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A COMPARATIVE EPIDEMIOLOGICAL EVALUATION OF TWO SUBSEQUENT EPISODES OF MAREK'S DISEASE ON THE SAME FARM

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Abstract

Marek's disease (MD) is highly economically impacting viral disease in chickens aged 4 weeks - 4 month. Various hypotheses were posed to explain its recurrent epidemiology and increasing virulence over the years. This study aimed at comparing the epidemiological indicators of two confirmed Marek's disease episodes (DIVA Real Time PCR) in two different series of Isa Brown pullets hatched one week apart on the same farm. The data indicated an increasing mortality due to MD from week 6 towards week 14, from 0.28 to 2.72% in the first series and 0.07 to 1.43% in the second one, with a cumulative mortality 3.26% and 2.28% respectively. There were no statistically significant differences between the weight gains of the two series on week 14 (peak of MD mortality), but it decreased in the first series (1398.7g versus 1478.00 g respectively) by week 16. Although the two episodes significantly differed in mortality ($p<0.05$), except a slight variation of the size of the flock, no other circumstances could have been identified as influential causes, the variability being attributed to differences in viral pathogenicity.

Key words: *Marek's disease, Isa Brown pullets, mortality, variability, viral pathogenicity.*

INTRODUCTION

Stressors and the evolution of infectious diseases have a negative effect on the health and welfare of birds raised in intensive farming systems. Hatching, vaccinations, microclimate factors of the halls, food deficiencies affect the innate and adaptive immunity. Infectious diseases resulting in immunosuppression (Gumboro disease, chicken infectious anemia Marek's disease), can be directly correlated with increased susceptibility to viral, bacterial and parasitic diseases thus influencing post-vaccination immunity (Hoerr, 2010) and increased morbidity and mortality with significant economic losses (Lütticken, 1997; Woolhouse, 2011).

Marek's disease (MD) is a disease induced by a virus of the Herpesviridae family (OIE, 2010) which affects chickens, associated with lympho-proliferative syndromes (Baigent and Davison, 2004; 2006) with polymorphic clinical manifestations, and from a lesional point of view characterized by the appearance lymphoid tumors in the spleen, liver and nerves (Calnek, 1979; 2001). Functionally altered lymphocytes can no longer truly support humoral or cell-mediated immunity. It is important to differentiate between enteric viruses, reoviruses, retroviruses, adenoviruses or avian pneumoviruses, which are associated with cell depletion or lymphoid organ atrophy, affecting their function.

In infectious diseases in chickens, the immunosuppressive potential of the etiological agent is poorly characterized (Gong et al., 2013). After establishing the diagnosis by applying specific methods, to control the outbreak, it is imperative to assess the degree of immunosuppression. The effective application of external and internal biosecurity measures, the reduction of stressors, the application of specific and non-specific prophylaxis, the increase of disease resistance could have a major impact on the elimination of these diseases. Moreover, in the case of MD through

genetic selection it would be possible to eliminate this pathology (Hoerr, 2010; Zhang et al., 2015). In this framework, the aim of our study focused on evaluating the developmental model of MD in Isa Brown replacement chicks, hatched at one week difference, coming from an intensive breeding farm.

MATERIALS AND METHODS

The research included two series of Isa Brown chickens, raised on the same intensive raising enterprise, in different chicken houses. The first series (A) included 109,350 birds while the second (B) was somewhat larger, of 110,625 birds. All birds shared the same rearing technology, while the anti-Marek's disease was performed on day 1 (Innovax ILT/HVT + Nobilis Rismavac + Cryomarex Rispens + HVT), in the incubation unit of the farm.

The birds were closely monitored during their technological cycle from week 1 to week 16. The body weight in g was measured and compared to the range limits. Similarly, the body weight range, the growth curve, the weekly mortality, cumulated mortality and feed consumption were recorded.

Marek's disease appeared on week 6 in both series of birds. At necropsy, samples were collected from the nerves, spleens, ovaries, livers, feather pulp and gizzard of the diseased chickens on FTA (Flinders Technology Associates) cards. An FTA card is a chemically treated filter paper designed for the collection, preservation and shipment of biological samples for subsequent DNA and RNA analysis. Special chemicals lyse and inactivate bacteria and viruses and preserve their DNA and RNA for detection by PCR. The swabs collected from various organs need to be pressed against the FTA card. If the procedure is correct after placing the samples on the card, you will notice changes in the color of the card, initially pink, and modifying it to white. The cards are dried at room temperature, heated in the microwave for 20 seconds at 900W. Subsequently, the samples were analyzed by DIVA RT-PCR in the CEVA Phylaxia laboratories in Deventer, the Netherlands, and the histopathological analysis of the samples was performed in the same laboratory.

When the disease episode started, the mortality caused by Marek's disease was also recorded separately, based on the pathological changes noticed at necropsy, while all the other parameters were continuously recorded.

The birds received balanced fodder according to their age, and were watered *ad libitum*.

Preventive and control measures were applied according to the layer replacement technology.

RESULTS AND DISCUSSIONS

Well-known as a viral neoplastic disease of chickens, Marek's disease (MD) is defined by the neoplastic changes at mainly T cell levels, which can reside in immune suppression and also neurological clinical disease. The virus is shed mainly at the feather pulp level, and afterwards is dispersed by the dust particles in the chicken house, the respiratory process leading to initiation of the pathogenesis. Vaccination against Marek's disease, although a wide-spread preventive procedure, does only inhibit the clinical expression, not the shedding of the virus by the infected birds (Boodhoo et al., 2016). Thus, by use of the Rispens (CVI988) vaccine, the infection decreased as clinical prevalence, but not the persistence of the virus on the farm.

Although some researchers believe no major problems appear to be uncontrolled with existing anti Marek's disease vaccination and non-specific preventive technologies worldwide (Morrow and Fehler, 2004), there are some episodes difficult to diagnose and prevent form re-emerging.

On the investigated enterprise, the clinical expression of the disease was recorded as an acute form with birds showing transient paralysis of the limbs. The FTA card results indicated in both episodes, the presence of the Rispens and MDV1 strains in all samples tested by PCR. The histopathology tests revealed an infiltration with small lymphocytes, some lymphoblasts and plasma cells at the level of the sciatic nerve. No changes were observed in the brain of the birds.

Tables 1 and 2 present the descriptive epidemiological indicators of the Marek's disease episodes compared during the research period.

Table 1. Descriptive indicators of the Marek's disease episode A

Age weeks	Morta-lity	Cumu-lated Morta-lity no.	Cumu-lated morta-lity %	Marek morta-lity no	Cumu-lated Marek morta-lity no	Marek morta-lity %
1	560	560	0.51			
2	163	723	0.66			
3	42	765	0.70			
4	48	813	0.74			
5	66	879	0.80			
6	225	1104	1.01	187	187	**0.28**
7	485	1589	1.45	389	576	**0.86**
8	521	2110	1.93	427	1003	**1.49**
9	276	2386	2.18	243	1246	**1.85**
10	362	2748	2.51	282	1528	**2.27**
11	207	2955	2.70	145	1673	**2.49**
12	337	3292	3.01	94	1767	**2.63**
13	221	3513	3.21	42	1809	**2.69**
14	57	3570	3.26	17	1826	**2.72**
15	38	3608	3.30			
16	21	3629	3.32			
Aver	226.81	2140.25	**1.96**	202.89	1290.56	**1.92**
St. dev.	182.65	1206.85	1.10	145.19	591.42	0.88

The data recorded indicated a non-significantly ($p = 0.08$) increased overall cumulative mortality in episode A versus B (1.96 ± 1.10 versus 1.37 ± 0.67, Tables 1 and 2), noting a significant decrease in mortality due to MD in the two episodes (A and B) ($p = 0.01308$), which may occur due to the accommodation of the second series birds to the existing infectious pressure.

Table 2. Descriptive indicators of the Marek's disease episode B

Age weeks	Morta-lity	Cumu-lated Morta-lity no.	Cumu-lated morta-lity %	Marek morta-lity no	Cumu-lated Marek morta-lity no	Marek morta-lity %
1	650	650	0.59			
2	114	764	0.69			
3	38	802	0.72			
4	39	841	0.76			
5	47	888	0.80			
6	81	969	0.88	46	46	**0.07**
7	211	1180	1.07	171	217	**0.32**
8	387	1567	1.42	235	452	**0.67**
9	214	1781	1.61	138	590	**0.88**
10	221	2002	1.81	185	775	**1.15**
11	187	2189	1.98	69	844	**1.26**
12	159	2348	2.12	59	903	**1.34**
13	129	2477	2.24	42	945	**1.41**
14	40	2517	2.28	17	962	**1.43**
15	26	2543	2.30			
16	650	650	0.59			
Aver	199.56	1510.50	**1.37**	106.89	637.11	**0.948**
St. dev.	200.10	742.58	0.67	76.91	334.52	0.50

But a significant decrease in mortality due to Marek's disease in one-week apart two episodes, A and B ($p = 0.01308$), which could stand for the accommodation, to some extent, of the birds of the second series to the infectious pressure existing on the farm. This assumption is supported by the decrease in total death (cumulative mortality due to all causes) by almost 50% from episode A to B.

When compared to the previous literature data (6.0 to 15.3%, Witter et al., 1970; 2005), the values obtained in this survey are significantly lower (0.948-1.92%), maybe due to continuous implementation of vaccination procedures, which diminished the clinical reflection of the disease (Biggs, 2001).

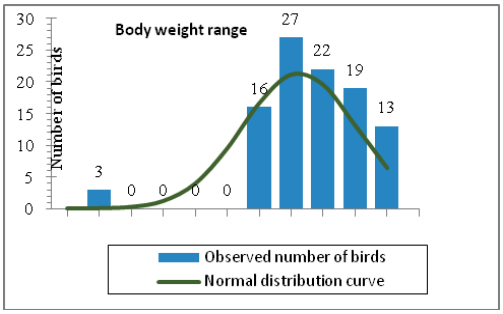

Figure 1. The body weight range recorded during episode A

The descriptors of weight range in the first series of birds are presented in Table 3.

The body weight range indictors were the same for both series, but there were slight differences in the normal distribution curve due to the disease episode.

Table 3. Variables considered to evaluate the body weight range in the first series of birds (A)

Number of birds weighed	100
Mean body weight	1153 g
Mean + 10%	1268 g
Mean – 10%	1038 g
Number of birds between 1,268.399 and 1,037.781 kg	3
Uniformity = ([100-3]/100) x 100 =	97%
Standard deviation	37 g
Coeficient of variation (CV), %	3.24%

In both series, the weight gain was only slightly altered, the difference between the two by the end of the episode in week 14 of the technology being of only 100 g. The more severe episode seemed to be self-limiting in a shorter time (15 weeks) as opposed to the milder one (16

weeks). No investigations on the further laying potential and the possible influence of the Marek's disease development in the two series were carried out.

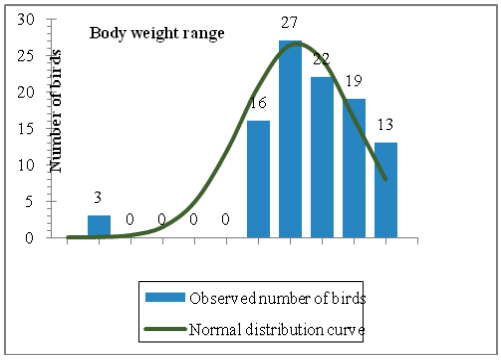

Figure 2. The body weight range recorded during episode B

In this enterprise, in the first six weeks, the infection spread, in spite of the vaccination program implemented on time and according to the recommendations of the vaccine producer, were sufficient to significantly ($p<0.05$) increase the mortality due to Marek's disease in series B, but not in series A, when comparing week 6 with week 7. In both series, the disease caused death for 5 weeks in a row, the number of birds dying of Marek's disease decreasing towards week 11. These observations are supported by the literature, according to which in commercial chicken houses virtually all birds become infected within the first few weeks of their life (CABI Datasheet, 2021). The results of the literature reveal the outbreak of MD disease from 7 to 31 weeks (Bercea, 1981). However, there are other studies that show an increased incidence of MD cases between 3-5 months and unlikely after the age of 8 weeks. The results of the literature reveal the outbreak of MD disease from 7 to 31 weeks (Bercea, 1981). However, there are other studies that show an increased incidence of MD cases between 3-5 months and unlikely after the age of 8 weeks.

More than one MDV strains were observed, including the vaccine Rispens strain in both A and B series. The incidence of non-pathogenic viruses seems to become higher with the increase in age of the birds (CABI Datasheet, 2021).

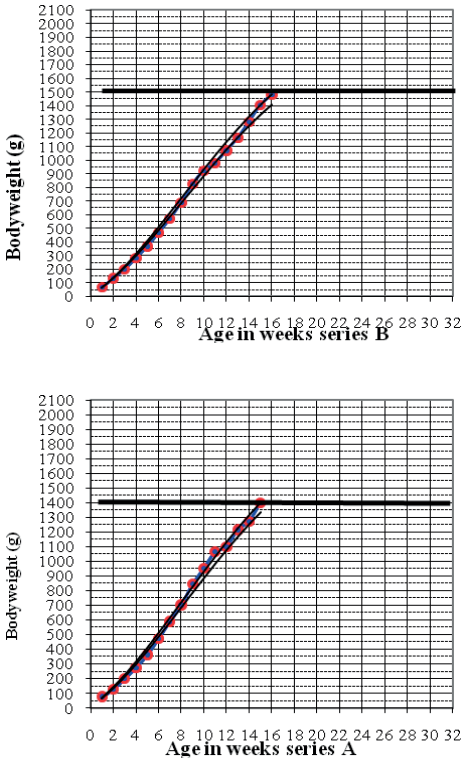

Figure 3. Growth curves for the two series which indicate the impact of the Marek's disease on weight gain in A and B

The etiological agent incriminated in the production of MD is an alpha-herpesvirus, it is a virus with oncogenic potential, it is highly contagious (Beigent et al., 2006). The incidence of this disease together with MD lymphoid leukosis of retroviral nature showed a significant increase between 1930 - 1950, with a negative effect on the birds health and welfare and implicitly with significant economic losses, globally the losses were estimated at 2 billion USD (Morrow and Fehler, 2004).The epithelial cells loaded with virus serve, by respiratory route, to transmit the disease to susceptible birds but also to contaminate the environment, the organic dust remaining infectious for several months. Affected birds could shed the virus throughout their lives, therefore precoucious diagnosis and early measures are of utmost importance. Sometimes transfer via various beetles in the bedding (*Alphitobius diaperinus,* classified in

the *Tenebrionidae* family), could also intervene in transmission (CABI Datasheets).

Due to the transformation into an intensive industry, in the case of poultry farming we are witnessing a reduction in genetic diversity and an increase in susceptibility to various diseases (Nair, 2005). The application of specific immunoprophylaxis in the case of MD is considered a relatively effective method in preventing the occurrence of this pathology (Atkins et al., 2004).Viral infections in chickens with immunosuppressive effects have a major impact on health and the economy. Although the application of specific immunoprophylaxis measures can successfully prevent the occurrence of epidemics, the emerging variants of the virus still cause increasing difficulties in controlling the disease. Because there are differences in susceptibility from a genetic point of view, such an increased level of genetic resistance could provide true means of preventing this disease. The development of genetic maps and the identification of genes that are responsible for resistance can contribute to the development of chicken lines resistant to this virus. Also, the identification of chicken lines based on the post-vaccine response may represent new possibilities for the appropriate selection of vaccination protocols (Bumstead, 1998). For effective prevention of MD, in-depth knowledge of the epidemiology and pathogenesis of the disease is a priority (Atkins et al., 2004). The immune response to MD can be regulated by the haplotype, so selection of the vaccine based on haplotype B is important. It is also important to identify and effectively eliminate stressors, which has a negative impact on the post-vaccination response (Atkins et al., 2004). For the effective control of the disease it is important to combine specific immunoprophylaxis with the increase of genetic resistance by applying appropriate selection programs (Nair, 2005). The results obtained from the study can be partially correlated with data from the literature (Adameşteanu et al., 1980).

Vaccination alone does not manage to control MD, especially if the management on the farm is poor. Infectious MDV present in dander, feathers and litter from infected flocks stays infectious for many months. Correct implementation of biosecurity measures including adequate disposal of cadavers and filthy litter, followed by appropriate disinfection of the chicken houses, strict control of bird and personnel movements help in avoiding the emergence of pathogens with increased virulence. The birds can easily get infected in a contaminated environment when being placed there while their immune system is immature (CABI Datasheet). Furthermore, the vaccination of such birds proves to be inefficient.

The increasing virulence of MDV strains worldwide requires for new specific preventive strategies. The possibility exists, supported by past and current research, for an increasing percentage of changes in virulence due to appearance of new variants. Lack of scientifically sound information for each and every episode of MD and its epidemiology dynamics can lead to further spread and further spatial expansion (Lopez et al., 2015; Lopez et al., 2019).

CONCLUSIONS

Based on the result of this study, it can be concluded that the increased incidence of MD cases is due to the lack of technological gap between different series of chickens. Effective control of MD cases can be ensured by the correct combination of technological and veterinary measures. Techniques that identify the viral pathotypes and allow the consequent monitoring of the vaccination efficacy along with personalized sanitary programs and establish more rigorous vaccination program against MDV and other viral pathogens.

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