliferative neoplasms has been widely accepted to fall into one of four distinct forms based on age and site of tumor development (Angelos and Thurmond, 2009). The adult multicentric form is also known as Enzootic Bovine Leukosis (EBL). The other three are grouped under the term sporadic bovine leukosis (SBL) and include the juvenile multicentric, thymic, and cutaneous forms. The cause of EBL is bovine leukemia virus (BLV), a retrovirus that integrates itself into the genome of bovine B cells with the potential to induce a lymphosarcoma and/or a persistent lymphocytosis

(Yin *et al.*, 2003). EBL affects cattle over 3 years of age and the clinical signs may vary depending on the location and development of neoplasia. Most reports describe a progressive loss of weight, decreased milk production, anorexia, diarrhea or constipation, which is associated with a generalized lymphadenopathy (Ohshima *et al.*, 1980; Reed, 1981; Thompson *et al.*, 1993). Cases with a history of posterior paralysis have been mostly reported in younger cattle with SBL (Grimshaw *et al.*, 1979; Doige, 1987; Oliver-Espinosa *et al.*, 1994) but it does not appear to be the main complain in adult cows with EBL. The following clinical case describes the presentation of progressive posterior paresis and ataxia caused by EBL.

Patient's examination

Anamnesis and clinical findings

A 6-year-old and 730 kg nulliparous Holstein female with an implanted rumen fistula for the collection of ruminal fluid was initially observed by the caretakers with slight ataxia and urinary incontinence. Upon physical examination by the veterinarian, a differential diagnosis list was made which in order of decreasing probabilities included: spinal cord lesion by a vertebral abscess or infection, parasitic reaction following deworming protocols, limphosarcoma, delayed neuropathy by neurotoxicants, and rare but possible forms of rabies, botulism, and bovine spongiform encephalopathy. Summarizing the clinical course, the slight ataxia progressed to paresis, paraplegia, and complete ataxia over a 21 day period, culminating in humane euthanasia.

Treatment schedule

During the first week, medical treatment with thiamine (3 mg/kg/day IM for 5 days, and dexamethasone (20 mg/day IM for 5 days, resulted in transient improvement followed by relapse and continued progression in severity of clinical signs. According to the daily observations by caretakers during the first 2 weeks, the animal was not febrile, remained bright, alert, and showed normal appetite and defecation. However, on the third week she became anorectic and had severe difficulty for rising and walking, making painful grunts whenever attempts were made to stand up or lay down. By the end of the third week, the cow was unable to stand on her hind legs and had complete loss of sensation of the posterior extremities. Haematological parameters and analysis of cerebrospinal fluid (collected by cistern puncture) did not reveal any abnormalities (Table 1). The animal was euthanized on day 21 after onset of clinical signs using an intracisternal overdose of xylazine, followed within minutes by lidocaine. Serum was then collected and submitted for the detection of antibodies against BLV envelope glycoprotein gp51 and was found positive (Table 1) using an enzyme-linked immunosorbent assay (Kit Svanova, Biotech AB, Sweden).

Table 1. Results of blood, serum and cerebrospinal fluid tests on a 6-year old Holstein cow with enzootic bovine leukemia*.

| Parameter | Result | Normal Range |
|--|-----------------|---------------------|
| Total leukocytes (x 10 ³ /μl) | 7.41 | 4.5 – 13.0 |
| Lymphocytes % (absolute x 10 ³ /μl) | 45 (3.00) | 40 – 60 (2.5 – 7.5) |
| Neutrophils % (absolute x 10 ³ /μl) | 49 (3.59) | 15 – 55 (0.6 – 4.0) |
| Monocytes % | 5 | 3 – 15 |
| Eosinophils % | 1 | 1 – 15 |
| Basophils % | 0 | 0 |
| Bands % | 0 | 0 |
| Red Blood Cells (x 10 ⁶ /μl) | 8.68 | 5.0 – 10.0 |
| Hemoglobin (g/dl) | 13.9 | 8.0 – 15.0 |
| Hematocrit (%) | 43.8 | 24 – 46 |
| Platelets (x 10 ³ /μl) | 299 | 230 – 690 |
| Total proteins (g/dl) | 7.0 | 6.0 – 8.0 |
| Creatinine (mg/dl) | 1.1 | 0.5 – 1.1 |
| Blood urea nitrogen (mg/dl) | 6 | 6 – 22 |
| Urea (mg/dl) | 12.8 | 12.8 – 47.0 |
| Glucose (mg/dl) | 68 | 50- 78 |
| Alkaline phosphatase (U/L) | 53 | 26 – 78 |
| Aspartate aminotransferase (U/L) | 199 | 57 – 108 |
| ELISA† (bovine leukaemia virus) | + (S/P = 95.5%) | Negative <15% |
| Cerebrospinal fluid | | |
| pH | 8.0 | |
| Specific gravity | 1.004 | |
| Color | Cristal clear | |
| Lymphocytes/neutrophils | Absent | |
| Protein | Traces | |

*Blood sample was collected on day 14 from the onset of clinical signs, except for the analysis of cerebrospinal fluid and BLV antibodies that were obtained on the day of euthanasia.

†ELISA = enzyme-linked immunosorbent assay (Kit Svanova Biotech AB, Sweden).

Findings at necropsy

Gross post-mortem findings revealed the presence of 4 to 6 small nodular masses, ranging in size from 1 to 5 cm, which were embedded in the walls of the small intestines but did not seem to adhere to adjacent tissues and did not appear to have perforated the mucosa or caused visible ulcers, or areas of haemorrhage. On cut surface, these masses were cream-colored with scarce areas of superficial haemorrhage and had a semi-firm consistency. The abomasal wall had several similar nodules as well as areas of diffuse wall thickening associated with an irregularly shaped eroded reddened mucosa. The adipose tissue surrounding segments of the lumbar spinal cord was interspersed with darker grey firm areas and small haemorrhages.

The mediastinal and mesenteric lymph nodes examined were not enlarged nor were other abnormal lymph nodes detected by gross examination. No masses were observed in other organs including the spleen, liver, brain, lungs, heart and kidneys.

Nodular masses and sections of the spinal cord were fixed in 10% formalin, embedded in paraffin, sectioned at 4 μm , and stained with hematoxylin & eosin. There was a diffuse infiltration of neoplastic lymphocytes in the abomasal submucosa and muscular layers (Figures 1, 2 and 3). Histological examination of the tissue surrounding the dura mater of the lumbar spine revealed abundant infiltration of pleomorphic lymphocytes (Figure 4).

The neoplastic cells aggregated as space occupying infiltrations of the connective and adipose tissue in extradural portions of the affected segments of the spinal cord and spinal nerve roots. Focal haemorrhages were present within neuropyle of the spinal cord, with mild axonal swelling observed in ventral cord horns as well as in spinal nerves. The changes were considered to be the result of pressure from the space-occupying neoplastic masses within the vertebral canal.

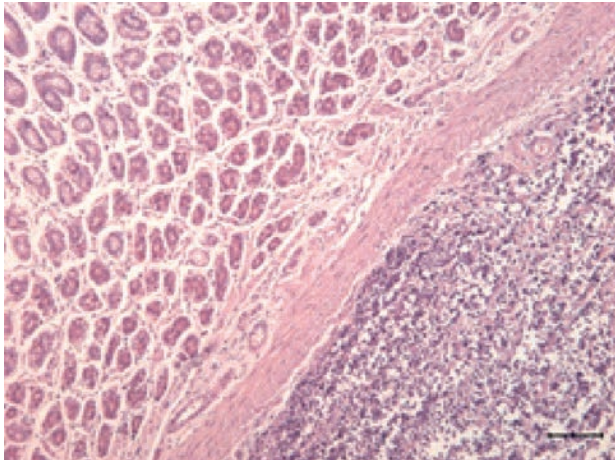


Figure 1. Bovine enzootic leukosis. Diffuse infiltration of neoplastic lymphocytes in the abomasal submucosa (Bar = 100 μm).

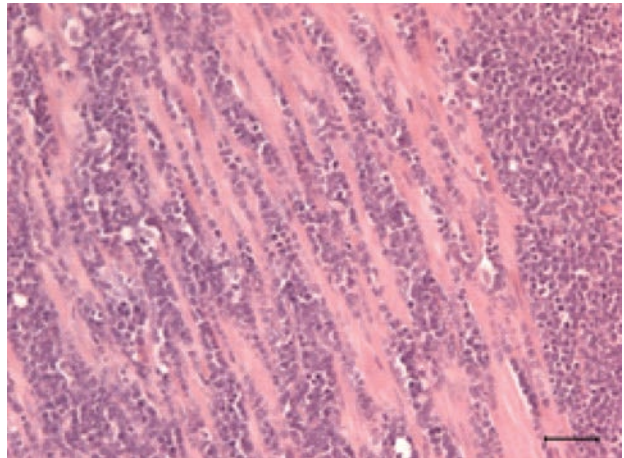


Figure 2. Enzootic Bovine Leukosis. Neoplastic cells infiltrating the abomasal muscular layer (Bar = 50 μm).

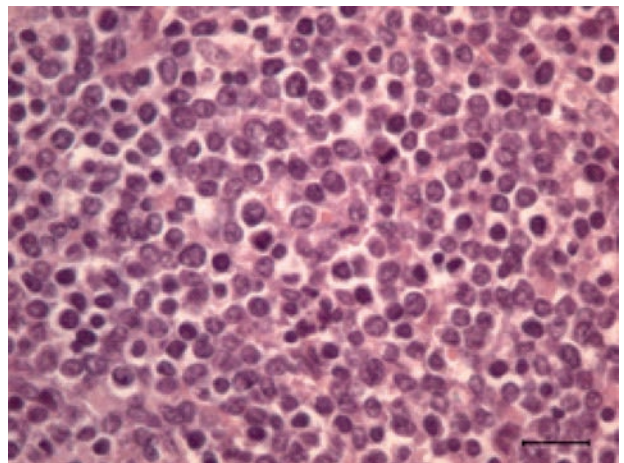


Figure 3. Enzootic Bovine Leukosis. Tumoral masses are characterized by the presence of well differentiated lymphocytes interspersed by lymphoid cells with big nuclei containing chromatin lumps, scarce cytoplasm, and few mitotic figures (Bar = 20 μm).

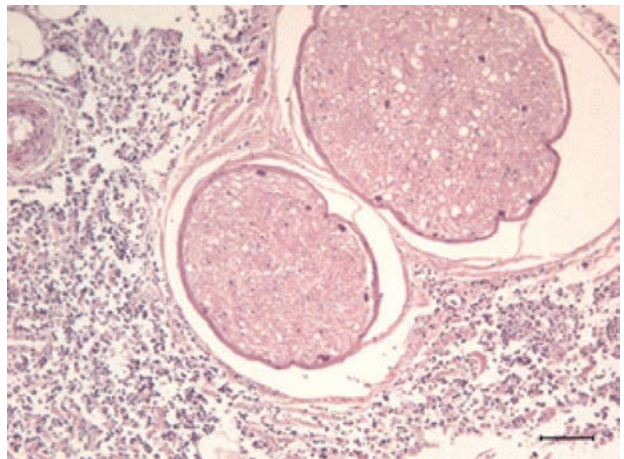


Figure 4. Nerve roots at the lumbosacral intersection. There are diffuse accumulations of neoplastic lymphocytes dilating myelomeninges of the cauda equina. Swollen axons are present within nerve bundles (Bar = 100 μm).

Discussion

Neither clinical nor paraclinical examinations confirmed the diagnosis during the clinical course of the disease in this animal. Approximately 30% of cows that seroconvert to BLV develop a persistent lymphocytosis (Angelos and Thurmond, 2009), but this animal was not leukemic and peripheral lymphocyte counts were within the normal range ($2.5 - 7.5 \times 10^3 \mu\text{L}$). In these cases, it has been shown that a careful morphological examination of the blood smear may distinguish between atypical mononuclear cells from other normal lymphocytes, thus providing an early diagnosis (Ohshima *et al.*, 1980). The present cow had very few and small lesions detectable at necropsy for what would be expected with typical EBL, and they could have been easily missed by a quick and superficial rectal palpation.

In a pathology study (Ohshima *et al.*, 1982) of 13 bovines euthanized in the early stages of EBL based on persistent lymphocytosis and/or atypical mononuclear cells, but no definitive enlargement of the lymph nodes, it was found that the histologic lesions preceding the tumor detection were marked follicular hyperplasia accompanied by atypical lymphoblastic cells in the sinuses and paracortical areas of the lymph nodes. In the current case, it is likely that insufficient time elapsed for tumors to grow-up to a size of antemortem clinical detection because of the rapid progression of the spinal cord compromise and posterior paralysis. Careful microscopic observations of lymph node biopsies may have revealed early generalized neoplastic infiltrations without gross enlargement of the lymph nodes, but was not performed in this case.

This cow came from a dairy herd of 250 cows in the high tropics of Antioquia, Colombia. Routine serological screening of aborted cows in this herd revealed that 6 out of 10 cows were seropositive for BLV during 2009. This is important because several reports have evaluated the economical implications of different rates of BLV infection in dairy herds (Pelzer, 1997; Rhodes *et al.*, 2003). For a prevalence of 50%, herds with 100 cows are predicted to have 0.66 cases of lymphosarcoma per year, with an expectation of 1-2 cases every 2 years (Rhodes *et al.*, 2003). However, the mean

annual cost of a subclinical infection due to premature culling and loss in milk production at 50% prevalence was estimated at \$6,400 (Rhodes *et al.*, 2003; Thurmond *et al.*, 1985). Considering that various epidemiological studies in major dairy areas of Colombia are quoting prevalence ranging between 21 and 62% (Alfonso *et al.*, 1998; Betancur *et al.*, 2008) basic control programs would likely be of significant economical benefit to producers.

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