Central nervous system syndrome associated with Marek's disease in Hubbard broilers

Syndroom van het centrale zenuwstelsel geassocieerd met de ziekte van Marek bij hubbard-vleeskuikens

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ABSTRACT

Marek's disease (MD) has a high economic impact in poultry production worldwide. MD consists of several pathologic syndromes of which the most commonly encountered are lymphoproliferative syndromes such as acute MD. Sporadically, MD leads to central nervous system syndromes characterized by non-neoplastic brain pathology. In this case report, an onset of neurological signs on two different broiler farms, housing 'slow-growing' Hubbard JA 757 broilers, is described. On the first farm, clinical signs were observed from 42 days of age onwards and on the second farm, respectively from 42 (stable 1) and 30 days (stable 2) of age onwards. The neurological symptoms consisted of acute central nervous system signs, which started with flaccid neck paralysis. Histopathological examination revealed multifocal perivascular cuffing of CD3 positive mononuclear cells in the brain tissues. These findings pointed towards the development of transient paralysis. qPCR analysis confirmed the diagnosis. In broiler flocks, which only have a limited life span, vaccination is not common practice. In flocks of slow-growing breeds, outbreaks of MD might become more important again. Therefore, vaccination of slow-growing broilers against MD seems recommended.

SAMENVATTING

De ziekte van Marek (MD) heeft een grote economische impact op de pluimveeproductie wereldwijd. MD bestaat uit verschillende pathologische syndromen, waarvan de meest voorkomende lymfoproliferatieve syndromen zijn, zoals acute MD. Sporadisch leidt MD tot syndromen van het centrale zenuwstelsel die worden gekenmerkt door niet-neoplastische hersenaandoeningen. In het voorliggende casusrapport worden de neurologische symptomen op twee verschillende vleeskuikenbedrijven beschreven, waar langzaam groeiende hubbard JA 757-vleeskuikens werden gehouden. Op het eerste bedrijf werden de klinische symptomen waargenomen vanaf een leeftijd van 42 dagen en op het tweede bedrijf op een leeftijd van 42 dagen (in stal 1) en 30 dagen (in stal 2). De neurologische symptomen bestonden uit acute tekenen in het centrale zenuwstelsel met initieel slappe nekverlamming. Histopathologisch onderzoek onthulde multifocale perivasculaire cuffing van CD3-positieve mononucleaire cellen in het hersenweefsel. Deze bevindingen wezen op de ontwikkeling van transiënte paralyse. qPCR-analyse bevestigde de diagnose. Bij koppels vleeskuikens, die slechts een beperkte levensduur hebben, is vaccinatie niet gebruikelijk. In koppels van langzaam groeiende rassen kunnen uitbraken van MD weer belangrijker worden. Daarom lijkt vaccinatie van langzaam groeiende vleeskuikens tegen MD aanbevolen.

INTRODUCTION

Marek's disease (MD) is a disease with a high economic impact in poultry production worldwide (Payne and Venugopal, 2000). Chickens are exposed to Marek's disease virus (MDV) around the globe and the virus is present in a large proportion of flocks (Dunn and Gimeno, 2013). The economic losses are due to lower production losses such as lower feed conversion rates, weight loss, condemnations of carcasses at slaughter and higher mortality rates (Rozins et al., 2019). Indirect economic impact is related to vaccination costs, maintaining high biosecurity levels and by inducing immunosuppression, which makes chickens more susceptible to secondary infections (Gimeno and Schat 2018; Rozins et al., 2019).

MD is caused by a strictly cell-associated virus, belonging to the genus Mardivirus in the subfamily Alphaherpesvirinae of the order Herpesvirales. There are three Marek's disease virus (MDV) serotypes, grouped in three species: Gallid herpesvirus 2 (serotype 1), Gallid Herpesvirus 3 (serotype 2) and Meleagrid herpesvirus 1 (serotype 3) (ICTV, 2020). Serotype 1 Marek's disease virus strains are oncogenic and, based on their virulence, divided into pathotypes (mild MDV, virulent MDV, very virulent MDV and very virulent plus MDV). Serotype 2 strains are non-oncogenic. Both serotypes have been isolated in chickens (Schat and Nair, 2009). The third serotype has been isolated in turkeys and is also non-oncogenic (Gimeno, 2008).

MD consists of several pathologic syndromes (Calnek, 2001) of which the most commonly encountered are lymphoproliferative syndromes. Acute MD, the most frequently reported lymphoproliferative syndrome, is characterized by the formation of lymphomas in visceral organs, such as liver, spleen and kidneys. Affected chickens show non-specific symptoms, such as anorexia, weight loss, depression, diarrhea and (sudden) death. Histologically, these tumors are dominantly composed of proliferating T-lymphoblasts and T-lymphocytes (Schat and Nair, 2009).

Chickens affected with fowl paralysis syndrome present peripheral nerve dysfunction due to lymphoproliferative lesions in the nerves. Depending on which nerves are affected, chickens show different symptoms. Paralysis of the legs and wings is a common clinical sign, but dysfunction of parts of the gastrointestinal and respiratory tract can also occur when the N. vagus is involved. Affected nerves are enlarged and histopathologic examination shows infiltration of T-lymphocytes (Schat and Nair, 2009).

Additional manifestations with lymphoproliferative components are skin leukosis and ocular lesions. In skin leukosis, the feather follicles are affected with nodular lesions leading to possible condemnation at slaughter in broiler chickens. Lymphoproliferative infiltration of the iris results in gross ocular lesions, including loss of pigmentation and pupil irregularities (Calnek, 2001).

Figure 1. Two chickens showing flat paralysis of the neck.

Although seldomly encountered in the field, also non-lymphoproliferative syndromes associated with MD have been described. Lymphodegenerative syndromes, resulting in increased diseases susceptibility, are only characterized by degenerative lesions and inflammation. Also vascular syndromes have been reported to lead to atherosclerosis (Fabricant et al., 1978). Other features of MD, sporadically encountered, are central nervous system syndromes which are characterized by non-neoplastic brain pathology (Kenzy et al., 1973; Gimeno and Witter, 1999)

In this case report, a clinical feature of central nervous system syndrome associated with Marek's disease in Hubbard JA757 broilers originating from two different broiler farms, is described.

MATERIALS AND METHODS

Case history

On a first farm (farm 1), housing 90.000 Hubbard JA 757 broilers divided over six stables, neurological signs were observed from the age of six weeks onwards. Diseased chickens were encountered in three stables. Three months later, in a second farm (farm 2) located nearby (10 kilometer), housing 22.500 Hubbard JA 757 broilers divided over two stables, an onset of the same clinical signs were noticed at 30 (stable 1) and 46 days (stable 2) of age. Slaughter of the chickens on both farms was planned between 50 and 53 days of age. The chickens housed at the first farm were bought from hatchery Morren BV (Lunteren, the Netherlands). The birds housed at the second farm originated from hathery Probroed (Meppel, the Netherlands). Affected birds showed an acute onset of central nervous system (CNS) signs with typically flaccid paralysis of the neck (limber neck) (Figure 1). In addition, paresis and unstable gait were

encountered. The number of affected birds increased slowly with 0.05% per day. Mortality rate was not increased. The birds were vaccinated on day 1 against infectious bronchitis with Poulvac® QX (Zoetis, Zaventem, Belgium) and Poulvac® IB primer (Zoetis, Zaventem, Belgium), against Newcastle disease with Nobilis® ND Clone 30 (MSD AH, Boxmeer, the Netherlands) and on farm 1, also against coccidiosis with Evant® (Hipra, Ghent, Belgium). On day 14, the birds received booster vaccination against Newcastle disease with Avinew® Neo (Boehringer Ingelheim, Elsense, Belgium) and on day 18, the birds were vaccinated against infectious bursal diseases virus with Cevac® IBD L (Ceva, Brussels, Belgium).

Necropsy

At 46 days of age, five broilers from farm 1, presenting neurological signs as described above, were delivered at the Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University for postmortem examination. Necropsy procedures were as follows: the birds were euthanized by means of cervical dislocation. After external inspection, the birds were weighed, carcasses were plucked, skinned and sternum was removed. Macroscopic investigation of all tissues and internal organs was performed. In addition, cytological examination of lungs, liver, spleen, kidneys en brains was performed using Hemacolor® staining and microscopic examination of smears from the intestinal content was performed for parasitological control. Organs were collected for microbiological and histopathological examination as described below. At 46 days of age, five clinically affected broilers from farm 2 were necropsied on farm. Organs for histopathological examination and polymerase chain reaction (PCR) analysis were collected on farm as described below.

Microbiology

Samples of brains from three birds/farm were cultured using commercial growth media [Columbia agar containing 5% sheep blood (Oxoid, Hampshire, UK), McConkey agar (Oxoid, Hampshire, UK), Briljant Green agar (Oxoid, Hampshire, UK) and Sabouraud agar (Oxoid, Hampshire, UK)] for bacteriological and mycological analysis.

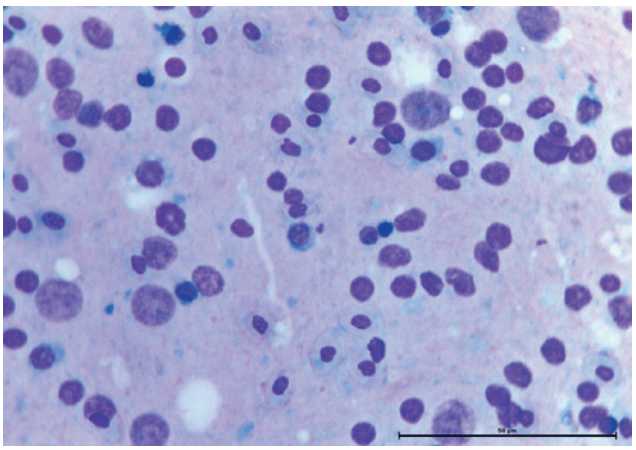

Figure 2. Cytology of cerebellum showing clear presence of mononuclear cells (Hemacolor®, 10 x 100).

Histopathology

Samples of brains and peripheral nerves from three birds/farm were fixed for 24 hours in 10%-buffered formalin and embedded in paraffin. Sections of the tissue (4 micrometer (µm)) were stained with hematoxylin and eosin as well as by a cluster of differentiation 3 (CD3) immunohistochemical staining and microscopically examined.

PCR

Samples of brain tissue from three birds (farm 2) were pooled and sent to a commercial diagnostic lab (Poulpharm, Izegem, Belgium) for quantitative polymerase chain reaction (qPCR) analysis for MDV detection.

RESULTS

Necropsy

During postmortem investigations, specific gross lesions could not be observed in any of the examined birds. Microscopic examination of smears from the intestinal content proved negative for parasites. Cytologic examination of the lungs, liver, spleen and kidney did not reveal any abnormalities. However, cytology of the brain showed clear presence of mononuclear cells (Figure 2).

Microbiology

Bacterial or fungal growth was not observed excluding bacterial or fungal origin of the central nervous symptoms observed.

Histopathology

Histopathological examination of brain tissues revealed a multifocal perivascular cuffing of mononuclear cells (Figure 3A). The cuffing consisted of lymphoid cells and blast-type cells. The latter cells had an indistinct cytoplasm and a round or oval vesicular nucleus containing dark clumped chromatin. Also in the white and grey matter, as in the meninges, a moderate infiltration of mononuclear cells was observed. The mononuclear populations proved CD3 positive (Figure 3B). Mild lymphocytic infiltrations were also encounterd in the *N. Ischiadicus*, which also proved CD3 positive.

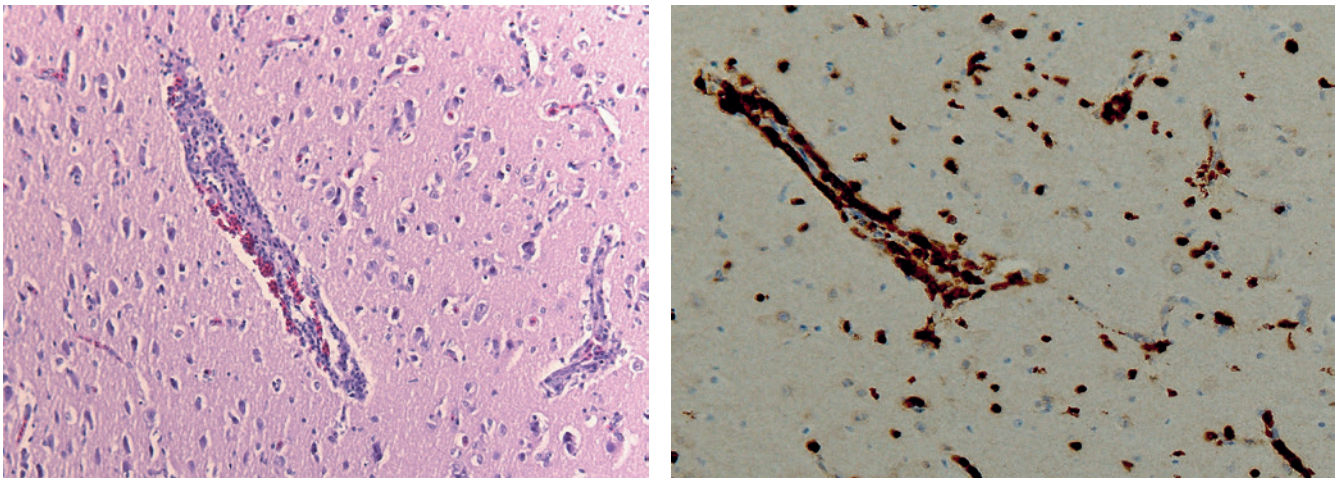

Figure 3. Histopathological examination of brain tissue (cerebrum). A. Perivascular cuffing of mononuclear cells. The cuffing consists of lymphoid cells and blast-type cells (HE®, 10x20). B. The mononuclear population (perivascular and in the grey matter) proved CD3 positive (10x20).

PCR

qPCR analysis proved positive for MDV (Cycle threshold (Ct) value = 26,3).

DISCUSSION

The clinical symptoms encountered in the present case were flaccid paralysis of the neck, paresis and unstable gait. Neurological disorders in broilers are a clinical feature that can be caused by numerous factors, including non-infectious and infectious diseases. With regard to non-infectious causes, clinical signs observed in the flock could be compatible with ionophore antibiotics intoxication or vitamin E/selenium deficiency (Fulton 2013; Klasing, 2013). Since the chickens from farm 1 were produced under a concept broiler label (Gildehoen, 2020), which doesn't allow the use of anticoccidials, ionophore intoxication was very unlikely. Neurological signs can also be caused by several viral, bacterial or mycotic pathogens, e.g. avian influenza (AI), newcastle disease (NCD), avian encephalomyelitis (AE), MD, botulism, *Salmonella*, *Enteroccus sp*., *Aspergillus sp.*, etc. Paralytic signs associated with botulism typically progress cranially from the legs to the neck (Skarin et al., 2013). In this case, initially the neck was affected after which the paralysis became more generalized. Culture of brain tissue proved negative, excluding bacterial or mycotic encephalitis. Also, based on the cytology of the brain, a viral etiology was suspected. With regard to the observed morbidity and mortality rates in the flock, AI and NCD were unlikely (Miller and Koch, 2013; Swayne et al., 2013) and the chickens were too old (46 days of age) for AE (Suarez, 2013). Because of this, MDV as etiological cause was presumed.

Paralysis associated with MDV infection is usually attributed to peripheral nerve lesions with gross enlargement of nerves with lymphoid infiltrates, which is a common feature of MD (fowl paralysis) (Schat and Nair, 2009). However, such lesions in the peripheric nerves were not encountered (macroscopically nor microscopically) in the present case. Yet, histopathological investigations of brain tissue showed multifocal perivascular cuffing of lymphoid and blast-type cells, resulting in vasculitis. Additional CD3 staining characterized the mononuclear population as T-cells. These lesions are characterstic for a rare syndrome involving the brain, resulting in central nervous signs (CNS) associated with infection of MDV and described as transient paralysis (TP) (Zander, 1959; Kenzey et al., 1973).

Field cases of TP have been occasionally reported (Walker and Grattan, 1968; Wight, 1968; Cho et al., 1970; Glavits et al., 1990, Gimeno et al., 1996). Like in the present cases, these reports typically note the development of a flaccid paralysis that initially affects neck muscles and later tends to become generalized. Microscopically, the principal CNS lesion consists of mild, but persistent perivascular cuffing, usually accompanied by gliosis. Clinical signs result from the development of vasogenic brain edema secondary to vasculitis (Cho et al., 1970; Swayne et al., 1988; Swayne et al., 1989b; Gimeno and Witter, 1999).

Widespread vaccination has drastically reduced the incidence of Marek's disease (Gimeno, 2008). Also, clinical TP almost dissapeared after widespread vaccination against MD in the poultry industry in the early 1970s (Witter, 1990). At present, outbreaks are rare and observed in unvaccinated flocks (Glavits et al., 1990; Gimeno et al., 1996). In Belgium, currently eight licensed vaccines are available on the market, which contain the cell-bound, attenuated strain Rispens, the recombinant strain RN1250 or are living recombinant vaccines based on the herpesvirus of turkeys (HVT) in combination with other pathogen(s) (Newcastle, ILT and/or IBDV) (Vetcompendium,

2022). Laying hens and breeders are routinely vaccinated in ovo or immediately after hatch (intramuscularly or subcutaneously), and a combination of the recombinant HVT and Rispens vaccine is advised (WVPA Belgium, 2015). However, in broiler flocks, which only have a limited life span, vaccination is not common practice. The reason for this is that in chickens affected by Marek's disease, clinical signs can appear as early as four weeks of age, but are usually only seen between ten and twenty weeks (Schat and Nair, 2009).

In the current cases, the chickens on the affected farms were slow-growing Hubbard JA 757 broilers (Aviagen, Roermond, the Netherlands), which are slaughtered at ≥ fifty days of age at end weights of 2.2 tot 2.5 kilogram (kg). This in contrast to conventionally kept broilers (Ross or Cobb), which are slaughtered at an earlier age (≤42 days of age). Despite their longer life span, which makes these chickens more prone for the onset of clinical sings, the current flocks were not vaccinated against MDV.

Although in some cases, recovery from paralysis is incomplete (Swayne et al., 1988 and 1989a), most cases report that birds clinically recover completely from TP within 24-48 hours. However, in the present cases, affected birds were culled and the rest of the flock was slaughtered four days after the birds were sent for necropsy. Therefore, the transient feature of paralysis in the affected birds could not be assessed.

After the diagnosis, farm hygiene and biosecurity measures were improved and next flocks were vaccinated against MDV in the hatchery using a HVT-based vaccine (Innovax-ND-IBD®, Intervet int). Since then, new MD outbreaks have not been encountered in next flocks produced on the respective farms. One should keep in mind that, vaccinated birds can still become infected and shed wild-type virus. It is hypothesized that these leaky anti-disease vaccines might lead to an enhanced host survival but not prevent viral shedding. As such, MDV vaccination of hens or offspring prolongs the infectious periods of hyperpathogenic strains and the amount of virus they shed into the environment (Gandon et al., 2001). Selection pressure within-host favoring virulent variants for their ability to evade immunity and vaccine-induced relaxation of between-host selection against virulence could together generate a very potent selection for more virulent strains (Read et al., 2015).

CONCLUSION

Widespread vaccination has drastically reduced the incidence of MD in industrial poultry. Yet, in broiler flocks, which only have a limited life span, vaccination is not common practice. Because of welfare reasons, there's a current tendency to switch production using slow-growing broilers breeds which are slaughtered at older age. In these flocks, outbreaks of MD might become more important again, as illustrated in this case report, in which an atypical clinical feature of MD, resulting in central nervous system syndrome, is described in two farms housing Hubbard JA 757 broilers. Therefore, vaccination against MD of slow-growing broiler flocks seems higly recommended. However, it is crucial to consider if, even with ideal vaccination practices, immunization will be sufficient to protect against strains of increasing virulence in the future.

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