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CASE REPORT
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Marek's Disease in Roosters in the Potiguar Semi-arid Region

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ABSTRACT

***Background*:** Marek's disease (MD) is a transmissible disease in chickens caused by *Gallid alphaherpesvirus* 2 (GaHV-2). The infection is characterized by lymphocyte cellular infiltrates in peripheral nerves and other organs and tissues, including the skin; which can lead to dysfunction causing progressive asymmetric paresis and complete spastic paralysis of body extremities. Dermatitis and cardiac myositis caused by GaHV-2 in free-range chickens has rarely been described in Brazil. This reports the occurrence of the disease with a confirmatory molecular diagnosis in free-range poultry showing signs of dermatitis, poor performance, and cachexia and no mortality in the semi-arid Potiguar region.

***Cases*:** Twenty roosters of the Shamo lineage, among a brood of 42 birds, had a history of progressive weight loss and skin lesions. Two birds with poor body condition, erythema, and scaling of the skin in the head and cervical regions were sent for clinical care. All birds were between 12 and 18 months of age and were vaccinated against Newcastle disease and Fowlpox with only a few receiving vaccines against MD and Gumboro disease. According to the owner's report, some birds were previously kept outdoors, and when they were transferred to a small shed with little air circulation, they began to develop clinical signs after approximately 15 days. The first signs of the disease were also reported to have appeared 2.5 months before clinical care and, in the meantime, several treatments were instituted without success. Owing to the general condition of the animals and inconclusive clinical suspicion, the birds were subjected to euthanasia and necropsy. Tissue samples were collected for histopathological and polymerase chain reaction analyses to search for the GaHV-2 DNA *meq* gene. The main clinicopathological findings were erythema (47%, 20/42) and desquamation of skin and mild, prominent white multifocal areas in the heart. Histopathology revealed infiltration of pleomorphic lymphoblastic cells in the skin, heart, and sciatic nerve. The amplification of the *L-meq* and *meq* oncoprotein genes in these organs and in the liver, confirmed the infection by GaHV-2, consistent with that of a field strain.

***Discussion*:** MD was confirmed based on the macroscopic and histological lesions, and with the detection of GaHV-2 DNA in the affected tissues. The unusual clinical presentation represented an initial challenge for diagnosis. The clinical history was important to lead to the suspicion of MD, as roosters initiated clinical signs 15 days after they were transferred to a small shed with poor air circulation. This probably favored the high viral concentration and disease transmission among susceptible birds in the brood because the feather follicle is the primary site of viral replication for transmission; and desquamation of infected epithelial cells favor airborne horizontal transmission to susceptible chickens. The roosters had not been vaccinated against MD, which probably favored the infection, as vaccination is known to be a fundamental approach for MD control for effective growth of the poultry industry. Clinical findings and lesions, together with viral molecular detection, were fundamental for the diagnosis, a premise for the application of adequate prevention and control measures for the disease in breeding. This is the first report of MD with a confirmatory molecular diagnosis in northeastern Brazil.

Keywords: viral diseases, *Gallid alphaherpesvirus* 2, neoplasms, polymerase chain reaction, histopathology.

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INTRODUCTION

Marek's disease (MD) affects chickens and is characterized by cellular infiltrates of the lymphocyte lineage in peripheral nerves and several other organs and tissues [4]. The disease is transmissible and caused by *Gallid alphaherpesvirus* 2 (GaHV-2) [12].

The signs associated with MD vary according to specific syndromes. Birds with lymphomas in multiple organs may be clinically normal or lethargic and comatose before death. Nonspecific signs such as weight loss, pallor, anorexia, and diarrhea can be observed, especially in birds where the disease course is prolonged [12].

Some animals can become persistently infected with GaHV-2 without developing clinical disease; therefore, the diagnosis must consider clinical signs, macroscopic or microscopic lesions and virus detection [12]. The use of polymerase chain reaction and immunohistochemistry in the lesions, considerably improve the possibility of correlating the lesion with the etiological agent [4].

Skin lesions are not reported frequently. Some authors reported the occurrence of subclinical MD in vaccinated free-range poultry, with tumor lesions in different organs and skin thickening, but without description of neoplastic cells in the latter [1].

MD is studied globally in poultry producing countries. However, studies in Brazil are scarce [15], especially in the northeast region of the country. This paper report cases of MD in roosters with unusual clinical signs of the disease, describe and discuss the clinicopathological information related to the management of the rearing, which in association with molecular tests, allowed the confirmatory diagnosis of the disease in semi-arid region of Brazil.

CASES

Two roosters (*Gallus gallus domesticus*) were taken to the Veterinary Hospital of the Universidade Federal Rural do Semi-Árido, located in the semi-arid Potiguar region. The history was of progressive emaciation and skin lesions in the thoracic limbs, which according to the owner, were initially nodular and later became ulcerated and crusted. The 20 affected birds had similar clinical signs and belonged to a brood of 42 roosters of the Shamo lineage. All birds were between 12 and 18 months of age and were vaccinated against Newcastle disease and Fowlpox with only a few receiving vaccines against MD and Gumboro disease. According to the owner's report, some birds were previously kept outdoors, when they were transferred to a small shed with poor air circulation, they began to develop clinical signs after approximately 15 days. The first signs of the disease were also reported to have appeared 2.5 months before clinical care. Based on this, several unsuccessful treatments were instituted, including antimycotics, multivitamins, topical phytotherapy, and antitoxic.

The clinical evaluation revealed poor body condition (score 2/5), erythema, and scaling of the skin, especially in the head and neck region, and in one rooster, there was a diffuse loss of feathers in the head and neck region, and a cyanotic comb. Whole blood samples were collected for blood count and serum biochemistry, which revealed hypocalcemia, hypophosphatemia, and hyperuricemia. During clinical care, a parasitological examination of the skin was performed by direct evaluation of the feathers and of skin scrapings from the lesions, under a white light microscopy, which were negative for ectoparasites. Considering the general condition of the animals and the inconclusive clinical suspicion, the roosters were euthanized and submitted to necropsy for *post mortem* examination.

During necropsy, sections of the skin, heart, liver, intestines, and sciatic nerves were aseptically collected and stored at -20°C for further DNA extraction and molecular testing. In addition, sections of 0.5 cm in thickness, of the same organs were collected and fixed in 10% formalin for histopathological examination. After 72 h of fixation in 10% formalin[1], the cleaved tissues were dehydrated in increasing concentrations of alcohol[1], cleared in xylene[1], and embedded in paraffin to obtain 4 µm-thick sections, which were stained with hematoxylin[2] and eosin[3] for analysis at light microscopy.

On macroscopic evaluation, the main lesions were restricted to the skin, heart, and liver. Chickens had a retracted, dehydrated, and cyanotic comb, diffuse aptheria in the head and neck region associated with erythema and skin exfoliation (Figure 1A), in addition to apteria on the dorsal surface of the wings associated with multifocal erythema and multifocal areas of yellowish-white thickening of the skin. In the epicardium, there were multifocal to coalescing millimetric white areas, slightly elevated, which extended into the myocardium (Figure 1B). In addition, there was mode-

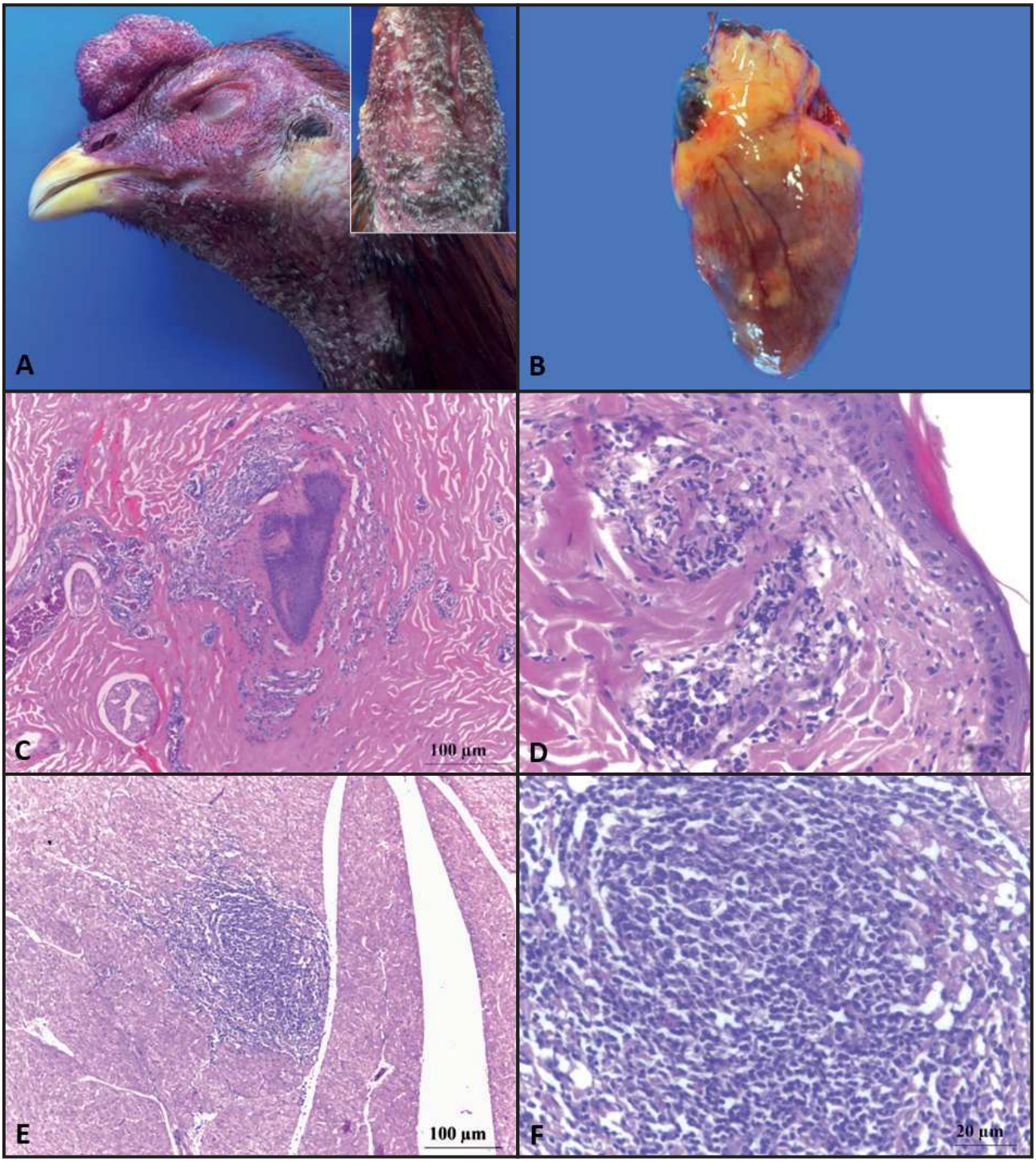

Figure 1. A 1.5-year-old Rooster. A- Skin of the head and neck region with aptheria, scaling, and erythema. B- Heart: left ventricular epicardium with yellowish-white multifocal to coalescing areas slightly elevated. C- Skin: infiltrates of cells identified as lymphocytes and lymphoblasts surround a feather follicle. Fibrosis in the dermis [HE]. D- Skin: infiltrate of lymphocytes and some lymphoblasts in the dermis, thinning of the epidermis with degenerating cells and orthokeratotic hyperkeratosis [HE]. E- Heart: focal infiltrate of pleomorphic lymphoblastic cells in a circumscribed pattern [HE]. F- High magnification of the lesion presented in (E) showing pleomorphic lymphoblastic cells [HE].

rate dilatation of the right atrium and afferent vessels. In the liver, there was moderate hepatomegaly owing to congestion and slightly elevated multifocal areas on the surface and parenchyma. The intestinal serosa and mesenteric vessels were also moderately congested. In one chicken, there was a large number of nematodes in the small intestine and cecum corresponding to *Ascaridia* sp. and *Heterakis* sp., respectively.

Histopathological examination of the skin revealed multifocal atrophy of feather follicles associated with moderate infiltration of pleomorphic lymphoblastic cells, which was also seen around the

follicles. Dermal vessels were moderately hyperemic and there was moderate thickening of the collagen layer in the dermis (Figure 1C). Furthermore, multifocal lymphoblastic cell infiltrates with moderate to intense pleomorphism and mild to moderate multifocal lymphoplasmacytic infiltrates in the dermis (Figure 1D) were observed. In the myocardium, multifocal to coalescent areas with loss of cardiomyocytes and moderate to intense infiltrates of pleomorphic lymphoblastic cells were observed, which were organized in a diffuse or circumscribed pattern (Figure 1 E & F).

Minimal perivascular infiltration of lymphoblastic cells with mild pleomorphism was observed in the epineurium of the sciatic nerves. In the liver, there was marked diffuse hyperemia, sinusoidal dilatation, and moderate proteinosis. In the small intestine serosa, there were multifocal granulomas with eosinophil infiltration, and in the cecum, eosinophil infiltration was associated with the presence of eggs and larvae of *Heterakis gallinarum*.

To search for GaHV-2 genetic material, the total DNA was extracted from tissue fragments [5,16]. The extracted DNA was analyzed for quantity and purity using a Nanodrop spectrophotometer[4] and then stored at -20°C. Total DNA was subjected to polymerase chain reaction for the detection of GaHV-2 genome using previously described oligonucleotide primers [11], which amplify the *L-meq* gene (763 bp) and the *meq* oncoprotein gene (583 bp), thus differentiating pathogenic field strains from vaccine strains.

GaHV-2 *L-meq* gene and *meq* oncoprotein gene DNA were detected in the skin, heart, liver, and sciatic nerve samples, and genetically related to virulent field strains.

DISCUSSION

MD was confirmed based on the macroscopic and histological lesions, and with the detection of GaHV-2 DNA in the affected tissues. The unusual clinical presentation represented an initial challenge for diagnosis. To the best of our knowledge, this is the first report of MD with a confirmatory molecular diagnosis in Northeastern Brazil.

The birds belonged to the Shamo breed, which are considered sports-type birds with combatant characteristics [7]; however, according to the owner, the roosters described in this report were bred for breed conservation purposes. Roosters had not been vaccinated against MD, which probably favored the infection, although cases of the disease have been reported recently in vaccinated birds in Brazil [1]. Vaccination is known to be a fundamental approach for MD control for the growth of the poultry industry [15], and in Brazil, vaccination against the disease is mandatory in commercial chickens, and is administered on the 18th day of incubation or on the 1st day of life [6]. However, the sanitary control of birds in extensive systems is less rigorous than in commercial systems, increasing the risk of disease [1]. In a study on the occurrence of MD in chickens raised in conventional and alternative production systems, Fossum *et al*. [9] observed the occurrence of MD only in the latter.

MD is considered highly contagious, and the feather follicle is the primary site of viral replication for transmission, through the desquamation of infected epithelial cells, enabling airborne horizontal transmission to susceptible chickens [13]. Increased skin desquamation was the most evident clinicopathological change in the roosters in the present study, in which GaHV-2 DNA was detected. According to the clinical history, roosters initiated clinical signs 15 days after they were transferred to a small shed with poor air circulation, which probably favored the high viral concentration and disease transmission among susceptible birds in the brood.

Another aspect regarding cutaneous lesions refers to the accentuated infiltration of lymphoblasts in the feather follicles with invasion of the dermis, and consequently, the formation of ulcerations [12]. Lymphoblastic infiltrations can lead to the suspicion of fowl pox [10], which was the initial suspicion in the birds in this report, as based on the history, and revealed by histopathology. Histopathological analysis of the skin revealed lesions consistent with MD, centered in the dermis and replacement of the feather follicle structures causing atrophy of the follicle and epidermis [14].

In the classic form of MD, the pathogenesis is mainly related to the capacity of neoplastic transformation of T lymphocytes [12] and the characteristic (classical) changes occur after lymphoblastic infiltration in peripheral nerves, mainly sciatic, brachial, and vagus nerves. These infiltrations induced axonal loss and degeneration, leading to unilateral leg paralysis with the consequent position of the extended pelvic limb(s), which is a classic clinical sign of MD. This clinical sign was not observed in the roosters reported

here, wherein the birds did not show any degree of pelvic limb paralysis, which may be related to the fact that the lymphocyte infiltrate was observed only in the epineurium, without associated axonal lesions. However, GaHV-2 DNA was detected in the sciatic nerves, suggesting its presence be associated to aggregates of infiltrated lymphocytes. Cutaneous involvement without peripheral nerve damage has been previously reported in broiler chicken [8], layer chicken, and quail (*Coturnix coturnix japonica*) [3].

The viral DNA was also detected in the heart, in which the macroscopic and microscopic changes observed corroborated the diagnosis of MD, as lymphoid tumors associated with this disease are common in the heart [2]. Microscopically, MD virus-induced lymphomas are characterized by infiltration of pleomorphic lymphoid cells into the pericardium or myocardium [2], as observed in this case.

This is the first report of MD with the molecular diagnosis of GaHV-2 in Northeastern Brazil. The epidemiological data and anatomopathological changes observed demonstrate the importance of molecular and histopathological analysis for the diagnosis, as the roosters did not present peripheral neurological signs. In this case, the detection of GaHV-2 DNA contributed to confirmatory diagnosis, showing that the association of diagnostic techniques can be valuable to confirm the occurrence of MD. Diagnosis will enable the application of appropriate prevention and control measures. Furthermore, the detected virus was genetically related to virulent field strains, highlighting the importance of vaccinating the new generations of susceptible chickens.

MANUFACTURERS

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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