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Severe Outbreak of Avian Encephalomyelitis in Laying Hens in Northeastern Brazil

ABSTRACT

This paper describes the epidemiological, clinical and pathological aspects of an avian encephalomyelitis (AE) outbreak in commercial laying hens in Northeastern Brazil. The disease affected 23,409 layers (from day one until 100 weeks) leading to mortality of 16.5% (5,049/30,600) of the flock. Twenty laying hens were clinically examined, and blood samples were collected and tested by Enzyme-Linked Immunosorbent Assay (ELISA). Five laying hens with neurological signs were necropsied and samples of the brain, cerebellum, spinal cord, sciatic nerve, heart, ceca tonsils, proventriculus and liver were collected, fixed in formalin buffered solution and processed according to routine methods for histological examination. Clinical signs were observed in approximately 60% of the chicken flock and included depression, ataxia, head and neck tremors, head tilt, motor incoordination and corneal opacity and of lens. All laying hens presented seroconversion for AE, with a geometric mean titer (GMT) of 6,854 and CV of 9.6%. At necropsy, there were bilateral opacity of the cornea and lens and the blood vessels of the cerebral cortex were hyperemic. The main microscopic lesions in the central nervous system consisted of red and shrunken neurons in the cerebral cortex, cerebellum and spinal cord. Significant areas of gliosis were observed in the cerebral cortex and in the molecular layer of the cerebellum. Hyperplasia of the proventricular lymphoid tissue and the ceca tonsils were observed with different degrees of intensity. In Northeastern Brazil, there haven't been previous reports of AE in commercial laying hens and this outbreak possibly occurred by inefficient immunization of laying breeders at the genetic supply company.

INTRODUCTION

Avian encephalomyelitis (AE) is caused by avian encephalomyelitis virus (AEV), classified within the family Picornaviridae, genus *Tremovirus* which exhibits tropism by central nervous system of chickens, turkeys, pheasants, quails and pigeons (Tannock & Shafren, 1994; Bakhshesh *et al.*, 2008). This disease was first described in the United States in 1930 by Jones (1932), who observed head tremors in chicks, and was later described in Europe, Canada, Japan and Australia (Tannock & Shafren, 1994). In Brazil, the disease was described in 1964, in broilers from the state of São Paulo (Bueno *et al.*, 1964) and later in Rio de Janeiro and Espírito Santo in 1972 (Silva *et al.*, 1972). Since then, AE was apparently controlled due to the use of vaccination. Recently, 58 confirmed cases of this disease in southern Brazil, between 2011 and 2013, has concerned the poultry industry (Freitas & Back, 2015).

The strains of avian encephalomyelitis virus (AEV) are antigenically uniform, but there is variation in tropism and virulence, which can



be enterotropic with horizontal transmission or neurotropic with vertical transmission (Martins & Silva, 2009). The infection by the AEV occurs mainly through the fecal-oral route and its replication takes place in the intestinal epithelial cells. The virus enters the bloodstream via Peyer's patches and lymphatic vessels, reaching other organs, such as the pancreas, heart, proventriculus and the central nervous system (Tannock & Shafren, 1994), causing clinical disease in susceptible chicks, usually aged between 1 and 35 days (Calnek *et al.*, 1997).

Clinical signs consist mainly of staggering gait, weakness, apathy and inability to stand up, followed by depression, ataxia, head tremors, and lens opacity (Back, 2010). Macroscopically lesions are difficult to visualize, although pale and punctiform areas can be found in the proventriculus and gizzard muscles (Calnek, 2008). Microscopically, the main lesion consists of nonsuppurative encephalomyelitis (Silva *et al.*, 1972; Tannock & Shafren, 1994).

There is no treatment for AE (Martins & Silva, 2009). Vaccination of meat and egg-type commercial breeders during the rearing phase is the most effective and economically viable tool to control the disease, since the humoral immunity transmitted to the progeny confers ensures the protection of the chicks against the challenges of the field (Martins & Silva, 2009).

In Brazil, more recent cases of AE were described in broilers up to 35 days of age (Freitas & Back, 2015). No AE outbreak was described in the Northeast of Brazil so far. This study aimed to describe the epidemiological, clinical and pathological aspects of an outbreak of avian encephalomyelitis in commercial laying hens in the Northeast of Brazil.

MATERIAL AND METHODS

Twenty laying hens of the affected flocks were clinically evaluated, and clinical signs were noted. Blood samples were collected from the ulnar vein in tubes without anticoagulant to obtain serum and subsequent serological analysis for avian encephalomyelitis. Serum samples were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) to determine antibody titers for AE by means of a commercial kit (IDEXX®) used according to the manufacturer's recommendations, wherein the cutoff value is 397.

Five adult laying hens presenting severe neurological signs were euthanized and necropsied. From these birds, samples of the brain, cerebellum, spinal cord, sciatic nerve, heart, colon, cecal tonsil, proventriculus

and liver were collected. After that, the samples were fixed in 10% formalin buffered solution and processed according to routine methods, included in histological paraffin, stained with hematoxylin and eosin and analyzed microscopically.

RESULTS

The outbreak occurred in a commercial laying hens farm located in the mesoregion of the Agreste of Pernambuco (semiarid region), Northeastern Brazil. The farm had a total of 350,000 birds divided in flocks. The breeding and rearing flocks are in a specific sector, away from the production sector, and all biosecurity measures are practiced. In this phase, the selection, beak trimming and vaccination process takes place. All flocks were immunized with obligatory vaccines required by the supervisory agencies, as well as immunized against AE and Bouda at 10 weeks of age.

The disease affected 76.5% of the flocks composed of about 30,600 birds since stage of chicks (1 day to 6 weeks), pullets (7 to 17 weeks) until laying hens' phase (older than 20 weeks).



Figure 1 – Clinical signs of avian encephalomyelitis in commercial laying hens in Northeast Brazil. (A) Bird in laying hen phase (43-week-old) exhibiting opacity of the cornea and lens. (B) Laying hen with motor incoordination characterized by hyperextension of pelvic members. (C, D) Laying hens with tremors, imbalance and difficulty in standing.

All 20 adult laying hens tested for AE presented seroconversion, with a Geometric Mean Titer (GMT) of 6,854 and CV value of 9.6%. In the flock consisted of chicks and pullets, 16.5% (5,049/30,600) presented clinical signs consisting of muscle tremors, motor incoordination and consequent starvation caused by



difficulty in water and feed intake, which evolved to spontaneous death.

In the laying hens' flocks, the major clinical signs consisted of depression, ataxia, opacity of the cornea and lens, and approximately 60% (18,360/30,600) of the flock was affected presenting bilateral opacities of the cornea and lens, tremors of head and neck, lateral deviation of the head and motor incoordination with falls towards the sides (Figure 1). In these laying hens, ataxia ranged from mild incoordination to lateral decubitus. There were cases with discreet neurological signs that consisted of mild head tremors throughout the production phase.

No significant changes were observed during the necropsy of adult birds. Microscopic findings in the central nervous system consisted of hyperemia from the blood vessels of the leptomenigeal and cerebral cortex. The neurons of the cerebral cortex, midbrain, and cerebellum had an eosinophilic, shrunken aspect and intensely basophilic, thin, sometimes pyknotic or karyolytic nuclei. Some neurons in the cerebral cortex and mesencephalic nuclei presented pyknotic nuclei, sometimes marginalized, and pale cytoplasm with no defined borders (ghost cells). In the cerebellum, there was also central chromatolysis of the Purkinje neurons (Figure 2A) and in both the gray matter of the cerebral cortex and the molecular layer of the cerebellum there was neuronophagia, lymphocytic inflammatory infiltrate in the perivascular spaces and significant microglial proliferation, both in nodular and diffuse form, sometimes containing astrocytes (Figure 2B). Focally diffuse areas of malacia containing macrophages, fibrillar gliosis and diffuse mononuclear inflammatory infiltration were also observed in the cerebral cortex (Figure 2C). In the spinal cord, neurons with central chromatolysis were also observed. Other lesions mainly characterized by hyperplasia of the lymphoid tissue of the proventriculus and of the lymphoid tissue associated with the mucosa of the ceca tonsils were observed with varying degrees of intensity. In these organs, there was still significant mononuclear infiltrate in the nervous ganglions of the mioenteric plexus (Figure 2D). Only in one of the layers hens, a discrete lymphocytic infiltrate was visualized in the myocardium.

DISCUSSION

The diagnosis of AE in laying hens from this study was based on epidemiological data, clinical signs, seroconversion and microscopic findings characteristic

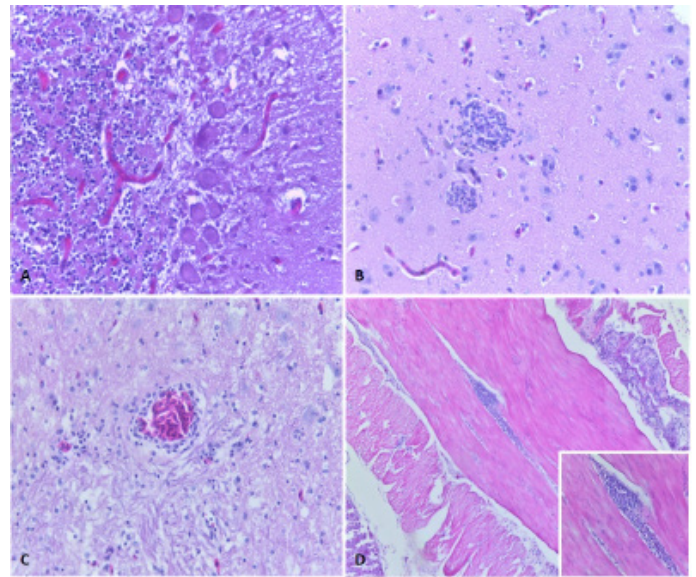


Figure 2 – Encephalomyelitis in commercial 43-week-old laying hen in Northeast Brazil. (A) Cerebellum with Purkinje neurons exhibiting central chromatolysis. Hematoxylin-Eosin, obj. 40x. (B) Cerebral cortex containing necrotic neurons and nodular microgliosis. Hematoxylin-Eosin, obj. 10x. (C) Cerebral cortex with focal areas of malacia, fibrillar gliosis and perivascular cuff. Hematoxylin-Eosin, obj. 10x. (D) Mononuclear inflammatory infiltrate in the nervous ganglions of the mioenteric plexus from proventriculus. Hematoxylin-Eosin, obj. 10x. *In set*, higher magnification of the previous image where a typical lymphocytic infiltrate is observed. Hematoxylin-Eosin, obj. 40x.

of the disease, like those reported in previous studies (Tannock & Shafren, 1994; Jana *et al.*, 2005; Asasi *et al.*, 2008; Freitas & Back, 2015). Histopathological findings observed in the central nervous system were characterized mainly by neuronal chromatolysis and necrosis, gliosis, neuronophagia and perivascular cuffs, indicating virus encephalitis, suggestive of AEV (Calnek, 2008; Martins & Silva, 2009; Freitas & Back, 2015).

Although birds of all ages can be affected by AEV, in this study birds with less than four weeks of age presented clinical signs, which was also observed by Jana *et al.* (2005) and Villareal (2009). In many reported cases of AE in adult birds, the main problem is related to the temporary drop in egg production of laying hens (5 to 10% of reduction) (Tannock & Shafren, 1994; Martins & Silva, 2009). However, the neurological signs were observed in chickens of all ages in the present outbreak. The presence of neurological signs in old birds is an uncommon finding and may have occurred due to viremia during the rearing or laying hens phases. The high ELISA titers with 100% of seroconversion supports this findings.

So far, in Brazil, there are no recent reports of AE in commercial laying hens. However, AE was described in several broiler farms in the Southern of Brazil showing that the AEV had spread in a short time between 2011 to 2013 in that region (Freitas & Back, 2015) and in



Bahia, high seroconversion was reported in hens that were tested by ELISA (Cruz *et al.*, 2014). In Alagoas and Pernambuco, cases of AE were also observed in broilers by our research group (Unpublished data). Considering these data, it is assumed that the AEV is circulating in the Brazilian poultry population. Thus, the possibility of the emergence of new strains of *Tremovirus* for which there is no cross-immunity with the vaccination currently used should be better investigated (Freitas & Back, 2015). However, it is more likely that the cases of AE described in this study are due to mistakes during the vaccination process of the light fowl that do not allow adequate transmission of immunity to the progeny resulting in clinical disease.

The occurrence of flocks of breeders that were certainly vaccinated, whose progeny present clinical signs of encephalomyelitis, may be related to the rearing period where there are practices of food restriction, clinical and subclinical coccidiosis, enteritis and other adverse conditions that cause immunosuppression and can affect the result of vaccination in terms of protection (Martins & Silva, 2009).

In general, the absence of AE in poultry farms is mainly due to the wide use of live or inactivated vaccines that are effective for disease prevention (Villareal, 2009). However, with the occurrence of recent cases of this disease in the country and because the virus has great stability in the environment, leading contaminated areas to be infected for long periods, poultry producers and veterinarians should be aware of suspected cases.

Other viral diseases, such as Newcastle disease and Marek disease, should be considered in the differential diagnosis of AE. In Marek disease, lymphocytic infiltration is observed in peripheral nerves and could have also lymphoid infiltration in the organs (Martins & Silva, 2009). In Newcastle disease, birds infected with mesogenic and neurotropic velogenic viruses present neurological signs including muscle tremors, limb paralysis and torticollis. Microscopically the lesions consist of lymphoid necrosis and nonsuppurative encephalomyelitis with neuronal degeneration, gliosis, perivascular cuffs, and endothelial cell hypertrophy. These lesions usually occur in the cerebellum, brainstem, spinal cord (Alexander, 2003).

Based on the clinical, serological and pathological findings, it was possible to diagnose an outbreak of avian encephalomyelitis in commercial laying hens from breeders, poorly immunized against this disease. This is the first report of an outbreak of avian encephalomyelitis in Northeastern Brazil.

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